

Gut microbiota and neoadjuvant chemoradiotherapy in locally advanced rectal cancer: a review of current evidence and emerging insights

Zhiwei Wu, Zihan Yang, Chengzhen Lyu , Bo Sun, Ruikai Zhang, Hongbo Li and Jian Chen

Ther Adv Med Oncol

2026, Vol. 18: 1–16

DOI: 10.1177/
17588359251413948

© The Author(s), 2026.
Article reuse guidelines:
sagepub.com/journals-
permissions

Abstract: Locally advanced rectal cancer (LARC) presents a significant burden on lower gastrointestinal diseases, with current treatment strategies primarily involving neoadjuvant chemoradiotherapy (nCRT) followed by radical surgery. However, patient responses to nCRT exhibit significant variability, highlighting the need for personalized therapeutic approaches. Emerging evidence suggests that the gut microbiota plays a critical role in influencing both treatment outcomes and toxicity in LARC patients. Intestinal dysbiosis has been linked to LARC progression and may affect the efficacy and adverse effects of nCRT. This narrative review critically evaluates the current literature on the relationship between gut microbiota and nCRT in LARC. Certain microbial taxa, such as *Alistipes* spp., *Akkermansia muciniphila*, and *Faecalibacterium prausnitzii*, have been associated with enhanced therapeutic responses, while others, such as *Fusobacterium nucleatum* and Enterotoxigenic *Bacteroides fragilis*, may contribute to treatment resistance and exacerbate adverse effects. We also discuss novel mechanisms by which specific gut microbiota and their metabolites modulate nCRT response distinct from conventional immune regulation, alongside emerging strategies for microbiota modulation, including dietary interventions, probiotics, prebiotics, and fecal microbiota transplantation. Despite challenges in standardizing microbiota analysis and fully understanding the precise mechanisms, microbiota-targeted interventions offer a promising avenue for personalized treatment in LARC, with the potential to improve patient outcomes and quality of life.

Correspondence to:

Jian Chen
Department of
Gastrointestinal Surgery,
The Second Affiliated
Hospital of Zhejiang
University School of
Medicine, Jiefang Road,
Hangzhou 310009, China
zrchenjian@zju.edu.cn

Zhiwei Wu
Department of
Gastrointestinal Surgery,
The Second Affiliated
Hospital of Zhejiang
University School of
Medicine, Hangzhou, China

Zihan Yang
Chengzhen Lyu
Department of
Gastroenterology, State
Key Laboratory of Complex
Severe and Rare Diseases,
Peking Union Medical
College Hospital, Chinese
Academy of Medical
Sciences and Peking Union
Medical College, Beijing,
China

Bo Sun
Ruikai Zhang
Hongbo Li
Division of Colorectal
Surgery, Peking Union
Medical College Hospital,
Chinese Academy of
Medical Sciences and
Peking Union Medical
College, Beijing, China

Plain language summary

Gut bacteria and treatment before surgery for advanced rectal cancer: a review

What did we study? We reviewed current research to understand how the gut microbiota, which is the community of bacteria living in the intestines, may affect how patients with locally advanced rectal cancer respond to neoadjuvant chemoradiotherapy given before surgery.

Why is this important? Patients respond very differently to chemoradiotherapy. Some benefit greatly, while others experience limited tumor response or significant side effects. Being able to predict who will respond well could help doctors personalize treatment, avoid unnecessary toxicity, and improve quality of life.

How did we do it? We analyzed published clinical and experimental studies that examined links between gut microbiota composition, treatment response, treatment

side effects, and biological mechanisms in rectal cancer patients receiving chemoradiotherapy.

What did we find? Studies show that patients with certain beneficial gut bacteria, such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Alistipes* species, tend to have better treatment responses and fewer side effects. In contrast, harmful bacteria like *Fusobacterium nucleatum* are often associated with treatment resistance and worse toxicity. Gut bacteria and their metabolites can influence immune activity, tumor behavior, and sensitivity to radiation and chemotherapy. Approaches such as dietary changes, probiotics, prebiotics, and fecal microbiota transplantation may help modify these effects.

What does this mean? The gut microbiota has the potential to become both a predictor of treatment response and a target for supportive therapies in rectal cancer. Microbiota-based strategies could support more personalized and effective treatment in the future, although further clinical studies are needed before routine use.

Keywords: dysbiosis, gut microbiota, locally advanced rectal cancer, microbiota modulation, neoadjuvant chemoradiotherapy

Received: 2 June 2025; revised manuscript accepted: 19 December 2025.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy.¹ Rectal cancer, accounting for approximately one-third of CRCs, poses a significant global health concern.² It ranks among the leading causes of cancer-related mortality, with 339,022 related deaths reported in 2020, representing 3.4% of all cancer deaths worldwide.³ Early-stage rectal cancer can often be treated effectively with surgery alone, but as the disease progresses, treatment becomes increasingly complicated.⁴ Locally advanced rectal cancer (LARC) refers to tumors that have invaded nearby structures or lymph nodes but have not yet metastasized to distant organs.⁵ The management of LARC typically necessitates a multidisciplinary approach to improve outcomes and reduce the risk of recurrence. Current guidelines recommend neoadjuvant chemoradiotherapy (nCRT) combined with radical surgery and post-operative adjuvant therapy as the standard treatment for patients with LARC.⁶ Effective responses to nCRT can significantly improve local disease control, and, in some cases, patients who achieve a clinical complete response post-nCRT treatment may shift clinical strategies from radical surgery to a “watch and wait” approach.⁶ However, patient responses to nCRT are highly variable. It frequently leads to side effects such as fatigue,

gastrointestinal disturbances, depressive symptoms, and more severe conditions like radiation proctitis and myelosuppression, all of which can severely impact quality of life.⁷

The gut microbiota, a complex community of approximately 100 trillions of microorganisms residing in the human gastrointestinal tract, plays a pivotal role in maintaining health and modulating disease processes.⁸ Dysbiosis, or imbalance in the gut microbial composition, has been implicated in the pathogenesis of numerous diseases, notably CRC.⁹ Emerging evidence have indicated that disruption of gut microbiota has been increasingly linked to the development of CRC.^{10–17} However, landmark studies have demonstrated that microbial dysbiosis is involved not only in carcinogenesis but also in shaping tumor biology and treatment responsiveness.^{18–21} In the context of LARC, dysbiosis has been gradually suggested as a factor that may predict the response to nCRT^{22–24} and modulate both its efficacy and toxicity.^{25,26} Certain microbial taxa may enhance therapeutic responses by activating immune pathways or metabolizing chemotherapeutic agents, thus improving the therapeutic outcomes of nCRT. Conversely, some may contribute to treatment resistance, promote chronic inflammation, or exacerbate side effects such as gastrointestinal

damage and radiation-induced proctitis.^{27–29} Collectively, these findings highlight the potential for the gut microbiota to influence treatment responses in LARC, suggesting that microbiota dysbiosis may act as both a biomarker for therapy response and a modifiable factor influencing the effectiveness and toxicity of nCRT. Therefore, elucidating the interplay between dysbiosis and nCRT in LARC may offer exciting prospects for improving patient outcomes.

In this narrative review, we aim to critically assess the current evidence on the relationship between gut microbiota and nCRT in LARC. We will explore how gut microbial composition predicts response to nCRT and affects treatment efficacy and toxicity, delve into the underlying mechanisms of these interactions, and evaluate emerging strategies for microbiota modulation. By synthesizing recent findings, we seek to highlight opportunities for integrating gut microbiota considerations into personalized therapeutic approaches, ultimately enhancing patient outcomes and minimizing adverse effects associated with nCRT.

