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REVIEW

Harnessing the microbiome: a new frontier in lung cancer immunotherapy

Kexin Feng[1,2,3]*, Jun Wang[1,2,3]*, Shuai Wang[1,2,3]*, Zewen Sun[1,2,3], Mantang Qiu[1,2,3], Zuli Zhou[1,2,3], Yun Li[1,2,3], Kezhong Chen[1,2,3]

[1]Department of Thoracic Surgery, Peking University People's Hospital, Beijing 100044, China; [2]Thoracic Oncology Institute, Peking University People's Hospital, Beijing 100044, China; [3]Research Unit of Intelligence Diagnosis and Treatment in Early Non-small Cell Lung Cancer, Chinese Academy of Medical Sciences, Peking University People's Hospital, Beijing 100044, China

ABSTRACT Lung cancer is a fatal and the most common malignancy globally. Despite the significant therapeutic benefits of immune checkpoint inhibitors (ICIs), 60%–80% of patients respond poorly to immunotherapy. The identification of reliable predictive biomarkers is essential for implementing precision medicine strategies. In recent years the microbiome has emerged as a promising predictor of immunotherapy outcomes. The influence of the microbiome on lung cancer immunotherapy was systematically and comprehensively reviewed. Moreover, the distinctive microbiome profiles among patients with lung cancer and the correlation with treatment effectiveness are discussed. We investigated the core mechanisms of the interactions between the microbiome and the tumor microenvironment, assessed the usefulness of microbial metabolites as predictive biomarkers, and discussed strategies for microbiome-targeted interventions. Furthermore, we evaluated the current limitations in research methodology and highlighted future research directions, offering novel insights, including multi-site integrated biomarker approaches, site-specific intervention strategies, metabolite-based functional biomarkers, and lung cancer-specific microbiome considerations for developing personalized immunotherapy.

KEYWORDS Lung cancer; immunotherapy; microbiome; biomarkers; gut-lung axis; microbial metabolites

Introduction

Current status and challenges in lung cancer immunotherapy

Lung cancer ranks as the leading cause of cancer-related mortality worldwide with approximately 2.4 million new diagnoses and 1.8 million deaths projected to occur in 2024[1]. Although immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment paradigms, only 20%–40% of patients with non-small cell lung cancer (NSCLC) receive clinical benefits from immunotherapy with 60%–80% exhibiting suboptimal responses[2,3]. This limited efficacy highlights the need for accurate predictive biomarkers.

Programmed death ligand 1 (PD-L1) expression is the most frequently used predictor of immunotherapy efficacy, despite limited predictive capability[4]. Even among individuals exhibiting high PD-L1 expression, approximately 40%–50% do not respond favorably to ICI monotherapy[5-8]. Other promising biomarkers, such as tumor mutational burden (TMB) and microsatellite instability, have demonstrated predictive potential but face significant challenges in lung cancer applications[9]. The heterogeneity of lung cancer with diverse histologic subtypes and molecular alterations further complicates biomarker development. Therefore, the discovery of novel, reliable biomarkers to enhance patient selection and improve therapeutic precision has become a pressing priority.

Microbiome as an emerging biomarker for immunotherapy

The microbiome encompasses the collective genetic material of microorganisms, including bacteria, fungi, viruses, and archaea, which co-exist in equilibrium with the human body

*These authors contributed equally to this work.
Correspondence to: Kezhong Chen
E-mail: mdkzchen@163.com
ORCID ID: https://orcid.org/0000-0002-9723-6153
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and has been associated with the pathogenesis of numerous diseases[10]. Advanced high-throughput sequencing techniques and cutting-edge bioinformatics have revealed complex relationships between the microbiome and tumor initiation, progression, and therapeutic responses[11-13].

The microbiome has emerged as a promising immunotherapy biomarker owing to several distinct advantages, as follows[14]: **non-invasive sampling**, microbiome specimens can be obtained *via* fecal or salivary samples; **enhanced stability**, microbiome profiles demonstrate greater stability than conventional biomarkers (such as PD-L1 expression and TMB); **dynamic monitoring capability**, longitudinal monitoring indicates changes throughout the treatment course; and **interventional potential**, the microbiome provides potential targets for combined therapeutic strategies.

Gopalakrishnan et al.[15] reported significant associations between the composition of the gut microbiome and successful anti-PD-1 treatment in patients with melanoma and concluded that microbiome modulation enhances immunotherapy outcomes in murine models. Routy et al.[16] subsequently validated these observations in 249 patients with cancer, including 140 patients with NSCLC, by documenting the adverse impact of antibiotics on anti-PD-1 treatment and identifying crucial bacterial species, such as *Akkermansia muciniphila* (AKK), that potentially improve immunotherapy efficacy. More recent investigations have further expanded our understanding of microbiome-immunotherapy interactions in lung cancer. Jin et al.[17] demonstrated a significant correlation between gut microbiome diversity and anti-PD-1 treatment outcomes in a cohort of 100 patients with NSCLC receiving immunotherapy. Peters et al.[18] described distinctive microbiome signatures associated with different histologic subtypes of lung cancer, suggesting microbiome-based stratification strategies. Advanced multi-omics approaches have recently enabled the integration of microbiome data with other biological parameters, further enhancing the predictive power of microbiome-based biomarkers.

Establishing causal relationships requires additional mechanistic studies, germ-free animal model validation, microbial metabolite functional research, and prospective intervention trials. This review will clearly distinguish between associative and causal evidence when discussing microbiome-immunotherapy relationships, providing more accurate guidance for clinical translation.

Multi-site microbiome interaction concept and research evolution

The evolution of microbiome research in lung cancer has undergone remarkable transformations over the past decade, shifting from the traditional "sterile lung" paradigm to the recognition of complex microbial communities within the respiratory system and the interactions with distant sites (**Figure 1**).

Historical development

Early microbiome research in cancer primarily focused on gastrointestinal malignancies, in which direct microbial-epithelial interactions were evident. The respiratory tract, long considered relatively sterile beyond the upper airways, received limited attention until culture-independent sequencing technologies revealed diverse microbial communities throughout the respiratory system[19]. The Human Microbiome Project, launched in 2007, catalyzed interest in mapping microbiome profiles across various body sites, although the lung was initially excluded from this initiative[20].

Dickson et al.[21] challenged the "sterile lung" dogma, demonstrating that the healthy lung contains 10^4–10^5 bacterial cells per gram of tissue. This revelation sparked interest in exploring the lung microbiome in various respiratory conditions, including lung cancer. Early studies by Yu et al.[22] in 2016 documented distinctive microbiome alterations in lung cancer tissues compared to adjacent normal tissues, establishing the foundation for lung cancer microbiome research.

Current research landscape

Contemporary microbiome research in lung cancer has evolved into a multidisciplinary field incorporating microbiology, immunology, oncology, and computational biology. Several key developments characterize the current landscape.

First, the recognition of multi-site microbiome interactions has expanded research beyond single-site analyses. While initial studies focused primarily on gut or lung microbiomes in isolation, current approaches have increasingly examined the interplay between oral, airway, lung, and gut microbiomes in lung cancer pathogenesis and treatment response. This multi-site perspective acknowledges that microbial communities throughout the body form an interconnected ecosystem with collective influence on systemic immunity and cancer progression.

Second, the integration of microbiome data with other biological parameters has enhanced our understanding of microbiome-host interactions. Multi-omics approaches combining metagenomics, metabolomics, transcriptomics, and immunophenotyping provide comprehensive insights into the mechanistic links between the microbiome and lung cancer. These integrated analyses have revealed complex networks of interactions between microbial communities, host immune cells, and tumor cells that collectively influence disease progression and treatment outcomes.

Third, the focus has shifted from descriptive studies documenting microbial alterations to mechanistic investigations exploring causal relationships. Advanced experimental

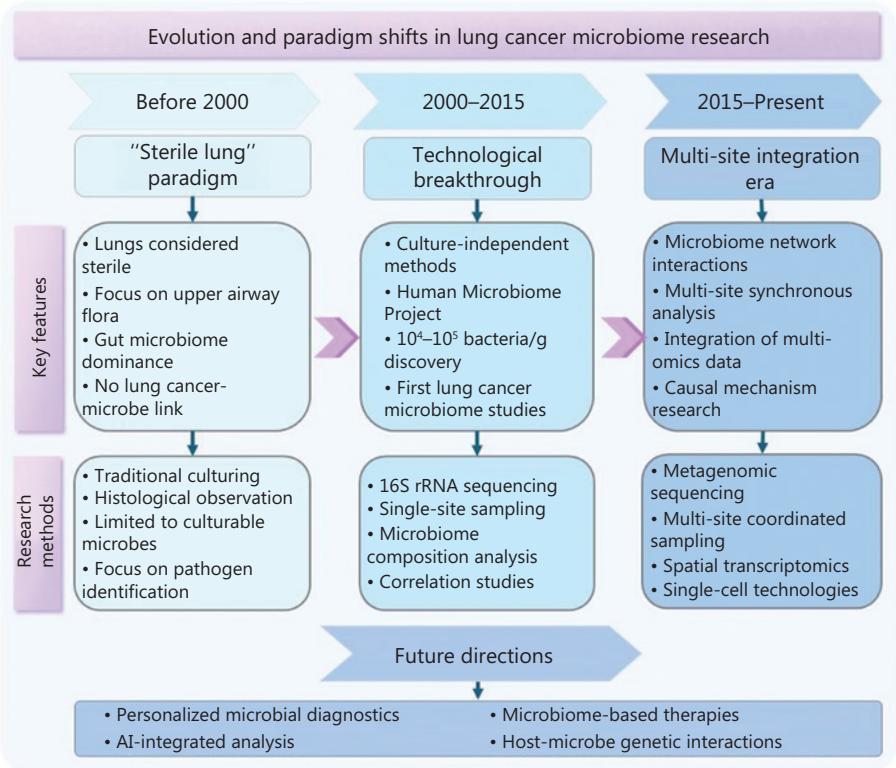


Figure 1 Evolution and paradigm shifts in lung cancer microbiome research. This figure illustrates the historical development and conceptual evolution of microbiome research in lung cancer from traditional views to contemporary multi-site approaches. (1) Pre-2000 "sterile lung" paradigm: The lungs were widely considered sterile, with microbiome studies primarily focused on the upper airway and gut flora. Research methods were limited to traditional culturing and histological observation, identifying only culturable pathogens with no recognized link to lung cancer. (2) 2000-2015 technological breakthrough: The advent of culture-independent techniques, notably 16S rRNA sequencing driven by initiatives like the Human Microbiome Project, led to the detection of low-biomass bacterial communities in the lungs (approximately 10^4–10^5 16S rRNA gene copies/g) and initiated the first studies linking microbiota with lung cancer. Research became focused on microbial composition and correlation analysis, though typically using single-site sampling. (3) 2015-present multi-site integration era: The field shifted toward systems biology approaches, exploring microbiome-host interactions across multiple anatomic sites. This era features metagenomic sequencing, spatial transcriptomics, multi-omics integration, and the application of single-cell technologies to uncover causal mechanisms. The figure also highlights future directions, including personalized microbial diagnostics, microbiome-based therapies, AI-integrated analysis, and host-microbe genetic interactions, underscoring the potential for precision medicine in lung cancer care. AI, artificial intelligence; 16S rRNA, 16S ribosomal RNA.

models, including gnotobiotic animals, *ex vivo* organoid cultures, and *in vitro* co-culture systems, allow researchers to manipulate specific microbial communities and observe direct effects on tumor behavior and immune responses. These mechanistic studies provide crucial insights for developing microbiome-based therapeutic strategies.

Multi-site microbiome interaction concept

The emerging concept of multi-site microbiome interactions represents a paradigm shift in lung cancer microbiome research. This framework recognizes that microbiomes at different body sites communicate and influence each other through various pathways, collectively affecting lung cancer development and treatment response.

The respiratory tract microbiome exists in a continuum from the oral cavity through the airways to the lung parenchyma with microbial density progressively decreasing along this gradient. Microaspiration of oral microbiota represents a primary source of lung microbiome with distinct oral microbial signatures potentially serving as early indicators of lung cancer risk or treatment response[19].

Concurrently, the gut microbiome influences lung cancer through systemic mechanisms, including immune modulation, metabolite production, and neural pathway regulation[23]. Recent evidence suggests bidirectional communication between gut

and lung microbiomes with respiratory infections potentially altering gut microbial composition, and gut dysbiosis influencing susceptibility to respiratory diseases[24].

This multi-site perspective has significant implications for biomarker development and therapeutic interventions. Integrated analyses of oral, airway, lung, and gut microbiomes may provide more comprehensive predictive information than single-site analyses, particularly in lung cancer in which the disease directly interfaces with respiratory tract microbiomes while being systemically influenced by gut microbiota[25].

Transition to multi-site microbiome analysis

The evolution toward multi-site microbiome analysis has been facilitated by technologic advances in sequencing, bioinformatics, and systems biology approaches. These developments enable simultaneous examination of microbiome profiles across different body sites and the correlation with clinical outcomes[26].

Recent studies have demonstrated the potential value of integrated multi-site microbiome analyses compared with single-site approaches. In NSCLC, combining microbiome signatures from different anatomical sites may capture complementary host–microbe interactions and improve the prediction of immunotherapy response compared with analyses based on a single site alone.

Methodologically, multi-site analysis presents unique challenges in sample collection, processing, and data integration. Standardized protocols for concurrent sampling of oral, airway, and gut microbiomes are being developed with bronchoscopy-based procedures allowing simultaneous collection of airway samples during routine diagnostic procedures[27]. Advanced computational methods, including multi-layer network analyses and machine learning algorithms, facilitate integration of heterogeneous microbiome datasets from different body sites[28].

This transition toward multi-site analysis represents a more holistic approach to understanding the role of the microbiome in lung cancer, acknowledging the complex interplay between different microbial communities in influencing disease progression and treatment response.

In this comprehensive review, recent research involving the microbiome as a lung cancer immunotherapy biomarker was systematically examined with a focus on four essential dimensions: multi-site microbiome profiles in patients with lung cancer; microbe-tumor-immune interaction networks; the microbiome and microbial metabolites as potential predictors of immunotherapy efficacy and adverse events; and microbiome analysis-based precision combination treatment approaches. We aimed to establish a theoretical framework for evaluating the microbiome as a predictive biomarker for lung cancer immunotherapy to support the advancement of personalized precision immunotherapeutics.

Oral microbiome in lung cancer immunotherapy

Oral microbiome characteristics in lung cancer patients

The oral microbiome profile has a crucial role in lung cancer. Oral microbial diversity is markedly diminished in patients with lung cancer, especially among non-smokers. Decreased oral microbiome α-diversity is a potential indicator of increased lung cancer risk, particularly in non-smokers[29].

