

Review

Rethinking Colorectal Cancer Microbiome: From Universal Biomarkers to Patient-Stratified Signatures

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Abstract

The gut microbiome has emerged as one of the most promising sources of non-invasive biomarkers for colorectal cancer (CRC). Over the past decade, fecal metagenomic studies have consistently identified a core CRC-associated signature enriched with oral-typical, biofilm-forming species, most notably *Fusobacterium nucleatum*, *Parvimonas micra*, *Peptostreptococcus stomatis*, and *Bacteroides fragilis*. The recent landmark pooled analysis by Piccinno et al., which combined 3741 metagenomes from 18 international cohorts, offers the most methodologically solid confirmation of this signature to date. It achieved a leave-one-dataset-out area under the curve (AUC) of around 0.85 and expanded resolution to previously unclassified species-level genome bins (SGBs) and strain-level phylogenies. In this narrative review, we critically evaluate the evidence supporting current universal CRC microbiome signatures, explore the mechanistic basis of the oral-to-gut microbial axis and the immunometabolic tumor microenvironment, and argue that increasing evidence indicates the field is nearing a point where investigating patient-level heterogeneity could be the most valuable next step. Because a strong average CRC signal has been convincingly established, an important next direction is to examine how much these signatures' impact varies among individual patients, considering tumor molecular subtype, immune environment, metabolic profile, and host genetics. We review emerging evidence of such patient-level heterogeneity, outline analytical methods to assess it, and discuss its importance for developing microbiome-based screening, prognostics, and therapeutic strategies in CRC.



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This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.**Keywords:** colorectal cancer; gut microbiome; precision oncology; oral-to-gut axis; tumor microenvironment

1. Introduction

Colorectal cancer (CRC) remains the second leading cause of cancer-related death worldwide, with an estimated 1.9 million new cases annually [1]. Despite significant advances in surgical treatment, systemic therapy, and endoscopic screening, survival rates for advanced stages remain low, and population-level adherence to conventional screening methods (colonoscopy and fecal immunochemical testing) is limited by cost, invasiveness,

and patient compliance [2–4]. Therefore, the development of non-invasive, accurate, and scalable biomarkers for early CRC detection, risk assessment, and treatment prediction remains a critical clinical priority. The gut microbiome has gained significant attention as a potential source of such biomarkers. The human colon hosts one of the densest and most complex microbial ecosystems in biology, and there is now strong evidence that CRC-associated dysbiosis is not just a secondary effect of tumor biology but actively contributes to carcinogenesis, immune evasion, and disease progression [3,5–7]. Over the past decade, a series of landmark metagenomic studies and meta-analyses have identified a consistent set of CRC-associated taxa, laying the groundwork for microbiome-based diagnostic tests.

The main goal of this narrative review is to critically examine this growing body of evidence. We argue that the field now faces a productive tension between two key needs: the clinical appeal of a universal, usable CRC microbiome signature on one side and the biological reality of significant patient-level differences on the other. Therefore, we review the evidence for both, identify the mechanisms driving individual variability, and propose a research plan focused on patient-specific microbiome biomarkers.

2. Materials and Methods

This narrative review is based on a systematic search of PubMed/MEDLINE and Web of Science conducted in March 2026, using the following MeSH terms and free-text keywords: “colorectal cancer”, “gut microbiome”, “microbiota”, “metagenomics”, “biomarker”, “oral-to-gut axis”, “tumor microenvironment”, “CMS subtypes”, “patient stratification”, “precision oncology”, combined with “*Fusobacterium nucleatum*”, “*Parvimonas micra*”, “*Peptostreptococcus stomatis*”, “*Bacteroides fragilis*”. Searches were limited to publications in English. Priority was given to studies published from 2015 onward, with seminal earlier works included when foundational. Reference lists of included articles were hand-searched for additional relevant studies. Given the narrative scope of this review, no formal PRISMA flow was applied; however, the selection of evidence was guided by a focus on cross-cohort validation, mechanistic depth, and clinical relevance, with explicit acknowledgment of study limitations throughout.