Literature search strategy

A structured literature search was conducted to identify relevant studies examining the relationship between gut microbiota and nCRT response in LARC. The search covered publications from inception to September 2025 across three electronic databases: *PubMed*, *Embase*, and *Web of Science*. The following core search terms and their combinations were used: “rectal cancer,” “locally advanced rectal cancer,” “neoadjuvant chemoradiotherapy,” “gut microbiota,” “microbiome,” “treatment response,” and “radiotherapy sensitivity.” Boolean operators (AND/OR) and Medical Subject Headings (MeSH) were applied where appropriate. Reference lists of included articles and relevant reviews were also screened to identify additional eligible studies.

Studies were included if they met the following criteria: (i) investigated gut microbiota in the context of LARC or nCRT; (ii) involved human subjects and/or preclinical models exploring mechanistic associations; and (iii) were published as full-text original research articles or review articles in English. Conference abstracts, editorials, case reports, and non-peer-reviewed sources were excluded. Given the emerging nature of this field, both clinical observational studies and mechanistic

preclinical studies were considered to provide a comprehensive overview of current evidence. Data were extracted narratively with attention to study design, cohort characteristics, microbiome assessment methods, and key findings.

The gut microbiota and colorectal cancer

The gut microbiota is crucial for maintaining intestinal health, playing a fundamental role in digestion, nutrient absorption, immune system modulation, and protection against pathogens.³⁰ This complex community of microorganisms—including bacteria, archaea, viruses, and fungi—contributes to the integrity of the intestinal barrier and the development of the host immune system.³¹ A balanced gut microbiota is essential for preventing colonization by pathogenic organisms and for modulating inflammatory responses.³² However, increasing evidence indicates that disruptions to microbial balance are closely linked to colorectal carcinogenesis through metabolic, inflammatory, and immune-mediated mechanisms.

Gut microbiota composition varies between healthy individuals and CRC patients

In healthy individuals, the gut microbiota is predominantly composed of bacteria from the phyla *Firmicutes* and *Bacteroidetes*, which together constitute over 90% of the gut microbial population. Notably, these two phyla are especially abundant in the colon. Other common phyla include *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, and *Fusobacteria*.³³ At the genus level, common bacteria include *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, *Clostridium*, and *Eubacterium*.⁸ This diverse microbial ecosystem maintains intestinal homeostasis through various functions such as fermenting dietary fibers into short-chain fatty acids (SCFAs), synthesizing vitamins, and regulating immune responses.³⁴

CRC is one of the most extensively studied diseases in relation to the gut microbiota. Accumulating evidence demonstrates marked alterations in gut microbiota composition, diversity, and functional capacity in CRC patients compared with healthy individuals. Studies have shown an increased abundance of pathogenic bacteria such as *Fusobacterium nucleatum*, *Bacteroides fragilis*, and pathogenic strains of *Escherichia coli* in the gut microbiota of CRC patients.^{12,35} These microbial shifts are not merely associative; mechanistic studies indicate that

pro-carcinogenic bacteria promote tumorigenesis by inducing chronic inflammation, releasing genotoxic metabolites, altering epithelial signaling pathways, and suppressing anti-tumor immunity.²⁹ Conversely, there is often a decrease in beneficial commensal bacteria like *Lactobacillus* and *Bifidobacterium*, which bear anti-inflammatory and anti-carcinogenic properties.³⁶ The imbalance between harmful and beneficial microbes contributes to a microenvironment conducive to carcinogenesis. Notably, geographic, dietary, and lifestyle differences strongly influence baseline microbiota composition, which may partially account for inconsistent microbial signatures reported across CRC cohorts.

Relationship between gut microbiota and the tumor immune microenvironment in CRC

The gut microbiota profoundly influences the tumor immune microenvironment in CRC. Microbial metabolites, such as SCFAs and secondary bile acids, can modulate local immune responses by affecting the differentiation and function of immune cells like T cells, dendritic cells, and macrophages.^{37,38} Certain pathogenic bacteria, such as *Fusobacterium nucleatum* and Enterotoxigenic *B. fragilis*, can create a pro-inflammatory environment by activating nuclear factor-kappa B and other signaling pathways. This leads to the recruitment of immunosuppressive cells and the inhibition of anti-tumor immunity.^{39,40} For instance, *F. nucleatum* promotes immune suppression by inducing myeloid-derived suppressor cells, while Enterotoxigenic *B. fragilis* produces inflammatory toxins that activate the Wnt/ β -catenin pathway, enhancing tumor growth and immune evasion, as shown in Table 1. Collectively, these microbial activities shape an immunosuppressive tumor milieu that facilitates cancer progression and reduces responsiveness to anti-cancer therapies.

CRC, colorectal cancer; CTLs, cytotoxic T lymphocyte; IL, interleukin; MDSCs, myeloid-derived suppressor cells; NF- κ B, nuclear factor-kappa B; SCFAs, short-chain fatty acids; Tregs, regulatory T cells.

Conversely, commensal bacteria such as *Lactobacillus* spp., *Bifidobacterium* spp., and *Faecalibacterium prausnitzii* enhance anti-tumor immunity by promoting regulatory T cell and cytotoxic T lymphocyte balance, strengthening epithelial barrier integrity, and reducing inflammation. Commensal bacteria also produce

metabolites, such as butyrate and lactic acid, that strengthen the intestinal barrier and prevent the translocation of pathogenic bacteria and their toxins, thus reducing inflammation and promoting immune responses.⁵⁸ Importantly, recent studies highlight that microbiota-immune cross-talk may influence not only tumor development but also treatment response, including immunotherapy and chemoradiotherapy outcomes. Understanding this interplay is critical for developing microbiota-targeted interventions aimed at boosting immune responses and improving therapeutic outcomes in CRC or LARC patients.

Gut microbiota as a predictor of nCRT response in LARC

The variability in patient responses to nCRT for LARC underscores the need for reliable predictive biomarkers. In this review, gut microbiota mainly refers to luminal microbial communities measured from fecal samples, which are the most commonly studied and clinically accessible source. At the same time, tumor progression and treatment response may also be shaped by tumor-associated or intratumoral microbiota, namely microorganisms detected within tumor tissue or in close proximity to cancer cells, typically assessed from biopsy specimens. Although these compartments are biologically connected, they may influence tumor biology and nCRT outcomes through different routes. Luminal microbiota may affect host metabolism, systemic immune regulation, and treatment tolerance, whereas intratumoral microbiota may more directly interact with tumor cells and modulate the local immune microenvironment.

Recent research increasingly suggests that the composition of the microbiota could serve as a promising predictor of nCRT response in these patients, as shown in Table 2. However, the predictive value of the microbiota remains inconsistent across clinical studies, largely due to variations in study design, sequencing platforms, sample types (stool vs biopsy), patient demographics, and analytical methods. Identifying microbial profiles associated with favorable or unfavorable treatment responses can help tailor personalized treatment strategies, ultimately improving patient outcomes.

CR, complete responders; LARC, locally advanced rectal cancer; nCRT, neoadjuvant chemoradiotherapy.

Table 1. Specific pathogenic and commensal bacteria and their role in tumor immune microenvironment of CRC.