Recent large-scale epidemiologic evidence from Vogtmann et al.[29] represents the most comprehensive study to date examining oral microbiome-lung cancer associations. A total of 1306 incident lung cancer cases were identified from 3 major prospective cohorts using a case-cohort design. The study revealed that higher alpha diversity was associated with lower lung cancer risk [Shannon index hazard ratio = 0.90, 95% confidence interval (CI) = 0.84–0.96] with associations particularly strong for squamous cell carcinoma and among former smokers.

A study examining the bronchoalveolar lavage samples of 39 patients with lung cancer and 36 participants without cancer revealed elevated levels of *Streptococcus* and *Veillonella* in the lower respiratory tract of patients with lung cancer, indicating a direct relationship between oral microbiota composition and lung cancer. A comparative investigation involving 172 healthy controls matched with 75 non-smoking female patients with lung cancer found that lower oral microbiome diversity was significantly linked to higher lung cancer occurrence in the non-smoking patients [odds ratio (OR) = 2.14, 95% CI: 1.26–3.63, $P < 0.01$][30]. This finding suggested that oral microbial dysbiosis may contribute to lung carcinogenesis through mechanisms independent of tobacco exposure.

In addition, oral *Helicobacter pylori* can reach the lungs *via* aspiration or hematogenous dissemination, inducing chronic mucosal inflammation and contributing to tumorigenesis[31]. Yan et al.[32] analyzed salivary samples and identified significant abundance of *Fusobacterium*, *Veillonella*, *Capnocytophaga*, and *Porphyromonas gingivalis* with concurrent depletion of *Neisseria* and *Rothia*. Notably, *Porphyromonas gingivalis* abundance is closely correlated with lung cancer staging and poor prognosis, establishing *P. gingivalis* abundance as a potential diagnostic and prognostic biomarker.

Different histologic lung cancer subtypes exhibit distinct oral microbiome profiles. Vogtmann et al.[29] performed a large-scale prospective analysis of 1,306 lung cancer cases across 3 US cohorts and demonstrated that increased oral *Streptococcus* abundance was significantly associated with elevated lung cancer risk with the strongest associations occurring among patients with squamous cell carcinoma. These findings suggested that different histologic subtypes harbor distinct

microbiome profiles, potentially reflecting subtype-specific pathogenic mechanisms and host-microbe interactions.

Oral-lung axis: mechanisms and pathways

The "oral-lung axis" represents an important pathway through which oral microorganisms may influence pulmonary carcinogenesis. Oral bacteria can reach the lungs *via* several mechanisms, as follows: micro-aspiration, which occurs frequently during sleep; direct inhalation of oral aerosols; hematogenous dissemination from periodontal disease sites; and translocation *via* saliva-contaminated mucus[33]. Once in the lungs, these microorganisms may induce local inflammation, produce genotoxic substances, or modulate the immune microenvironment, thereby contributing to carcinogenesis or influencing treatment response[34].

Oral microbial metabolites, particularly short-chain fatty acids (SCFAs), can enter and affect the pulmonary state through airway and digestive tract pathways. Studies suggested that oral SCFAs may alleviate allergic airway disease, indicating that oral microbial metabolites may indirectly influence lung cancer immunotherapy effectiveness by modulating the pulmonary immune microenvironment.

Oral microbiome as a predictive biomarker for immunotherapy

Recent studies have begun to explore the relevance of oral microbiome characteristics to immunotherapy outcomes. Huang et al.[35] combined salivary microbiome sequencing with untargeted metabolomics analysis and revealed notable distinctions in the salivary microbiome composition between treatment responders and non-responders to ICIs. Treatment responders had abundant *Neisseria* and *Actinomyces*, whereas non-responders had abundant *Granulicatella adiacens* and *Streptococcus oralis*. These bacterial profile differences were associated with PD-L1 expression levels and progression-free survival (PFS) outcomes. Metabolomic analysis identified lipids and lipid-like molecules as the most significant differentially expressed metabolites between both patient groups with the majority exhibiting upregulation in non-responders and negative correlation with PD-L1 expression. This multi-omics integration approach provides comprehensive insights into oral microbiome-immunotherapy associations.

Circulating microbiome deoxyribonucleic acid (cmDNA) analysis has revealed that some oral-associated bacteria, such as *Actinomyces* and *Acinetobacter*, are significantly enriched in lung cancer patients and closely correlate with immune checkpoint inhibitor efficacy. Chen et al.[36] developed predictive models using 119 significant microbial species that achieved good performance in early-stage lung cancer prediction, establishing circulating microbiome DNA as a promising non-invasive biomarker approach.

Oral microbiome-based intervention strategies

These findings suggested that the oral microbiome may serve as a non-invasive biomarker for lung cancer risk assessment, disease monitoring, and prediction of immunotherapy responses. However, standardization of sampling techniques and analytic methodologies remains a challenge for clinical application.

Oral microbiome modulation offers several unique advantages for clinical application compared to gut microbiome interventions. Saliva collection is more readily accepted by patients than fecal collection, which enables better compliance for longitudinal monitoring. In addition, oral microbiome changes may more rapidly reflect treatment responses due to direct oral-lung axis connections, allowing for more timely treatment adjustments.

Targeted oral interventions include antimicrobial mouthwashes designed to selectively inhibit pathogenic bacteria, while preserving beneficial flora, oral probiotic formulations containing specific *Lactobacillus* and *Bifidobacterium* strains, and oral metabolite supplements rich in beneficial compounds, like SCFAs that can reach the lungs through airways.

Emerging approaches to oral microbiome-based intervention include "salivary microbiota transplantation" protocols, like fecal microbiota transplantation (FMT) and engineered oral delivery systems for beneficial microbial metabolites. However, standardization of sampling techniques and analytic methodologies remains a challenge for clinical application.

It should be noted that the above studies primarily revealed associations between the oral microbiome and lung cancer, as well as immunotherapy effectiveness. To establish causal relationships, more mechanistic studies, germ-free animal model validation, and prospective intervention trials are needed for support. This distinction between correlation and causation remains crucial for the appropriate clinical translation of oral microbiome-based biomarkers.

Airway microbiome in lung cancer immunotherapy

Airway microbiome characteristics in lung cancer patients

The airway microbiome exhibits distinctive alteration patterns in patients with lung cancer and may serve as a bridge between the oral cavity and lung parenchyma in the continuum of respiratory tract microbiota. Recent technological advances in bronchoscopic sampling have enabled more precise characterization of the airway microbiome at different respiratory tract levels.

Kim et al.[37] performed comprehensive bronchoalveolar lavage fluid (BALF) analysis comparing 24 lung cancer patients with 24 benign lung disease patients using 16S rRNA gene amplicon sequencing. The study revealed that the α- and β-diversity distribution differed significantly between the groups (P = 0.001). Firmicutes represented the most abundant phylum in lung cancer patients (33.39% ± 17.439), whereas Bacteroidota predominated in benign lung disease patients (31.132% ± 2.5%). Yan et al.[32] were the first to clarify the complex relationship between salivary microbiota and lung cancer. The Yan et al.[32] investigation determined that *Flavobacteriales*, *Burkholderiales*, *Campylobacterales*, *Spirochaetales*, *Veillonellaceae, Capnocytophaga*, *Corynebacterium*, and *Veillonella* were abundant in patients with lung cancer, whereas *Neisseria* was depleted. Druzhinin et al.[38] identified distinctive taxa in the sputum of patients with lung cancer, including *Actinobacillus*, *Peptostreptococcus*, *Oligella*, *Ruminococcus*, *Elisabethkingia*, *Psychrobacter*, and *Malusibacillus*. In particular, *Bergeyella* was significantly higher in the lung cancer sputum samples than in healthy control samples, while *Atopobium*, *Oribacterium*, and *Treponema* were significantly depleted. *Haemophilus* was more abundant in patients with lung cancer. *Bergeyella zoohelcum* exhibited a notable abundance, whereas *Atopobium rimae*, *Treponema amylovorum*, and *Prevotella* (*P. histicola* and *Prevotella* spp. *oral clone DO014*) were significantly depleted.

Spatial meta-transcriptomic analysis by Wong-Rolle et al.[39] demonstrated that the intratumoral bacterial burden was progressively reduced from airways-to-tumor cells-to-normal lung tissue, suggesting that airway microbiota likely serves as the primary source of bacteria identified within lung tumors. This gradient distribution pattern provides important insights into the potential origin and migration pathways of cancer-relevant microbes. A notable abundance of *Streptococcus*, *Veillonella*, and *Haemophilus*, and comparative depletion of *Prevotella* were observed in the airways of patients with lung cancer[40,41]. Environmental factors substantially affect airway microbiota composition with specimens from the lower respiratory tract being less susceptible to contamination and maintaining closer proximity to lung tissue, making lower respiratory tract bacterial examination particularly important for understanding fundamental lung cancer mechanisms[42]. Smoking history profoundly impacts airway microbiome characteristics with persistent effects. Tsay et al.[43] showed that smoking history in lung cancer patients, regardless of cessation status, was associated with long-term *Streptococcus* enrichment in the airway microbiome, promoting phosphatidylinositol 3-kinase (PI3K) signaling pathway activation and enhancing tumor proliferative potential. This finding established direct molecular links between airway microbiome alterations and cancer progression pathways.

Spatial distribution and immune microenvironment interactions

The airway microbiome demonstrates complex spatial relationships with immune cells and tumor tissues. Advanced spatial analysis techniques revealed that bacteria preferentially colonize specific microniches within the respiratory tract with distinct distribution patterns correlating with local immune cell populations and inflammatory states. Liu et al.[44] developed a protected specimen brush technique that significantly reduces upper airway contamination, allowing more accurate profiling of the lower airway microbiome. Using this approach, Liu et al.[44] identified site-specific microbial signatures associated with different lung cancer stages, with progressive dysbiosis correlating with disease advancement.

Some airway bacteria can induce varying levels of cytokines, including interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor-alpha (TNF-α). Respiratory microbiota rich in oral-associated taxa, such as *Prevotella*, relate to Th17-mediated immune responses in healthy subjects and lung cancer patients. These phenomena demonstrate that airway microecosystems significantly influence pathogenesis through inflammation stimulation and immune response modulation.

Airway microbiome as a predictive biomarker for immunotherapy

The airway microbiome offers unique advantages as a predictive biomarker due to direct anatomic proximity to lung tumors and accessibility through routine bronchoscopic procedures[45]. Multi-site integration studies demonstrated superior predictive value compared to single-site analyses as reported by Lu et al.[46] and Reddy et al.[47]

Recent systematic reviews analyzing airway microbiome composition across different lung cancer stages revealed that early-stage patients maintain higher airway microbial diversity compared to advanced-stage patients, suggesting that airway microbial diversity may serve as a disease progression predictor.

A multicenter prospective observational study (UMIN000046428) involving 400 lung cancer patients aims to identify airway microbiome predictive biomarkers of immunotherapy response using artificial intelligence. The ongoing investigation will provide crucial validation for airway microbiome clinical applications.

Airway microbiome-based intervention strategies

Airway microbiome modulation offers direct access to the lung tumor microenvironment through inhalation-based

delivery systems. Emerging approaches include nebulized probiotic formulations designed to deliver viable beneficial bacteria directly to airways using specialized carrier technologies to ensure bacterial survival in airway environments.

The pH modulation agents can be inhaled to create favorable microenvironments for beneficial bacterial growth and T cell activation[48]. Surfactant modulation represents another innovative approach with novel surfactant formulations designed to maintain lung function, while optimizing microbial community structure[49].

Engineered bacterial therapies specifically targeting lung tumors show promise with bacteria designed to proliferate selectively in tumor hypoxic environments and release immunostimulatory factors or cytotoxic substances intratumorally. However, safety considerations and standardized delivery protocols require further development.

Current airway microbiome-lung cancer immunotherapy relationship studies are primarily based on cross-sectional surveys and retrospective analyses, establishing statistical associations rather than causal relationships. How airway microbiomes influence immune checkpoint inhibitor efficacy through specific molecular mechanisms requires verification through germ-free animal models, microbiome-host interaction mechanistic studies, and prospective intervention trials.

The complex, dynamic nature of airway microbiome-immunotherapy relationships necessitates sophisticated analytical approaches capable of capturing non-linear patterns and interaction effects. Machine learning algorithms detecting non-linear relationships demonstrate improved predictive accuracy compared to traditional statistical methods, although causal inference remains challenging.

The intratumoral microbiome in lung cancer immunotherapy

Intratumoral microbiome characteristics

The lung microbiome directly reflects the intra-tumor microenvironment (TME) microbial ecology and exhibits distinctive features in patients with lung cancer. Contrary to the historical view of the lungs as a sterile environment, current evidence demonstrates the presence of diverse microbial communities in healthy and diseased lung tissues.

The microbiome of healthy lung tissue comprises *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*[22]. However, the microbiome in cancerous lung tissue exhibits significant alterations that closely correlate with lung cancer-specific factors. These alterations are characterized by decreased α-diversity, shifts in community structure, and enrichment of specific bacterial taxa.

Histologic lung cancer subtypes influence intra-tumoral microbiota composition. Adenocarcinomas have abundant environmental bacteria, including *Thermus* and *Legionella*, whereas squamous cell carcinomas have abundant *Acidovorax* and *Klebsiella*. Small cell lung cancer (SCLC) has a unique anaerobic bacterial signature that is characterized by abundant *Fusobacterium* and *Bacteroides*. These differences may reflect the distinct metabolic characteristics and microenvironmental conditions of different lung cancer subtypes.

Different histologic and molecular lung cancer subtypes exhibit distinct microbiome characteristics. *Prevotella* is abundant in SCLC, whereas *Pseudomonas*, *Thermomonas*, and *Bacteroides* are abundant in NSCLC[50]. Distinct microbial profiles are observed at different stages in lung adenocarcinoma (LUAD). *Bacteroidetes* and *Firmicutes* are more abundant in invasive adenocarcinoma (IAC) than adenocarcinoma *in situ* and minimally invasive adenocarcinoma[51]. Conversely, *Bosea* spp. and *Microbacterium paludicola* are less abundant in IAC, suggesting a protective role in early-stage disease. *Granulicatella* and *Actinobacillus* are abundant in early-stage NSCLC, whereas *Actinomyces* is more abundant in advanced stages[52].

Specific microbial profiles are associated with different lung cancer stages and types and specific gene mutations, such as epidermal growth factor receptor (EGFR) mutations. *Parvimonas* is abundant in adenocarcinoma with EGFR mutations[52], while *Pseudomonas aeruginosa* abundance is less likely, indicating a potential interaction between microbial presence and gene alterations[53]. TP53-mutant tumors typically harbor a high overall bacterial burden, potentially due to p53 loss-induced epithelial barrier dysfunction[54].