3. The Converging Evidence for a Universal CRC Microbiome Signature

The progression of fecal microbiome research in CRC has involved ongoing refinement and validation across different cohorts. Early targeted studies identified higher levels of *Fusobacterium nucleatum* and *Bacteroides fragilis* in CRC tissue and stool, but their small sample sizes, usually fewer than 200 participants, prevented definitive conclusions about their general applicability [8–11]. A major turning point occurred with the use of whole-metagenome shotgun sequencing in larger, multi-ethnic groups. Yu et al. found that metagenomic classifiers trained on fecal microbiome data could distinguish CRC from controls across various geographic backgrounds, and that combined microbial gene signatures performed better than any single taxonomic marker (area under the curve [AUC] 0.72–0.77 in cross-cohort validation) [12]. Later meta-analyses by Wirbel et al. and Thomas et al., which pooled data from eight and five studies, respectively, for model training, showed that taxonomic and functional CRC signatures that are broadly applicable globally could be extracted from harmonized metagenomes, with pooled models reaching AUCs of 0.80–0.84 [13,14]. Yachida et al. added an important perspective by profiling the gut microbiome and metabolome throughout the adenoma-carcinoma sequence, revealing that specific microbial and metabolomic changes—including depletion of butyrate-producing *Lachnospiraceae* and expansion of *Fusobacterium* and *Peptostreptococcus* species—accumulate gradually from healthy mucosa to adenoma and invasive CRC [15]. This staged model reinforced the idea that microbiome changes are not merely associated with but could also

serve as early causes of cancer. The study by Piccinno et al. summarizes these findings on an unprecedented scale: its leave-one-dataset-out (LODO) framework, which trains on all cohorts except one and tests on the withheld dataset, offers a rigorous measure of generalizability. The fact that even classifiers based solely on oral SGBs achieve an AUC of about 0.85 highlights the strength of the oral-typical CRC signal [16]. Key landmark metagenomic cohort studies that have defined the universal CRC microbiome signature, including study design, cohort size, sequencing approach, identified principal taxa, and major methodological limitations, are summarized in Table 1.

Table 1. Landmark metagenomic studies establishing a reproducible colorectal cancer-associated microbiome signature.

Study	Journal	N Metagenomes	Key Contribution	AUC/Key Finding
[12]	<i>Gut</i>	128 + validation	First cross-population fecal metagenomic classifier with qPCR confirmation	0.72–0.84
[13]	<i>Nat. Med.</i>	969 (8 cohorts)	Core 29-species CRC signature; multi-study training improves cross-study generalization	~0.80
[14]	<i>Nat. Med.</i>	969 (5 + 2 cohorts)	Cross-cohort reproducibility; enrichment of oral taxa; choline degradation pathway	0.84
[15]	<i>Nat. Med.</i>	616	Stage-specific microbiome and metabolome shifts from adenoma to CRC	Adenoma-CRC continuum
[16]	<i>Nat. Med.</i>	3741 (18 cohorts)	Largest pooled analysis; strain-level resolution; 19 novel SGBs; <i>Fn</i> clade diversity	~0.85 (LODO)

AUC: area under the receiver operating characteristic curve; CRC: colorectal cancer; *Fn*: *Fusobacterium nucleatum*; LODO: leave-one-dataset-out; qPCR: quantitative polymerase chain reaction; SGB: species-level genome bin.

A critical review of this literature must recognize several key limitations. First, most studies have used retrospective, cross-sectional designs with varied stool collection and DNA extraction methods; the extent to which pre-analytical variability affects reproducibility estimates remains unclear. Second, the main metagenomic classifiers are designed and tested mainly in European and East Asian populations, and their effectiveness in under-represented groups, including sub-Saharan African, South Asian, and Indigenous populations, has not been thoroughly examined. Third, and most importantly, AUC metrics from population-based classifiers conceal significant differences among individuals in both the direction and extent of microbiome changes: a model that performs well overall may still perform poorly with specific patient groups. This final issue is the main focus of the following sections.

4. The Oral-to-Gut Microbial Axis: A Key Mechanism with Context-Dependent Consequences

One of the most mechanistically consistent findings in CRC microbiome research is the persistent enrichment of oral-like taxa, especially periodontal pathogens and biofilm-forming communities, in CRC-associated stool and mucosal samples. The oral cavity serves as a microbial reservoir from which bacteria are constantly swallowed, and emerging evidence indicates that certain taxa can survive transit, colonize the distal gut, and become part of pro-tumorigenic biofilms [17]. Flemer et al. analyzed the oral, mucosal, and fecal microbiota of 234 individuals with CRC, colorectal polyps, or healthy controls, and found shared bacterial networks dominated by *Peptostreptococcus*, *Parvimonas*, *Fusobacterium*, and *Streptococcus* species in both oral and colonic samples from CRC patients. Notably, they also identified a Lachnospiraceae-dominated microbiota type that was inversely linked to oral biofilm colonization of the colon, suggesting that protective commensals may confer

colonization resistance against CRC-associated oral taxa, a finding with direct implications for personalized risk assessment [18].

