

Review

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Review

Human Microbiome as Clinical Biomarkers for Respiratory Tract Cancers

Running title: Microbiome as biomarkers for respiratory tract cancers.

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Abstract

Respiratory tract cancers (RTCs), including lung, laryngeal, nasopharyngeal, and tracheal cancers, are among the most common cancer types. These cancers show difficulties in the case of early diagnosis and treatment monitoring. The microbiome, the community of microbes living in a single area of the body, is associated with cancer and offers potential for developing non-invasive diagnostic tools and probiotic therapies. Microbiome dysbiosis affects both the tumor and the immune microenvironment. The major pathways of RTCs, including EGFR, ALK, KRAS, STAT3, WNT, etc., are affected by the dysbiosis that occurs during cancer progression. Several microbial species have also been associated with treatment outcomes of immune checkpoint inhibitors (ICIs), chemotherapy, and anti-PD-1 therapy. Microbes such as *Alistipes indistinctus*, *Alistipes shahii*, *Barnesiella viscericola*, *Streptococcus salivarius*, *Parabacteroides*, and *Faecalibacterium*, along with genera like *Bifidobacterium*, *Collinsella*, *Veillonella*, and families/orders like *Actinomycetales*, *Odoribacteraceae*, and *Selenomonadales*, have shown positive associations with overall survival, progression-free survival, and improved treatment responses. Conventional biomarkers for RTCs have greatly improved diagnosis and monitoring. Still, they suffer from limitations such as low sensitivity in early-stage, intrusive sample procedures, and a lack of specificity. In contrast, gut, airway, and oral microbiota have emerged as promising non-invasive biomarkers with established associations to cancer progression, metabolic pathways, immune responses, and treatment monitoring. Moreover, non-invasive sampling methods like stool, sputum, and oral swabs offer improved patient comfort and early detection opportunities. In this mini review, we explore global research on human microbiomes as potential diagnostic or therapeutic biomarkers and their associations with RTCs.

Keywords: respiratory tract cancers; lung cancer; nasopharyngeal cancer; microbiome; lung microbiome; stool; metagenomics; biomarker; clinical biomarker; prognostic biomarker; diagnostic biomarker

1. Introduction

Respiratory tract cancers (RTCs), the prevalent types of cancers,[1] include lung cancer (LC), laryngeal cancer (LAC), nasopharyngeal cancer (NPC), etc. Besides these major types, LCs can be classified as small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). NSCLC can also

be subdivided into squamous cell carcinoma (SCC), adenocarcinoma (ADC), large cell carcinoma (LCC), etc.[2] While these cancers show difficulties in early diagnosis and treatment monitoring,[3] with the help of omics, finding associations of cancers with microbiomes is now more feasible than ever. Thus, we can find non-invasive diagnostic/prognostic biomarkers that might have therapeutic application as well.[4] This mini review aims to explore research on human microbiomes as potential diagnostic or treatment biomarkers and their associations with RTCs.

2. Microbiomes and Their Relevance as Biomarkers

The term microbiome/microbiota was coined by Joshua Lederberg in 2001.[5] However, the related research accelerated after the initiation of the Human Microbiome Project in 2008. The contemporary bioinformatics advancements played a crucial role, too. Several microbiomes (gut, tissue, oral, lung, etc.) related to different body parts have been sequenced so far, and the association of gut and lung microbiomes with RTCs is now well known.[6,7]

Existing biomarkers for RTCs include circulating tumor DNA, oncogenic mutations, protein markers, and imaging-based markers, etc.[8,9] While they improved diagnosis and treatment surveillance, but had limited sensitivity in early-stage illness, intrusive sampling procedures, or a lack of specificity, etc. In contrast, the microbiome has emerged as a non-invasive novel biomarker with proven associations to cancer progression, metabolic pathways, immune responses, and treatment monitoring.

2.1. Association of Cancer Pathways and Microbiomes in RTCs

The major pathways include EGFR, ALK, KRAS, STAT3, MAPK, WNT, etc.[10] Most of the cancer diagnoses and treatments are based on these pathways. And they are significantly associated with and affected by the microbial dysbiosis. (Figure 1)

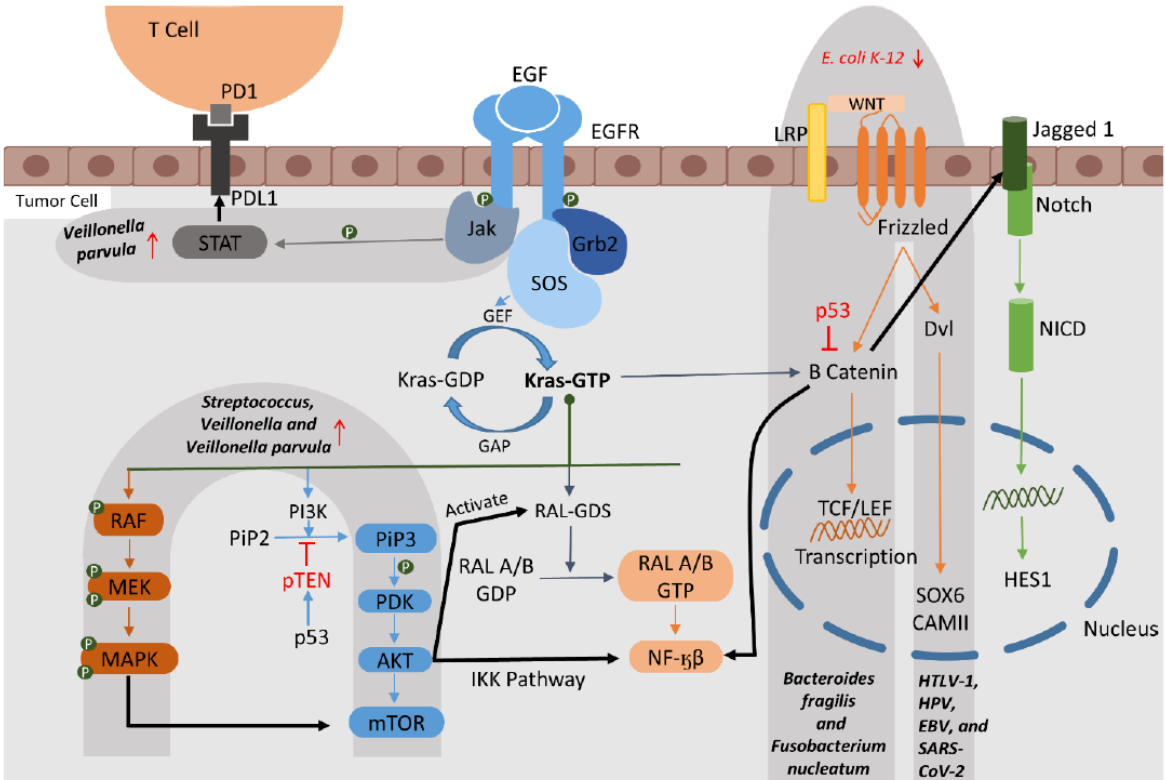


Figure 1. Microbes affecting cancer pathways.

2.1.1. The EGFR (Epidermal Growth Factor Receptor) Pathway

The EGFR gene codes for a tyrosine kinase receptor activated by EGF binding. The NSCLC cases (40-60%) have EGFR mutations, primarily exon-19 deletions (Ex19del) or an exon-21-point mutation (L858R). Exons 18 (e.g., G719X) and 20 (e.g., T790M) are affected by less frequent mutations, resulting in elevated kinase activity and pathway hyperactivation.[11]

The papillomavirus, *Bacteroidetes*, along with genera like *Parvimonas*, *Prevotella*, *Mycoplasma*, etc., affects the EGFR pathway. The EGFR-TKI treatment can induce skin toxicity, showing increased abundances of species like *E. coli*, *S. haemolyticus*, *S. aureus*, *S. enterica*, etc., and lower abundances of *Corynebacterium sp.*, *Prevotella copri*, and *S. epidermidis*. [12] Other abundant microbes in NSCLC are *Rhizopus oryzae*, *Natronolimnobius innermongolicus*, *Staphylococcus sciuri*, etc.[13] (Table 1)

Table 1. Microbes associated with cancer-related pathways, transcription factors, and metabolites.

Microbe	Pathways	Transcription_F actors	Metabolites	Cancer_T ype	Association_S tatus
Papillomavirus	EGFR, MAPK	SOX4, TCF3, ETV4, and FOXM1	N/A	LC	reported in literature
Parvimonas	EGFR	N/A	N/A	NSCLC	reported in literature
Prevotella	EGFR, MAPK, ERK, PI3K	N/A	Acylcarnitines, Lysophospholipi ds, Beta-Santalyl Acetate, Xanthines, and Theobromine, nervonic acid/all- trans-retinoic acids, Butyrate, Folate, Propionate, Acetaldehyde, Deoxycholic Acid, and Biotin	LC	reported in literature
Mycoplasma	EGFR	N/A	N/A	LC	reported in literature
E. coli	EGFR, WNT, PI3K/AKT/m TOR, Notch, and TGF-beta	SNAI3, KLF4,	Cysteinylyl-Valine, 3-Chlorobenzoic acid, and 3,4- Dihydroxyphenyl ethanol	LC	probable association/ reported in literature
haemolyticus	EGFR	N/A	N/A	NSCLC	reported in literature
S. aureus	EGFR	N/A	Sphingosine, Cyclohexane	LC	reported in literature
S. enterica, Corynebacterium sp., Prevotella copri, S. epidermidis, Rhizopus oryzae, Natronolimnobius innermongolicus, Staphylococcus sciuri	EGFR	N/A	N/A	LC	reported in literature

Streptococcus	KRAS, ERK, PI3K	N/A	Butyrate, Folate, Propionate, Acetaldehyde, Deoxycholic Acid, and Biotin	LC	reported in literature
Veillonella	KRAS, ERK, IL-17, PI3K, MAPK, and ERK	N/A	N/A	LC	reported in literature
HTLV-1, HPV, EBV, SARS-CoV-2	MAPK, non-canonical WNT, IFN, VEGF	SOX4, TCF3, ETV4, and FOXM1	N/A	LC	reported in literature
Bifidobacterium	MAPK, TNF	N/A	N/A	LC	reported in literature
Wolbachia	MAPK, ERK and PI3K	N/A	N/A	LC	reported in literature
Helicobacter pylori	VEGF	N/A	N/A	LC	reported in literature
Akkermansia muciniphila	VEGF	N/A	Gln (glutamic acid, succinic acid, and malic acid) and adenosine (AMP, ADP, UMP, GMP, and uric acid)	LC	reported in literature
Enterococcus hirae	VEGF	N/A	N/A	LC	reported in literature
Bacteroides fragilis	Wnt/ β -catenin signaling	N/A	N/A	LC	reported in literature
Fusobacterium nucleatum	Wnt/ β -catenin signaling	HOXC6	N/A	LC	reported in literature
Hericium erinaceus (fungus)	N/A	FOXM1	N/A	LC	reported in literature
Faecalibacterium prausnitzii	N/A	NF-KB and IL-8	N/A	LC	reported in literature
Synergistes, Megasphaera, Clostridioides, Prevotellaceae, Halocella	N/A	N/A	glycerophospholipids, Acylcarnitines, Lysophospholipids, Beta-Santalyl Acetate, Xanthines, and Theobromine.	LC	reported in literature
Erysipelotrichaceae_UCG_003, Clostridium	N/A	N/A	glycerophospholipids	LC	reported in literature
Rikenellaceae	N/A	N/A	pentanoic and butyric acids	LC	reported in literature
Granulicatella	N/A	N/A	SCFAs (propionic,	LC	reported in literature

			butyric, acetic, and valeric acids), lysine, and nicotinic acid,		
Ruminococcus gnavus, Lachnospira, Firmicutes, Fusicatenibacter	N/A	N/A	L-valine, quinic acid, 3-hydroxybenzoic acid,1-methylhydantoin,3,4-dihydroxydrocinamic acid, and 3,4-dihydroxybenzene acetic acid	LC	reported in literature