Bacterial species	Bacteria type	Mechanism in tumor immune microenvironment	Effect on tumor immune microenvironment	References
<i>Fusobacterium nucleatum</i>	Pathogenic	Induces NF- κ B activation, triggers pro-inflammatory cytokine production (e.g., IL-6, TNF- α), and promotes immune suppression by inducing MDSCs	Promotes tumor progression, immunosuppression	41, 42
Enterotoxigenic <i>Bacteroides fragilis</i>	Pathogenic	Produces <i>B. fragilis</i> toxin that activates the Wnt/ β -catenin signaling pathway; induces IL-17 production by CD4 ⁺ T cells, creates a pro-inflammatory tumor environment, and impairs anti-tumor immunity	Promotes tumorigenesis and immune suppression	43, 44
<i>Lactobacillus</i> spp.	Commensal	Produces lactic acid and SCFAs (e.g., acetate), which can stimulate T cell differentiation, enhance CTLs function, and improve immune responses	Promotes anti-tumor immunity	45–47
<i>Bifidobacterium</i> spp.	Commensal	Produces butyrate that strengthens the intestinal barrier, enhances antigen presentation by dendritic cells, and modulates T cell responses	Enhances anti-tumor immunity and maintains gut homeostasis	48–50
<i>B. fragilis</i> (non-toxigenic)	Commensal	Produces SCFAs that strengthen the intestinal barrier and regulate immune responses; enhances gut homeostasis	Enhances immune function and maintains gut health	51, 52
<i>Faecalibacterium prausnitzii</i>	Commensal	Produces butyrate and acts as an anti-inflammatory agent by suppressing pro-inflammatory cytokines such as IL-6 and TNF- α	Reduces inflammation and enhances anti-tumor immunity	53, 54
<i>Akkermansia muciniphila</i>	Commensal	Enhances mucus layer and gut barrier integrity; promotes differentiation of Tregs that modulate immune responses	Helps maintain immune balance and tumor suppression	55–57

Microbial signatures associated with nCRT response

Several studies have pinpointed specific microbial taxa correlated with the effectiveness of nCRT in patients with LARC.^{23,59,64} Patients who achieve a pathological complete response (pCR) often present with a distinct gut microbiota composition compared to non-responders. A commonly reported feature is increased baseline microbial diversity among responders, which may reflect a more resilient and functionally adaptable microbial ecosystem. For instance, Fan *et al.*⁶⁴ reported that both the alpha diversity and richness of the microbiota were notably higher in pCR groups than in non-pCR groups. Similarly, Sánchez-Alcoholado *et al.*⁶⁵ found that responder patients had significantly higher microbial diversity and richness compared to non-responder patients.

These findings suggest that a more diverse microbiota might enhance mucosal barrier function and modulate systemic immunity, potentially improving the efficacy of treatment.

In terms of alterations of bacteria taxa, Yi *et al.* found that nCRT treatment significantly alters the microbial composition, characterized by a reduction in diversity and unevenness. Specifically, *Fusobacterium*, *Peptostreptococcus*, *Parvimonas*, and *Porphyromonas* demonstrated a significant decrease following nCRT, whereas *Lactobacillus*, particularly *Streptococcus*, showed a marked increase.²³ Additionally, certain bacteria such as *Roseburia*, *Dorea*, and *Anaerostipes*, known for their ability to produce butyrate and modulate immune responses, have been correlated to better nCRT outcomes.²³ Conversely, an elevated

Table 2. Microbial signatures linked to nCRT response and toxicity in LARC.

Taxa	Country (year)	Sample (method)	Timepoint	Association	References
<i>Shuttleworthia</i>	China (2020)	Stool (16S rRNA)	Baseline	Enriched in responders	59
Several <i>Clostridiales</i> (e.g., <i>Faecalibacterium</i> , <i>Murimonas</i> , <i>Lachnospiraceae incertae sedis</i>)			Baseline	Enriched in non-responders	
<i>Bifidobacterium</i> , <i>Butyricicoccus</i> , <i>Clostridium</i> XIVa, <i>Hungatella</i> , <i>Bacteroides vulgatus</i>			During nCRT	Higher in patients without severe diarrhea	
<i>Fusobacterium</i> , <i>Peptostreptococcus</i> , <i>Parvimonas</i> , <i>Porphyromonas</i>	China (2021)	Stool (16S rRNA)	On-treatment change vs baseline	Decrease during nCRT	23
<i>Lactobacillus</i> , <i>Streptococcus</i>				Increase during nCRT	
<i>Roseburia</i> , <i>Dorea</i> , <i>Anaerostipes</i>			Baseline	Enriched in responders	
<i>Coriobacteriaceae</i> , <i>Streptococcus</i> , <i>Fusobacterium</i>				Enriched in non-responders	
<i>Dorea</i> , <i>Anaerostipes</i> , <i>Clostridium</i> XVIII				Predict response	
<i>Eisenbergiella</i> , <i>Granulicatella</i> , <i>Ralstonia</i>				Predict non-response	
<i>Hungatella</i> , <i>Flavonifractor</i> , <i>Methanospaera</i>	Brazil (2022)	Stool (16S rRNA)	Baseline	Enriched in responders	60
<i>Enhydrobacter</i> , <i>Paraprevotella</i> , <i>Finegoldia</i>				Enriched in non-responders	
<i>Collinsella</i> , <i>Alistipes</i> , <i>Christensenella</i> , <i>Faecalibacterium</i> , <i>Ruminococcus</i> , <i>Pavimonas</i> , <i>Akkermansia</i>	Germany (2023)	Tumor biopsy (16S rRNA)	Baseline	Predict response	61
<i>Faecalibacterium</i> sp900539945, <i>Phocaeicola dorei</i> , <i>Gemmiger qucibialis</i>	China (2024)	Stool (metagenomics)	Baseline	Higher in good responders	62
<i>Ruminococcus</i> spp.	Switzerland (2025)	Tumor biopsy (16S rRNA)	Baseline	Predict CR	63
<i>Fusobacterium</i> , <i>Porphyromonas</i> , <i>Oscillibacter</i>				Predict non-CR	

presence of specific pathogenic bacteria, such as *Coriobacteriaceae*, *Streptococcus*, and *Fusobacterium*, has been linked to resistance to nCRT.²³ For instance, *F. nucleatum* is known to promote tumor progression by suppressing immune cytotoxicity, which may diminish the efficacy of nCRT.⁶⁶ Furthermore, a recent metagenome shotgun

sequencing performed by Chen et al.⁶² revealed that *Faecalibacterium* sp900539945, *Phocaeicola dorei*, and *Gemmiger qucibialis* were found significantly abundant in LARC patients with good response. Understanding these microbial signatures and their potential effects on tumor biology could be instrumental in predicting patient

responses to nCRT and guiding more personalized treatment approaches for LARC.

Predictive microbiota-based biomarkers

The identification of microbiota-based biomarkers offers the potential to predict individual responses to nCRT. Integrating microbiota analysis into clinical practice could lead to more personalized and effective treatment plans for LARC patients. Several microbial genera have been identified as potential biomarkers for predicting response to nCRT. Shi *et al.*⁵⁹ employed statistical analysis of metagenomic profiles (STAMP) and linear discriminant analysis of effect size (LEfSe) to identify *Shuttleworthia* as enriched in responders, while several bacteria taxa in the *Clostridiales* order (*Faecalibacterium*, *Murimonas*, and *Lachnospiraceae incertae sedis*) were found to be enriched in non-responders. Yi *et al.*²³ developed a random forest model incorporating 10 microbial biomarkers that achieved an AUC of 93.57% in the training cohort and 73.53% in the validation cohort, highlighting the potential of *Eisenbergiella*, *Granulicatella*, and *Ralstonia* as microbiota-based predictors of non-response; however, external validation remains limited. Takenaka *et al.*⁶⁰ used LEfSe to identify specific genera, such as *Hungatella*, *Flavonifractor*, and *Methanospaera*, as potential predictive markers for nCRT response in responder LARC patients, while non-responders exhibited a higher abundance of genera like *Enhydrobacter*, *Paraprevotella*, and *Finegoldia*.⁶⁰ Additionally, Sun *et al.*⁶¹ established a predictive classifier and found that *Collinsella* (AUC = 0.652), *Alistipes* (AUC = 0.702), *Christensenella* (AUC = 0.702), *Faecalibacterium* (AUC = 0.658), *Ruminococcus* (AUC = 0.652), *Pavimonas* (AUC = 0.669), and *Akkermansia* (AUC = 0.689) were significantly associated with the responses to nCRT. And a recent study by Roesel *et al.*⁶³ analyzed diagnostic biopsies from LARC patients and identified significant differences in intratumoral microbiota between complete responders (CR) and non-complete responders (non-CR). Specifically, a consistent association was observed by LEfSe between the presence of *Ruminococcus* species and CR, whereas higher abundances of *Fusobacterium*, *Porphyromonas*, and *Oscillibacter* were linked to non-CR. These findings highlight the significant microbial profile differences between responders and non-responders, emphasizing the role of specific bacterial communities in predicting treatment outcomes. Nevertheless, across studies, the

overlap in identified microbial biomarkers is minimal, indicating that population-specific, tumor microenvironment-dependent, and diet-related factors strongly influence microbial signatures.