The oral microbiome has also been proposed as an independent screening compartment. A 2025 study showed that oral microbiota signatures predicted the prognosis of colorectal carcinoma, with specific periodontal taxa correlating with survival outcomes independent of classical clinicopathological variables [18]. A systematic review further documented that several oral pathogens associated with CRC, including *Porphyromonas gingivalis* and *Treponema denticola*, exert pro-tumorigenic effects through multiple mechanisms [19,20]. An important implication, so far incompletely explored, is that the magnitude of oral-to-gut microbial introgression is likely modulated by host factors including oral hygiene, periodontitis severity, salivary flow, and mucosal immune defenses. These host determinants vary substantially between individuals and may therefore contribute to the inter-patient heterogeneity in oral-typical CRC microbiome signatures observed in Piccinno et al.'s data, where the oral-to-gut score was significantly higher in CRC than in controls but showed wide individual variation.

Biofilm formation is a key mechanism in this process. Mucosal biofilms rich in *F. nucleatum*, *B. fragilis*, and related taxa increase epithelial permeability by shedding E-cadherin and activating NF- κ B/IL-6, which then triggers STAT3-mediated pro-inflammatory signaling. These biofilms create a local immune environment that favors tumor initiation and growth [21–23]. Importantly, biofilm-competent microbial communities are more commonly associated with right-sided CRC and microsatellite-stable (MSS) tumors, suggesting that the cancer-causing effects of typical oral biofilms differ across CRC subtypes [24–26]. Whether the same biofilm-forming microbes have equally strong carcinogenic effects in microsatellite-unstable (MSI-H) tumors, which already feature high mutational loads and significant immune infiltration, or if their role is countered by the host's immune response in these cases, remains an important and unanswered clinical question.

The main mechanistic features of the oral-to-gut microbial axis and the host factors that influence interindividual differences in CRC microbiome signatures are summarized schematically in Figure 1.