Spatial heterogeneity and TME interactions

Wong-Rolle et al.[55] were the first to quantitatively demonstrate that intra-tumoral bacteria are not randomly distributed across the immune landscape. The pioneering work revealed that bacterial concentrations are significantly higher in tumor cells than T cells, macrophages, B cells, plasma cells, natural killer cells, dendritic cells, and surrounding stroma. Bacterial burden exhibits a gradient distribution that is highest in the airway, followed by tumor cells, and lowest in the adjacent normal lung tissue and tertiary lymphoid structures. Furthermore, Wong-Rolle et al.[55] reported that bacterial burden was strongly correlated with oncogenic β-catenin expression and cell growth and epithelial-mesenchymal transition pathways.

Intra-tumoral bacteria are localized and not randomly distributed in specific intra-TME microniches. These often less vascularized and highly immunosuppressive niches promote cancer progression by supporting immune and epithelial cell functions. The bacterial burden is higher in tumor cells than in immune cells and stroma in lung cancer[39]. Galeano Niño et al.[56] demonstrated that intratumoral bacteria exhibit a highly organized spatial distribution within specific tumor microniches, which are associated with reduced vascularization, local immunosuppression, and increased tumor cell invasion. In

lung cancer, Wong-Rolle et al.[39] further showed that bacterial burden is higher in tumor cells than in immune cells and stroma, and is strongly correlated with oncogenic β-catenin expression as well as pathways involved in cell growth and epithelial–mesenchymal transition. Hypoxic regions within tumor microenvironments create distinctive ecological niches conducive to specific bacterial populations. Anaerobic bacteria in these regions synthesize metabolites, such as succinate and lactate, which stabilize hypoxia-inducible factor 1-alpha (HIF-1α) in both tumor and immune cells, fostering immunosuppressive phenotypes in macrophages and dendritic cells, while augmenting regulatory T cell (Treg) functionality.

Pro-inflammatory bacteria often co-localize with activated T cells at invasive tumor margins, potentially enhancing local immune responses. Conversely, immunosuppressive bacteria associate more frequently with Tregs and M2 macrophages in tumor core regions, potentially promoting immune evasion (**Figure 2**).

Different lung cancer subtypes exhibit distinct metabolic profiles. LUAD is associated with glycolysis and lactate production that can alter the TME and affect immune cell infiltration. Deng et al.[57] demonstrated that glycolysis-lactate risk and 18-microbe prognostic scores can predict prognosis and immunotherapy response in LUAD. In NSCLC, inter-subpopulation metabolic cooperation, such as reliance on oxidative phosphorylation versus glycolysis, facilitates collective tumor invasion and metastasis.

Intratumoral microbiome as a predictive biomarker

Intratumoral microbiome composition correlates significantly with immune checkpoint inhibitor efficacy. Zhang et al.[58] reported that intratumoral microbiota impacts first-line treatment efficacy and survival in NSCLC patients free of lung infection with specific gram-negative bacterial presence associating with better treatment responses, while certain gram-positive bacteria correlate with treatment resistance.

Battaglia et al.[59] analyzed paired tumor tissue biopsy samples and showed that immunotherapy significantly decreased bacterial diversity in treatment responders compared to non-responders. *Fusobacterium* abundance had a significant negative correlation with durable clinical benefit. Multivariate Cox proportional hazard models demonstrated that persistently high *Fusobacterium* abundance was significantly associated with reduced overall survival (OS) and PFS.

The expression signatures of cytotoxicity, interferon-γ, and major histocompatibility complex class II genes were significantly lower in tumors with higher *Fusobacterium* abundance, providing mechanistic insights into microbiome-immunotherapy resistance relationships.

Recent advances in single-cell technologies combined with spatial transcriptomics have revealed intricate relationships between specific bacterial taxa and host cells within TMEs. These approaches demonstrated that some bacteria are preferentially associated with some cell types, potentially influencing function and phenotype.

The spatial heterogeneity of intratumoral microbiomes presents significant challenges for clinical sampling and biomarker development in lung cancer. Conventional tumor biopsy procedures, whether through bronchoscopy, transthoracic needle biopsy, or surgical resection, typically obtain samples from limited tumor regions, potentially missing the complex spatial distribution of therapeutically relevant microorganisms.

Biopsy sampling bias represents a critical concern for microbiome-based biomarker reliability. Given that bacterial burden varies significantly between tumor center and periphery, and that different microbial communities associate with distinct immune microenvironments, single-site biopsies may provide incomplete or misleading information about the overall tumor microbiome landscape. For example, a biopsy obtained primarily from the tumor core might overrepresent immunosuppressive bacterial species, while underestimating pro-inflammatory microorganisms concentrated at invasive margins.

These spatial considerations have direct implications for clinical decision-making. Microbiome-based predictions of the immunotherapy response could vary substantially depending on biopsy location within the same tumor. Patients whose biopsies inadvertently sample microbe-poor regions might be incorrectly classified as unlikely to respond to immunotherapy, while samples from microbe-rich areas might receive overly optimistic prognoses.

To address these challenges, several strategies merit consideration in clinical practice. Multi-site sampling protocols during bronchoscopy or surgical resection could provide more comprehensive microbiome profiles, although this approach must balance diagnostic accuracy with procedural safety and feasibility. Integration of microbiome analysis with spatial imaging techniques, such as positron emission tomography/computed tomography (PET-CT) or magnetic resonance imaging (MRI), might help identify optimal sampling sites based on metabolic activity or vascular perfusion patterns that correlate with microbial distribution. In addition, development of non-invasive approaches, such as circulating microbial DNA analysis or exhaled breath microbiome profiling, could complement tissue-based sampling to provide more complete microbiome characterization.

Gut microbiome in lung cancer immunotherapy

Gut microbiome characteristics in lung cancer patients

Patients with lung cancer exhibit significant alterations in gut microbiome α- and β-diversity compared to healthy individuals. The altered diversity correlates with immune dysfunction

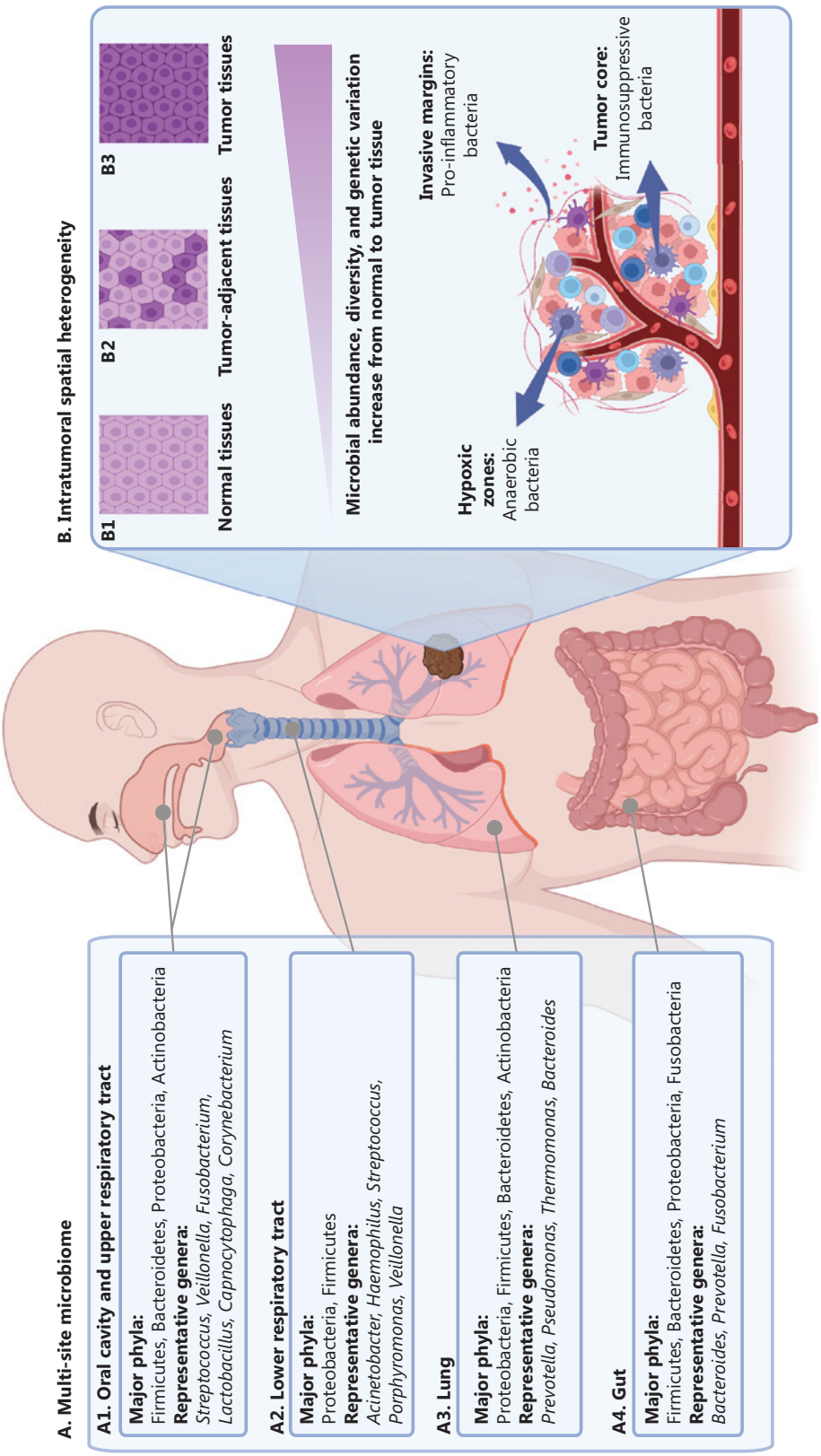


Figure 2 Multi-site microbiome characteristics and intratumoral microbial spatial heterogeneity in lung cancer. This figure demonstrates the characteristic microbiome profiles across multiple anatomic sites in lung cancer patients and the spatial distribution of microbes within tumors. Panel A (multi-site microbiome): Depicts microbial communities at four key anatomical sites. A1 (oral cavity and upper respiratory

tract): As sites exposed to the external environment, the oral cavity and upper respiratory tract typically show the highest microbial biomass and diversity with representative oral-associated genera, such as *Streptococcus*, *Veillonella*, and *Fusobacterium*. A2 (lower respiratory tract): The lower respiratory tract generally shows reduced microbial biomass/diversity compared to the upper airway with representative genera, including *Acinetobacter*, *Haemophilus*, and *Streptococcus*. A3 (lung): The lung is typically a low-biomass site with communities often reported to be enriched for Proteobacteria at the phylum level and representative genera, such as *Prevotella* and *Pseudomonas*. A4 (gut): The gut shares broad phylum-level composition with the lung (e.g., Firmicutes, Bacteroidetes, and Proteobacteria) but differs substantially at the genus level (e.g., *Bacteroides* and *Prevotella*). Panel B (intratumoral spatial heterogeneity): Details the tumor microenvironment cross-section. B1 (normal tissue): Baseline microbial diversity with low bacterial burden. B2 (tumor-adjacent tissue): Intermediate bacterial abundance with transitional microbial profiles. B3 (tumor tissues): Highest bacterial burden with three distinct microniches (hypoxic zones harboring anaerobic bacteria that promote immunosuppression through succinate and lactate production, invasive margins containing pro-inflammatory bacteria co-localizing with activated CD8+ T cells, and central regions dominated by immunosuppressive bacteria associated with Treg cells and M2 macrophages). CD8+, cluster of differentiation 8-positive; M2, alternatively activated macrophages; Treg, regulatory T cells.

and systemic inflammation, potentially affecting the pulmonary microenvironment through alterations in mucosal immune function. Hakozaki et al.[60] conducted an ancillary study based on the JCOG2007 (NIPPON) phase III clinical trial. A total of 270 patients with advanced NSCLC were enrolled. Significant associations between the gut microbiota and chemoimmunotherapy efficacy and toxicity were determined through 16S rDNA sequencing analysis. High abundance of butyrate-producing bacteria, such as *Fusicatenibacter* [median OS: 23.7 *vs*. 23.1 months, hazard ratio (HR) = 0.61], *Butyricicoccus* (not reached *vs*. 18.4 months, HR = 0.48), and *Blautia* (not reached *vs*. 19.2 months, HR = 0.48), correlated with longer survival, while genera, such as *Ruminococcaceae UBA1819* (15.3 months *vs*. not reached, HR = 2.01) and *Prevotellaceae NK3B31* (16.4 months *vs*. not reached, HR = 1.62) predicted poorer outcomes. Patients with low microbial α-diversity had a significantly increased risk of ≥ grade 4 severe adverse events (32.8% *vs*. 15.7%, OR = 2.6). This association was more pronounced in the nivolumab plus ipilimumab combination chemotherapy (NIC) arm (OR = 3.8 *vs*. 1.7 in PC arm). Notably, some genera had regimen-specific effects with *Fusicatenibacter* and *Butyricicoccus* demonstrating stronger protective effects in the NIC arm (HR = 0.56 and 0.52, respectively), suggesting that gut microbiota profiles could serve as important biomarkers for guiding individualized treatment selection.

Lung cancer patients exhibit characteristic compositional shifts at the phylum level. Liu et al.[61] observed an elevated *Bacteroidetes*-to-*Firmicutes* ratio in lung cancer patients, a pattern associated with chronic inflammatory states. More detailed analyses revealed increased relative abundance of *Proteobacteria*, particularly in advanced lung cancer patients, potentially reflecting intestinal barrier dysfunction and bacterial translocation. Concurrently, *Actinobacteria* abundance was reduced, possibly leading to decreased production of beneficial metabolites.

The gut microbiome of lung cancer patients displays complex alteration patterns at the genus and species levels. Notable changes in the gut microbiome include the following: significant reductions in *Bifidobacterium*, especially *B. longum* and *B. adolescentis*, critical species involved in maintaining intestinal barrier integrity and immune balance[62]; decreased *Faecalibacterium*, particularly *F. prausnitzii*, an important butyrate producer the reduction of which may affect anti-inflammatory factor production and Treg function; altered levels of AKK, which typically shows reduced abundance in lung cancer patients compared to healthy controls and influences host immune responses through mucin metabolism and intestinal barrier regulation[63]; reduced *Roseburia* and *Ruminococcus*, important butyrate producers that may diminish local anti-inflammatory capacity and immune regulatory functions when decreased; and increased abundance of specific pathogenic bacteria, including *Clostridioides* difficile and some *Enterobacteriaceae* members, such as *Escherichia coli* and *Klebsiella pneumoniae*, which are associated with intestinal inflammation and barrier dysfunction.

Different histologic and molecular subtypes of lung cancer demonstrate distinctive gut microbiome characteristics, reflecting the complex tumor-host interactions. Peters et al.[64] identified subtype-specific microbial signatures in 153 lung cancer patients. LUAD patients exhibited higher ratios of *Bacteroidetes* and *Firmicutes*, relative abundance of propionate-producing bacteria, such as *Propionibacterium*, and marked increases in bile acid metabolism-related taxa, such as *Bilophila*. In contrast, lung squamous cell carcinoma (LUSC) patients were shown to have an increased abundance of *Proteobacteria* members, particularly *Pseudomonas* and *Citrobacter*, significant enrichment of *Klebsiella pneumoniae*, and more active microbial metabolic pathways related to oxidative stress. SCLC patients had unique anaerobic bacterial community features, significant enrichment of *Fusobacterium* and *Bacteroides*, and more prominent amine and sulfide metabolism pathways.