Gut microbiota and nCRT-induced toxicity

The relationship between gut microbiota and the toxicity of nCRT in LARC has gained increasing attention due to its potential role in modulating treatment outcomes. nCRT can induce significant gastrointestinal side effects, including diarrhea, mucositis, and intestinal permeability changes. The diversity and composition of the gut microbiome are thought to be key factors influencing these toxicities. Several studies have demonstrated that loss of microbial diversity and changes in bacterial composition are associated with therapy-related toxicities in cancer patients.^{67–71} However, a recent study by Shi *et al.*⁵⁹ investigating rectal cancer patients undergoing nCRT found no significant differences in the richness and diversity of the gut microbiome between patients with no or mild diarrhea and those with severe diarrhea. These findings suggest that other factors, such as specific bacterial taxa, might be more influential in modulating nCRT toxicity. When specific bacterial taxa were examined, significant differences in the relative abundances of several taxa were observed between the two groups. Notably, *Bifidobacterium*, several bacteria in the *Clostridia* class, including *Butyrivibrio*, *Clostridium* XIVa, and *Hungatella*, as well as the *Bacteroides* genus (particularly *Bacteroides vulgatus*), were enriched in patients with no or mild diarrhea. These microbial communities may play a protective role in mitigating the severity of diarrhea induced by nCRT, highlighting the potential for microbiome-based interventions to reduce treatment-related toxicities.

Specific mechanisms of gut microbiota influencing the efficacy of nCRT in LARC

The interaction between gut microbiota and nCRT is multifaceted. The gut microbiota plays a crucial role in modulating systemic immunity, influencing inflammation, and affecting drug metabolism, all of which can impact the response to nCRT. Building on the earlier overview of microbiota-associated immune effects within the tumor microenvironment in CRC, this section focuses on mechanisms that are more directly relevant to nCRT efficacy in LARC and minimizes repetition of general immune concepts. In

particular, we highlight emerging nCRT-related pathways beyond broad immune modulation, including microbiota-mediated effects on radio-sensitivity, tumor metabolism, DNA damage and repair, and direct interactions with chemotherapeutic agents.

Teng et al.²⁵ reported that the presence of *B. vulgatus* was found to upregulate genes related to nucleotide biosynthesis, potentially aiding tumor survival and contributing to therapy resistance. The study also suggests that *B. vulgatus* can protect cancer cells from the cytotoxic effects of 5-fluorouracil-induced chemotherapy or radiation treatment. Furthermore, Zhou et al.²⁶ discovered that methylglyoxal (MG), a metabolite derived from the gut microbiota, particularly from *Lactobacillus*, enhances the radiotherapy response by stimulating the generation of intracellular reactive oxygen species (ROS) and reducing tumor hypoxia. Mechanistically, MG activates the cGAS-STING pathway, increases DNA double-strand breaks, and promotes immunogenic cell death through ROS-induced endoplasmic reticulum stress, which leads to enhanced infiltration of CD8⁺ T cells and natural killer cells into the tumor immune microenvironment. Moreover, MG has shown potential as an effective radiosensitizer and immunomodulator, with the combination of MG and anti-PD1 therapy producing long-lasting complete responses in both irradiated and non-irradiated tumor sites, which offers a promising avenue for improving treatment outcomes in LARC patients. These findings highlight a potential therapeutic avenue for microbiota-derived metabolites to synergize with immune checkpoint blockade and radiotherapy.

Strategies for modulating gut microbiota to improve nCRT outcomes

The growing understanding of how gut microbiota interacts with nCRT has led to the exploration of strategies for microbiota modulation aimed at improving treatment outcomes and reducing toxicities. These approaches can be broadly categorized into dietary interventions, probiotics, prebiotics, and fecal microbiota transplantation (FMT).

Dietary interventions

Diet plays a crucial role in shaping the gut microbiota. Studies suggest that a high-fiber diet can increase the abundance of SCFA-producing

bacteria including *Lactobacillus*, *Bifidobacterium*, and *Akkermansia*, potentially enhancing immune function and improving the response to nCRT.^{72,73} Diets rich in fiber from fruits, vegetables, and whole grains may not only improve microbiota composition but also reduce the side effects of nCRT, such as diarrhea and mucositis, by promoting gut health and immune homeostasis. For patients with diarrhea, a diet rich in soluble fiber that helps absorb water can be beneficial.⁷⁴

While dietary fiber generally benefits gut health, a recent study has raised concerns about high-dose soluble fiber intake. Research in mice has shown that high doses of soluble fiber but not insoluble fiber can promote colorectal tumorigenesis by dysregulating gut microbiota and metabolites.⁷⁵ This effect was associated with increased tumor number and load, as well as alterations in gut microbial composition, including enrichment of *Bacteroides uniformis* and depletion of *Bifidobacterium pseudolongum*.

Probiotics and prebiotics

Probiotics are live beneficial microorganisms that play a crucial role in maintaining health, capable of restoring microbial balance, and alleviating chemotherapy-induced adverse events such as diarrhea and oral mucositis in patients undergoing chemoradiotherapy.^{76,77} In recent years, the role of probiotics, particularly strains like *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*, has garnered clinical attention for their ability to mitigate chemotherapy-induced adverse events, especially chemotherapy-induced diarrhea (CID).⁷⁸ The mechanisms through which probiotics alleviate CID include regulating dysbiosis, modulating inflammatory cytokine production, enhancing immunity, and improving the gut mucosal barrier.^{45,79–81} Among these, strains like *Lactobacillus* and *Bifidobacterium* have shown promise in reducing CID and protecting the intestinal barrier.⁸² In a randomized clinical trial by Österlund et al.,⁸³ *Lactobacillus* GG supplementation was found to potentially reduce the frequency of severe diarrhea and abdominal discomfort in CRC patients undergoing 5-fluorouracil-based chemotherapy. However, a more recent clinical study reported contradictory results, finding no significant effect of probiotic supplementation (*Lactobacillus* and *Bifidobacterium*) on the severity of CID in CRC patients.⁸⁴ Additionally, Golkhalkhali et al.⁸⁵ reported that CRC patients treated with a combination of *Lactobacillus*,

Bifidobacterium, and omega-3 fatty acids exhibited clinically significant improvement in CID. Mohebian et al.⁸⁶ reported that yogurt combined with probiotics including *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus* spp. could lead to better outcome in CRC patients suffering from CID. Overall, probiotics play a significant role in alleviating the side effects associated with nCRT, particularly in improving symptoms such as CID.

Prebiotics, non-digestible food components that promote the growth and activity of beneficial gut microorganisms, have also been investigated for their potential in modulating gut microbiota and improving outcomes in patients undergoing nCRT. Prebiotics, such as inulin, fructooligosaccharides (FOS), and galactooligosaccharides, have been shown to selectively stimulate the growth of beneficial gut bacteria, which may help in restoring microbial balance and supporting gut health during nCRT.⁸⁷ By enhancing the growth of probiotics like *Lactobacillus* and *Bifidobacterium*, prebiotics may work synergistically to further improve gut microbiota composition, reduce inflammation, and strengthen the gut mucosal barrier.^{88,89} Recent studies have indicated that prebiotics may enhance the beneficial effects of probiotics in mitigating chemotherapy-induced side effects, including diarrhea and mucositis, by promoting a more favorable gut environment.^{90,91} For instance, *Lactobacillus fermentum* together with the prebiotic FOS alleviated 5-fluorouracil-induced intestinal mucositis and improved intestinal barrier function.⁹² Additionally, probiotics combined with prebiotics can influence the efficacy of anti-CRC treatments by affecting the colonization of pathogenic bacteria, such as *Clostridium difficile* and *Staphylococcus aureus*, in the intestine.⁹³ They also regulate intestinal immunity and affect the production of SCFAs.⁹³ Therefore, the combined use of prebiotics and probiotics holds great potential for enhancing gut health of patients undergoing nCRT, ultimately leading to better treatment outcomes and improved quality of life.