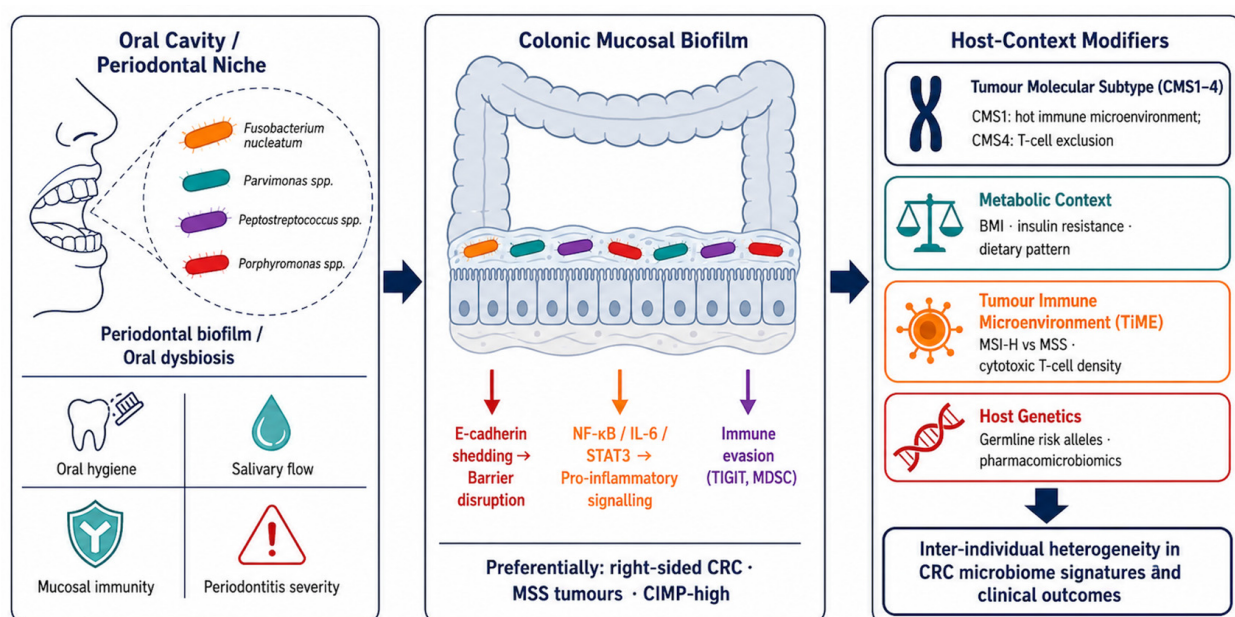


Figure 1. Schematic representation of the oral-to-gut microbial axis and host-context modulation in colorectal cancer (CRC). (**Left panel**) the oral cavity and periodontal niche as a reservoir for CRC-associated taxa (*Fusobacterium nucleatum*, *Parvimonas spp.*, *Peptostreptococcus spp.*, *Porphyromonas*

spp.) and host factors influencing oral dysbiosis (oral hygiene, salivary flow, mucosal immunity, periodontitis severity). (**Center panel**) downstream effects at the colonic mucosal biofilm level, including E-cadherin shedding and barrier disruption, NF- κ B/IL-6/STAT3-driven pro-inflammatory signaling, and immune evasion through TIGIT and myeloid-derived suppressor cell (MDSC) induction; these effects are more common in right-sided, microsatellite-stable (MSS), and CpG island methylator phenotype (CIMP)-high CRC. (**Right panel**) host factors that generate individual differences in CRC microbiome signatures and clinical outcomes, such as tumor molecular subtype (CMS1–4), metabolic factors (BMI, insulin resistance, dietary habits), tumor immune microenvironment (TiME; MSI-H vs. MSS; cytotoxic T-cell density), and host genetics (germline risk alleles; pharmacomicrobiomics).

5. *Fusobacterium Nucleatum*: A Paradigm of Host-Context-Dependent Microbial Oncogenesis

No single organism better illustrates the complexity of microbiome–CRC interactions than *Fusobacterium nucleatum* (*Fn*). It is the most consistently enriched taxon in CRC stools across independent metagenomic cohorts, is detectably more abundant in primary tumors than in adjacent normal mucosa, correlates with lymph node metastasis and poor prognosis, and has been associated with tumor cell proliferation in mechanistic studies. In vitro and animal models suggest that *Fn* promotes invasion and chemotherapy resistance through FadA-mediated Wnt/ β -catenin and E-cadherin signaling [8,21,27–31]. It is important to note that while mechanistic data from in vitro and animal studies support a causal role for *Fn* in chemoresistance and immune evasion, applying these findings to human clinical outcomes requires caution: observational human studies show correlation, not causation, and reverse causation or confounding by tumor stage cannot be ruled out. Piccinno et al. expand this understanding by identifying multiple *Fn* clades with distinct associations with CRC stage and metastasis, reinforcing that *Fn* is not a single, uniform entity but a diverse clade whose clinical impact depends on both intra-species variation and host context [16]. Studies using the molecular pathological epidemiology (MPE) framework have shown that *Fn* preferentially colonizes MSS tumors with BRAF mutations and the CpG island methylator phenotype (CIMP) high. *Fn*-positive tumors exhibit a unique immune phenotype marked by reduced cytotoxic T-cell infiltration and increased immune checkpoint molecule expression [27,32]. Notably, the immunosuppressive effect of *Fn* appears confined to specific immune niches within the tumor core rather than margins and does not consistently translate into worse overall survival across all patients. This suggests a disconnect between microbial enrichment, immune suppression, and clinical outcomes that a univariate biomarker model cannot capture [32]. Recent studies further detail how *Fn* modulates the tumor immune microenvironment (TiME), including suppression of NK cell cytotoxicity via TIGIT/CD155 interactions, induction of myeloid-derived suppressor cells, and direct interference with the effectiveness of checkpoint immunotherapy [33,34]. Overall, these findings imply that the oncogenic influence of *Fn*, and by extension other CRC-associated taxa, is heavily influenced by the host tumor's immune and molecular landscape. Consequently, the same microbial abundance may carry vastly different clinical risks in an MSI-H tumor with a highly active immune microenvironment versus an MSS tumor with T-cell exclusion.