2.1.2. The KRAS (Kirsten Rat Sarcoma Viral Oncogene Homolog) Pathway

A GTPase protein encoded by the KRAS oncogene controls proliferation, survival, and differentiation. KRAS mutations inhibit GTP hydrolysis and over-activate pathways frequently in lung adenocarcinoma (LUAD) (20–40%). G12D mutation is linked with PI3K-AKT activation and co-mutations in STK11, KEAP1, TP53, and CDKN2A/B.[14]

The association of *Streptococcus*, *Prevotella*, and *Veillonella* with upregulation of ERK and PI3K pathways in NSCLC has been reported.[13,15] (Table 1)

2.1.3. The MAPK (Mitogen-Activated Protein Kinase) and ERK Pathway

A serine/threonine kinase related to MAPK, regulating cell survival, differentiation, and proliferation, is encoded by the BRAF gene. Melanoma, colorectal (CRC), thyroid, ovarian, and NSCLC are associated with BRAF mutations, particularly the V600E. Protein interactions, disrupted by the mutation, lead to pathway overactivation and worse outcomes for NSCLC patients, such as lower progression-free survival (PFS) and overall survival (OS) rates. Additional inactivating mutations (D594X, T599I, and G466X) and activating mutations (G469X, K601E, and L597X) have also been found. It is yet unknown how BRAF mutations affect the way targeted treatments respond.[16]

Lower airway microbiota, especially *Veillonella parvula*, are associated with the upregulation of IL-17, PI3K, MAPK, and ERK pathways and influence chemokine signaling pathway, TNF, JAK-STAT, and PI3K-AKT signaling pathways. Several viruses [human T-cell leukemia virus 1 (HTLV-1), human papillomavirus (HPV), Epstein-Barr virus (EBV), and SARS-CoV-2] trigger the MAPK, non-canonical WNT, and IFN pathways. TNF α is associated with *Bifidobacterium*, while *Wolbachia*, *Prevotella*, and *Streptococcus* upregulated ERK and PI3K pathways in LC.[17–19] (Table 1)

2.1.4. The VEGF (Vascular Endothelial Growth Factor) Pathway

With the help of VEGFR-1 and VEGFR-2 receptors, VEGF drives endothelial cell proliferation, blood supply, angiogenesis, and vasculogenesis. Although RTCs are minimally dependent on angiogenesis, NSCLC, SCLC, and other adenocarcinomas all show aberrant VEGF expression. With prostaglandin E2 (PGE2), raised cyclooxygenase-2 (COX-2) levels in NSCLC promote EGFR signaling, which stimulates VEGF expression and angiogenic potential. In NSCLC, overexpression of VEGF is a prognostic marker that is linked negatively to a reduced survival rate. Angiogenesis is directed by several microbes including *Helicobacter pylori*, *Akkermansia muciniphila*, *Enterococcus hirae*, and HPV. [20] (Table 1)

2.1.5. The WNT Pathways

Wnt/ β -Catenin (Canonical Wnt pathway) is part of the major WNT pathways. WNT, a protein family, is involved in cell survival, formation, and renewal of organs and stem cells. In the canonical

pathway, inhibition of β -Catenin degradation by active WNT signaling results in the regulation of several genes. Non-canonical WNT pathways are β -Catenin independent. The Wnt/Calcium pathway specially Wnt5a is involved in LC progression.[21,22]

In CRC, *Bacteroides fragilis* and *Fusobacterium nucleatum* can activate Wnt/ β -catenin signaling.[23] The WNT pathways also influence Notch and Sonic Hedgehog pathways.[21,22] The Notch pathway is associated with lung cell development, and cell fate determination. The concrete evidence of associations between these pathways and microbes is still missing. (Table 1)

2.2. Association of Transcription Factors (TFs) and Microbiomes in RTC

TFs are pivotal in tumor development and progression, immunological response, immune cell infiltration, and the cell cycle etc. Recent studies showed TFs can predict patient prognosis, regulate drug resistance, and associate with gene expressions.[24–26] A study on NSCLC patients identified 725 associated TFs, including 200 upregulated TFs. Among them, five TFs (SETDB2, SNAI3, SCML4, ZNF540, and ETV1) were identified as potential prognostic markers (Survival-related TFs).[24]

Although the association of the microbiome (*E. coli*, butyrate-producing bacterium SM4/1, and a species of *Oscillatoria*) with expression of SNAI3 is reported, other TFs' associations have not been reported yet.[27]

Another study found seventeen overregulated and sixteen downregulated TFs in LC. According to the transcriptional regulatory networks of LC, the main positive regulators are SOX4, FOXM1, ETV4, HOXC6, and E2F3, and the main negative regulators are SOX17, KLF4, and ZBTB16. These TFs (SOX4, TCF3, ETV4, and FOXM1) are found to be associated with HTLV-1, HPV, EBV, and SARS-CoV-2 in different cancers, including LC.[17] Additionally, *Fusobacterium nucleatum* is found to be associated with MNDA, HOXC6, SOX4, FOXF1, and TAL1. [28]

Fungal proteins from *Hericius erinaceus* have antitumor effects through dysbacteriosis, causing inhibition of some bacterial growth, while increasing the growth of probiotic bacteria (*Bifidobacterium*, *Gemellales*, *Blautia*, *Sutterella*, *Anaerostipes*, *Roseburia*, *Lachnobacterium*, *Lactobacillus*, and *Desulfovibrio*). The fungus influences the expression of FOXM1, too.[29]

Other than these TFs, nuclear factor kappa-B (NF-KB) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) are also associated with LCs, and *Faecalibacterium prausnitzii* was found to inhibit NF-KB activation and IL-8 secretion.[18] These associations between TFs and microbiomes show promise for combined biomarker detection. (Table 1)

2.3. Association of Metabolites and Microbiomes in RTC

Microbial metabolites, such as Serum Metabolites (glycerophospholipids, glycerolipids, lysophospholipids, etc.), Short Chain Fatty Acids (SCFAs), Polyamines, Reactive Oxygen Metabolites (ROS), Aldehydes, Terpenes, Ketones, Secondary Bile Acids (SBAs), Volatile Organic Compounds (VOCs), Sphingosine, etc., are involved in inflammation, immune responses, cell growth, apoptosis, tumor progression, and thus regulate the RTCs.[30,31]

Zhao et al. (2021), revealed that Acylcarnitines, Lysophospholipids, Beta-Santalyl Acetate, Xanthines, and Theobromine have associations with 21 genera, including *Synergistes*, *Megasphaera*, *Clostridioides*, *Prevotellaceae*, *Halocella*, etc. Zhao et al. also revealed an association of glycerophospholipids with microbes such as *Erysipelotrichaceae_UCG_003*, *Clostridium*, and *Synergistes* in LC[32].

Another study on NSCLC patients showed that SCFAs (pentanoic and butyric acids) have a strong association with *A. muciniphila*, *Rikenellaceae*, *Bacteroides*, *Peptostreptococcaceae*, *Mogibacteriaceae*, and *Clostridiaceae* [abundant in healthy controls (HCs)]. On the other hand, patients undergoing anti-PD1 treatment had a different microbial richness, including a higher incidence of *Rikenellaceae*, *Prevotella*, *Streptococcus*, *Lactobacillus*, *Bacteroides plebeius*, *Oscillospira*, and *Enterobacteriaceae* in stool. The presence of SCFAs (propionic, butyric, acetic, and valeric acids), lysine, and nicotinic acid, and bacteria like *Granulicatella* are associated with better treatment response. In non-responders, metabolites such as tridecane and 2-pentanone were potential indicators. In addition, Butanal is a

common gut aldehyde formed by the bacterial breakdown of leucine and has a strong association with inflammation and cancer.[33–35]

Qian et al. used Fecal Microbiota Transplant (FMT) and identified genera like *Prevotella*, *Gemmiger*, and *Roseburia* as significantly upregulated. And nervonic acid/all-trans-retinoic acids are negatively associated with *Prevotella*. When FMT replenished the *Prevotella* community, the levels of the nervonic acid/all-trans-retinoic acid were improved. Moreover, eight differentially expressed proteins were associated with IL-8 and NF- κ B pathways, and CRP, LBP, and CD14 were reported as NSCLC biomarkers. The analysis also showed 49 significant metabolites changed between NSCLC and HCs. In NSCLC, m-Coumaric acid, 13-l-hydroperoxylinoleic acid, allocholic acid, 13S-hydroxyoctadecadienoic acid, palmitic acid, 5-hydroxyindoleacetic acid, eicosadienoic acid, pyroglutamic acid, 3-(2-hydroxyphenyl)propanoic acid, nervonic acid, arachidic acid, l-glutamic acid, and oxoadipic acid exhibited lower concentrations. In NSCLC tumor samples, hippuric acid, adipic acid, 5-amino pentanoic acid, 16-hydroxy hexadecanoic acid, and citric acid were also reduced significantly. However, the levels of p-aminobenzoic acid, azelaic acid, 4-hydroxycinnamic acid, L-lactic acid, N-acetyl- α -neuraminic acid, gluconic acid, hydroxyphenyl acetic acid, gamma-aminobutyric acid, adenylyl succinic acid, citramalic acid, glutaric acid, and propionic acid were increased.[36]

The study on Ginseng polysaccharides (GPs) as therapeutic interventions for LC revealed that GPs can increase the SCFA-producing bacteria *Muribaculum* in mice models and maintain gut homeostasis. The degradation of polysaccharides by microbes produces the SCFAs, and they facilitate communication between the microbiome and immune cells. Besides SCFAs, metabolites such as L-tryptophan and L-kynurenine are also influenced by GPs. Increased and decreased production of L-tryptophan and L-kynurenine, respectively, and kynurenine/tryptophan ratio were observed, indicating the influence of GPs in tryptophan metabolism with the help of gut microbes. In anti-PD-1 treatment responses, responders showed an abundance of bacteria such as *Bacteroides vulgatus*, *Parabacteroides distasonis*, *bacterium* LF-3, and *Sutterella wadsworthensis*, and had better survival rates.[37]

Akkermansia muciniphila-influenced symbiotic bacteria (*Bacteroides*, *Dubosiella*, *Methylovirgula*, and *Acidobacteriaceae*) exhibited significant correlations with discriminative metabolites in the respective pathways; the pathway of Gln (glutamic acid, succinic acid, and malic acid) and adenosine (AMP, ADP, UMP, GMP, and uric acid) metabolism of the metabolic network in Lewis LC tissue.[38]

Another Review mentioned the effect of bacterial metabolites and toxins on inducing genomic instability in the host. Butyrate, Folate, Propionate, Acetaldehyde, Deoxycholic Acid, and Biotin can potentially modify epigenetics. *Lactobacillus*, *Bifidobacteria*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Prevotella*, and *Fusobacterium* are required for the formation and activities of the metabolites mentioned earlier. In NSCLC patients receiving anti-PD-1 immunotherapy, the responders exhibited higher serum acetic acid, propionic acid, and butyric acid.[39]

Lipid metabolites (Leukotriene B4 and prostaglandin E2) are upregulated in mice with LC. In addition, high levels of adrenic, palmitic, stearic, and oleic acids were also seen. Moreover, isohumulone (peroxisome proliferator-activated receptor gamma activator) and resolvin (an ω -3 polyunsaturated fatty acid) both have anti-cancer effects. The abundance of *Lachnospiraceae*_UCG-006 and the decrease of L-valine showed a strong association with LC patients, indicating a potential biomarker.[40,41]

Fecal metabolites (quinic acid, 3-hydroxybenzoic acid, 1-methyl hydantoin, 3,4-dihydroxydrocinnamic acid, and 3,4-dihydroxy benzene acetic acid) showed reduced levels in LC. The study also found correlations with most metabolites, where *Ruminococcus gnavus* showed a negative correlation, and *Lachnospira*, *Firmicutes*, and *Fusicatenibacter* showed positive correlation[42].