Fecal microbiota transplantation

FMT is a therapeutic procedure that involves transferring fecal matter from a healthy donor to a patient to restore microbial diversity and function in the recipient's gut.⁹⁴ This approach has proven effective in treating conditions such as *C. difficile* infection,⁹⁵ which remains the primary approved indication for FMT. Beyond this, FMT

is being explored for broader applications, including its potential role in cancer therapy.

Recent studies suggest that FMT may help re-establish a healthy microbiota in cancer patients undergoing nCRT, potentially enhancing the treatment response and reducing toxicities.⁹⁶ For example, FMT has been shown to alleviate radiation-induced gastrointestinal toxicity, with several clinical studies demonstrating its ability to improve dysbiosis.^{97,98} Furthermore, FMT has been shown to reduce intestinal inflammation and improve the gut microbiome in patients with CRC by decreasing the abundance of cancer-promoting bacteria.^{89,94} Additionally, FMT has been shown to decrease the abundance of the *Firmicutes* phylum and increase the abundance of the *Bacteroidetes* phylum, thereby restoring the gut microbiome's diversity and composition and shifting it toward a more balanced state that favors healthy microbial functions.^{97,99}

In animal studies, Cui et al.¹⁰⁰ found that FMT from healthy donors helped to restore gastrointestinal function, improve survival rates, and promote epithelial integrity without accelerating tumor growth. Dong et al.¹⁰¹ found that FMT potentiated the tumoricidal effects of radiation and alleviated intestinal toxicity in CRC mice models by accumulating *Roseburia intestinalis* in the gastrointestinal tract. FMT has also been shown to increase levels of beneficial metabolites, such as SCFAs, which are associated with improved gut barrier function and reduced inflammation.^{102,103}

Given these findings, FMT emerges as a promising adjunctive therapy for improving outcomes in cancer patients undergoing nCRT. However, more research is needed to fully understand its role in enhancing therapeutic efficacy, particularly in terms of modulating immune responses and influencing treatment-related side effects.

Discussion

The gut microbiota's role in modulating the response to nCRT in patients with LARC is increasingly recognized. Specific microbial signatures, including the abundance of certain bacterial taxa like *Alistipes* spp., are associated with improved responses to nCRT, while others, like *F. nucleatum*, are linked to resistance. Recent studies have emphasized the predictive value of microbiota-based biomarkers in nCRT respo

nse^{23,59,60–62} and have also suggested that the gut microbiota may be useful in predicting treatment-related toxicity. For instance, *Clostridium* spp. and *Alistipes* spp. have been found to be significantly more abundant in patients who do not experience adverse events compared to those who do.¹⁰⁴ These findings align with the study by Shi et al., which highlighted *Alistipes* spp. as a potential predictor of nCRT response.⁶¹ Such specific microbial signatures, with the ability to predict nCRT response or adverse effects, could pave the way for personalized therapeutic strategies in the treatment of LARC in real-world clinical settings.^{105,106} However, the current evidence remains heterogeneous, and the translation of microbial signatures into clinically actionable biomarkers requires validation across diverse populations, standardized methodologies, and prospective clinical trials.

Contradictory findings across studies further complicate biomarker development and underscore the limits of genus-level associations. Using 16S rRNA profiling, Shi et al.⁵⁹ reported *Faecalibacterium* to be more abundant in non-responders than responders, with levels declining post-nCRT among responders. This appears at odds with the broader literature describing *Faecalibacterium* (notably *F. prausnitzii*) as a butyrate-producing taxon with anti-inflammatory and immune-regulatory effects.^{107,108} In contrast, a shotgun metagenomic study by Chen et al.⁶² identified *Faecalibacterium* sp900539945 enriched in good responders, suggesting that strain-level heterogeneity—and not the genus per se—may drive response signals. Similarly, the dual role of *Streptococcus* species in study by Yi et al.²³ further complicates microbiota modulation efforts. While certain strains such as *Streptococcus thermophilus* exert anti-inflammatory effects,¹⁰⁹ others, like *Streptococcus agalactiae*, possess virulence factors that may suppress immune responses and hinder nCRT effectiveness.¹¹⁰ Together, these contrasts argue for strain-resolved, function-anchored analyses (e.g., metagenomics/metatranscriptomics with metabolomics), and standardized reporting of sample type and timepoint; without this resolution, clinical translation risks being misleading or ineffective.

Beyond taxonomic correlations, recent mechanistic studies emphasize that microbial influence on nCRT extends beyond immune modulation. While the beneficial effects of gut microbiota metabolites, such as butyrate, in

modulating inflammation and supporting immune responses have been well-established, emerging evidence demonstrates direct microbial interactions with tumor metabolic pathways and treatment-induced cell death mechanisms. For example, *B. vulgatus* upregulates nucleotide biosynthesis genes, contributing to cancer cell survival and therapy resistance.²⁵ In contrast, *Lactobacillus*-derived metabolites MG enhances radiotherapy outcomes by activating the cGAS-STING pathway and promoting immunogenic cell death.²⁶ These findings indicate that microbial metabolites can act as radiosensitizers or resistance factors, opening new opportunities for combining microbiota-targeted agents with chemoradiotherapy and immunotherapy. However, studies examining these mechanisms in LARC remain scarce, underscoring a critical research gap.

Research has predominantly focused on the gut microbiota, but the tumor-associated microbiome is now emerging as an equally important determinant of treatment response. The intratumoral microbiota directly interfaces with tumor cells and the immune microenvironment, thereby shaping therapeutic sensitivity. Recent integrated microbiome-immune profiling studies in LARC demonstrated distinct intratumoral microbial patterns between responders and non-responders.^{61,63} To move beyond associative signals, future work should prioritize paired designs that collect diagnostic LARC tumor biopsies and contemporaneous baseline stool samples for shotgun metagenomics ($\pm$ metatranscriptomics), enabling concordance/discordance mapping of taxa and functions across tumor and lumen. Such paired datasets would clarify which stool features reliably mirror tumor biology, improve classifier generalizability, and identify stool-based surrogates for hard-to-sample intratumoral features. Complementary approaches—spatial profiling of tumor microbiota (e.g., 16S/ISH or spatial metagenomics) alongside single-cell or bulk immune transcriptomics, longitudinal sampling pre-/mid-/post-nCRT, and standardized protocols for tissue handling and DNA/RNA extraction—should be embedded to strengthen mechanistic inference and cross-cohort reproducibility. Ultimately, integrating tumor- and stool-derived microbial features with host genomics, immune contexture, and clinical covariates in multiscale models may enhance predictive accuracy beyond stool-only biomarkers and better inform organ-preserving strategies in LARC.

Strategies for modulating the gut microbiota offer promising avenues to enhance nCRT outcomes. Dietary interventions, particularly high-fiber diets, increase beneficial bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Akkermansia*, and may support immune function and reduce nCRT-related toxicities. However, dietary composition must be carefully tailored, as the effects of fiber vary depending on solubility, and excessive soluble fiber may promote colorectal tumorigenesis under certain microbial conditions. Probiotics and prebiotics have garnered attention for their potential to alleviate nCRT-induced side effects. Certain strains in *Lactobacillus* and *Bifidobacterium* can reduce CID and enhance the gut mucosal barrier. The combination of probiotics and prebiotics is an exciting approach, as prebiotics may enhance the effects of probiotics, improving the gut environment during nCRT. As these strategies are better understood, they could be seamlessly integrated into standardized treatment protocols for LARC, making microbiota-based interventions an ancillary component of care. In addition, FMT has shown promise in restoring microbial diversity, reducing toxicities, and potentially improving treatment responses in cancer patients undergoing nCRT. Although FMT is a powerful microbiota-modifying intervention, its optimal timing, donor selection criteria, long-term effects, and oncologic safety remain unclear and require evaluation in controlled clinical trials.