6. Host Metabolic Context as a Modulator of Microbiome–CRC Risk

The link between obesity, metabolic syndrome, and CRC is well known epidemiologically, but the gut microbiome acts as a key mechanistic link between these conditions, often overlooked in biomarker research. Gut dysbiosis simultaneously promotes obesity-related metabolic issues and CRC development through shared pathways: chronic low-grade inflammation caused by bacterial lipopolysaccharide (LPS) translocation, changes in short-chain fatty acid (SCFA) production, direct genotoxin production by growing *Escherichia coli*

populations under high-fat diets, and microbiome-driven insulin resistance via disruption of bile acid metabolism [35]. Importantly, several taxa that are consistently enriched in CRC metagenomes, including *Fn*, enterotoxigenic *B. fragilis* (ETBF), and specific *Clostridiales* species, are also found at higher levels in obese, insulin-resistant people, regardless of CRC status [35–37]. This raises the concern that current universal CRC microbiome classifiers, trained on general populations, might be systematically biased: in an overweight, metabolically dysregulated patient, bacteria marked as CRC-associated may actually reflect the metabolic environment rather than cancer development itself.

The interaction between host metabolic health and microbiome–CRC risk has been directly studied in population cohort research. Data from several independent studies show that specific microbial signatures are linked to both CRC risk and obesity phenotypes, and their association with cancer risk is significantly reduced after adjusting for metabolic factors [38–40]. A 2024 metabolic interaction study specifically modeled how a high-fat diet promotes genotoxin-producing *E. coli* in the context of gut inflammation, demonstrating that the carcinogenic effects of certain microbiome changes depend on the host’s dietary and inflammatory background rather than being constant [41]. These findings strongly support the inclusion of metabolic covariates, such as BMI, insulin resistance markers, dietary patterns, and statin/metformin use, in any future model for microbiome-based CRC risk stratification and highlight the limitations of models that view all patients as metabolically identical [42,43].

Beyond metabolic medications, the significant impact of drugs that greatly alter the gut microbiome, especially antibiotics, on the reliability of CRC microbiome biomarkers warrants clear attention. Long-term antibiotic use in early to middle adulthood has been prospectively linked to a higher risk of colorectal adenoma, with women who used antibiotics for 2 months or more between ages 40 and 59 showing a multivariable OR of 1.69 (95% CI 1.24–2.31) for future adenoma compared to non-users, and these associations appear stronger for proximal than distal lesions [44]. Importantly, the CRC-associated taxa that form the core of current metagenomic classifiers, such as *F. nucleatum*, *Parvimonas micra*, and *Peptostreptococcus stomatis*, are precisely those vulnerable to antibiotic disruption. This means that in a patient with a history of frequent antibiotic use, these taxa might be reduced not because CRC is absent, but due to treatment-induced dysbiosis. Besides antibiotics, Vich Vila et al. demonstrated across three separate cohorts that 19 out of 41 common drug classes were linked to notable changes in gut microbiome composition or metabolic activity, with proton-pump inhibitors, metformin, antibiotics, and laxatives having the most significant effects. This emphasizes the importance of adjusting for polypharmacy in microbiome biomarker studies [43]. Future models for biomarker development should therefore include detailed medication history, covering antibiotic class, total dose, and recent exposure, as covariates during classifier training and validation.

7. Tumor Molecular Subtype and Microbiome: Beyond Stage and Location

A rarely integrated aspect of patient heterogeneity in microbiome biomarker studies is the tumor’s molecular subtype. CRC is a highly heterogeneous disease, and the consensus molecular subtypes (CMS) framework segments tumors into CMS1 (MSI-immune), CMS2 (canonical/WNT-MYC), CMS3 (metabolic), and CMS4 (mesenchymal/TGF- β), representing biologically distinct entities with markedly different immune environments, outcomes, and treatment susceptibilities [45]. It is important to note that current evidence for CMS–microbiome links mainly comes from single-cohort studies and should be considered hypothesis-generating rather than definitive. Purcell et al. were among the first to systematically connect CRC tumor microbiomes to CMS classification, revealing that

oral-associated taxa like *Fn*, *P. gingivalis*, and *Porphyromonas* species were more common in CMS1 tumors, while CMS2 tumors showed enrichment in *Selenomonas* and *Prevotella* species, and CMS4 tumors exhibited other unique microbial associations [46]. These results suggest that the same metagenomic presence of *Fn* carries different mechanistic implications depending on the tumor's CMS subgroup; in CMS1/MSI-H tumors, where immune infiltration and mutation burden are high, its role may differ from that in CMS4/MSS tumors, where the immunosuppressive and mesenchymal-invasive environment could enhance its oncogenic effects [32,47].