Another interesting study on Time-restricted feeding (TRF) and LC provided valuable insights. TRF increased *Lactobacillus* and *Bacillus*, which were correlated with most differential metabolites and six genera (*Lactobacillus*, unclassified_c__*Bacilli*, *Bifidobacterium*, *Bacillus*, *Ileibacterium*, and *Candidatus_Saccharimonas*).[43]

A separate study reported 40 metabolites showing different abundances between HCs and LC patients. Out of them, 14 were upregulated and 26 were downregulated in LC. Finally, they suggested three metabolites (Cysteiny1-Valine, 3-Chlorobenzoic acid, and 3,4-Dihydroxyphenyl ethanol) and nine species (*s__Lactobacillus murinus*, *s__uncultured bacterium g__norank f__Muribaculaceae*, *s__Pelomonas saccharophila*, *s__uncultured Olsenella_sp g__Atopobium*, *s__Corynebacterium glutamicum*, *s__uncultured rumen_bacterium g__Prevotella*, *s__Massilia timonae*, *s__Lactobacillus delbrueckii subsp__bulgaricus*, and *s__Lactobacillus reuteri*) as potential biomarkers. Additionally, Sphingosine inhibited the growth of *S. aureus*, *A. baumannii*, *H. influenzae*, *E. coli*, *F. nucleatum*, *S. sanguinis*, etc.[44].

In a Mendelian Randomization Study, fifteen plasma metabolites were found to be associated with LC, including eight and seven metabolites with a positive and protective effect, respectively.[45] In addition, Docosapentaenoic acid (DPA) was found to be associated with major LC subtypes. Finally, the study found associations of 13 taxa and 15 metabolites in LC risk, where eight taxa and fourteen plasma metabolites were associated with LUAD, four taxa and ten metabolites with SCC, and seven taxa and sixteen metabolites with SCLC. The metabolites include DPA, Serine, Pyridoxate, etc., and taxa include *Bacteroidales*, *Ruminococcaceae*, *Collinsella*, *Lentisphaerae*, etc. [45]

Another study on a postbiotic (MS-20) (comprised of microbial metabolites) showed inhibitory actions against CRC and LC growth in combination with PD-1 antibody in mice. After the treatment with MS-20, the abundances of *Ruminococcaceae* and *Bacteroidaceae* increased, and *Clostridium colinum*, *Tyzzarella spp*, and *Clostridium leptum* were decreased[46].

Early-stage LC patients showed a high abundance of *Bacillus* and a lower abundance of *Bifidobacterium* and *Faecalibacterium*. [18].

Finally, VOCs are also found to be associated with microbiome dysbiosis and cancer detection. VOCs such as methyl butane and Acetone were significantly decreased, while Cyclohexane and Xylene were increased in patients. Another study showed that Cyclohexane levels and infections by *E. coli*, *P. aeruginosa*, and *S. aureus* are associated.[47] Microbial-related VOCs like Isopropanol, Butane, Butanol, Methanol, Methyl acetate, Butandione, Cyclohexanone, Toluene, Benzaldehyde, Propanal, and O-Xylene are also associated with LC.[35] (Table 1)

3. Microbiomes as Diagnostic and Treatment Biomarkers: Recent Empirical Data

As of February 2025, we found only 174 papers in PubMed on the “microbiome” as a “diagnostic biomarker” for LC. Out of them, only 63 original articles were finally included for this study. The rest of them are either reviews, only abstracts, not covering LC or microbiome, only protocol papers, or inaccessible to us.

For LAC and NPC, we included only 1 and 3 papers from PubMed after initial exclusion. And no original studies were found related to Bronchial, Sinus, Pleural, and Tracheal cancers. The summary of these papers is shown in Table 2.

Table 2. Summary of original publications covering RTCs and microbiome as diagnostic/treatment biomarkers.

Study (Year)	Sample Type	Microbiome Source	Key Taxa Identified	Cancer Type / Stage	Context (Detection/Treatment)	Diagnostic / Prognostic Value	Methods Used	Cohort Size	PMIDs
Liu et al. (2019)	Fecal samples	Gut microbiome	Lower abundance in LC: <i>Firmicutes</i> , <i>Actinobacteria</i> . Higher abundance in LC: <i>Proteobacteria</i> , <i>Verrucomicrobia</i> . The ratio of two phyla (<i>Firmicutes</i> to <i>Bacteroidetes</i>) was decreased in lung cancer group 2.14 (CYF), 1.64 (CEA) and 2.18 (NSE)	Lung cancer (grouped by biomarkers: CYFRA21-1, NSE, CEA)	Detection	Alpha diversity: NSE (and CEA) vs healthy control (Shannon -35.99 P=0.0369, -31.16 P=0.0369; Simpson 38.1944, P =0.0272, 33.11364, P =0.0386). J index (39.4028 P=0.0217 and 33.07955 P=0.0437. Beta diversity: (ANOSIM, r = 0.288, P = 0.001)	16S rRNA gene sequencing, KEGG/COG pathway analysis, alpha/beta diversity metrics	30 lung cancer patients (divided into 3 groups) and 16 healthy controls.	31595156
Routy et al. (2018)	Fecal samples(human & mice)	Gut Microbiome	<i>A. municipihila</i> , <i>E. hirae</i> restored antibiotic induced dysbiosis, showed better treatment outcome. <i>Alistipes indistinctus</i> was also efficient in avatar mice to restore ICI efficacy. <i>Ruminococcus</i> spp., <i>Eubacterium</i> spp. also found to be enriched in NSCLC.	Advanced non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), and urothelial carcinoma	Treatments/ effects of FMT in ICI activity in mice model	<i>A. muciniphila</i> (p= 0.004)	Metagenomic shotgun sequencing, fecal microbiota transplantation (FMT), flow cytometry, mouse tumor models	Human: 249 patients (validation cohort of 239). Mouse: Multiple groups (5–12 mice per group).	29097494
et al. (2021)	Stool samples and serum samples for metabolites	Gut microbiota from fecal matter	Higher in LC: <i>Enterococcus</i> , <i>Veillonella</i> , <i>Megasphaera</i> , <i>Clostridioides</i> . Higher in Healthy Control (HC): <i>Faecalibacterium</i> , <i>Eubacterium</i> , <i>Phascolarctobacterium</i> .	Lung cancer	Detection	<i>Tenericutes</i> (P <0.0001) and <i>Cyanobacteria</i> (P = 0.0183) were significantly more abundant in HC group, whereas <i>Halanaerobiaeota</i> (P = 0.0202) were in LC group. <i>Actinomyces</i> (P <0.0051), <i>Veillonella</i> (P = 0.0057), <i>Megasphaera</i> (P =0.0149), <i>Enterococcus</i> (P = 0.0183) and <i>Clostridioides</i> (P = 0.0202) were more	16S rRNA gene sequencing, LC-MS.	Microbiome: 41 LC patients, 40 healthy controls (HC). Metabolomics: 30 LC patients, 30 HC (final analysis: 27 LC, 29 HC).	34527604

					abundant in LC than HC Glycerophospholipids (LysoPE 18:3 (AUC, 0.908), LysoPC 14:0 (AUC, 0.895), LysoPC 18:3 (AUC, 0.893), AcylGlcADG 66:18; AcylGlcADG (22:6/22:6/22:6) (AUC, 0.906), Acylcarnitine 11:0 (AUC, 0.854) and Hypoxanthine (AUC, 0.769) all at P<0.001. The diagnostic potential of glycerophospholipids for LC was superior to that SCC (AUC, 0.539; P = 0.56), NSE (AUC, 0.536; P = 0.58) and CYFRA21-1 (AUC, 0.592; P = 0.16).				
Vernocchi et al. (2020)	Stool samples	Gut microbiota from fecal samples	Higher in controls: <i>Akkermansia muciniphila</i> (possible probiotic), <i>Rikenellaceae</i> , <i>Bacteroides</i> , <i>Peptostreptococcaceae</i> , <i>Mogibacteriaceae</i> , <i>Clostridiaceae</i> , <i>Dialister</i> , <i>Coriobacteriaceae</i> , <i>Prevotellaceae</i> . Higher in NSCLC patients: <i>Granulicatella</i> (associated with treatment response). <i>Metaboiltes</i> , higher in controls: acids (pentanoic, butyric), aldehydes (benzaldehyde, benzenacetaldehyde, 3-methyl- butanal, 2-butenal), ketones	Non-small cell lung cancer (NSCLC)	treatment	p ≤ 0.05, Network analysis and WGCNA (Weighted Gene Co-expression Network Analysis).	16S rRNA gene sequencing (microbiome). GC-MS and NMR spectroscopy (metabolomics).	11 NSCLC patients (4 non- responders, 7 responders to anti-PD1 therapy). 8 healthy controls (CTRLs).	33227982