Despite notable progress, substantial limitations hinder clinical translation. Significant heterogeneity exists in study settings, including timing of sample collection, stool versus tissue sampling, choice of sequencing (16S vs metagenomics), and analytical pipelines, all of which restrict comparability. Population-specific microbiome structures driven by diet, lifestyle, genetics, and environmental exposure further limit generalizability, given that most findings originate from East Asian and European cohorts. Without standardized workflows and multi-center, longitudinal validation, microbiota-based biomarkers may remain population-dependent and non-generalizable.

Overall, the potential for microbiota-based interventions to enhance the efficacy of nCRT and reduce treatment-related toxicities is undeniable. Future research should focus on refining microbiota profiling techniques to identify reliable biomarkers of nCRT response, exploring the mechanisms of microbiota-tumor interactions,

and conducting well-designed clinical trials to evaluate the effectiveness of microbiota modulation strategies in LARC patients. Additionally, the incorporation of microbiota-based therapies into multimodal treatment regimens for LARC could provide significant benefits in terms of improving patient outcomes and quality of life.

Conclusion

In conclusion, gut microbiota shapes both response and toxicity to nCRT in LARC, with distinct taxa and functional pathways emerging as candidate biomarkers to identify responders, guide risk stratification, and inform supportive care. Beyond immune modulation, microbial metabolites can directly alter radiosensitivity and therapy-induced stress responses, revealing actionable mechanisms. Microbiota-targeted strategies, including dietary interventions, probiotics/prebiotics, and FMT, are promising adjuncts for enhancing efficacy and mitigating gastrointestinal toxicity, but require rigorous, standardized validation. Key gaps include heterogeneity in sampling and analytics, population-specific effects, limited LARC-focused mechanism studies, and a paucity of interventional trials, including those assessing the intratumoral microbiome. Integrating microbiome profiling with immunologic, metabolomic, and genomic data in prospective, multi-center studies will be critical to deliver reproducible biomarkers and safe, effective interventions. These advances could enable microbiome-informed precision therapy that improves outcomes and quality of life for patients with LARC.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contributions

Zhiwei Wu: Conceptualization; Investigation; Writing – original draft; Writing – review & editing.

Zihan Yang: Conceptualization; Investigation; Writing – original draft.

Chengzhen Lyu: Conceptualization; Visualization.

Bo Sun: Conceptualization; Visualization.

Ruikai Zhang: Conceptualization; Visualization.

Hongbo Li: Conceptualization; Visualization.

Jian Chen: Conceptualization; Writing – review & editing.

Acknowledgements

The authors would like to thank Dr Jian Chen for his valuable guidance and insightful suggestions throughout the preparation of this review.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Not applicable.

ORCID iD

Chengzhen Lyu  <https://orcid.org/0009-0002-9222-7327>

References

1. Keum N and Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. *Nat Rev Gastroenterol Hepatol* 2019; 16: 713–732.
2. Siegel RL, Wagle NS, Cercek A, et al. Colorectal cancer statistics, 2023. *CA Cancer J Clin* 2023; 73: 233–254.
3. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; 71: 209–249.
4. Koukourakis IM, Kouloulis V, Tiniakos D, et al. Current status of locally advanced rectal cancer therapy and future prospects. *Crit Rev Oncol Hematol* 2023; 186: 103992.
5. He K, Li L, Li A, et al. Genomic features, evolutionary patterns and minimal residual disease at surgical margins as novel prognostic/predictive biomarkers in locally advanced rectal cancer. *Clin Transl Med* 2023; 13: e1286.
6. Benson AB, Venook AP, Al-Hawary MM, et al. Rectal cancer, version 2.2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2022; 20: 1139–1167.
7. Xu Y, Zou H, Shao Z, et al. Efficacy and safety of different radiotherapy doses in neoadjuvant chemoradiotherapy in patients with locally advanced rectal cancer: a retrospective study. *Front Oncol* 2023; 13: 1119323.
8. Hou K, Wu ZX, Chen XY, et al. Microbiota in health and diseases. *Signal Transduct Target Ther* 2022; 7: 135.
9. Ternes D, Karta J, Tsenkova M, et al. Microbiome in colorectal cancer: how to get from meta-omics to mechanism? *Trends Microbiol* 2020; 28: 401–423.
10. Gao Z, Guo B, Gao R, et al. Microbiota disbiosis is associated with colorectal cancer. *Front Microbiol* 2015; 6: 20.
11. Li J, Zhang AH, Wu FF, et al. Alterations in the gut microbiota and their metabolites in colorectal cancer: recent progress and future prospects. *Front Oncol* 2022; 12: 841552.
12. Feng Q, Liang S, Jia H, et al. Gut microbiome development along the colorectal adenoma-carcinoma sequence. *Nat Commun* 2015; 6: 6528.
13. Yu J, Feng Q, Wong SH, et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* 2017; 66: 70–78.
14. Sobhani I, Tap J, Roudot-Thoraval F, et al. Microbial dysbiosis in colorectal cancer (CRC) patients. *PLoS One* 2011; 6: e16393.
15. Wang T, Cai G, Qiu Y, et al. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME J* 2012; 6: 320–329.
16. Ahn J, Sinha R, Pei Z, et al. Human gut microbiome and risk for colorectal cancer. *J Natl Cancer Inst* 2013; 105: 1907–1911.
17. Zeller G, Tap J, Voigt AY, et al. Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol Syst Biol* 2014; 10: 766.
18. Kim SH and Lim YJ. The role of microbiome in colorectal carcinogenesis and its clinical potential as a target for cancer treatment. *Intest Res* 2022; 20: 31–42.
19. Kim J and Lee HK. Potential role of the gut microbiome in colorectal cancer progression. *Front Immunol* 2022; 12: 807648.
20. Yachida S, Mizutani S, Shiroma H, et al. Metagenomic and metabolomic analyses reveal

- distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat Med* 2019; 25: 968–976.
21. Emoto S, Inoue R, Murai S, et al. A pilot study of gut mucosal and faecal microbiota in rectal cancer: associations with histological response and adverse events following preoperative chemoradiotherapy. *Colorectal Dis* 2025; 27: e70106.
 22. Sun Y, Zhang X, Jin C, et al. Prospective, longitudinal analysis of the gut microbiome in patients with locally advanced rectal cancer predicts response to neoadjuvant concurrent chemoradiotherapy. *J Transl Med* 2023; 21: 221.
 23. Yi Y, Shen L, Shi W, et al. Gut microbiome components predict response to neoadjuvant chemoradiotherapy in patients with locally advanced rectal cancer: a prospective, longitudinal study. *Clin Cancer Res* 2021; 27: 1329–1340.
 24. Yang Z, Ma J, Han J, et al. Gut microbiome model predicts response to neoadjuvant immunotherapy plus chemoradiotherapy in rectal cancer. *Med* 2024; 5: 1293–1306.e4.
 25. Teng H, Wang Y, Sui X, et al. Gut microbiota-mediated nucleotide synthesis attenuates the response to neoadjuvant chemoradiotherapy in rectal cancer. *Cancer Cell* 2023; 41: 124–138.e6.
 26. Zhou H, Wang L, Lin Z, et al. Methylglyoxal from gut microbes boosts radiosensitivity and radioimmunotherapy in rectal cancer by triggering endoplasmic reticulum stress and cGAS-STING activation. *J Immunother Cancer* 2023; 11: e007840.
 27. Sevcikova A, Izoldova N, Stevurkova V, et al. The impact of the microbiome on resistance to cancer treatment with chemotherapeutic agents and immunotherapy. *Int J Mol Sci* 2022; 23: 488.
 28. Ramos A and Hemann MT. Drugs, bugs, and cancer: *Fusobacterium nucleatum* promotes chemoresistance in colorectal Cancer. *Cell* 2017; 170: 411–413.
 29. Garrett WS. Cancer and the microbiota. *Science* 2015; 348: 80–86.
 30. Sommer F and Backhed F. The gut microbiota—masters of host development and physiology. *Nat Rev Microbiol* 2013; 11: 227–238.
 31. McCallum G and Tropini C. The gut microbiota and its biogeography. *Nat Rev Microbiol* 2024; 22: 105–118.
 32. Rooks MG and Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol* 2016; 16: 341–352.
 33. Jandhyala SM, Talukdar R, Subramanyam C, et al. Role of the normal gut microbiota. *World J Gastroenterol* 2015; 21: 8787–8803.
 34. Kau AL, Ahern PP, Griffin NW, et al. Human nutrition, the gut microbiome and the immune system. *Nature* 2011; 474: 327–336.
 35. Wong CC and Yu J. Gut microbiota in colorectal cancer development and therapy. *Nat Rev Clin Oncol* 2023; 20: 429–452.
 36. O’Keefe SJ. Diet, microorganisms and their metabolites, and colon cancer. *Nat Rev Gastroenterol Hepatol* 2016; 13: 691–706.
 37. Schulthess J, Pandey S, Capitani M, et al. The short chain fatty acid butyrate imprints an antimicrobial program in macrophages. *Immunity* 2019; 50: 432–445.e7.
 38. Campbell C, McKenney PT, Konstantinovskiy D, et al. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature* 2020; 581: 475–479.
 39. Yu T, Guo F, Yu Y, et al. *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell* 2017; 170: 548–563.e16.
 40. Mima K, Nishihara R, Qian ZR, et al. *Fusobacterium nucleatum* in colorectal carcinoma tissue and patient prognosis. *Gut* 2016; 65: 1973–1980.
 41. Kostic AD, Chun E, Robertson L, et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013; 14: 207–215.
 42. Zhang Y, Zhang L, Zheng S, et al. *Fusobacterium nucleatum* promotes colorectal cancer cells adhesion to endothelial cells and facilitates extravasation and metastasis by inducing ALPK1/NF-kappaB/ICAM1 axis. *Gut Microbes* 2022; 14: 2038852.
 43. Dadgar-Zankbar L, Shariati A, Bostanghadiri N, et al. Evaluation of enterotoxigenic *Bacteroides fragilis* correlation with the expression of cellular signaling pathway genes in Iranian patients with colorectal cancer. *Infect Agent Cancer* 2023; 18: 48.
 44. Geis AL, Fan H, Wu X, et al. Regulatory T-cell response to enterotoxigenic *Bacteroides fragilis* colonization triggers IL17-dependent colon carcinogenesis. *Cancer Discov* 2015; 5: 1098–1109.
 45. Ren S, Zhang X, Guan H, et al. *Lactobacillus acidipiscis* induced regulatory gamma delta T cells and attenuated experimental autoimmune encephalomyelitis. *Front Immunol* 2021; 12: 623451.

46. Fong W, Li Q, Ji F, et al. *Lactobacillus gallinarum*-derived metabolites boost anti-PD1 efficacy in colorectal cancer by inhibiting regulatory T cells through modulating IDO1/Kyn/AHR axis. *Gut* 2023; 72: 2272–2285.
47. Cervantes-Barragan L, Chai JN, Tianero MD, et al. *Lactobacillus reuteri* induces gut intraepithelial CD₄(+)CD₈alphaalpha(+) T cells. *Science* 2017; 357: 806–810.
48. Ruiz L, Delgado S, Ruas-Madiedo P, et al. Bifidobacteria and their molecular communication with the immune system. *Front Microbiol* 2017; 8: 2345.
49. Strisciuglio C, Miele E, Giugliano FP, et al. Bifidobacteria enhance antigen sampling and processing by dendritic cells in pediatric inflammatory bowel disease. *Inflamm Bowel Dis* 2015; 21: 1491–1498.
50. Sivan A, Corrales L, Hubert N, et al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* 2015; 350: 1084–1089.
51. Hu J, Chen J, Xu X, et al. Gut microbiota-derived 3-phenylpropionic acid promotes intestinal epithelial barrier function via AhR signaling. *Microbiome* 2023; 11: 102.
52. Zhou Q, Shen B, Huang R, et al. *Bacteroides fragilis* strain ZY-312 promotes intestinal barrier integrity via upregulating the STAT3 pathway in a radiation-induced intestinal injury mouse model. *Front Nutr* 2022; 9: 1063699.
53. Lopez-Siles M, Duncan SH, Garcia-Gil LJ, et al. *Faecalibacterium prausnitzii*: from microbiology to diagnostics and prognostics. *ISME J* 2017; 11: 841–852.
54. Zhang M, Zhou L, Wang Y, et al. *Faecalibacterium prausnitzii* produces butyrate to decrease c-Myc-related metabolism and Th17 differentiation by inhibiting histone deacetylase 3. *Int Immunol* 2019; 31: 499–514.
55. Xu Y, Duan J, Wang D, et al. *Akkermansia muciniphila* alleviates persistent inflammation, immunosuppression, and catabolism syndrome in mice. *Metabolites* 2023; 13: 194.
56. Jiang Y, Xu Y, Zheng C, et al. Acetyltransferase from *Akkermansia muciniphila* blunts colorectal tumorigenesis by reprogramming tumour microenvironment. *Gut* 2023; 72: 1308–1318.
57. Liu Y, Yang M, Tang L, et al. TLR4 regulates RORgammat(+) regulatory T-cell responses and susceptibility to colon inflammation through interaction with *Akkermansia muciniphila*. *Microbiome* 2022; 10: 98.
58. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013; 504: 446–450.
59. Shi W, Shen L, Zou W, et al. The gut microbiome is associated with therapeutic responses and toxicities of neoadjuvant chemoradiotherapy in rectal cancer patients—a pilot study. *Front Cell Infect Microbiol* 2020; 10: 562463.
60. Takenaka IKTM, Bartelli TF, Defelicious A, et al. Exome and tissue-associated microbiota as predictive markers of response to neoadjuvant treatment in locally advanced rectal cancer. *Front Oncol* 2022; 12: 809441.
61. Sun L, Qu J, Ke X, et al. Interaction between intratumoral microbiota and tumor mediates the response of neoadjuvant therapy for rectal cancer. *Front Microbiol* 2023; 14: 1229888.
62. Chen H, Zeng M, Batool SS, et al. Metagenomic analysis reveals effects of gut microbiome in response to neoadjuvant chemoradiotherapy in advanced rectal cancer. *Genomics* 2024; 116: 110951.
63. Roesel R, Strati F, Basso C, et al. Combined tumor-associated microbiome and immune gene expression profiling predict response to neoadjuvant chemo-radiotherapy in locally advanced rectal cancer. *Oncimmunology* 2025; 14: 2465015.
64. Fan Q, Shang F, Chen C, et al. Microbial characteristics of locally advanced rectal cancer patients after neoadjuvant chemoradiation therapy according to pathologic response. *Cancer Manag Res* 2021; 13: 2655–2667.
65. Sanchez-Alcoholado L, Laborda-Illanes A, Otero A, et al. Relationships of gut microbiota composition, short-chain fatty acids and polyamines with the pathological response to neoadjuvant radiochemotherapy in colorectal cancer patients. *Int J Mol Sci* 2021; 22: 9549.
66. Serna G, Ruiz-Pace F, Hernando J, et al. *Fusobacterium nucleatum* persistence and risk of recurrence after preoperative treatment in locally advanced rectal cancer. *Ann Oncol* 2020; 31: 1366–1375.
67. Manichanh C, Varela E, Martinez C, et al. The gut microbiota predispose to the pathophysiology of acute postradiotherapy diarrhea. *Am J Gastroenterol* 2008; 103: 1754–1761.
68. Wedlake L, Thomas K, McGough C, et al. Small bowel bacterial overgrowth and lactose intolerance during radical pelvic radiotherapy: an observational study. *Eur J Cancer* 2008; 44: 2212–2217.