A multi-omics integration study published in 2025 by Wang et al. explicitly modeled the interactions among gut microbiota composition, tumor transcriptomics, and immunotherapy response in 274 CRC patients. It identified two major integrative subtypes (CS1 and CS2) with distinct microbiome profiles, immune cell infiltration patterns, and survival outcomes. The machine-learning-derived MCMLS score, which combines both genomic and microbiome features, outperformed any single-omics model for predicting immunotherapy response across six independent validation datasets [48]. If this finding is replicated in other cohorts, it would be significant: it suggests that the microbiome's role as a biomarker of immunotherapy response is not separate from tumor genomics but instead is part of an interactive multi-omics network, with its clinical predictive power emerging only when both layers are evaluated together. Simultaneously, a 2025 analysis using explainable AI (XAI) and SHAP-based interaction network analysis, trained on 16S rRNA data from 453 patients, identified microbial hub interactions among *Peptostreptococcus*, *Fusobacterium*, and other taxa as key determinants of CRC risk within specific high-risk subgroups. This provides a new analytical framework for identifying context-dependent microbial risk factors that population-level models often overlook [49].

8. Clinical Translation: Screening, Prognosis, and Therapeutic Modulation

The main clinical use of CRC microbiome biomarkers is non-invasive screening, which could complement or serve as an alternative to fecal immunochemical testing (FIT) in average-risk populations. The diagnostic accuracy of current pooled models (LODO AUC $\approx$ 0.85) is clinically significant and, importantly, provides early-stage discrimination that exceeds that of FIT alone [50–52]. However, implementing this in real-world screening programs will require performance data within specific clinical subgroups, including age, sex, ethnicity, metabolic status, and medication profile, to prevent systematic disparities in test performance across different patient groups [51,53].

Beyond screening, microbiome profiling offers a potentially transformative window into prognosis and treatment prediction. A prospective study published in 2026 found that patients with CRC who developed locoregional recurrence had a distinct preoperative fecal microbial composition compared with those who did not, suggesting that pre-surgical microbiome profiling could help stratify recurrence risk independently of traditional pathological factors [54]. It should be noted that these associations are observational; causality has not been established, and prospective interventional studies are needed before pre-surgical microbiome profiling can be recommended in clinical practice. For treatment prediction, the most promising area is immunotherapy: as discussed earlier, *Fn* abundance, oral-to-gut scores, and multi-omics integrative subtypes all show links with immune checkpoint inhibitor response in CRC, especially in MSS tumors where response rates are currently low and biomarker-guided patient selection is critically important [55,56]. The therapeutic modification of the CRC microbiome, through targeted bacteriophage therapy, microbiome-directed dietary interventions, or probiotic use, is an emerging field that also requires patient stratification, since the

effectiveness and safety of microbial manipulation are likely influenced by the baseline host–microbiome state and tumor immune environment [57,58].

Alongside test performance metrics, the fair deployment of microbiome-based CRC screening requires a clear focus on socioeconomic and lifestyle factors that influence microbiome composition. Elements like physical activity, smoking, alcohol use, and diet quality each independently affect the gut microbiome and CRC risk. A diet low in fiber and high in red and processed meats, which is more common among lower socioeconomic groups, is one of the most well-documented environmental factors linked to CRC [38]. The consistent presence of CRC-related bacteria, such as *F. nucleatum* and *B. fragilis*, in people with unhealthy diets, regardless of cancer status, raises concerns that microbiome-based classifiers may perform differently across socioeconomic groups. This could lead to more false positives in lower-income populations with poor diets, and false negatives in those whose microbiomes are disturbed by antibiotic overuse or inadequate nutrition [38]. There are already disparities in access to CRC diagnosis based on ethnicity and socioeconomic status: in the English NHS, non-white groups are less likely to get diagnosed through urgent early-detection referral routes and have higher rates of early-onset CRC than white populations, highlighting how structural healthcare inequalities increase diagnostic delays [53]. Beyond technical accuracy, microbiome-based risk assessments also raise important social acceptability issues. Sharing microbial risk scores with patients could cause health anxiety or misinterpretation. Additionally, using this data in insurance or employment decisions poses bioethical concerns that healthcare governance must actively address. Most of these issues, however, remain largely unexplored in current studies and represent a key area for future health policy research.