			(acetone, 2-heptanone, 2-octanone), terpenes (g-terpinene, 3-carene) and p-cresol.					
Greathouse et al. (2018)	Lung tumor tissue, non-tumor adjacent tissue, immediate autopsy (ImA) lung tissue, and hospital biopsy (HB) lung tissue.	Lung tissue-associated microbiome, TCGA lung cancer database, and Control lung tissues (Immediate autopsy, Hospital Biopsy).	<i>Acidovorax</i> , <i>Brevundimonas</i> , <i>Comamonas</i> , <i>Tepidimonas</i> , <i>Rhodoferrax</i> , <i>Klebsiella</i> , <i>Leptothrix</i> , <i>Polaromonas</i> , <i>Anaerococcus</i> were differentially abundant in SCC vs AD (Student's t-test; MW $P < 0.05$) <i>Acidovorax</i> , <i>Klebsiella</i> , <i>Rhodoferrax</i> , <i>Comamonas</i> , and <i>Polaromonas</i> that differentiated SCC from AD were also more abundant in the tumors harboring TP53 mutations <i>Acidovorax</i> , <i>Ruminococcus</i> , <i>Oscillospira</i> , <i>Duganella</i> , <i>Ensifer</i> , <i>Rhizobium</i> were distinguishable in smokers (current or former) vs non-smokers ever (Kruskal-Wallis p value < 0.05).	Non-small cell lung cancer (NSCLC), specifically squamous cell carcinoma (SCC) and adenocarcinoma (AD); stages I–IV.	Detection	significant differences in beta diversity between all tissue types (PERMANOVA $F = 2.90$, $p = 0.001$), tumor and non-tumor (PERMANOVA $F = 2.94$, $p = 0.001$), and adenocarcinoma (AD) versus squamous cell carcinoma (SCC) (PERMANOVA $F = 2.27$, $p = 0.005$), between tumor and non-tumor (PERMANOVA $F = 3.63$, $p = 0.001$) and AD v SCC (PERMANOVA $F = 27.19$, $p = 0.001$).	16S rRNA gene sequencing (Illumina MiSeq). RNA-seq (TCGA data validation). Fluorescent in situ hybridization (FISH) for <i>Acidovorax</i> detection. PacBio sequencing for full-length 16S rDNA.	NCI-MD study: 143 tumor cases, 144 non-tumor adjacent tissues, 33 ImA controls, 16 HB controls. TCGA validation: 1,112 tumor and non-tumor RNA-seq samples.
Zheng et al. (2020)	Fecal samples	Gut microbiota	Enriched in cancer: <i>Proteobacteria</i> , <i>Ruminococcus</i> , <i>Bacteroides thetaiotaomicron</i> . Reduced in cancer: <i>Faecalibacterium</i> , <i>Bifidobacterium</i> , <i>Streptococcus infantis</i> . 13 OTU-based biomarkers were chosen for cancer detection (AUC=97.6%)	Early-stage non-small cell lung cancer (NSCLC), including adenocarcinoma (ADC), squamous cell carcinoma (SCC), and large cell carcinoma (LCC). Stages 0, I, and II.	Detection	AUC = 97.6% in discovery (SVM prediction), 76.4% in validation	16S rRNA sequencing. Support-Vector Machine (SVM) for prediction. PICRUST for metabolic pathway analysis.	Discovery cohort: 42 patients, 65 controls. Validation cohort: 34 patients, 40 controls.
Dohlman et al. (2023)	Tumor tissues, normal tissues,	Fungal DNA (mycobiome) from tumor and	<i>Candida</i> species (e.g., <i>C. albicans</i> , <i>C. tropicalis</i>) in GI cancers. <i>Blastomyces</i> in lung	GI cancers: Head-neck (HNSC), esophagus (ESCA), stomach (STAD), colon (COAD), rectum	Detection	<i>Candida</i> ($p=0.00423$) significantly and uniquely enriched in stomach tumor	ITS sequencing and culture validation. Random forest	883 sequencing runs from 767 tumor samples

	and blood samples	matched normal tissues	<i>cancer. Malassezia in breast cancer.</i>	(READ). Non-GI cancers: Lung (LUSC), breast (BRCA), brain (LGG).		samples, Blastomyces (p = 0.0088) similarly enriched in lung tumors, and e Cyberlindnera was significantly enriched in normal tissue (p = 0.0000215).	classifiers for biomarker identification.	(671 patients). Independent validation cohorts (e.g., 3 CRC samples for ITS sequencing).
Leng et al. (2021)	Lung tumor tissues Noncancerous lung tissues (paired with tumors) Sputum samples	Lower respiratory tract	SCC-associated: <i>Acidovorax</i> , <i>Veillonella</i> , <i>Streptococcus AC-associated: Capnocytophaga</i> <i>Others: Helicobacter, Haemophilus, Fusobacterium</i>	Non-small cell lung cancer (NSCLC)	detection	Acidovorax and Veillonella were further developed as a panel of sputum biomarkers that could diagnose lung squamous cell carcinoma (SCC) with 80% sensitivity and 89% specificity. The use of Capnocytophaga as a sputum biomarker identified lung adenocarcinoma (AC) with 72% sensitivity and 85% specificity. The use of Acidovorax as a sputum biomarker had 63% sensitivity and 96% specificity for distinguishing between SCC and AC, the two major types of NSCLC	Droplet digital PCR (ddPCR) for bacterial DNA quantification. Logistic regression for biomarker panel selection. ROC curve analysis for diagnostic accuracy.	Cohort 1: 17 NSCLC patients + 10 cancer-free smokers. Cohort 2 (validation): 69 NSCLC patients + 79 cancer-free smokers.
Chen et al. (2022)	Plasma samples	Microbial-derived cell-free RNAs (cfRNAs) from human plasma	<i>Proteobacteria, Firmicutes, Actinobacteria, Torque teno viruses (TTVs), Orthohepadnavirus (e.g., HBV), Mycoplasma, Acholeplasma.</i>	Colorectal, stomach, liver, lung, and esophageal cancers; mostly early-stage	Detection	The average AUROC scores of human cfRNAs on testing sets across 100 bootstrap replicates were approximately 0.9, and microbial cfRNAs quantified by k-mer-based pipeline achieved	SMART-seq-based RNA sequencing, computational pipelines (k-mer and alignment-based), machine learning (random forest).	300 plasma samples

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						AUROCs from approximately 0.8 to above 0.9			
al. (2020)	Bronchoalveolar lavage fluid (BALF)	Lung microbiota from BALF	<i>TMT, Capnocytophaga, Sediminibacterium, Gemmiger, Blautia, Oscillospira</i>	Lung cancer; stages I–IV, limited stage, extensive stage	Detection	AUC =84.52% (95% CI: 74.06–94.97%) (the microbiome with clinical tumor markers). To distinguish LC vs benign pulmonary disease, AUC were 79.12% and 78.27%, using ten genera and clinical diagnostic markers.	16S rRNA sequencing, bioinformatics (QIIME 2, PICRUSt), random forest modeling.	54 patients (32 lung cancer, 22 benign pulmonary diseases)	32676331
Qin et al. (2022)	Stool	Gut microbiome	<i>Decreased in IA and MIA: Faecalibacterium, Prevotella, Roseburia, Subdoligranulum, Anaerotruncus. In MIA: Firmicutes. In AAH/AIS: Acidobacteria. Increased in AAH/AIS: Lachnocostridium, Parasutterella. In IA: Prevotella, Klebsiella, Eubacterium eligens. In MIA: Proteobacteria, Bacteroidetes, and Fusobacteria.</i>	Non-small cell lung cancer (NSCLC) subtypes: Atypical Adenomatous Hyperplasia/Adenocarcinoma in situ (AAH/AIS), Minimally Invasive Adenocarcinoma (MIA), and Invasive Adenocarcinoma (IA).	Detection	The Firmicutes/Bacteroidetes ratio in the HP group was 1.88, while in the lung cancer group, the ratio was 1.12 (AAH/AIS), 0.48 (MIA), and 0.95 (IA), respectively.	16S rRNA gene sequencing, alpha/beta diversity analysis, LEfSe, KEGG, and COG pathway analysis.	89 participants (28 healthy, 61 lung cancer patients)	35774470
Peters et al. (2022)	Tumor and distant normal lung tissue samples, peripheral blood buffy coat	Lung microbiome	<i>Worse RFS: Clostridia, Clostridiales, Bacteroidia, Bacteroidales. Better RFS: Alphaproteobacteria, Betaproteobacteria, Burkholderiales, Neisseriales. Worse DFS: Actinomycetales and Pseudomonadales</i>	Stage II non-small cell lung cancer (NSCLC)	Detection	Area under the time-dependent ROC curve for microbiome model was >0.8 on average for 12 to 96 months. For microbiome and gene model, slightly more higher AUC.	16S rRNA sequencing, nanoString® gene expression analysis, Cox regression, time-dependent ROC curves	46 stage II NSCLC patients (39 tumor samples, 41 normal lung samples)	36303210

Ni et al. (2023)	Stool and serum samples	Gut microbiota	Enriched in NSCLC: <i>Agathobacter</i> , <i>Blautia</i> , <i>Clostridium</i> , <i>Cetobacterium</i> Reduced in NSCLC: <i>Prevotella</i> , <i>Lachnospira</i> , <i>Catenibacterium</i> , <i>Desulfobacterota</i> , <i>Oxalobacter</i>	Early-stage non-small cell lung cancer (NSCLC)	Detection	Linear Discriminant Analysis (LDA) Effect Size (LEfSe), LAD score ≥ 3	16S rRNA gene sequencing (microbiome). LC-MS/MS metabolomics (serum). LEfSe, PLS-DA, and Spearman correlation analysis.	78 stool samples (43 early-stage NSCLC patients, 35 healthy controls), 63 serum samples (35 NSCLC and 28 healthy)	36743312
Chen et al. (2023)	Bronchoalveolar lavage fluid (BALF)	Lung microbiome	<i>Prevotella</i> , <i>Klebsiella</i> , <i>Mycobacterium</i> , <i>Gordonia</i> , <i>Sphingobium</i> , <i>Achromobacter</i> , <i>Pedobacter</i> , <i>Xanthomonas</i> . five genera were considered as potential markers for identification of lung cancer, including <i>Klebsiella</i> , <i>Mycobacterium</i> , <i>Pedobacter</i> , <i>Prevotella</i> , and <i>Xanthomonas</i> with threshold value of $MDA > 5$ or $MDG > 2$ 0.837	Lung cancer	Detection	($p < 0.05$, $LDA > 3$), The diagnostic performances of all genus classifier (receiver operating curve [AUC = 0.847]), five genus classifier (AUC = 0.898), five genus classifier + CEA (AUC = 0.837), five genus classifier+CYF21-1 (AUC = 0.898), and five genus classifier+NSE+CYF21-1 (AUC = 0.878) were similar. However, compared with the above classifiers, five genus classifier+NSE (AUC = 0.959) displayed superior diagnostic performances with 85.7% of specificity, 100% of sensitivity, and cut-off value of 0.386.	Metagenomic next- generation sequencing (mNGS), LEfSe, Metastat, Random Forest analysis, ROC curve evaluation.	90 BALF samples (30 Negative Control samples, 31 benign samples, and 29 malignant samples)	36480163
Liu et al. (2022)	Bronchoalveolar lavage fluid (BALF) and lung tissue flushing solutions.	Lung microbiota from BALF samples	3 metabolites and 9 species with significantly differences, might be potential diagnostic markers associated with LC <i>Streptococcus</i> , <i>Prevotella</i> , <i>Veillonella</i> , <i>Haemophilus</i> , <i>Fusobacterium</i> , <i>Lactobacillus</i> ,	Lung cancer (non-small cell lung cancer, NSCLC)	Detection	ROC based on the combination of three metabolites (AUC > 0.91). ROC for the combination of 9 species screened by LASSO (AUC > 0.88)	16S rRNA amplicon sequencing, metagenomics, metabolomics (LC-MS/MS), ROC analysis, LEfSe, LASSO.	43 patients (30 lung cancer, 13 non-lung cancer controls)	36457513