These subtype-specific gut microbiome features align with the metabolic preferences, oxidative stress levels, and immune microenvironment characteristics of different lung cancer subtypes, suggesting that tumor biological properties may shape gut microbial ecology through systemic factors[65].

The gut microbiome demonstrates dynamic changes across different stages of lung cancer development, reflecting the complex interactions between tumor progression and host responses. Longitudinal studies have revealed characteristic temporal patterns.

Diversity gradually decreases in early-stage lung cancer, although not reaching significant levels. Specifically, *Roseburia* and *Bifidobacterium* begin to decline and butyrate production-related functional genes show reduced abundance. Conditionally pathogenic bacteria gradually increase during middle-stage lung cancer, particularly some *Enterobacteriaceae* members. Microbial network complexity decreases with reductions in keystone taxa and functional redundancy diminishes with increased metabolic network vulnerability[66]. Advanced lung cancer stages exhibit increased volatility in microbiome composition with significantly reduced stability, increased colonization risk by specific pathogens, such as *C. difficile*, and markedly altered metabolite profiles with more pronounced systemic effects.

Spatial distribution patterns reveal that microbiome changes vary across different intestinal regions with proximal colon changes preceding the changes in the distal colon. Mucosa-associated microbiome changes precede changes in the fecal microbiome and some microorganisms, such as *Fusobacterium*, demonstrate tissue tropism that can be detected in lung metastases.

Gut-lung axis: mechanisms and systemic effects

The gut microbiome represents the largest microbial community in the human body and exhibits distinctive biological features in patients with lung cancer. The gut microbiome influences lung cancer development and treatment response *via* the gut-lung axis. Mendelian randomization studies by Ma et al.[67] and Li et al.[68] have begun establishing causal relationships between gut microbiota and lung cancer development, providing stronger evidence for causality. However, specific molecular mechanisms of the gut-lung axis and optimal intervention strategies require further causal research for clarification.

The "gut-lung axis" concept helps clarify the role of the microbiome in lung cancer immunotherapy. This bidirectional communication network mutually influences both environments *via* immune modulation and metabolite exchange. Gut microbiota and the metabolites affect the pulmonary immune microenvironment through the following mechanisms (**Figure 3**)[69]: **1) Microbial metabolites.** Bioactive metabolites generated by the gut microbiota (e.g., SCFAs) move to the lungs *via* the systemic circulation. **2) Immune regulation**. Immune regulation involves the gut-lung immune cell communication network. Intestinal dendritic cells move to pulmonary lymph nodes *via* the lymphatic system after capturing antigens, triggering specific immune responses. Gut-generated cytokines, such as IL-10 and IL-22, can reduce lung inflammation, whereas IL-6 and TNF-α can worsen lung damage. **3) Neural pathway regulation.** The gut microbiota and metabolites stimulate the vagal afferent fibers, and after central processing of the information, cause the vagal efferent fibers to release acetylcholine. Subsequently, the acetylcholine binds to α7 nicotinic acetylcholine receptors on lung macrophages, inhibiting NF-κB activation and pro-inflammatory factor release. Gut microbes can indirectly regulate pulmonary immune function *via* the influence on the hypothalamic-pituitary-adrenal axis and changes in glucocorticoid secretion. **4) Microbial translocation.** Gut dysbiosis-induced intestinal barrier dysfunction increases intestinal wall permeability, enabling bacteria or bacterial metabolites to enter the portal venous system and systemic circulation, and eventually reach the lungs. Moreover, gut microbiota alterations can affect pulmonary microbiota composition *via* oropharyngeal microbiota alterations. **5) Microbe-associated molecular patterns (MAMPs):** Pulmonary immune cells can identify intestinal microbial components, such as lipopolysaccharide, peptidoglycan, and flagellin, *via* pattern recognition receptors, such as Toll-like receptors (TLRs), NOD-like receptors, and C-type lectin receptors. Activation of these receptors initiates signaling pathways that control pulmonary inflammation, innate immune training, and adaptive immune responses, thus affecting the lung defense mechanisms.

Gut microbiome as a predictive biomarker for immunotherapy

Large-scale cohort studies have successfully developed and validated various microbiome-based prediction models for the immunotherapy response in lung cancer (**Table 1**). Nomura et al.[79] integrated metagenomic sequencing, metabolomics, and machine learning to develop a model for predicting the anti-PD-1 therapy response in NSCLC. Nomura et al.[79] identified a panel of 12 bacteria, including AKK, *Bifidobacterium longum*, and *Faecalibacterium prausnitzii*. The model exceeded the performance of conventional biomarkers such as PD-L1 expression and TMB, with an accuracy rate of 86% in predicting long-term clinical benefits. This finding was validated in an independent cohort comprising 118 patients with NSCLC [area under the ROC curve (AUC) = 0.84, 95% CI: 0.76–0.91, $P < 0.001$].

Zhu et al.[73] conducted a multi-center validation study incorporating data from 733 patients (including 338 with NSCLC) to establish an enterotype-based pan-cancer prediction system. Zhu et al.[73] identified five primary enterotypes (*Ruminococcus*, *Escherichia*, *Bacteroides*, *Clostridium*, and *Prevotella*), each associated with distinct immunotherapy prognoses. Patients with the *Ruminococcus* and *Bacteroides* enterotypes demonstrated superior treatment responses and survival benefits, while the *Clostridium* enterotype patients had worse outcomes.

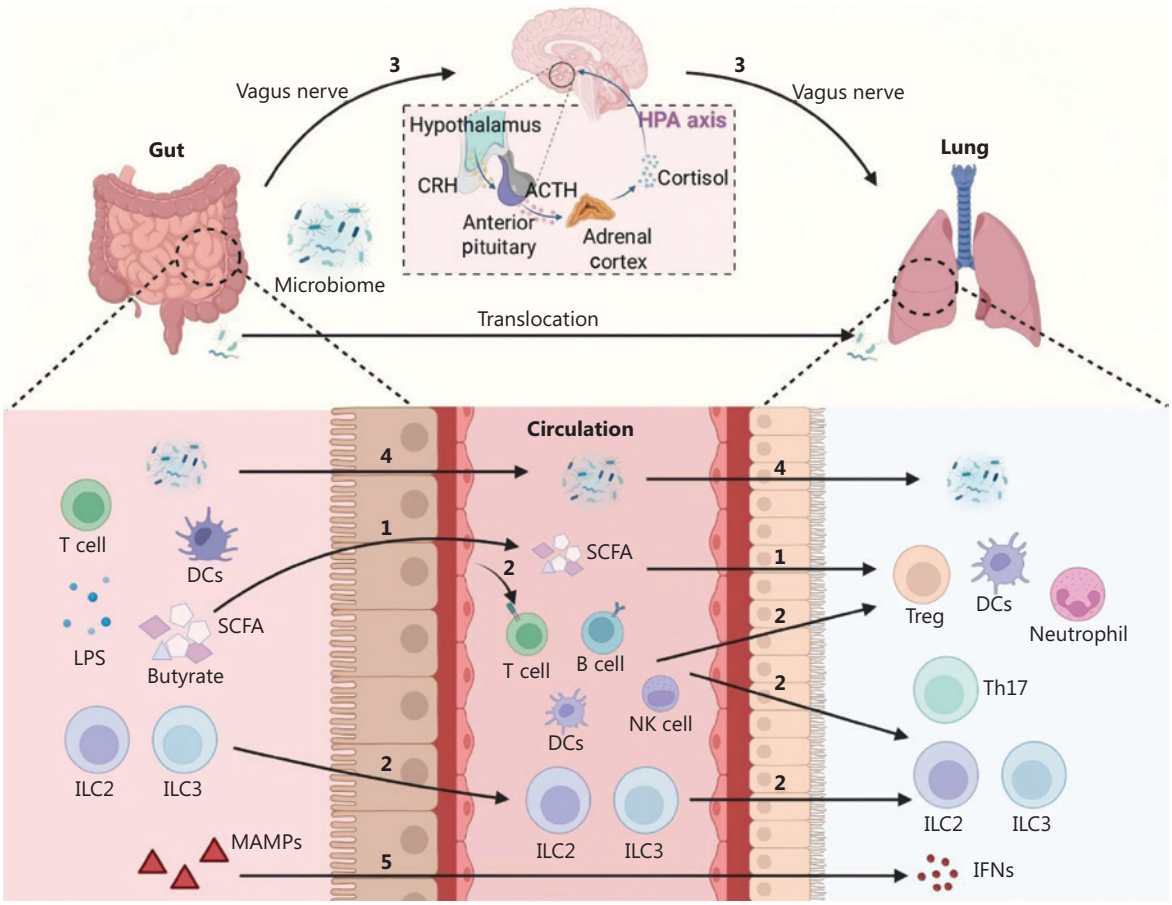


Figure 3 Gut-lung axis mechanisms in lung cancer immunotherapy. This figure illustrates the five major pathways through which the gut microbiome influences pulmonary immune responses and lung cancer progression. Pathway 1 (microbial metabolites): Gut bacteria produce SCFAs, including butyrate, propionate, and acetate, through dietary fiber fermentation. These metabolites enter systemic circulation *via* the portal vein, travel through the hepatic system, and reach the lungs where the metabolites modulate immune cell function and the tumor microenvironment. Pathway 2 (immune regulation): Microbial cues in the gut modulate antigen-presenting cells and lymphocytes, promoting the mobilization of gut DCs and T cells as well as innate lymphoid cells (ILC2/ILC3) into the circulation; these microbiome-imprinted immune components then disseminate to the lung to reshape immune composition, increasing or decreasing T-cell polarization (Treg/Th17) and altering innate compartments, including pulmonary DCs, neutrophils, and ILC2/ILC3. Pathway 3 (neural regulation): Gut microbiota and metabolites stimulate vagal afferent fibers, signals are processed in the brainstem, then vagal efferent fibers release acetylcholine that binds to α7nAChR on lung macrophages, inhibiting NF-κB activation. The hypothalamic-pituitary-adrenal axis is also modulated, affecting glucocorticoid secretion and systemic immunity. Pathway 4 (microbial translocation): Gut dysbiosis increases intestinal permeability, allowing bacteria and metabolites to translocate through the compromised epithelial barrier into the portal circulation, subsequently reaching systemic circulation and lung tissue. Pathway 5 (molecular pattern recognition): Pulmonary immune cells express pattern recognition receptors (TLRs, NLRs, and CLRs) that detect microbe-associated molecular patterns from gut bacteria, triggering downstream signaling cascades that regulate inflammation and adaptive immunity. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; DCs, dendritic cells; HPA, hypothalamic-pituitary-adrenal; IFNs, interferons; ILC2/ILC3, group 2/3 innate lymphoid cells; LPS, lipopolysaccharide; MAMPs, microbe-associated molecular patterns; SCFAs, short-chain fatty acids; Th17, T helper 17 cells; Treg, regulatory T cells.

This classification system was validated across multiple independent cohorts, achieving an 87% overall prediction accuracy and providing a robust foundation for clinical decision-making.

The NEOSTAR clinical trial yielded crucial insights into the role of AKK in treatment response, demonstrating that in patients with resectable NSCLC the neoadjuvant ipilimumab and nivolumab combination achieved a 50% response rate compared to 32.1% in patients with NSCLC treated with the nivolumab and chemotherapy combination. Notably, both treatment cohorts exhibited an abundance of AKK at

Table 1 Microbiome signatures for predicting immunotherapy efficacy

Treatment	Bacteria*	Effects	Samples	References
Anti-PD-1[†]	*Neisseria* and *Actinomyces*	Enhancing	Saliva	[35]
Anti-PD-1	AKK[‡]	Enhancing	Fecal	[70]
Anti-PD-1	AKK, *Bifidobacterium*, and *Bacteroides fragilis*	Enhancing	Fecal	[71]
Ipilimumab	*Rhodospirillales* and AKK	Enhancing	Fecal	[72]
Ipilimumab	*Holdemanella*, *Megasphaera*, *Haemophilus*, and *Sellimonas*	Inhibiting	Fecal	[72]
Anti-PD-1	*Faecalibacterium prausnitzii*, *Adlercreutzia equolifaciens*, *Allisonella histaminiformans*, *Eubacterium ramulus*, *Holdemania filiformis*, and *Blautia obeum/wexlerae*	Enhancing	Fecal	[73]
Anti-PD-1	*Klebsiella pneumoniae* and *Bacteroides massiliensis*	Inhibiting	Fecal	[73]
Anti-PD-1/PD-L1[§]	AKK, *Bifidobacterium bifidum*, *Roseburia intestinalis*, *Blautia species*, *Faecalibacterium prausnitzii*, *Intestinimonas butyriciproducens*, *Dysosmobacter welbionis*, and *Alistipes shahii*	Enhancing	Fecal	[74]
Anti-PD-1/PD-L1	*Erysipelatoclostridium ramosum*, *Enterococcus faecalis*, and *Escherichia coli*	Inhibiting	Fecal	[74]
Anti-PD-1/PD-L1	AKK, *Candidatus Gastranaerophilales bacterium MH-37*, *Clostridia bacterium*, and *crAssphage cr127-1*	Enhancing	Fecal	[75]
Anti-PD-1/PD-L1	*Aspergillus tamarii* and *H. hathewayi*	Inhibiting	Fecal	[75]
Anti-PD-1/PD-L1	AKK, *Bifidobacterium longum*, *Alistipes putredinis*, *Bradyrhizobium guangdongense*, *Plantactinospora sp. BC1*, *Corynebacterium stationis*, *Methanococcus vannielii*, *Lachnospiraceae*, and *Ruminococcaceae*	Enhancing	Fecal	[76]
Anti-PD-1/PD-L1	*Ruminococcus_unclassified*, *Nocardioides* spp. *dk3136*, *Candidatus thioglobus* spp. *NP1*, *Burkholderia anthina*, and *Brachybacterium* spp. *SGAir0954*	Inhibiting	Fecal	[76]
Anti-PD-1/PD-L1	*Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Sneathia*, and *Sutterella*	Enhancing	Fecal	[77]
Anti-PD-1/PD-L1	*Lactobacillus*, *Neisseria*, *Neisseriales*, and *Neisseriaceae*	Inhibiting	Fecal	[77]
Anti-PD-1/PD-L1	*Odoribacter*, *Gordonibacter*, *Candidatus Stoquefichus*, *Escherichia-Shigella*, *Collinsella*, and *Clostridium sensu stricto 1*	Enhancing	Fecal	[78]
Anti-PD-1/PD-L1	*Prevotella*, *Porphyromonas*, *Streptococcus*, and *Escherichia-Shigella*	Inhibiting	Fecal	[78]

[†]anti-PD-1, anti-programmed cell death protein 1; [‡]AKK, *Akkermansia muciniphila*; [§]PD-L1, programmed death-ligand 1; *, $P < 0.05$.

baseline[70]. AKK-positive patients exhibited higher objective response rates than AKK-negative patients with a 12-month survival rate of 59% *versus* 35%, respectively ($P < 0.01$), suggesting the potential of AKK as a biomarker[72].