9. Towards Patient-Stratified Microbiome Biomarkers: Analytical Frameworks

The shift from universal to patient-specific microbiome biomarkers is primarily an analytical and conceptual challenge, not a data limitation [59]. Four complementary frameworks look most promising. First, stratified and interaction-aware machine learning can determine if the same taxa maintain discriminatory power within molecular (e.g., CMS) or clinical (e.g., BMI, diabetes) subgroups rather than across a mixed population [60]; for example, Novielli et al.'s SHAP-based approach modeling *Fusobacterium*–*Peptostreptococcus* interactions within high-risk subgroups exemplifies this [49]. Second, *hierarchical mixed-effects models* can distinguish technical variability between studies from meaningful biological differences among patients, allowing testing of interactions between microbial features and host factors such as metabolic status, medication use, or germline risk alleles [61]. Third, *unsupervised multi-omics clustering* using integrative non-negative matrix factorization (NMF) or multi-omics factor analysis (MOFA) can uncover biologically relevant patient subgroups that are not visible in any single data layer, as shown in Wang et al.'s classification of two CRC subtypes [48]. Fourth, *pseudo-longitudinal trajectory modeling* of cohorts with matched samples across the adenoma-carcinoma sequence can shift the focus from static stage labels to transition probabilities between microbial community states, potentially revealing the microbiome's dynamic role in CRC progression influenced by the host [15]. The principal host-context modifiers that shape inter-individual heterogeneity in CRC microbiome signatures, their mechanistic basis, current level of evidence, and implications for patient-stratified clinical applications are summarized in Table 2.

Table 2. Analytical frameworks for patient-stratified CRC microbiome biomarkers.

Study	Journal	N Metagenomes
Stratified & interaction-aware ML	Tests taxon discriminatory power within CMS or clinical subgroups	Overfitting risk; requires large subgroups
Hierarchical mixed-effects models	Disentangles technical from biological inter-patient variability	Assumes linearity; requires compositional data transformation
Integrative multi-omics clustering	Reveals patient subgroups invisible from single data layers	Latent factor interpretability requires external validation
NMF-based class discovery	Unsupervised molecular pattern discovery in high-dimensional data	Optimal <i>k</i> (components) has no universal selection criterion
Pseudo-longitudinal trajectory modeling	Re-interprets stage-matched cohorts as microbial state transitions	Cross-sectional data cannot capture true within-individual dynamics

CMS: consensus molecular subtypes; ML: machine learning; NMF: non-negative matrix factorization.

10. Limitations and Research Priorities

Several limitations of the current evidence base should be explicitly acknowledged. First, most mechanistic insights into the CRC microbiome come from correlational studies rather than interventional ones; establishing causality instead of mere co-occurrence requires germ-free animal models, humanized microbiome transfer experiments, and prospective human intervention studies, which are still uncommon. Second, large existing metagenomic cohorts are mostly cross-sectional, limiting the ability to study microbiome dynamics within individuals concerning CRC onset and treatment response; creating truly longitudinal microbiome biobanks linked to clinical outcomes is a key goal. Third, most published research focuses on the bacterial microbiome, while the roles of the virome, mycobiome, and archaeome in CRC carcinogenesis and individual differences remain poorly understood. Fourth, standardizing stool collection, DNA extraction, library preparation, and bioinformatic processing across centers is essential before any microbiome-based diagnostic test can proceed through regulatory approval.

A further limitation that deserves explicit recognition is the near-exclusive focus of existing large metagenomic cohorts on adult-onset CRC. Early-onset CRC (EO-CRC), defined as CRC diagnosed before age 50, is increasing globally at a rate disproportionate to overall CRC trends, and the extent to which microbiome-based classifiers trained mainly on older patients generalize to younger individuals warrants explicit investigation. Qin et al. assembled the largest young CRC gut metagenome dataset to date and found that the CRC-associated microbiome signals had limited association with age across adulthood [62]. Key CRC-enriched taxa, such as *Clostridium symbiosum*, *Peptostreptococcus stomatis*, *Parvimonas micra*, and *Hungatella hathewayi*, were consistently and significantly enriched in both old- and young-onset patients, and microbiome-based classification models showed similar predictive accuracy for CRC status regardless of age group [62]. Notably, CRC patients in both age groups displayed higher alpha-diversity than controls, with no significant difference in alpha-diversity between young and old CRC. At the same time, only a small set of species showed age-dependent abundance variation, primarily *Prevotella* species (negatively correlated with age) and *Bifidobacterium dentium* (positively correlated), suggesting that while most CRC-associated signals are age-independent, a residual age-modulated compositional background exists that could introduce noise in classifier performance at the extremes of the age spectrum [62]. Complementary data from Adnan et al., using fecal shotgun metagenomics across 11 international studies, showed that microbe–host pathway interactions at the tumor site were significantly stronger in EO-CRC than in late-onset CRC, supporting a potentially more direct microbial contribution to tumorigenesis in younger pa-