			<i>Lactococcus, Oscillospirales, Christensenellaceae.</i>						
Zheng et al. (2021)	Bronchoalveolar lavage (BAL) fluid by bronchoscopy and lobectomy	Lung microbiota	<i>Streptococcus, Enterobacter, Mycobacterium, Lactobacillus rossiae, Bacteroides pyogenes, Paenibacillus odorifer, Pseudomonas entomophila, Chaetomium globosum.</i>	Non-small cell lung cancer (NSCLC); stages I-IV included	Detection	Distance based RDA revealed a higher correlation between microbial community composition (response variables) and explanatory variable sampling method (R2=0.061, p=0.003). This suggests the need of standardized sampling methods.	Shotgun metagenomic sequencing, NMDS, PCoA, Wilcoxon rank-sum test.	47 samples (15 non-cancer controls, and 32 NSCLC patients) (25 were form Bronchoscopy, 22 from lobectomy)	34083661
Zhao et al. (2021)	Stool samples	Gut microbiome	<i>Enriched in responders: Streptococcus mutans, Enterococcus casseliflavus, Acidobacteria and Granulicella. Enriched in nonresponders: Leuconostoc lactis, Eubacterium siraeum, Streptococcus oligofermentans, Megasphaera micronuciformis, and Eubacterium siraeum</i>	Locally advanced and advanced lung cancer (NSCLC and SCLC)	Treatment	p values < 0.05	Metagenomic sequencing, statistical analysis (Wilcoxon rank-sum, Spearman correlation), unsupervised clustering.	64 patients (33 responders, 31 nonresponders)	33111503
Kim et al. (2024)	Bronchoalveolar lavage fluid (BALF)	Lung microbiome	<i>Enriched in lung cancer: SAR202 clade (Chloroflexi), Firmicutes, Streptococcus. Enriched in benign diseases: Bacteroidota, Prevotella_7.</i>	Lung cancer stages III and IV	Detection	benign lung disease vs LC (micro AUC=0.98, macro AUC=0.99). pneumonia vs lung cancer (micro AUC=0.94, macro AUC=0.98).	16S rRNA sequencing, metagenomic analysis, random forest prediction model	48 patients (24 lung cancer, 24 benign lung diseases)	38242941
Hakozaki et al. (2020)	Fecal samples	Gut microbiome	<i>Enriched in longer survival cohort and not having ATB: Ruminococcaceae UCG 13, Agathobacter, Lachnospiraceae UCG 001. Enriched in cohort received ATB: Hungatella. Enriched in cohort with non-</i>	Advanced non-small cell lung cancer (NSCLC), stages III and IV, treatment with or without ATB	Treatments	p values < 0.05	16S rRNA sequencing, alpha/beta diversity analysis, LEfSe, DESeq2, random forest model.	70 patients (16 with prior antibiotics, 54 without)	32847937

			severe irAE: Lactobacillaceae and Raoultella Enriched in severe irAE: Agathobacter						
Otoshi et al. (2022)	Fecal samples and blood samples	Gut microbiome	positive/negative correlation to tumour: Faecalibacterium, Fusicatenibacter, Bacteroides, Bifidobacterium. Lower abundance in EGFR mutation-negative patients: Blautia.	Lung adenocarcinoma in female never-smokers; analyzed by TNM stage, T category, and tumor size.	Detection	R>= 0.5, and P <0.05 means positive significant correlation. R< 0.5, and P <0.05 means negative significant correlation	16S rRNA gene sequencing, PCoA, Bray-Curtis distance, correlation analysis	37 patients	35220256
Liu et al. (2023)	Blood samples (plasma) for cfRNA analysis	Microbial-derived cfRNAs from plasma (bacteria and viruses)	difference in proportion, Bacterial genera (e.g., Klebsiella Cutibacterium, Priestia and Halomonas.) and viral genera (e.g., Gorganvirus, Orthobunyavirus, Orthohantavirus and Betabaculovirus)	Non-small cell lung cancer (NSCLC), primarily early-stage	Detection	For VIP cutoff >1.8, In cross-validation test, AUC of RF= 0.907, AUC of LR= 0.885 In independent test, AUC of RF=0.703, AUC of LR=0.938	cfRNA-Seq, machine learning (random forest, logistic regression), immune repertoire analysis (TRUST4), microbiome profiling (Kraken2)	TP cohort: 35 NSCLC, 46 normal. SU cohort: 6 NSCLC, 6 normal. Inhouse cohort: 10 normal. Total: 41 NSCLC, 62 normal samples.	37692082
Zhou et al. (2023)	Blood samples	Circulating microbial DNA (cmDNA) from peripheral blood.	14 OS-related microbes, as prognostic risk factors (e.g., Candidatus_Babela, Methanotorris, Anaeromusa, etc.) and as prognosis favorable factors (e.g., Kozakia, Andromedalikevirus, Desulfuromonas, etc.).	Non-small cell lung cancer (NSCLC), all stages.	treatment outcome	ROC based on MAPS, OS AUC, 1 year=0.890, 3 years=0.920, 5 years=0.878 ROC based on nomogram without MAPS, OS AUC, 1 year=0.887, 3 years=0.745, 5 years=0.791 ROC based on nomogram with MAPS, OS AUC, 1 year=0.964, 3 years=0.850, 5 years=0.818	Cox regression, Kaplan-Meier, GSEA, immune infiltration analysis, drug sensitivity prediction (TIDE, oncoPredict).	109 NSCLC patients from TCGA	37950236
Chen et al. (2022)	Stool and serum samples	Gut microbiome	The key species were Roseburia (LDA score 4.22, p = 0.012), Lachnospira (LDA score 4.21, p = 0.001), Anaerostipes(LDA score 3.83, p = 0.007), and Lachnoclostridium (LDA score	Lung cancer	Detection	LDA>3.5, p<0.05	16S rRNA sequencing, LC/MS Non-Targeted Metabolomics	30 lung cancer patients and 15 healthy individuals	35586253

3.60, $p = 0.042$)inHC; <i>Lactobacillus</i> ES LC (LDA score 3.89, $p= 0.029$); and <i>Escherichia-Shigella</i> in NESLC (LDA score 4.53, $p = 0.010$).									
Sun et al. (2023)	Bronchoalveolar lavage fluid (BALF) and saliva (oral) samples	Lung and oral microbiomes from lung cancer patients.	<i>Predominant phyla: Firmicutes, Proteobacteria, Actinobacteria. Genera: Streptococcus, Veillonella, Prevotella, Pseudomonas. Species: Prevotella oralis, Gemella sanguinis, Streptococcus intermedius.</i>	Non-small cell lung cancer (NSCLC: adenocarcinoma and squamous cell carcinoma) and small-cell lung cancer (SCLC).	Detection	The study collected three samples from the same cancer patients, cancerous (C) and healthy (H) sites of the lung, and the oral (O) cavity. No significant difference of diversity between C and H. Shannon index ($P = 0.527$) and Chao1 index ($P =$ 0.428), e Bray-Curtis distances ($P = 0.39$).	Culturomics (bacterial culture) and 16S rRNA gene sequencing	25 patients with unilateral lobar masses (23 with lung cancer, 2 non-cancer controls)	37092999
Haberman et al. (2023)	Stool	Gut microbiome	<i>Decreased in lung cancer: Clostridiales, Lachnospiraceae, Faecalibacterium prausnitzii. Increased in lung cancer: Ruminococcus torques. Predictive of durable clinical benefit (DCB): Akkermansia muciniphila, Alistipes onderdonkii, Ruminococcus.</i>	Non-small cell lung cancer (NSCLC: adenocarcinoma, squamous cell carcinoma) and small-cell lung cancer (SCLC); stages IIB–IV	Detection	AUC = 0.74	16S rRNA gene amplicon sequencing, Random Forest modeling, PERMANOVA, and Kaplan-Meier survival analysis.	75 lung cancer patients and 31 healthy controls.	36737654
Jin et al. (2019)	Bronchoalveolar lavage fluid (BALF)	Lower respiratory tract (LRT) microbiome	<i>Decreased in lung cancer: Prevotella, Bacteroidetes, Fusobacteria. Unique to lung cancer: Bradyrhizobium japonicum. Enriched in lung disease: Acidovorax spp. 11 biomarker bacteria: Prevotella melaninogenica, Streptococcus sp. I-P16, Corynebacterium urealyticum, Acidovorax sp.</i>	Non-small cell lung cancer (NSCLC: adenocarcinoma, squamous cell carcinoma), small cell lung cancer (SCLC); stages I–IV.	Detection	AUC = 0.882 in training set, 0.796 in independent validation set	Metagenomics sequencing, Random Forest modeling, PCoA, PERMANOVA	Discovery set: 150 (91 lung cancer, 29 nonmalignant, 30 healthy). Validation set: 85.	31494531

			<i>KKS102, Pseudomonas aeruginosa, Streptococcus sanguinis, H. influenzae, Streptococcus pseudopneumoniae, Bacteroides salanitronis, Campylobacter concisus, and B. japonicum</i>						
Yang et al. (2018)	Saliva samples from non-smoking female lung cancer patients and healthy controls.	Salivary microbiome	<i>Higher in cancer patients: Sphingomonas, Blastomonas. Higher in controls: Acinetobacter, Streptococcus.</i>	Non-small cell lung cancer (NSCLC)	Detection	(ANOSIM, $r = 0.454$, $P < 0.001$, unweighted UniFrac; $r = 0.113$, $P < 0.01$, weighted UniFrac).	16S rRNA gene sequencing. Statistical analyses (LEfSe, PCoA, Spearman's correlation). PICRUSt for functional prediction.	247 participants (75 non-smoking lung cancer female patients, 172 healthy controls).	30524957
Roy et al. (2022)	Saliva samples from lung adenocarcinoma (LAC) patients and healthy controls	Salivary microbiome	<i>Elevated in LAC: Rothia mucilaginosa, Veillonella dispar, Prevotella melaninogenica, Prevotella pallens, Prevotella copri, Haemophilus parainfluenzae, Neisseria bacilliformis, Aggregatibacter segnis.</i>	Lung adenocarcinoma (LAC), stages included IV and T3N0M0/T4N2M1	Detection	Pilot Study: did not provide any diagnostic/prognostic value	16S rRNA gene sequencing (V3-V4 region). Illumina MiSeq platform. QIIME for analysis, PICRUSt for functional prediction	10 participants (5 LAC patients, 5 healthy controls)	35074976
Lee et al. (2016)	Bronchoalveolar lavage fluid (BALF)	Lower respiratory tract microbiota	<i>Phyla increased in LC: Firmicutes, TM7. Genera increased in LC: Veillonella, Megasphaera (potential biomarkers).</i>	Non-small cell lung cancer (NSCLC; adenocarcinoma, squamous cell carcinoma) and small cell lung cancer (SCLC). Stages included I-IV (advanced stages predominant).	Detection	AUC = 0.888, $p = 0.002$	16S rRNA sequencing (V1-V3 regions), Illumina HiSeq 2500. Statistical analysis (ROC curves, UniFrac, PCoA).	28 participants (20 lung cancer, 8 benign lesions)	27987594
Wang et al. (2022)	Stool and blood samples	Gut microbiome	<i>Bacteroides, Pseudomonas, Ruminococcus gnavus group</i>	Lung adenocarcinoma (LUAD), stages I-IV	Detection	AUC = 0.852 (for 16S-rRNA sequencing) and 0.841 (for metagenomics)	16s-rRNA sequencing, metagenomics, metabolomics, logistic regression.	100 participants (60 LUAD patients, 40 healthy controls)	35592677
Sarkar et al. (2023)	Fecal (stool) samples and	Gut microbiome	<i>Responders: Decreased: Odoribacter, Gordonibacter, Candidatus Stoquefichus,</i>	Non-small cell lung cancer (NSCLC), stages IIIA-IV	Detection	$P < 0.05$	16S rRNA sequencing, flow cytometry,	5 patients (3 responders, 2 non-respon	37350807