Microbiome signatures for predicting immunotherapy-related adverse events (irAEs)

IrAEs significantly limit ICI use in cancer treatment. ICIs may increase infection risk in patients with lung cancer *via* an overactive immune response, particularly reactivation of latent infections, including respiratory, urinary system, and skin infections. Between 27% and 34% of patients develop infections post-ICI treatment with age > 67 years being an independent risk factor (significantly increased OR)[80].

Gut microbiome composition exhibits predictive value for these irAEs. Specific microbial species alterations are closely linked to ICI-induced colitis (**Table 2**). Simpson et al.[83] reported that patients with a bacteria-dominated gut microbiome have an increased risk of early severe irAEs. Specifically, patients with a *Bacteroidaceae*-dominant microbiome, characterized by decreased microbiome stability and increased circulating activated B cells, experienced severe irAEs earlier than patients with a *Ruminococcaceae*-dominant microbiome, providing a time window for early intervention.

Liu et al.[81] performed a Mendelian randomization analysis and established a causal relationship between gut microbiota and irAEs, demonstrating that Lachnospiraceae significantly increased the risk of high- and all-grade irAEs ($P < 0.01$), whereas Akkermansia and Verrucomicrobiaceae were protective against severe irAEs ($P < 0.05$).

Hu et al.[82] developed a random forest-based classification model using microbial features to predict the risk of irAEs, incorporating 14 key microbial biomarkers. The model demonstrated excellent discriminative performance (AUC = 0.88) and the prediction scores were significantly correlated with OS. In addition, the negative correlation of irAEs risk with gut microbial diversity (e.g., α-diversity) suggested that high microbial diversity may reduce irAE risk *via* immune homeostasis regulation. These findings provided new strategies for predicting and managing irAEs and emphasize the importance of pre- and peri-treatment monitoring of the gut microbiome.

Some bacteria have been shown to have protective effects against immunotherapy-induced inflammation and antibiotics can substantially disrupt this microbial balance. Lurienne et al.[84] conducted a systematic review and meta-analysis that included 23 studies involving > 5500 patients and evaluated the impact of antibiotic exposure on ICI efficacy in NSCLC. The results showed that patients receiving antibiotics had a significantly reduced OS (HR = 1.69) and PFS (HR = 1.47). The effect was most pronounced for antibiotic exposure within 60 days pre- or post-ICI initiation with a reduced OS of 6.7 months, suggesting that antibiotics may influence ICI efficacy by altering the gut microbiome. Limitations of heterogeneity and retrospective design notwithstanding, the results support the cautious use of antibiotics in patients with lung cancer receiving immunotherapy, especially pre- and post-treatment.

Furthermore, some researchers have suggested that gut microbiota modulation *via* FMT, probiotics, or dietary interventions may reduce the occurrence of irAEs and enhance ICI efficacy. For example, commensal bacteria, such as AKK, may enhance anti-tumor immunity while reducing toxic reactions.

Microbial metabolites for predicting immunotherapy efficacy and irAEs

Microbial metabolic activity shows marked alterations in lung cancer patients, producing metabolite profiles that differ significantly from healthy individuals. These metabolites may influence distant organs, particularly the lungs, through systemic circulation.

Table 2 Microbiota and immune-related adverse events in patients with lung cancer receiving immunotherapy

Immunotherapy	irAEs[†]-related microbiota*	Effects	References
Anti-PD-1[‡] and anti-CTLA-4[§]	*Lachnospiraceae*, *Ruminiclostridium 6*, *Coprococcus 3*, *Collinsella*, *Eubacterium*, *Porphyromonadaceae*, and *Peptococcus*	Increase	[81]
Anti-PD-1 and anti-CTLA-4	*Akkermansia*, *Verrucomicrobiaceae*, *Ruminococcaceae UCG-004*, and *Defluviitaleaceae UCG-011*	Decrease	[81]
Anti-PD-1 and anti-CTLA-4	*Parabacteroides goldsteinii*, *Parabacteroides merdae*, *Ruminococcus bromii*, and *Ruminococcus champanellensis*	Increase	[82]
Anti-PD-1 and anti-CTLA-4	*Bacteroides xylanisolvens*, *Phocaeicola plebeius*, *Prevotella copri*, *Fusobacterium mortiferum*, and *Lactobacillus salivarius*	Decrease	[82]

[†]irAEs, immune-related adverse events; [‡]anti-PD-1, anti-programmed cell death protein 1; [§]Anti-CTLA-4, anti-cytotoxic T-lymphocyte associated protein 4; *****, $P < 0.05$.

As functional biomarkers directly reflecting microbiome metabolic activity, microbial metabolites may predict the immunotherapy response more accurately than taxonomic composition. Integrated metabolomics and microbiome analysis can identify metabolic signatures unique to immunotherapy responders, providing new approaches for precision medicine. In addition to identifying five gut microbiome enterotypes, Zhu et al.[73] reported that these enterotypes were associated with different bacterial compositions and unique metabolic characteristics. The microbial metabolite, phenylacetylglutamine (PAGln), was shown to negatively impact immune responses to PD-1 therapy by inhibiting T cell activation. Furthermore, multi-omics data and immunotherapy response characteristics were used to develop a signature based on nine microbial metabolites, which exhibited promise in predicting the immunotherapy response in 733 patients.

SCFAs and immune regulation

SCFAs facilitate the interaction between gut microbiota and immune system regulation. Nomura et al.[79] assessed SCFA concentrations in pre-treatment fecal and plasma samples from 52 patients with advanced solid tumors receiving nivolumab or pembrolizumab. The results revealed that increased fecal butyrate, propionate, and acetate levels were significantly associated with objective response and prolonged PFS. Notably, high fecal concentrations of acetic acid (≥270 μmol/g), propionic acid (≥90 μmol/g), and butyric acid (≥40 μmol/g) were each independently associated with significantly reduced risk of disease progression or death, with hazard ratios of 0.29 (95% CI, 0.15–0.54), 0.08 (95% CI, 0.03–0.20), and 0.31 (95% CI, 0.16–0.60), respectively. Elevated plasma isovaleric acid also demonstrated a significant association with improved PFS (HR, 0.38; 95% CI, 0.14–0.99). This association remained statistically significant, even after adjusting for factors, such as PD-L1 expression and TMB, indicating that SCFAs may serve as independent predictive biomarkers for immunotherapy efficacy. These results highlight the importance of SCFAs as bridge metabolites between gut microbiota and immune system regulation in lung cancer therapy. SCFAs influence immune responses *via* multiple mechanisms, as follows: **1) Direct action on immune cells.** SCFAs act on T cells and dendritic cells *via* G protein-coupled receptor 43 (GPR43) and GPR109A, promoting Treg cell differentiation and IL-10 production. **2) Epigenetic regulation**. SCFAs induce epigenetic modifications of immune-related genes as histone deacetylase (HDAC) inhibitors, enhancing anti-tumor immune responses. **3) Intestinal barrier protection.** SCFAs strengthen epithelial tight junctions, reducing intestinal permeability and preventing inflammatory factors from entering the bloodstream.

Botticelli et al.[85] reported that patients with NSCLC receiving nivolumab with early disease progression (in the initial 3 months of treatment) exhibited significantly reduced fecal SCFA levels ($P < 0.01$), supporting the ICI efficacy predictive value of SCFAs. Liu et al.[74] demonstrated that patients in the "benefit group" exhibited significantly higher levels of gut SCFAs. In particular, the "benefit group" had notably higher propionate ($P = 0.01$) and butyrate/isobutyrate ($P = 0.12$) levels than the "resistant group," a finding that was validated in an independent cohort (propionate, $P < 0.001$; butyrate/isobutyrate, $P = 0.002$). In addition, metabolomic analysis confirmed that the "benefit group" had significantly higher fecal acetate ($P = 0.03$), propionate ($P = 0.09$), and butyrate ($P = 0.02$) levels, which were strongly associated with a longer PFS and lower tumor progression risk. Furthermore, the study identified key enzymes in the butyrate production pathway that were significantly enhanced in the "benefit group," and confirmed a positive correlation between beneficial bacteria and butyrate-producing enzymes. These results provide evidence that microbial metabolites, especially SCFAs, can significantly influence the long-term efficacy of immunotherapy by regulating T cell activity and the TME.

Tryptophan metabolism pathway and immune regulation

Tryptophan metabolic products hold promise in predicting immunotherapy efficacy. Jia et al.[86] showed that indole-3-propionic acid (IPA), a small-molecule metabolite produced by gut microbiota, can continuously mobilize T cells to kill tumors, confirming that IPA effectively enhances ICI efficacy across multiple malignant tumor models. Tryptophan metabolites influence immunotherapy through the following mechanisms: **1) Aryl hydrocarbon receptor (AhR) pathway activation.** Indole compounds, such as indole-3-carboxaldehyde, (3-IAld), activate the AhR, modulating T cell differentiation and inflammatory responses. **2) Intestinal barrier integrity.** 3-IAld augments the intestinal barrier function, reducing ICI-induced colitis risk. **3) Treg cell balance.** IPA enhances effector T (Teff) cell activity, while inhibiting Treg cell differentiation, optimizing the anti-TME.

Tryptophan metabolism demonstrates unique predictive characteristics in lung cancer. A study conducted by Nomura et al.[79] showed that the plasma kynurenine:tryptophan ratio strongly predicts the immunotherapy response in NSCLC patients (AUC = 0.81, $P < 0.001$), potentially reflecting the balance between immunosuppressive and immunostimulatory tryptophan metabolites. This ratio correlated with tumor indoleamine 2,3-dioxygenase (IDO) expression, suggesting a mechanistic link between tumor metabolism and systemic tryptophan metabolites.

Bile acids and other metabolites

Host-microbiome co-metabolites and bile acids are important molecules for fat digestion and act as signaling molecules in different physiologic processes, including immune regulation. Bile acids regulate immune cell function *via* farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (TGR5), influencing ICI efficacy[87]. In particular, TGR5 can inhibit CD8+ T cell function by promoting M2 polarization of tumor-associated macrophages (TAMs), while FXR expression is negatively correlated with PD-L1 expression, potentially affecting ICI efficacy[88]. The correlation of Treg cell expansion and inflammation suppression with secondary bile acids provides a theoretical foundation for bile acid metabolism-based intervention strategies.

Altered bile acid profiles have been associated with immunotherapy outcomes in lung cancer patients. Ma et al.[89] reported that decreased glycoursodeoxycholic acid (GUDCA) and increased taurodeoxycholic acid (TDCA) levels correlate with better response to PD-1 inhibitors in NSCLC ($P < 0.01$). These bile acid alterations were linked to enhanced CD8+ T cell function and reduced immunosuppressive myeloid cell activity, potentially explaining the association with treatment outcomes.

Bacterial polyamines impact immunotherapy efficacy as well. Bacterial polyamine application in immunotherapy is a new research frontier. Recent research indicates that some human microbiome bacterial species, particularly intratumoral bacteria, synthesize peptides that can be identified by the immune system. These peptides may serve as potential tumor antigens by being presented on the surface of tumor cells, thereby activating immune responses. Bacterial polyamines, such as spermidine, spermine, and putrescine, regulate intestinal barrier integrity and maintain intestinal homeostasis. These polyamines can augment the tight junctions between mucosal epithelial cells, reduce intestinal permeability, and alleviate irAEs, such as ICI-induced colitis.

Immune cell function regulation is significantly influenced by polyamines, which support the differentiation and maintenance of memory T cells, modulate Treg cell functions, and impact the antigen presentation capabilities of dendritic cells, thereby highlighting the potential as adjuvant factors for enhancing ICI efficacy. In particular, supplementation with specific bacteria-produced polyamines enhanced the antitumor effect of PD-1 inhibitors in melanoma and lung cancer models. Preclinical models have indicated that polyamine blockade therapy can reduce immunosuppression in the TME and enhance the ICI efficacy[90].

Other novel metabolic biomarkers, including 2-pentanone and tridecane, and increased alkanes, methyl ketones, and p-cresol in patients with early-progressing NSCLC, may potentially serve as predictive biomarkers that augment metabolomic monitoring strategies.

These findings collectively highlight the potential of microbial metabolites as predictive biomarkers for efficacy and toxicity in lung cancer immunotherapy, potentially enabling more personalized treatment approaches that maximize benefits while minimizing adverse events.

Microbiome intervention strategies and clinical applications: lung cancer-specific considerations

Microbiome analysis for patient stratification

Lung cancer demonstrates significant unique characteristics in microbiome stratification that distinctly differentiate lung cancer from other solid tumors. Lung cancer patients require a multi-site integrated analytical approach that comprehensively evaluates oral, airway, and gut microbiomes, unlike other cancers that rely solely on gut microbiome analysis. Jin et al.[17] demonstrated that combining oral and gut microbiome characteristics can improve prediction accuracy by up to 15%, while lower respiratory tract microbial samples collected *via* bronchoscopy show a stronger correlation with the local TME. Lung cancer-specific stratification must consider the following multiple unique factors: smoking status (current/former/never smokers) significantly influences microbiome composition; history of diagnostic antibiotic exposure (commonly used during lung lesion diagnosis to differentiate infections from tumors); histologic subtypes (adenocarcinoma/squamous cell carcinoma/SCLC) closely correlate with microbiome features; and driver gene mutation status, such as EGFR/activin receptor-like kinase (ALK). The relationship between the microbiome and immunotherapy efficacy in lung cancer patients exhibits an "inverted U-shaped" pattern (moderate abundance of AKK correlates with optimal efficacy), distinguishing lung cancer from the linear correlation observed in melanoma patients. Luo et al.[76] identified distinct dynamic microbiome patterns in EGFR-mutant *versus* wild-type patients with specific bacterial signatures predicting immunotherapy response following tyrosine kinase inhibitor (TKI) failure. This molecular subtype-specific microbiome variation represents a unique consideration in lung cancer biomarker development.

Products of the tryptophan metabolism pathway, such as IPA, demonstrate stronger predictive value compared to SCFAs. These characteristics support the necessity of developing lung cancer-specific microbiome stratification strategies rather than simply adopting stratification models from other cancer types.

Microbiome intervention strategies

Microbiota-based interventions primarily include probiotic supplements to replenish beneficial bacterial populations,

FMT to reshape the intestinal microenvironment by transplanting healthy donor microbiota, dietary modifications rich in fiber and fermented foods to promote microbial diversity and balance, as well as engineered bacteria designed for tumor-targeted delivery (**Figure 4**; **Table 3**). These approaches demonstrate varying degrees of efficacy in regulating gut health, immune function, and metabolism, but clinical applications still require individualized assessment.