tients [63]. Together, these findings suggest that while universal CRC microbiome classifiers appear broadly transferable across age groups, dedicated EO-CRC studies, stratified by ethnicity and potential germline predisposition, remain important to characterize the residual age-dependent biological heterogeneity and to validate test performance in populations that remain under-represented in existing training datasets.

Another source of biomarker variability beyond individual differences is intra-individual temporal variation in gut microbiome composition. As Zeng et al. reported, the gut microbiome experiences both daily and seasonal fluctuations throughout adult life [64]. In adults, about 10–15% of bacterial OTUs exhibit daily oscillatory patterns, with Bacteroidota and Bacillota showing opposite circadian rhythms: Bacteroidota peaking at night and Bacillota peaking during the day. Longitudinal studies across geographically diverse populations have documented seasonal shifts in the relative abundance of Bacteroidota, Bacillota, and Actinomycetota, driven by changes in diet and environmental exposures, with Bacteroidota displaying particularly notable seasonal variation [64]. Diet changes, antibiotic use, and temporary illness episodes can also cause significant microbiome disruptions, and the permanence of these effects seems to follow a dose–response pattern based on the intensity and duration of the perturbation [64]. These patterns suggest that fecal microbiome biomarkers collected at a single time point carry inherent variability within individuals, making test–retest reproducibility studies, which ideally sample the same person across different seasons and health states, potentially necessary before microbiome-based diagnostics can be used in clinical screening. The question of whether the CRC-specific signal remains stable despite these background microbiome fluctuations remains unresolved and requires targeted longitudinal cohort studies.

Key research priorities emerging from this review include: (1) conducting a systematic, CMS-stratified re-analysis of existing pooled metagenomics datasets; (2) integrating host metabolic, immunological, and pharmacological data into microbiome biomarker models; (3) developing validated, multi-omics prognostic signatures that combine tumor genomics and microbiome profiles; (4) performing prospective studies with sufficient power to detect subgroup-specific microbiome–treatment interactions; (5) harmonizing microbiome biobanking protocols internationally to facilitate meta-analyses with clinical outcome data; and (6) establishing standards for the temporal reproducibility of fecal microbiome biomarkers, including minimum test–retest intervals and seasonal correction protocols.

11. Conclusions

The CRC-associated microbiome signal is strong, consistent, and now verified on an unprecedented scale. The field has the fundamental knowledge and analytical tools to go beyond simply determining if the microbiome is a biomarker of CRC. It can instead systematically explore who is affected, how, and under what host conditions its clinical impact is maximized. Moving from universal signatures to patient-specific biomarkers is not just a refinement but a crucial step toward genuinely personalized microbiome medicine for CRC. This approach aims to be equitable, mechanistically based, and ultimately clinically useful, assuming the analytical and translational challenges discussed here are thoroughly addressed.

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Abbreviations

The following abbreviations are used in this manuscript:

CRC	Colorectal cancer
FIT	Fecal immunochemical testing
AUC	Area under the curve
LODO	Leave-one-dataset-out
SGB	Species-level genome bin
MSS	Microsatellite-stable
MSI-H	Microsatellite instability-high
Fn	<i>Fusobacterium nucleatum</i>
MPE	Molecular pathological epidemiology
CIMP	CpG island methylator phenotype
TiME	Tumor immune microenvironment
NK	Natural killer (cells)
TIGIT	T cell immunoreceptor with Ig and ITIM domains
LPS	Lipopolysaccharide
SCFA	Short-chain fatty acid
ETBF	Enterotoxigenic <i>Bacteroides fragilis</i>
BMI	Body mass index
EO-CRC	Early-Onset Colorectal Cancer
CMS	Consensus molecular subtypes
XAI	Explainable artificial intelligence
SHAP	SHapley Additive exPlanations
NMF	Non-negative matrix factorisation
MOFA	Multi-omics factor analysis

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