	peripheral blood samples		<i>Escherichia-Shigella, Collinsella; increased: Clostridium sensu stricto 1. Non-responders: Increased: Prevotella, Porphyromonas, Streptococcus, Escherichia-Shigella; decreased: Akkerm</i>				immunohistochemistry ders, 1 male, 4 female)		
Luan et al. (2024)	Fecal samples	Gut microbiome	<i>Subdoligranulum, Romboutsia, Blautia, Bacteroides, Fusicatenibacter</i> Biomakers: 2 bacteria (<i>Subdoligranulum, and Romboutsia</i>), 4 metabolites (<i>Stearoylethanolamide, Serylthreonine, Xestoaminol C and Farnesyl acetone</i>)	Lung cancer stages I–IV	Detection	AUC = 0.9	16S rRNA gene sequencing, LC-MS metabolomics, PLS-DA, ROC analysis	55 LC patients and 28 benign pulmonary nodules patients	38166904
Seixas et al. (2021)	Bronchoalveolar lavage fluid (BALF)	Lung microbiome	<i>Streptococcus, Prevotella are associated with LC, Haemophilus with COPD, Pseudomonas, Staphylococcus with ILD.</i>	Non-small cell lung cancer (NSCLC), adenocarcinoma (ADC), squamous cell carcinoma (SCC), small-cell lung cancer (SCLC)	Detection	p<0.05	16S rRNA gene sequencing, bioinformatics analysis (DADA2, DESeq2), alpha/beta diversity metrics	106 BALF samples (49 LC, 40 non-LC, 17 comorbidity-controlled: 8 LC*, 7 COPD, 10 ILD)	34294826
Lu et al. (2021)	Fecal and sputum samples	Gut and lung microbiota	<i>Pseudomonas aeruginosa, Haemophilus, Streptococcus, Actinomyces, Faecalibacterium</i>	Non-small cell lung cancer (NSCLC), stages I–IV	Detection	AUC = 0.896	16S rRNA sequencing, machine learning (random forest), LEfSe analysis, PICRUST2 for functional prediction	121 participants (87 NSCLC patients, 34 healthy controls)	34787462
Kwok et al. (2023)	Malignant pleural effusions (MPE), Pleural fluid, background sample s (sterile surgical equipment swabs, reagent controls), skin swabs	Pleural fluid microbiota from malignant and non-malignant effusions.	<i>MPE-lung and mesothelioma: increase of Rickettsiella, Ruminococcus, Enterococcus and Lactobacillales MPE-other: increase of Methylobacterium Benign: increase of Prevotella and Bacillus Paramalignant: increase of Deinococcus Mortality in MPE-lung:</i>	Non-small cell lung cancer (NSCLC), mesothelioma, other metastatic cancers (e.g., gastrointestinal)	Detection	p<0.05, for mortality, MPE-lung (AUC range: 0.59 to 0.66) MPE-other (AUC range: 0.66 to 0.94) Mesothelioma (AUC range: 0.5 to 0.81)	16S rRNA sequencing, ddPCR, LEfSe, random forest classifiers, DMM modeling	165 subjects (pleural fluid samples), plus 58 background and 21 skin samples	36755121

increase of <i>Methylobacterium</i> , <i>Blattabacterium</i> , and <i>Deinococcus</i>									
Dora et al. (2023)	Stool samples	Gut microbiota from advanced-stage NSCLC patients treated with anti-PD1 immunotherapy	<i>Alistipes shahii</i> , <i>Alistipes finegoldii</i> , <i>Barnesiella viscericola</i> (associated with long progression free survival, PFS); <i>Streptococcus salivarius</i> , <i>Streptococcus vestibularis</i> , <i>Bifidobacterium breve</i> (associated with short PFS)	Advanced-stage NSCLC (stage IIIB/IV), including adenocarcinoma, squamous cell carcinoma, and NSCLC-NOS	Detection	taxonomic profile is best suited for PFS (AUC=0.74), pathway profile predicts better the PD-L1 phenotype of patients (AUC=0.87)	Shotgun metagenomic sequencing, Lasso/Cox regression, Random Forest machine learning, PERMANOVA, UMAP visualization.	Discovery cohort (n=62), Validation cohort (n=60)	37197440
Gomes et al. (2019)	Bronchoalveolar lavage fluid (BALF), tumor tissue RNAseq reads	Lung microbiota	<i>Proteobacteria</i> (dominant), <i>Enterobacteriaceae</i> (linked to worse SCC survival) Associated with ADC: <i>Acinetobacter</i> , <i>Propionibacterium</i> , <i>Phenylobacterium</i> , <i>Brevundimonas</i> and <i>Staphylococcus</i> Associated with SCC: <i>Enterobacter</i> , <i>Serratia</i> , <i>Kluyvera</i> , <i>Morganella</i> , <i>Achromobacter</i> , <i>Capnocytophaga</i> and <i>Klebsiella</i>	NSCLC subtypes—adenocarcinoma (ADC) and squamous cell carcinoma (SCC)	Detection	p<0.05	16S rRNA sequencing (pooled), RNAseq unmapped read analysis, LEfSe, PCoA, survival analysis	103 BALF samples (49 LC, 54 controls) + 1009 TCGA cases (509 ADC, 500 SCC)	31492894
Zhou et al. (2023)	Saliva, cancerous tissue (CT), paracancerous tissue (PT)	Oral (saliva) and lung (CT, PT)	<i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Bacteroidetes</i> (dominant phyla); <i>Promicromonosporaceae</i> , <i>Chloroflexi</i> (enriched in CT); <i>Enterococcaceae</i> , <i>Enterococcus</i> (enriched in PT)	Lung adenocarcinoma (stages I–III)	Detection	AUC = 0.74	16S rRNA sequencing, QIIME, PICRUSt, LEfSe, ROC analysis	43 patients	37641037
Takada et al. (2022)	Clinical data from NSCLC patients (no physical samples like tissue or saliva)	Gut microbiome	<i>Probiotics analyzed: Clostridium butyricum</i> , <i>Bifidobacterium</i> , antibiotic-resistant lactic acid bacteria	Advanced non-small cell lung cancer (NSCLC), all stages (treatment lines varied)	Treatment	C-index 0.57–0.62, p<0.05	Retrospective analysis, Kaplan-Meier survival, Cox regression, drug score validation.	293 patients	35780526

Han et al. (2019)	Metagenomic sequencing data	Gut microbiome	<i>Akkermansia muciniphila</i> , <i>Eggerthella lenta</i> , <i>Bifidobacterium</i>	Non-small-cell lung cancer (NSCLC) and renal cell carcinoma (RCC)	Detection	AUCs: 0.83–0.96 for liver cirrhosis; 0.81–0.91 for NSCLC/RCC immunotherapy response	Subtractive assembly (CoSA), machine learning (SVM, Random Forest), read mapping (Bowtie 2)	microbiome datasets: 93 (T2D), 181 (cirrhosis), 65 (NSCLC), 62 (RCC)	30864326
Liu et al. (2023)	fecal sample	gut mycobiome	<i>Increased in LUAD:</i> <i>Basidiomycota</i> , <i>Saccharomyces</i> , <i>Aspergillus</i> , and <i>Apiotrichum</i> <i>Decreased: Ascomycota</i> , <i>Candida</i>	Early stage lung adenocarcinoma	Detection	AUC: 0.935, AUC of validation cohorts: 0.9538 (Beijing), 0.9628 (Suzhou), and 0.8833 (Hainan)	ITS2 sequencing, OTU clustering, Feature selection (Boruta algorithm), Supervised ML (Random Forest, SVM, NB, LR, KNN), Diversity analysis	299 participants, 181 LUAD and 118 HCs). Validation cohort, Internal (Beijing, n=44 LUAD, 26 HC) and external (Suzhou, n=17 LUAD, 19 HC, and Hainan, n=15 LUAD, 12 HC), and discovery cohort (Beijing, n=105 LUAD, 61 HC).	37904139
Zhang et al. (2021)	Stool samples (gut microbiota) and paired sputum samples (respiratory microbiota)	Gut and respiratory microbiota from metastatic NSCLC patients treated with anti-PD1 immunotherapy	<i>Enriched in responders:</i> <i>Desulfovibrio</i> , <i>Actinomycetales</i> , <i>Bifidobacterium</i> ,	Metastatic NSCLC (stage IV)	Treatment	individual AUC (p<0.05) for gut microbiome range: 0.67-0.78 for respiratory microbiome: 0.67-0.77	16S rRNA sequencing, LEfSe, PCoA, Kaplan-Meier survival analysis, Spearman correlation	75 patients (25 responders, 50 non- responders), 75 stool and 57 sputum samples	34028936
Song et al. (2020)	Stool samples	Gut	<i>Parabacteroides</i> , <i>Methanobrevibacter</i> (PFS ≥6 months); <i>Veillonella</i> , <i>Selenomonadales</i> , <i>Negativicutes</i> (PFS <6 months)	Advanced non-small cell lung cancer (NSCLC), stages IIIB–IV	treatment	p<0.05	Metagenomic shotgun sequencing (Illumina HiSeq), bioinformatics analysis (α/β-diversity, LEfSe, KEGG pathways).	63 patients	32329229