Strain-specific probiotic interventions for enhanced immunotherapy

Probiotic intervention is a relatively safe and easily implementable strategy for microbiome modulation. Probiotics

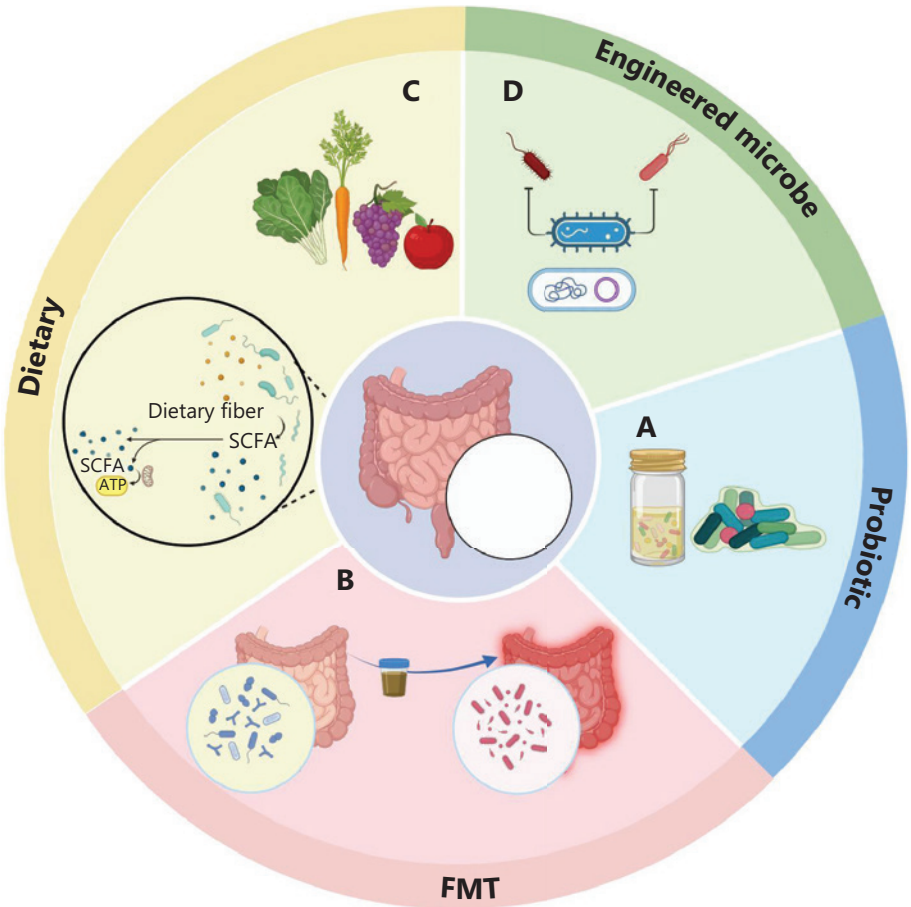


Figure 4 Microbiome-based therapeutic interventions for lung cancer immunotherapy. This figure presents four major strategies for microbiome modulation to enhance immunotherapy efficacy in lung cancer patients. Panel A (probiotic interventions): Shows oral administration of specific probiotic strains. A1: Single-strain probiotics (*Akkermansia muciniphila* and *Bifidobacterium* spp.) are ingested and colonize the gut. A2: Multi-strain consortia work synergistically through complementary mechanisms. A3: Probiotics enhance immunotherapy by increasing CD8+ T cell infiltration, promoting interferon-γ production, and modulating checkpoint molecule expression. Panel B (FMT): Illustrates the FMT process. B1: Healthy donor screening and fecal sample collection. B2: Sample processing and encapsulation or preparation for delivery. B3: Administration *via* capsules or colonoscopy to recipient. B4: Donor microbiota engraftment reshapes recipient gut ecosystem, enhancing anti-tumor immunity. Panel C (dietary interventions): Depicts dietary modulation effects. C1: High-fiber diet increases beneficial SCFA-producing bacteria. C2: Omega-3 fatty acids promote anti-inflammatory microbial communities. C3: Fermented foods introduce beneficial microbes directly. C4: Dietary changes lead to increased SCFA production, enhanced T cell function, and reduced immune-related adverse events. Panel D (engineered microbial therapies): Shows synthetic biology approaches. D1: Genetically modified bacteria designed to target tumor microenvironment. D2: Engineered strains produce immunostimulatory molecules (cytokines and checkpoint inhibitors) locally within tumors. D3: Bacteria programmed for synchronized lysis release therapeutic payloads. D4: Spatial colonization of hypoxic tumor regions maximizes local drug delivery, while minimizing systemic toxicity. CD8+, cluster of differentiation 8-positive; FMT, fecal microbiota transplantation; IFN-γ, interferon-gamma; SCFAs, short-chain fatty acids.

Table 3 Microbiota-targeting strategies in cancer treatment

Interventions	Strategies	Model	Effects*	References
Probiotics and FMT†	MS-20	Xenograft mouse model	Favorable	91
Probiotics	*Lactobacillus*, *Clostridium butyricum*, and *Bifidobacterium*	Meta-analysis	Favorable	92
Probiotics	Antibiotic-resistant lactic acid bacteria	Retrospective study	Favorable	93
Probiotics	*Bifidobacterium bifidum*	Mouse model	Favorable	57
Probiotics	*Akkermansia muciniphila*	Mouse model	Favorable	94
FMT	*Prevotella*, *Lactobacillus salivarius*, and *Bacteroides*	Mouse model	Favorable	95
FMT	NA	Mouse model	Favorable	16
FMT	*Muribaculaceae*, *Ligilactobacillus*, *Escherichia-Shigella*, *Parabacteroides*, *Lachnospiraceae_NK4A136_group*, *Helicobacter*, and *Blautia*	Mouse model	Favorable	77
FMT	NA	NCT06403111	Favorable	96
Dietary	Increased dietary fiber	Observational study	Favorable	97
Engineered microbial therapies	*Escherichia coli* Nissle 1917 strain	Mouse model	Favorable	98
Engineered microbial therapies	Photosynthetic bacteria	Mouse model	Favorable	99

†FMT, fecal microbiota transplantation; *, $P < 0.05$.

are promising therapeutic adjuvants in lung cancer immunotherapy. Recent studies revealed that various probiotics can enhance immunotherapy efficacy by modulating host immune responses.

Abundant *Bifidobacteria* have been observed in the fecal microbiota of NSCLC treatment responders[100]. Notably, *Bifidobacterium bif-K57* enhances anti-tumor immune responses *via* peptidoglycan synthesis, while *B. pseudolongum* activates the T cell-specific A2AR signaling pathway *via* inosine synthesis[101]. Collectively, these mechanisms enhance the intra-TME immune responses and improve ICI efficacy.

Clinical trials have uncovered the extraordinary potential of *Clostridium butyricum 588* (CBM588) in improving ICI efficacy in patients with NSCLC receiving proton pump inhibitors and significantly prolonging PFS in patients with metastatic renal cell carcinoma (RCC), while demonstrating no additional toxicity when combined with ICIs. The advantages of CBM588 include butyrate production, favorable safety profile, and ameliorating antibiotic-associated dysbiosis.

AKK has garnered considerable attention as an emerging probiotic. AKK recruits tumor-specific cytotoxic T cells *via* the TLR2 signaling pathway, while the outer membrane protein (Amuc) activates immune responses and exhibits significant anti-tumor effects when combined with IL-2[102]. However, precise dosage control is crucial because a relative abundance > 5% may paradoxically diminish ICI efficacy.

Beneficial microorganisms help in strengthening anti-tumor defenses through several pathways during cancer treatment. These beneficial bacteria help activate helper and killer T lymphocytes, boost memory T cell numbers, and reduce suppressive Treg cell populations. In addition, these microbes reshape chemical messenger networks by stimulating the production of pro-inflammatory signals, such as interferon-gamma (IFN-γ) and IL-2, managing IL-12-dependent defense reactions, and carefully modulating intra-tumoral chemical balance. Microbes further assist by enhancing antigen presentation by activating specialized CD103+ dendritic cells and improving the expression of key recognition molecules on cell surfaces. Multi-strain approaches often yield better results than single-strain approaches. For example, a specific mixture of 11 different bacterial strains has exhibited a remarkable ability to enhance ICI efficacy with research indicating that maximum benefits require the concerted working of all components. Such multi-strain approaches work *via* various complementary mechanisms, as follows: gut accumulation of IFN-producing activated killer T cells is increased; overall anti-cancer immune activity is strengthened; and restructuring of a more favorable intestinal microbial community is facilitated.

However, several challenges persist in the clinical application of probiotics. Primarily, these challenges include quality control issues, such as a lack of standardized production protocols, inconsistent product quality, and difficult viability

maintenance. In addition, personalized treatment is challenged by significant inter-patient variability in probiotic responses, difficulties in determining optimal dosages, and the need for optimized combination therapy. Microbial metabolites (e.g., SCFAs, tryptophan metabolites, and bile acids) also hold promise as functional biomarkers. Probiotics may be a "double-edged sword" in cancer immunotherapy modulation. Probiotic efficacy is further influenced by patient heterogeneity, including baseline gut microbiota composition, dietary habits, and genetic factors, leading to variable patient responses, including exhibiting no benefits or even experiencing adverse effects. Furthermore, the lack of standardization complicates efficacy assessment, limits clinical trial data, and renders unclear long-term outcomes. These findings highlight the importance of microbiome-centered precision intervention strategies.

FMT

FMT involves transferring fecal matter from healthy donors to recipients to re-establish normal gut microbiota functionality. FMT modifies the gut microbiota composition in recipients markedly, augmenting immune cell activity and enhancing anti-tumor immune responses. Globally, numerous on-going clinical studies are examining FMT effects on pan-cancer immunotherapy responses, including melanoma, lung and colorectal cancers, and RCC.

Clinical studies

Pioneering research has established the foundation for FMT applications in cancer immunotherapy. Routy et al.[16] transplanted gut microbiota from immunotherapy-responsive patients into tumor-bearing mice lacking these bacterial communities. The results demonstrated that mice receiving FMT exhibited significantly enhanced systemic immunity and anti-tumor responses, mirroring the responses observed in donor patients. This finding provided critical evidence supporting FMT applications in clinical settings.

Human clinical trials have further validated the potential of FMT. The trials included 10 patients with melanoma that had failed to respond to anti-PD-1 immunotherapy and FMT donors in remission for > 1 year. The study reported positive responses in 3 patients, including 1 complete and 2 partial remissions with a PFS > 6 months. Considering these patients had previously exhibited complete non-responsiveness to treatment, this outcome suggests that FMT can effectively remodel the tumor immune microenvironment, although the 30% response rate might appear modest.

FMT has expanded to multiple solid tumors, including hepatocellular carcinoma and gastrointestinal cancers. FMT with nivolumab has achieved disease control rates in gastrointestinal cancers of up to 40%, suggesting broader therapeutic potential[95]. FMT with pembrolizumab and axitinib was reported to markedly enhance therapeutic outcomes in patients diagnosed with metastatic RCC with 66.7% of patients maintaining a PFS status at the 1-year mark, in contrast to 35% of the placebo cohort[97]. These results indicate that FMT possesses broad potential for enhancing immunotherapy efficacy across diverse tumor types.

Currently, multiple clinical trials are evaluating the role of FMT in lung cancer immunotherapy. Trial NCT04521075 is evaluating nivolumab with FMT capsules in melanoma and NSCLC[103], while trial NCT05008861 is evaluating anti-PD-1/PD-L1 immunotherapy with FMT capsules in NSCLC[104]. These ongoing studies will provide additional evidence-based support for FMT applications in lung cancer treatment.

Mechanism underlying FMT

FMT enhances anti-tumor immune responses through multiple mechanisms. Microbial transplantation alters the intestinal environment of the recipient, augmenting CD8+ T cell activity and cytotoxicity, which are critical mediators of effective anti-tumor responses. Microbial metabolites, particularly SCFAs, regulate the immune system by influencing T cell differentiation and function. Bacterial antigens may cross-react with tumor antigens through molecular mimicry, enhancing anti-tumor immune surveillance.

Japanese researchers have identified 11 distinct gut bacterial strains that elevate CD8+ T cell levels and exhibit anti-cancer properties. Recent investigations have further identified specific bacterial strains that significantly impact immunotherapy responses[98]. For example, *Prevotella* boosts CD8+ T cell activity, while promoting anti-tumor immune responses. Conversely, *Lactobacillus salivarius* and various *Bacteroides* spp. may inhibit T cell activation, potentially weakening FMT and anti-PD-1 therapy efficacy. Although FMT holds promise in enhancing cancer immunotherapy, several challenges remain. Detailed evaluations of long-term safety, especially in immunocompromised patients, are needed. Patient-specific differences in treatment effects highlight the need to better comprehend the nuanced relationship between the gut microbiome and immune function. Future research should focus on drafting personalized therapeutic strategies that account for the unique microbial environment of each individual, while formulating more stringent protocols for selecting optimal donors in microbiome transplant scenarios.

Donor selection strategies

The clinical success of FMT increasingly depends on appropriate donor selection strategies. Recent studies have revealed significant heterogeneity in donor efficacy with appropriate donor selection potentially exerting determinative influences on treatment outcomes. In a breakthrough study, a hepatocellular carcinoma patient who initially showed no response to FMT treatment exhibited significant tumor regression after donor replacement, clearly establishing donor selection as a critical factor influencing treatment outcomes[98]. Researchers

noted that some donors achieved engraftment rates as high as 67%, while other donors demonstrated markedly lower rates, a disparity potentially associated with the presence of specific microbes, such as *Bifidobacterium*[105].

Donor selection should be based on multiple criteria. First, microbiome composition represents a key consideration with donors harboring abundant beneficial bacteria, such as AKK, *Bifidobacterium*, and *Faecalibacterium*, typically associated with superior treatment outcomes. Second, the strategy of obtaining fecal samples from immunotherapy-responsive patients is preferable to sourcing from healthy but immunotherapy-naïve donors because the TME may exhibit unique responses to specific microbial communities. Third, the baseline microbiome status of the recipient influences engraftment efficacy, with research indicating that patients with lower baseline microbial diversity may be more responsive to FMT, providing a theoretical foundation for personalized selection. Finally, different cancer types may demonstrate varying response patterns to specific microbial characteristics, necessitating the adjustment of donor selection criteria for specific tumor types.

Limitations and future directions

Despite the significant potential demonstrated by FMT in cancer immunotherapy, several important limitations warrant thorough consideration. First, most current FMT cancer studies have limited sample sizes, typically < 20 patients, which restricts the statistical power and generalizability of results. Small-sample studies may fail to capture subtle differences in treatment effects and inadequately assess response patterns across patient subgroups.