Jiang et al. (2024)	Fecal samples	Gut microbiota	<i>Faecalibacterium</i> , <i>Bifidobacterium</i> , <i>Butyrivicoccus</i> , <i>Klebsiella</i> , <i>Streptococcus</i> , <i>Blautia</i> , <i>Fusobacteria</i> , <i>Proteobacteria</i>	Non-small cell lung cancer (NSCLC), early stage (I–III) and brain metastasis (stage IV)	Detection	AUC=0.884	16S rRNA sequencing, gas chromatography for SCFAs, bioinformatics analysis (LEfSe, PICRUSt)	115 participants (35 healthy, 40 early-stage NSCLC, 40 brain metastasis)	38304459
Derosa et al. (2024)	Fecal samples	Gut microbiota	<i>SIG1</i> (37 species, e.g., <i>Enterocloster</i> , <i>Streptococcaceae</i>). <i>SIG2</i> (45 species, e.g., <i>Lachnospiraceae</i> , <i>Faecalibacterium prausnitzii</i>) Finally 21 species were used in the qPCR-TOPOSCORE including <i>A. muciniphilia</i> , <i>Blautia wexlerae</i> , <i>F. prausnitzii</i>	Non-small cell lung cancer (NSCLC). Renal cell carcinoma (RCC). Urothelial cancer (UC). Melanoma and colorectal cancer (validation)	treatment	AUC=0.6 in NSCLC cohort (discovery+validation, n=499)	Shotgun metagenomics. qPCR-based assay. Co-abundance network analysis.	920 cancer patients (NSCLC, RCC, UC) + healthy volunteers	38906102
Otálora-Otálora et al. (2024)	Transcriptomic datasets from lung, colon, and gastric cancer tissues and normal tissues.	Oral-gut-lung axis microbiota, including viruses (HTLV-1, HPV, EBV, SARS-CoV-2) and bacteria (e.g., <i>Helicobacter pylori</i>)	HTLV-1, HPV, EBV, SARS-CoV-2, <i>Helicobacter pylori</i> , <i>Entamoeba histolytica</i> , <i>Salmonella enterica</i>	Lung cancer (NSCLC, SCLC), colon cancer, gastric cancer (intestinal/diffuse types)	Detection/treatment	No diagnostic or prognostic values were reported	Bioinformatic pipeline using R libraries (Limma, DESeq2), DAVID, RTN, CoRegNet, and gene network analysis	25 datasets (10 lung, 10 gastric, 5 colon cancer datasets)	39228892
Zeng et al. (2024)	GWAS summary data, plasma metabolites, gut microbiota data.	gut microbiota and plasma metabolome	<i>Bacteroidia</i> , <i>Bifidobacteriaceae</i> , <i>Streptococcus</i> , <i>Slackia</i> , <i>Ruminococcaceae</i> UCG005 <i>causal effect on LC</i> (13 taxa and 15 plasma metabolites), LUAD (8 taxa and 14 metabolites), SCC (4 taxa and 10 metabolites), SCLC (7 taxa and 16 metabolites)	Lung cancer subtypes: LUAD, SCC, SCLC	detection	p<0.05, Odd ratios	Mendelian Randomization (IVW, Wald ratio), sensitivity tests, enrichment/mediation analysis	18,000 individuals (GM), 7,824 europeans (metabolites), LC meta-analysis	38793035

Wang et al. (2024)	Bronchoalveolar lavage fluid (BALF)	Lower respiratory tract microbiome	LC: <i>Lactobacillus acidophilus</i> , <i>Streptococcus mitis</i> Lung infection group: <i>Lactobacillus</i> , <i>Streptococcus</i> , <i>Corynebacterium</i> , <i>Pseudomonas</i> , <i>Staphylococcus</i> , <i>Veillonella</i> , <i>Neisseria</i> , <i>Acinetobacter</i> , and <i>Klebsiella</i> others group: <i>Actinomyces</i> , <i>Prevotella</i> , <i>Fusobacterium</i> , <i>Leptotrichia</i> , <i>Corynebacterium</i> , and <i>Rothia</i>	Lung adenocarcinoma (n=16), squamous cell carcinoma (n=4), small cell carcinoma (n=1); stages I–IV	Detection	AUC=0.985, accuracy = 98.46%, sensitivity= 95.24%, and specificity = 100.00%; P< 0.001)	Metagenomic NGS, qPCR, multivariate logistic regression	158 patients with diffuse lung parenchymal lesions.	39185086
Feng et al. (2024)	Human feces	Gut microbiota	<i>Akkermansia muciniphila</i> , <i>Lachnospiraceae bacterium</i> NSJ-38, <i>Streptococcus</i> , <i>Enterococcus</i> , <i>Acutalibacter timonensis</i> , and <i>Lachnospiraceae bacterium</i> COE1	Non-small cell lung cancer (NSCLC), stages IIIB and IV (human); murine Lewis lung carcinoma model	Detection	p<0.05	Shotgun metagenomics, flow cytometry, statistical analysis (R packages)	9 healthy humans, 12 NSCLC patients, 24 mice (12 LCM, 12 HCM)	38612577
Ren et al. (2024)	Saliva samples from pulmonary nodule (PN) patients and healthy controls (HCs)	Oral microbiota	<i>Fusobacterium</i> , <i>Porphyromonas</i> , <i>Parvimonas</i> , <i>Peptostreptococcus</i> , <i>Haemophilus</i>	Pulmonary nodules (PNs), early-stage precursors for lung cancer (no specific cancer stage)	Detection	AUC=0.80, p<0.05	16S rRNA sequencing, random forest model, KEGG/COG analysis, Wilcoxon/LEfSe tests	173 PN patients + 40 HCs = 213 total	38643115
Chen et al. (2025)	Fecal samples	Gut microbiota	4 genera used for the model: <i>Bacteroides</i> , <i>Parabacteroides</i> , <i>Prevotella</i> , <i>Flavonifractor</i>	Lung Adenocarcinoma	Detection	LUAD with QP Syndrome, AUC=0.989 (16S-rRNA), 1.00 (metagenomics)	16s-rRNA sequencing, LEfSe, logistic regression, metagenomics	110 participants (30 healthy, 80 LUAD)	38847243
Tesolato et al. (2024)	Fecal samples	Gut microbiota	CRC: <i>Parvimonas</i> , <i>Gemella</i> , <i>Eisenbergiella</i> , <i>Peptostreptococcus</i> , <i>Lactobacillus</i> , <i>Salmonella</i> , <i>Fusobacterium</i> . NSCLC:	Colorectal Cancer (CRC) and Non-Small Cell Lung Cancer (NSCLC); stages I–IV	Detection	AUC=0.840 (CRC), AUC=0.747 (NSCLC)	16S rRNA sequencing, QIIME2, LEfSe, logistic regression, ROC analysis	77 participants (38 CRC, 19 NSCLC, 20 controls)	38540316

DTU089, Ruminococcaceae									
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Zeng et al. (2024)	Saliva samples	Salivary microbiota	Top 6 species: <i>Fusobacterium</i> , <i>Solobacterium</i> , <i>Actinomyces</i> , <i>Porphyromonas</i> , <i>Atopobium</i> , <i>Peptostreptococcus</i>	Persistent pulmonary nodules	Detection	AUC=0.877 (all microbial species), 0.872 (Top 6 microbial species)	16S rRNA sequencing, machine learning (LightGBM), PICRUST2	483 participants (141 healthy, 342 pPN)	39609902
Mao et al. (2025)	Cancerous and adjacent normal tissues	Intratumoral microbiota from NSCLC and normal lung tissues	11 HAM genera in tumour (<i>Anaerovorax</i> , <i>Marivivens</i> , <i>Donghicola</i> , <i>Lachnospira</i> , <i>Dubosiella</i> , <i>Lactobacillus</i> , <i>Methylobacterium</i> , <i>Akkermansia</i> , <i>Paenibacillus</i> , <i>Aerococcus</i> and <i>Cloacibacterium</i>) and 1 HAM genera in normal tissue (<i>Campylobacter</i>) <i>Peptococcus</i> ; protective (9 genera) and harmful (3 genera) microbial clusters For survival (OS and PFS) prognostic model: <i>Xanthobacter</i> , <i>Pantoea</i> , <i>Oscillospira</i> , <i>Hydrogenispora</i> , <i>Peptococcus</i> and <i>Glycomyces</i>	Non-small cell lung cancer (NSCLC), subtypes LUAD and LUSC, stages II–IV	Detection/treatment	AUC=0.862 (NSCLC diagnostic model, 5 genera), p<0.05 AUC=0.9916 (1 yr), 1.0000 (3 yrs) and 0.9649 (5 yrs) (Survival prognostic model), p<0.05	16S rRNA sequencing, transcriptome sequencing, Cox regression, LASSO, Mendelian randomization	30 NSCLC patients	39754314
Su et al. (2024)	Tumor tissues from LUAD patients	Intratumoral microbiota from LUAD tumor tissues (TCGA RNA-seq data)	<i>Pseudoalteromonas</i> , <i>Luteibacter</i> , <i>Caldicellulosiruptor</i> , <i>Loktanella</i> , <i>Serratia</i>	Lung adenocarcinoma (LUAD); stages I (early) vs. II–IV (advanced)	Detection	AUC = 0.70	RNA-seq, DESeq2, random forest, co-abundance networks, GO enrichment	491 patients (267 early, 224 advanced)	38721596
Dora et al. (2024)	Fecal samples	Gut microbiome	<i>Actinomycetota</i> , <i>Euryarchaeota</i> , <i>Bacillota</i> , <i>Bifidobacterium</i> , <i>Collinsella</i>	Advanced-stage non-small cell lung cancer (NSCLC; stage IIIB/IV)	treatment	AUC= 0.878 and Accuracy= 78.1% (RF model, long vs short PFS) AUC= 0.85 and accuracy= 75.6% (SVM model) AUC= 0.84 and Accuracy= 75%	Metatranscriptomics, de novo assembly (Trinity), DESeq2, machine learning (RF, SVM, XGBoost)	29 NSCLC patients undergoing ICI therapy	39563352

						(GBDT, XGBoost model) combined risk score (AUC=0.89)			
Yang et al. (2025)	Tumor tissues (from TCGA and GEO datasets)	Lung microbiome	18 genera including <i>Azotobacter</i> , <i>Buchnera</i> , <i>Corynebacterium</i> , <i>Delftia</i> , <i>Mycoplasma</i> , etc.	Lung squamous cell carcinoma (LUSC)	detection	AUC= 0.690 (1 yr), 0.736 (3 yrs) and 0.771 (5 yrs) (Survival prognostic model), p<0.05 AUC= 0.549 (1 yr), 0.63 (3 yrs) and 0.67 (5 yrs) (mRNA prognostic signatures)	LASSO, Cox regression, XGBoost, RNA-Seq, external validation	470 patients (TCGA training cohort)	39915534
Vicente-Valor et al. (2025)	Tumor and non-tumor tissues, fecal samples	Colorectal tissues (CRC), lung tissues (NSCLC), feces	<i>Fusobacterium</i> (CRC); <i>Cryobacterium</i> , <i>Tepidicella</i> , <i>Geodermatophilus</i> , <i>Nakamurella</i> (NSCLC)	Colorectal Cancer (CRC) and Non-Small Cell Lung Cancer (NSCLC); stages I-IV	Detection	p<0.05, although results did not show much significant results for NSCLC	16S rDNA metagenomic sequencing, QIIME2, STATA IC16, LEfSe, PICRUST2	38 CRC, 19 NSCLC patients	39859429
Qian et al. (2022)	Fecal samples, serum samples, NSCLC tumor tissues, adjacent non-cancerous tissues, mouse tissues, blood samples	Gut microbiota	<i>Prevotella</i> , <i>Gemmiger</i> , <i>Roseburia</i> (upregulated); <i>Prevotella copri</i> (used as intervention)	Non-Small Cell Lung Cancer (NSCLC); early-stage and tumor tissues (stages unspecified)	Detection/treatment	p<0.05	16S rRNA sequencing, LC-MS metabolomics/proteomics, FMT in mice, bioinformatics (PICRUST, LEfSe)	55 NSCLC patients, 15 healthy controls (human); 40 NSCLC tissue pairs; mice groups (n=5-6)	35735103
Lu et al. (2023)	Stool samples	Gut microbiome	Abundance in HC: <i>Firmicutes</i> , <i>Clostridia</i> , <i>Bacteroidaceae</i> , <i>Bacteroides</i> , <i>Lachnospira</i> LC: <i>Ruminococcus gnavus</i> SCC: <i>Proteobacteria</i> , <i>Gammaproteobacteria</i> , <i>Enterobacteriaceae</i> ADC: <i>Fusicatenibacter</i> , <i>Roseburia</i>	Lung cancer (non-small cell carcinoma: adenocarcinoma [ADC] and squamous cell carcinoma [SCC]), stages I-IV	Detection	p<0.05	16S rRNA sequencing, GC/LC-MS metabolomics	81 participants (52 LC, 29 HC)	37577375
Cameron et al. (2017)	Sputum samples	Respiratory tract (upper bronchial tract/lungs)	<i>Granulicatella adiacens</i> , <i>Streptococcus viridans</i> , <i>Streptococcus intermedius</i> , <i>Escherichia coli</i> , <i>Acinetobacter junii</i> , <i>Mycobacterium tuberculosis</i>	Non-small cell lung cancer (NSCLC) subtypes: squamous cell, adenocarcinoma, large cell carcinoma; stages unspecified	Detection	R2 value, p<0.05	Metagenomic sequencing, 16S rRNA qPCR, MG-RAST analysis	10 participants (4 LC+, 6 LC-), pilot study	28542458

Yu et al. (2023)	Oral rinse samples and tumor/control laryngeal tissue samples	Oral microbiota (from rinse), laryngeal microbiota (from tissue)	<i>Fusobacterium</i> , <i>Acinetobacter</i> , <i>Pseudomonas</i> , <i>Klebsiella</i> , <i>Flavobacterium</i> , <i>Mycoplasma</i> in tumor tissues, <i>Saccharopolyspora</i> , <i>Actinobacillus</i> in oral rinse of LSCC patients, <i>Ralstonia</i> , <i>Streptococcus</i> , <i>Rothia</i> , <i>Lactobacillus</i> in tumor tissues	Laryngeal squamous cell carcinoma (LSCC), early to advanced stages (clinical detail available but not staged by AJCC)	Detection	AUC = 0.857 in test set (oral rinse), 0.864 training	16S rRNA gene sequencing (V3–V4), Bioinformatics with QIIME, Wilcoxon test, PERMANOVA, PCoA, Random forest classifier, 10-fold cross-validation, ROC curve analysis	153 totals (77 LSCC patients, 76 vocal polypcontrols)	37408030
Qiao et al. (2022)	Tumor biopsy samples	Intratatumoral microbiota	<i>Corynebacterium</i> , <i>Staphylococcus</i> , <i>Prevotella</i> , <i>Porphyromonas</i> (higher in relapsed tumors)	Nasopharyngeal carcinoma (NPC)	Treatment	HR (DFS): 2.90 (training), 3.32 (internal), 2.24 (external)	16S rRNA sequencing, qPCR for bacterial load quantification, FISH & IHC for validation, SNV-based strain origin tracing, Host transcriptomics (RNA-seq), Survival analysis (Kaplan-Meier, Cox models), Pathway enrichment (GSEA)	802 total patients: - 241 (training, fresh frozen) - 233 (internal validation) - 232 (external validation) - 96 discovery (relapse/no relapse) - 20 additional patients (multi-omics profiling)	35834269
Zhong et al. (2022)	tissue samples	Intratissue microbiome (NPC tumor and chronic nasopharyngitis tissues)	<i>Epulopiscium</i> , <i>Terrisporobacter</i> , and <i>Turicibacter</i>	Nasopharyngeal carcinoma (NPC)	Detection/ Treatment	AUC= 0.842 for differentiating NPC from chronic nasopharyngitis AUC= 0.956 for diagnosis of R from NR for NPC treatments	16S rRNA gene sequencing, bioinformatics analysis (alpha/beta diversity, LEfSe), immunohistochemistry, and statistical modeling (ROC, Cox regression)	64 participants (50 NPC patients and 14 controls)	35677160
Lu et al. (2024)	Nasopharyngeal and middle meatus swabs	Commensal microbiota (nasopharynx and middle meatus)	<i>Pseudomonas</i> , <i>Cutibacterium</i> , <i>Finegoldia</i> , <i>Paracoccus</i>	Nasopharyngeal carcinoma (NPC) / Stages I–IV	Detection	AUC = 0.86 (NPC vs nNPC groups)	16S rRNA sequencing, Machine learning (RFECV, XGBoost), PICRUST2, DESeq2	25 participants (10 NPC, 15 non-NPC)	39704233

The majority of studies used the gut microbiome. Several studies combined the data from microbiome, metabolites, tumor markers, etc., to obtain better performance. Some even tried multiple microbiomes together, too.[48,49]

For presenting the prognostic/diagnostic efficacy, AUC values were used predominantly along with “r”, “HR”, “LDA”, “R²”, “C-index”, or simple “p values”. An AUC value greater than 0.7 indicates an acceptable value, and 0.9 indicates a highly reliable value for any diagnostic tool. Only two studies had AUC values less than 0.7 [50,51], and several studies achieved AUC values equal to or greater than 0.9 as well. [18,19,44,52–55] Some studies found higher AUC (~0.9) in the discovery data set, but lower AUC (~0.7) in the independent or validation dataset.[18,56]

Several machine learning models (MLMs) were also used in these studies, and the random forest (RF) model was the most frequent. The other MLMs, such as SVM, Lasso, XGBoost, etc., were also used individually or together.

Among microbial populations, *Akkermansia muciniphila* was associated with HCs or better treatment outcomes, even restoring antibiotic-induced dysbiosis along with *E. hirae*. [57] One study [33] even suggested the bacterium as a probiotic, too. Besides, *Prevotella*, *Bifidobacterium*, *Faecalibacterium*, etc., are also associated with positive outcomes. Based on available omics data, some studies used certain probiotics and analyzed their effects on treatment or survival. For example, Takada et al. (2022) [58] used *Clostridium butyricum*, *Bifidobacterium sp.*, and antibiotic-resistant lactic acid bacteria as probiotics, finding moderate C-index values (0.57–0.62). Similarly, Qian et al. (2022) used *Prevotella copri* as an intervention. [36]

In treatment outcome, *Alistipes indistinctus*, *Actinomycetota*, *Euryarchaeota*, *Bacillota*, *Bifidobacterium*, and *Collinsella* showed effects on ICI treatment. [57] Other microbes (*Alistipes shahii*, *Alistipes finegoldii*, *Barnesiella viscericola*, *Streptococcus salivarius*, *Streptococcus vestibularis*, *Parabacteroides*, *Methanobrevibacter*, *Veillonella*, *Selenomonadales*, *Negativicutes*, *Desulfovibrio*, *Actinomycetales*, *Bifidobacterium*, *Odoribacteraceae*, *Anaerostipes*, *Rikenellaceae*, *Faecalibacterium*, etc.) showed association with better survivals (OS or PFS), or better response to chemotherapy, or association with anti-PD1 immunotherapy, etc. [59,60]

Overall, microbes increased and associated to LC include different species (*Bacteroides thetaiotaomicron*, *Eubacterium spp.*), genera (*Acidovorax*, *Agathobacter*, *Bacteroides*, *Bifidobacterium*, *Blautia*, *Capnocytophaga*, *Cetobacterium*, *Clostridioides*, *Clostridium*, *Enterococcus*, *Faecalibacterium*, *Fusicatenibacter*, *Fusobacterium*, *Granulicatella*, *Haemophilus*, *Klebsiella*, *Lactobacillus*, *Lactococcus*, *Megasphaera*, *Mycobacterium*, *Pedobacter*, *Prevotella*, *Ruminococcus*, *Streptococcus*, *Veillonella*, *Xanthomonas*), family (*Christensenellaceae*), order (*Oscillospirales*), and phylum (*Proteobacteria*, *Verrucomicrobia*, *Firmicutes*) etc. [19,32,44,48,61–66]

Moreover, NPC is associated with *Corynebacterium*, *Staphylococcus*, *Prevotella*, *Porphyromonas*, *Epulopiscium*, *Terrisporobacter*, *Turcibacter*, *Pseudomonas*, *Cutibacterium*, *Finegoldia*, and *Paracoccus*, and LAC with *Fusobacterium*, *Acinetobacter*, *Pseudomonas*, *Klebsiella*, *Flavobacterium*, *Mycoplasma*, *Ralstonia*, *Streptococcus*, *Rothia*, and *Lactobacillus* in tumor tissues and *Saccharopolyspora*, and *Actinobacillus* in oral rinse. [67–69]

4. Emerging Next-Generation Approaches

Besides omics approaches, some other emerging approaches are coming forward. Two such approaches are Liquid Biopsy, or using circulating microbial DNA (c-mDNA) or cell-free RNA (cfRNA) from blood samples [20,31,34,55], and analyzing the mycobiome. [54,70]

Nowadays, imaging approaches (tomography of gut microbiota) are getting polished for diagnostic use. These include positron emission tomography (PET), single-photon emission computed tomography (SPECT), and computed tomography (CT). While CT alone cannot detect microbiota, but with PET and SPECT imaging can establish a non-invasive biomarker technique both for diagnosis and treatment monitoring. These assays use tracers [¹⁸F-FDS (targets sugar metabolism of *Enterobacteriaceae*), ¹⁸F-FDG, D-[¹⁸F]-CF₃-ala (targets cell wall peptidoglycan), ²⁻¹⁸F-PABA (targets folate biosynthesis), etc.] for detection. [71–75]

5. Conclusion

Human microbiome as biomarkers shows high diagnostic potential for differentiating HCs and RTCs, cancer types, and monitoring treatment. Standardized protocols for sample collection, extraction, sequencing, access to easy-to-use analytic pipelines/tools, open-access databases, etc., are essential for clinical adaptation and reproducibility. However, the lack of reference ranges and the high population variations call for future cohort studies to determine whether microbes contribute to or originate from tumor development.

In conclusion, integrating microbiome data into cancer diagnostics and treatment holds great promise. Although we couldn't access some original studies, the overall evidence suggests they would have shown a similar picture.

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Statement of translational relevance: Existing diagnostic detection of respiratory tract cancers relies mostly on approaches, such as tissue sampling by biopsy, liquid biopsy for the detection of oncogenic mutations, serum protein profiling, etc. Besides diagnosis and prognosis, monitoring treatment also requires accessible, non-invasive biomarkers related to survival and precision medicine strategies. Recent studies discovered that the human microbiome (such as the lung and gut microbiomes) possesses distinct microbial signatures for cancer detection, treatment outcome monitoring, and patient survival. Multi-omics profiling of these microbial communities using samples such as stool, oral swabs, or sputum offers a non-invasive way for early detection of these cancers, real-time outcome monitoring, and prognostic classifications. However, clinical translation requires standardization of these approaches. Without such efforts and further cohort studies, the effects of different samples and sampling methods, variable analytical tools, and limitations across the population diversity will not be understood properly. This review comprehensively reports the current research on using microbiome signatures as clinical biomarkers for respiratory tract cancers, evaluates the diagnostic and prognostic performances, and identifies critical gaps that must be addressed to develop validated, commercially viable assays for future clinical implementation.

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