Donor variability represents the second major challenge confronting FMT. Significant differences in microbial composition between donors lead to inconsistent and unpredictable treatment effects. The specific microbial characteristics or combinations that reliably predict FMT success remain incompletely defined, complicating the development of standardized treatment protocols. Even samples from the same donor collected at different time points may exhibit significant variations, further increasing the variability of treatment outcomes.

Safety concerns cannot be overlooked. Although FMT demonstrates good tolerability in most cases, potential serious risks exist. Two patients have been reported who developed broad-spectrum β-lactamase-producing *Escherichia coli* bacteremia following FMT, resulting in one fatality. This finding underscores the necessity for rigorous donor screening. In addition, immunocompromised patients, such as cancer patients, may face additional risks, requiring more stringent safety monitoring.

Finally, regulatory framework uncertainties present challenges for the widespread application of FMT. The current regulatory focus of the FDA for FMT centers on *Clostridioides difficile* infection treatment with cancer immunotherapy applications lack clear guidelines[106]. Regulatory differences across countries and regions also increase the complexity of multicenter clinical trials, potentially delaying the clinical translation of this therapeutic approach.

To overcome the current challenges facing FMT in cancer immunotherapy, future research should advance along several key directions. First, developing standardized microbial consortia can reduce donor dependence and enhance treatment consistency. Through precise definition and production of specific beneficial microbial combinations, more controllable and predictable therapeutic effects can be achieved while reducing pathogen transmission risks.

Second, establishing precision donor-recipient matching systems based on microbiome analysis is crucial for enhancing personalized treatment efficacy. Similar to tissue typing technologies in organ transplantation, microbiome compatibility assessment may become key to future FMT treatment success. This assessment requires developing more precise microbiome sequencing and analysis platforms with predictive algorithms to determine optimal donor-recipient combinations.

Comprehensive strategies integrating FMT with other microbiome-modulating interventions, such as probiotics and dietary interventions, also warrant exploration. For example, fiber-rich diets may enhance the growth of certain beneficial bacteria, thereby augmenting FMT efficacy. Specific probiotic supplementation may help stabilize the transplanted microbial community, prolonging therapeutic effects. Such combinatorial approaches may produce significant effects that single interventions cannot achieve.

Establishing more rigorous and standardized donor screening and safety monitoring protocols is essential for mitigating potential risks. This protocol includes more comprehensive pathogen screening, microbiome functional analysis, and establishing shared donor databases and adverse event reporting systems. Long-term follow-up studies are also indispensable for evaluating the persistent effects of FMT and potential delayed adverse reactions.

Finally, large-scale, multicenter randomized controlled trials are key to establishing the position of FMT in cancer treatment. These studies should include sufficient sample sizes, consider patient heterogeneity, and incorporate detailed microbiome and immune monitoring to reveal treatment response mechanisms and predictive factors. Collaboration between academia and industry is critical for overcoming resource and regulatory challenges and accelerating the clinical translation of this promising therapeutic approach.

Through these comprehensive efforts, FMT holds promise as an important strategy for enhancing cancer immunotherapy efficacy, offering new therapeutic hope for patients with refractory malignancies. However, achieving this goal requires balancing innovation with caution, ensuring that this complex and powerful therapeutic approach is both safe and effective.

Dietary interventions

Dietary habits shape microbial composition and activity with individual differences resulting in personalized effects of specific nutrients on host metabolism. Findings from animal model studies, population-based studies, and clinical trials highlight the essential role of dietary components in immunotherapy outcomes. Simpson et al.[83] explored the positive impact of gut microbiota linked to a fiber- and omega-3 fatty acid-rich diet on the effectiveness of neoadjuvant ICI in high-risk resectable metastatic melanoma. The findings revealed enhanced anti-tumor immune responses and decreased irAEs. Better immunotherapy outcomes and fewer adverse effects have been reported in patients whose gut microbiome features abundant *Ruminococcaceae*, AKK, and *Methanogenic archaea*. This beneficial connection partly resulted from an increased consumption of fiber and omega-3 rich foods. Patients consuming adequate fiber exhibit notably longer PFS during ICI therapy, possibly through the following key mechanisms: SCFAs altering T cell development pathways; adjusting the proportion between Teff and Treg lymphocytes; and enhancing memory capabilities in activated CD8+ T cells. Furthermore, laboratory experiments have revealed that high-fiber dietary patterns can reshape the gut microbiome. Such diet-driven changes enable STING-dependent type I IFN pathways to reprogram TAMs, ultimately boosting immunotherapy efficacy[91].

Individual responses to specific dietary factors are significantly influenced by baseline microbial communities, highlighting the complex interactions between diet, microbiota, metabolome, and immunity. Patients with microbiomes previously uninfluenced by fiber reportedly experience enhanced clinical benefits from immunotherapy after increased dietary fiber intake. Although dietary interventions are safe and cost-effective, accurately identifying responsive individuals and effectively leveraging the diet-microbiota-immune axis to optimize therapeutic outcomes remain its primary challenges.

Tumor-targeting engineered bacterial therapeutics

Although immunotherapy has revolutionized cancer treatment, demonstrating considerable advancements, challenges including limited response rates, acquired resistance, toxicity, and high costs persist. Engineered bacteria with specific tumor-targeting and programmable features offer novel strategies to enhance immunotherapy efficacy. The TME is distinct from normal tissues, characterized by hypoxia, necrotic cores, and disorganized vasculature, rendering the TME an ideal target for specific bacterial species. Obligate anaerobes, such as *Clostridium* and *Bifidobacterium*, are more likely to accumulate in hypoxic tumor regions. Facultative anaerobes, including *Salmonella*, *E. coli*, and *Listeria*, specifically target tumors *via* mechanisms, such as lingering in damaged blood vessels and chemotaxis toward tumor-emitted substances and preferential growth under favorable metabolic conditions[92].

Various synthetic biology and metabolic engineering strategies have been developed by leveraging bacterial tumor-targeting abilities. Engineered *E. coli* Nissle 1917 strains convert ammonia into L-arginine at tumor sites, enhancing intra-tumoral L-arginine levels, and facilitating T cell infiltration and working synergistically with PD-L1 inhibitors[93]. The auxotrophic *Salmonella typhimurium* A1-R strain achieves tumor targeting by selectively growing in the nutrient-rich TMEs.

Engineered immunomodulatory bacteria are designed to produce some chemokines. Bacteria with C-X-C motif chemokine ligand 16 (CXCL16) expression attract cytotoxic T lymphocytes to tumor sites, whereas bacteria with C-C motif chemokine ligand 20 (CCL20) expression help recruit dendritic cells[94]. The synergistic activity of these immune cells significantly enhances anti-tumor immune responses. Furthermore, recent advances include engineered bacteria that express CD47-targeting nanobodies, thereby augmenting the macrophage-mediated phagocytosis of tumor cells[77].

Novel delivery systems, such as the synchronized lysis circuit, have been engineered to enhance therapeutic outcomes. This technology enables bacteria to undergo controlled lysis upon reaching critical population density[96], facilitating therapeutic molecule release while preserving minimal surviving populations to ensure prolonged therapeutic efficacy. Empirical studies demonstrate that this approach impacts directly-injected tumors and elicits immune responses in distant, non-injected tumors as well.

Bacteria-mediated immune responses function at two levels. Locally, bacteria augment tumor-infiltrating immune cells, enhance antigen presentation, and bolster T cell-mediated anti-tumor activities. Systemically, bacteria affect non-injected tumors, inhibit metastasis, and contribute to establishing long-term immune memory. Han et al.[107] developed an *in situ* tumor vaccine strategy using engineered photosynthetic bacteria that facilitates tumor antigen release and efficiently transports bacteria to tumor-adjacent tissues and lymph nodes with intact immune function, inducing robust anti-tumor immune responses.

Although engineered bacteria exhibit distinct advantages in tumor immunotherapy, several challenges persist. Future research should focus on developing precision bacterial engineering techniques, optimizing delivery protocols, enhancing safety measures, expanding therapeutic indications, and exploring synergies with existing immunotherapies.

Integrated strategies: combining the microbiome with other immunotherapy approaches

A combination strategy, such as microbiome modulation with conventional immunotherapy, could yield better therapeutic results. This approach recognizes TME complexity and the diverse nature of microbiome-host interactions. Technological advancements, such as metagenomic sequencing and artificial intelligence (AI)-assisted analysis, provide classification of patients based on microbiome status. This finding establishes the basis for designing personalized treatment strategies, incorporating microbiome modulation along with ICIs, cancer vaccines, or cellular therapies.

Research in preclinical and early clinical stages has revealed that combining probiotic supplements or FMT with PD-1 inhibitors can markedly improve treatment outcomes and decrease adverse effects[108]. Dietary interventions customized to the initial microbiome profiles of patients can enhance immunotherapy outcomes[99].

Moreover, creating precision antibiotic treatments personalized for particular patient groups can maintain beneficial microbiota, while eliminating those that might adversely affect immunotherapy. Microbiome metabolites, such as SCFAs or tryptophan metabolites, may serve as promising adjuncts to immunotherapy. The approach of merging engineered microbes with current immunotherapeutics is a largely unexplored frontier with the potential to innovatively deliver therapeutic molecules and alter the TME.

Confounders and clinical considerations in lung cancer immunotherapy

Antibiotic use in lung cancer patients

Antibiotic use represents a critical factor affecting immunotherapy efficacy in lung cancer. The diagnostic process for lung cancer involves unique patterns of antibiotic use, presenting special challenges for these patients. When suspicious lung nodules are detected in clinical practice, antibiotic treatment is often initially administered to exclude infectious lesions. This "diagnostic antibiotic therapy" strategy is widely applied in the differential diagnosis of indeterminate lung nodules, particularly for cases with inflammatory manifestations. Physicians typically observe changes in nodules following antibiotic treatment. If the nodule disappears or significantly shrinks, an infectious disease is favored; if no significant change occurs, the likelihood of malignancy increases[109].

This diagnostic antibiotic usage pattern means many patients ultimately diagnosed with lung cancer have already undergone one or more cycles of antibiotic treatment before diagnosis. According to clinical practice, these antibiotics are typically broad-spectrum types, such as quinolones, β-lactams, or macrolides, which are precisely the drugs proven to have the most significant impact on the gut microbiome. Consequently, lung cancer patients may already have microbiome dysbiosis before starting immunotherapy, which could be an important but often overlooked factor contributing to variations in immunotherapy efficacy in lung cancer.

Furthermore, lung cancer patients frequently have comorbid chronic pulmonary diseases, such as chronic obstructive pulmonary disease (COPD) or bronchiectasis, which often require repeated antibiotic use to control chronic infections or acute exacerbations[110]. This pattern of long-term, intermittent antibiotic exposure has more complex effects on the microbiome, potentially leading to persistent microbiome dysbiosis and functional alterations.

Multiple large-scale studies have confirmed the negative impact of antibiotic use. Pinato et al.[111] conducted a study involving 196 NSCLC patients receiving ICIs that showed antibiotic use before and after immunotherapy significantly reduced OS (HR = 2.5, 95% CI: 1.7–3.7, $P < 0.01$). A systematic review and meta-analysis by Lurienne et al.[84] encompassing 23 studies and > 5,500 patients confirmed that patients receiving antibiotics had a significantly reduced OS (HR = 1.69) and PFS (HR = 1.47). This effect was most pronounced when antibiotic exposure occurred within 60 days before or after ICI initiation, resulting in a reduction in OS of approximately 6.7 months. Notably, this negative impact has been observed not only in immunotherapy alone but in patients receiving combined chemotherapy-immunotherapy (Chemo-IO).

Antibiotics have a varying impact on the microbiome. Broad-spectrum antibiotics, particularly fluoroquinolones, cause the most severe damage to key immunomodulatory bacteria, such as AKK and *Bifidobacterium*. These antibiotics interfere with immune responses through multiple mechanisms, including downregulation of ileal mucosal cell adhesion molecule-1, promoting gut recolonization, and migration of regulatory T cells to tumors. In addition, antibiotics reduce the number of Teff and memory T cells, directly affecting the ability of the immune system to recognize and kill tumor cells.

Several optimization strategies can be considered to reduce adverse effects on the microbiome given the unique characteristics of antibiotic use in the lung cancer diagnostic process. The primary principle is precise use of diagnostic antibiotics, strictly limiting use to lung nodules genuinely suspected of infection. Among nodules highly suspicious for malignancy, such as nodules with high PET-CT metabolism or typical malignant imaging features, unnecessary "diagnostic antibiotic trials" should be avoided. Minimally invasive biopsies and other methods can be considered to determine the nature of lung nodules more directly, reducing diagnostic antibiotic use. Selecting antibiotics with less impact on the microbiome is equally crucial. When diagnostic antibiotic trials are necessary, antibiotics should be prioritized with minimal

effects on the gut microbiome. Narrow-spectrum antibiotics should be considered to reduce widespread effects on gut commensal bacteria and prolonged use of broad-spectrum antibiotics, such as fluoroquinolones, should be avoided for diagnostic trials. Post-diagnostic microbiome recovery strategies are also essential. Patients diagnosed with lung cancer after diagnostic antibiotic treatment should receive targeted microbiome recovery plans. Microbiome status should be assessed and interventions implemented before immunotherapy begins, especially for patients who have received multiple cycles of antibiotics. Probiotics, prebiotics, and dietary interventions can be used to promote rapid microbiome recovery. Multidisciplinary collaborative decision-making is the safeguard for implementing these strategies, promoting cooperation among pulmonologists, oncologists, microbiologists, and infectious disease specialists to formulate optimal diagnostic and antibiotic use strategies. Standardized processes for lung nodule diagnosis should be established to reduce unnecessary antibiotic use and considerations of potential future immunotherapy effects should be incorporated into antibiotic treatment decisions.

Smoking-related microbiome alterations

The association between smoking history and the microbiome introduces distinct challenges as well. Tsay et al.[43] analyzed 298 participants and demonstrated that smoking status significantly influenced the composition of the airway microbiota. Current smokers exhibited distinct microbiome profiles compared to non-smokers ($P < 0.001$), particularly in the abundance of inflammation-associated bacterial communities. Tsay et al.[43] further confirmed that smoking affects airway and gut microbiome composition, significantly correlating with immunotherapy efficacy. The duration of smoking cessation influences microbiome recovery, providing important insights for developing personalized intervention strategies.

Impact of respiratory co-morbidities

Patients with lung cancer and co-morbidities, such as COPD or pneumonia, face distinctive hurdles during immunotherapy. These respiratory issues significantly reshape the microbial landscape of the airway. Patients with COPD typically exhibit increased bacterial burdens with some species (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) dominating, while overall microbial diversity decreases[110-114]. Such shifts potentially undermine immunotherapy effectiveness by altering local inflammation patterns, cytokine signatures, and mucosal immune responses. Furthermore, these microbiome disruptions may even influence systemic immunity through the gut-lung axis and metabolites, such as SCFAs[110]. The lack of comprehensive studies directly examining the effect of respiratory disease-related microbiome changes on immunotherapy outcomes in lung cancer requires urgent research attention. Clarifying these relationships could significantly improve response prediction, enable microbiome-targeted supportive therapies, and help customize treatment approaches for patients with lung cancer and respiratory diseases.

Cardiovascular disease considerations

Patients with lung cancer and cardiovascular diseases face a complex treatment scenario. Heart failure often leads to pulmonary edema, potentially disrupting the local microbiome. Risk factors, such as smoking and alcohol use, are common. Notably, ICIs may trigger heart-related side effects, while heart medications may alter immunotherapy results. The CANTOS trial revealed that patients with heart disease have distinctive immune characteristics and elevated inflammation markers[115]. In addition, multi-omics research suggests that statins have a meaningful role by potentially boosting outcomes for patients with acute coronary syndrome by reshaping the gut microbiota composition[116], specifically increasing beneficial microbes, such as *Bifidobacterium* and *Anaerostipes hadrus*, while decreasing harmful microbes, such as *Parabacteroides merdae*.

Future perspectives and research directions

Challenges in clinical validation and translation of microbiome biomarkers

The laboratory-to-clinical practice translation of microbiome biomarkers faces numerous roadblocks. The lack of uniform protocols for collecting, processing, and analyzing samples renders comparison studies infeasible. Varied sequencing platforms, data analysis, and statistical methods lead to contradictory findings that stall progress.

The ever-changing profiles of the microbiome are a challenge as well. Dietary choices, medications, environmental exposures, and genetic factors shape microbial communities. This complexity complicates the identification of reliable predictors because researchers must consider day-to-day variations and individual differences. Geographic and population differences contribute significantly to these inconsistencies. Microbiome signatures predictive of immunotherapy response in Western populations show markedly reduced accuracy when applied to Asian lung cancer cohorts, suggesting that population-specific factors, including genetic background, dietary patterns, and environmental exposures, may modulate microbiome-immunotherapy relationships in ways that limit biomarker generalizability. A multicenter

European analysis reported substantial inter-site variability in microbiome-response associations with findings from one center failing to replicate in other centers[117].

Current studies have only revealed connections rather than proving cause-and-effect relationships. This knowledge gap hinders our comprehension of microbial influence on cancer, limiting targeted intervention development. The distinction between correlation and causation represents one of the most fundamental challenges in microbiome research translation. Most microbiome-immunotherapy studies in lung cancer use observational designs that successfully identify statistical associations but cannot definitively establish whether microbiome alterations are causes, consequences, or merely coincidental markers of treatment outcomes.

Several factors complicate causal inference in microbiome research. First, the microbiome exists in complex ecological networks where individual taxa rarely function in isolation, making it difficult to attribute specific effects to particular microorganisms. Second, bidirectional relationships between microbiome and host immunity create feedback loops that obscure primary causal directions. Third, confounding variables, including diet, medications, genetic factors, and environmental exposures, may simultaneously influence both microbiome composition and immunotherapy responses, creating spurious associations.

To establish stronger causal evidence, future research should prioritize randomized controlled trials of microbiome interventions, mechanistic studies using gnotobiotic animal models, and Mendelian randomization approaches that use genetic variants as instrumental variables. Until such evidence emerges, clinical applications should acknowledge that microbiome-based strategies remain largely experimental, with efficacy based primarily on associative rather than mechanistic evidence.

Laboratory findings and animal studies often do not translate well to human patients, owing to the highly individualized nature of the microbiome. Several randomized controlled trials of probiotic supplementation during immunotherapy failed to demonstrate clinical benefits. Multi-strain probiotic formulations, despite showing promise in preclinical models, have not consistently improved treatment response rates or reduced irAEs in lung cancer patients receiving ICIs.

Rigorous clinical trial designs for microbiome-based treatments should include appropriate control groups, sufficient participant numbers, and long-term follow-up to confirm both safety and effectiveness across diverse patient groups. Furthermore, the multi-omics integration of microbiome data to comprehend microbiome-tumor-host relationships adds considerable analytical complexity. Standardized approaches may help address these multifaceted challenges. Large, multi-center prospective studies using standardized protocols help generate reliable results. Combining multi-omics with advanced AI methods might help identify more stable predictive markers, ultimately closing the laboratory-to-clinical use distance.

Technological and methodologic advances

Cutting-edge technologies are revolutionizing microbiome research and creating new possibilities. Third-generation sequencing platforms, such as Oxford Nanopore and PacBio, deliver in-depth microbial genetic information down to specific strains. Ultra-deep sequencing detects previously invisible low-abundance microbes, including fungi and viruses, that may significantly affect cancer treatment outcomes. Single-cell microbiomics allow the detailed study of individual microbial cells. Spatial microbiome mapping techniques reveal the precise intra-tumoral location of specific microbes and the interaction with nearby immune cells, offering new insights into microbe-host relationships. In addition, new computational platforms integrate multi-omics with microbiome data, thoroughly illustrating microbe-host interactions. These holistic analyses help decipher the complex microbiome and cancer biology connection. Artificial gut models and organ-on-chip technologies allow the study of microbe-host interactions under controlled conditions, bridging the gap between observation and causation. AI and machine learning approaches, including deep learning, natural language processing (NLP), and network analysis, have revolutionized complex microbiome dataset analysis and accurately predict treatment responses. Several breakthrough studies have demonstrated the practical application of these technological advances in lung cancer immunotherapy. Jin et al.[17] developed a multi-site integrated scoring system combining oral, airway, and gut microbiome analyses, achieving significantly superior predictive accuracy (AUC = 0.89) compared to single-site approaches (AUC = 0.71–0.78). This multi-site integration approach leverages the unique accessibility of respiratory tract samples during routine bronchoscopic procedures, offering practical advantages for lung cancer patients. Dynamic monitoring represents another significant methodologic advance with direct clinical implications. In a prospective study involving 112 NSCLC patients, Derosa et al.[118] revealed that microbiome stability during the initial 6 weeks of treatment provides stronger prognostic value than baseline abundance alone. Patients maintaining stable AKK and *Faecalibacterium prausnitzii* levels demonstrated a median PFS of 8.2 months compared to only 3.5 months in those experiencing significant microbial fluctuations (HR = 0.41, $P < 0.001$). These findings suggested that real-time microbiome monitoring could guide treatment continuation decisions and enable timely therapeutic adjustments.

Collectively, these innovations bolster microbiome research capabilities for discovering and validating immunotherapy biomarkers. With further advancement and accessibility, these technologies may accelerate breakthroughs in cancer treatment approaches.

Microbiome research advancing personalized immunotherapy

Microbiome research holds much potential for personalizing lung cancer immunotherapy strategies. A microbiome signature classification system development, which helps identify patient groups who benefit most from specific treatments, is a major step forward. These systems can incorporate diverse information, namely microbial communities, functional pathways, and metabolite profiles, comprehensively guiding clinical decisions. In addition, non-invasive microbiome monitoring tools could enable real-time treatment adjustments. Such approaches might reflect treatment responses and resistance development, allowing clinicians to modify protocols and timing to maximize benefits for individual patients. Customized dietary plans and probiotic treatments are other promising avenues. The design of individualized approaches based on a patient's existing microbiome profile and cancer subtype might enhance immunotherapy effectiveness, while reducing irAEs *via* targeted microbial adjustments. Precision antibiotic strategies that preserve beneficial bacteria while controlling harmful bacteria strike a crucial balance between infection management and immunotherapy success. Advanced approaches could include engineered bacterial therapies targeting specific tumor characteristics or producing beneficial compounds locally. In addition, developing dose-response models for microbiome interventions, whether FMT, probiotics, or dietary modifications, could help optimize individualized treatment protocols.

With evolving knowledge of microbiome-cancer relationships and advancing technologies, truly personalized

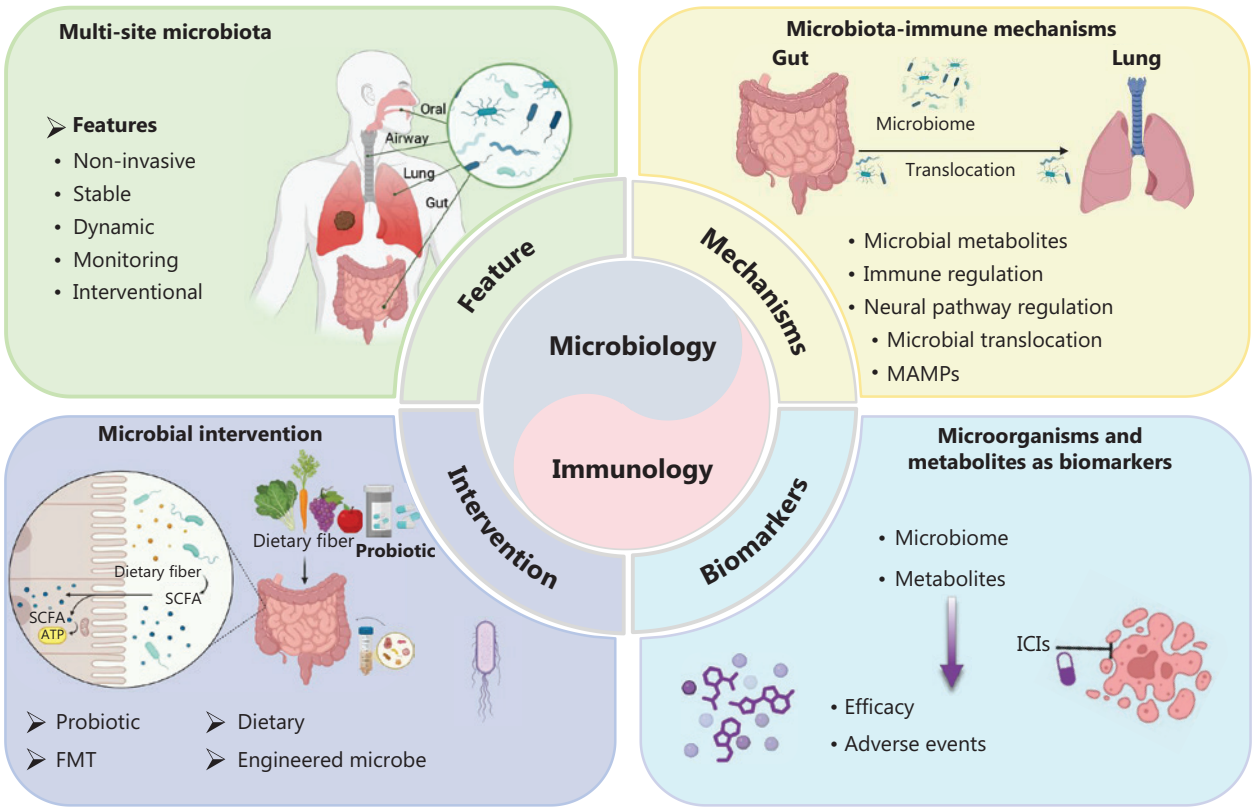


Figure 5 Comprehensive framework of microbiome applications in lung cancer immunotherapy. This figure summarizes key findings and clinical applications of microbiome research in lung cancer immunotherapy. Section 1 (microbiome characteristics): Presents the characteristics of microbial communities in the oral cavity, airways, lungs, and intestines, including non-invasive, stable, dynamic, monitoring, and interventional aspects. Section 2 (interaction mechanisms): This section highlights four main pathways: regulation of immune checkpoints through bacterial metabolites, influencing PD-1/PD-L1 expression; modulation of cytokine networks through microbial stimulation of pro-inflammatory and anti-inflammatory mediators; activation of T cells through bacterial antigen cross-reactivity; and metabolic processes that reprogram the tumor microenvironment. Section 3 (predictive biomarkers): Highlights microbiome features predictive of treatment outcomes and biomarkers associated with immune-related adverse events. Section 4 (therapeutic strategies): Outlines intervention methods, including probiotics, FMT, dietary adjustments, and engineered bacteria with arrows indicating the integration with standard immunotherapy. FMT, fecal microbiota transplantation; ICIs, immune checkpoint inhibitors; irAEs, immune-related adverse events; PD-1, programmed death protein 1; PD-L1, programmed death ligand 1; SCFAs, short-chain fatty acids.

microbiome-based immunotherapy has increasingly become feasible. The convergence of microbiome science, cancer treatment, and personalized medicine holds remarkable promise for improving future patient outcomes.

Conclusions

The microbiome characteristics in patients with lung cancer, the microbiome and TME interactions, the importance of microbial metabolites as predictive biomarkers, and microbiome-based personalized immunotherapy have been comprehensively analyzed in this review. The microbiome as a biomarker for lung cancer immunotherapy is a promising research frontier that may enable accurate patient stratification, revolutionize personalized treatment regimens, and transform outcome monitoring.

Alterations in oral, respiratory tract, pulmonary, and intestinal microbiome exhibit strong links to both cancer progression and immunotherapy effectiveness. Specific microbial taxa, such as AKK, *Bifidobacterium*, and *Faecalibacterium prausnitzii*, predict better treatment responses, while *Helicobacter pylori* and *Gammaproteobacteria* are associated with unfavorable outcomes. Microbial metabolites, such as SCFAs, tryptophan metabolites, and bile acids, hold promise as functional biomarkers, providing better predictions of treatment outcomes and irAEs risks than taxonomic composition. Notwithstanding significant progress, translating microbiome biomarkers to clinical practice faces multiple challenges, including methodologic standardization, causality establishment, clinical validation, and regimen personalization.

A thorough understanding of the microbiome-tumor-immune system relationship underpins novel treatment strategies. Microorganisms influence immunotherapy effectiveness *via* different mechanisms, including immune checkpoint expression regulation, cytokine network modification, T cell activation and recruitment, and TME metabolic profile alteration. The spatial heterogeneity of these mechanisms further increases the complexity and personalization requirements for microbiome-based interventions.

Microbiome-based intervention strategies, including probiotics, FMT, dietary modulation, and engineered bacteria, can potentially enhance immunotherapy efficacy, reducing irAE risks while improving response rates, thus supporting treatment options.

Despite these promising associations, it is crucial to acknowledge that microbiome research in lung cancer immunotherapy faces significant limitations. Inconsistent findings across studies, negative intervention trial results, and the predominance of correlational rather than causal evidence underscore the complexity of microbiome-host-tumor interactions. The field must embrace negative results as equally informative as positive findings because negative results help define the boundaries of microbiome influence on treatment outcomes and prevent overly optimistic expectations.

Future research should focus on microbiome function rather than merely composition, integrating multi-omics data to provide in-depth immunotherapy response prediction models, and developing personalized microbiome-based intervention strategies for specific patient populations, ultimately improving survival rates and quality of life for patients with lung cancer (**Figure 5**).

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Kezhong Chen.
Collected the data: Zewen Sun, Mantang Qiu, Zuli Zhou.
Performed the analysis: Yun Li.
Wrote the paper: Kexin Feng, Jun Wang, Shuai Wang.

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