69. Ferreira MR, Muls A, Dearnaley DP, et al. Microbiota and radiation-induced bowel toxicity: lessons from inflammatory bowel disease for the radiation oncologist. *Lancet Oncol* 2014; 15: e139–e147.
70. Wallace BD, Roberts AB, Pollet RM, et al. Structure and inhibition of microbiome beta-glucuronidases essential to the alleviation of cancer drug toxicity. *Chem Biol* 2015; 22: 1238–1249.
71. Wang A, Ling Z, Yang Z, et al. Gut microbial dysbiosis may predict diarrhea and fatigue in patients undergoing pelvic cancer radiotherapy: a pilot study. *PLoS One* 2015; 10: e0126312.
72. Bach Knudsen KE, Laerke HN, Hedemann MS, et al. Impact of diet-modulated butyrate production on intestinal barrier function and inflammation. *Nutrients* 2018; 10: 1499.
73. Chen L, Liu B, Ren L, et al. High-fiber diet ameliorates gut microbiota, serum metabolism and emotional mood in type 2 diabetes patients. *Front Cell Infect Microbiol* 2023; 13: 1069954.
74. Gill SK, Rossi M, Bajka B, et al. Dietary fibre in gastrointestinal health and disease. *Nat Rev Gastroenterol Hepatol* 2021; 18: 101–116.
75. Yang J, Wei H, Lin Y, et al. High soluble fiber promotes colorectal tumorigenesis through modulating gut microbiota and metabolites in mice. *Gastroenterology* 2024; 166: 323–337.e7.
76. Davoodvandi A, Fallahi F, Tamtaji OR, et al. An update on the effects of probiotics on gastrointestinal cancers. *Front Pharmacol* 2021; 12: 680400.
77. Das M. Probiotics for chemoradiotherapy-induced oral mucositis. *Lancet Oncol* 2019; 20: e14.
78. Thet D, Areepium N and Siritientong T. Effects of probiotics on chemotherapy-induced diarrhea. *Nutr Cancer* 2023; 75: 1811–1821.
79. Fioramonti J, Theodorou V and Bueno L. Probiotics: what are they? What are their effects on gut physiology? *Best Pract Res Clin Gastroenterol* 2003; 17: 711–724.
80. Liu Z, Qin H, Yang Z, et al. Randomised clinical trial: the effects of perioperative probiotic treatment on barrier function and post-operative infectious complications in colorectal cancer surgery—a double-blind study. *Aliment Pharmacol Ther* 2011; 33: 50–63.
81. Wilkins T and Sequoia J. Probiotics for gastrointestinal conditions: a summary of the evidence. *Am Fam Physician* 2017; 96: 170–178.
82. Lu D, Yan J, Liu F, et al. Probiotics in preventing and treating chemotherapy-induced diarrhea: a meta-analysis. *Asia Pac J Clin Nutr* 2019; 28: 701–710.
83. Osterlund P, Ruotsalainen T, Korpela R, et al. Lactobacillus supplementation for diarrhoea related to chemotherapy of colorectal cancer: a randomised study. *Br J Cancer* 2007; 97: 1028–1034.
84. Zaharuddin L, Mokhtar NM, Muhammad Nawawi KN, et al. A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. *BMC Gastroenterol* 2019; 19: 131.
85. Golkhalkhali B, Rajandram R, Paliany AS, et al. Strain-specific probiotic (microbial cell preparation) and omega-3 fatty acid in modulating quality of life and inflammatory markers in colorectal cancer patients: a randomized controlled trial. *Asia Pac J Clin Oncol* 2018; 14: 179–191.
86. Mohebian F, Mohammadzadeh Zarankesh S, Jenabian A, et al. The effectiveness of Yogurt+ probiotic on chemotherapy-related diarrhea in patients with colorectal cancer: a randomized clinical trial. *Middle East J Cancer* 2023; 14: 127–135.
87. Chen Y, Liao X, Li Y, et al. Effects of prebiotic supplement on gut microbiota, drug bioavailability, and adverse effects in patients with colorectal cancer at different primary tumor locations receiving chemotherapy: study protocol for a randomized clinical trial. *Trials* 2023; 24: 268.
88. Miarons M, Roca M and Salva F. The role of pro-, pre- and symbiotics in cancer: a systematic review. *J Clin Pharm Ther* 2021; 46: 50–65.
89. Kazmierczak-Siedlecka K, Daca A, Fic M, et al. Therapeutic methods of gut microbiota modification in colorectal cancer management—fecal microbiota transplantation, prebiotics, probiotics, and synbiotics. *Gut Microbes* 2020; 11: 1518–1530.
90. Horvat M, Krebs B, Potrc S, et al. Preoperative synbiotic bowel conditioning for elective colorectal surgery. *Wien Klin Wochenschr* 2010; 122(Suppl. 2): 26–30.
91. Moreira MM, Carrico M, Capelas ML, et al. The impact of pre-, pro- and synbiotics supplementation in colorectal cancer treatment: a systematic review. *Front Oncol* 2024; 14: 1395966.
92. Inamura K. Gut microbiota contributes towards immunomodulation against cancer: new frontiers in precision cancer therapeutics. *Semin Cancer Biol* 2021; 70: 11–23.

93. Fong W, Li Q and Yu J. Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer. *Oncogene* 2020; 39: 4925–4943.
94. Wang JW, Kuo CH, Kuo FC, et al. Fecal microbiota transplantation: review and update. *J Formos Med Assoc* 2019; 118(Suppl. 1): S23–S31.
95. Kelly CR, Fischer M, Allegretti JR, et al. ACG clinical guidelines: prevention, diagnosis, and treatment of *Clostridioides difficile* infections. *Am J Gastroenterol* 2021; 116: 1124–1147.
96. Van Dingenen L, Segers C, Wouters S, et al. Dissecting the role of the gut microbiome and fecal microbiota transplantation in radio- and immunotherapy treatment of colorectal cancer. *Front Cell Infect Microbiol* 2023; 13: 1298264.
97. Ding X, Li Q, Li P, et al. Fecal microbiota transplantation: a promising treatment for radiation enteritis? *Radiother Oncol* 2020; 143: 12–18.
98. Liu T, Su D, Lei C, et al. Treatment of radiation enteritis with fecal transplantation. *Am Surg* 2023; 89: 2999–3001.
99. Zheng YM, He XX, Xia HH, et al. Multi-donor multi-course faecal microbiota transplantation relieves the symptoms of chronic hemorrhagic radiation proctitis: a case report. *Medicine (Baltimore)* 2020; 99: e22298.
100. Cui M, Xiao H, Li Y, et al. Faecal microbiota transplantation protects against radiation-induced toxicity. *EMBO Mol Med* 2017; 9: 448–461.
101. Dong J, Wang B, Xiao Y, et al. *Roseburia intestinalis* sensitizes colorectal cancer to radiotherapy through the butyrate/OR51E1/RALB axis. *Cell Rep* 2024; 43: 113846.
102. Li Y, Dong J, Xiao H, et al. Gut commensal derived-valeric acid protects against radiation injuries. *Gut Microbes* 2020; 11: 789–806.
103. Xiao HW, Cui M, Li Y, et al. Gut microbiota-derived indole 3-propionic acid protects against radiation toxicity via retaining acyl-CoA-binding protein. *Microbiome* 2020; 8: 69.
104. Ma J, Sun S, Cheng X, et al. Unraveling the role of gut microbiome in predicting adverse events in neoadjuvant therapy for rectal cancer. *Hum Vaccin Immunother* 2024; 20: 2430087.
105. Yang Z, Gao J, Zheng J, et al. Efficacy and safety of PD-1 blockade plus long-course chemoradiotherapy in locally advanced rectal cancer (NECTAR): a multi-center phase 2 study. *Signal Transduct Target Ther* 2024; 9: 56.
106. Yang Z, Zhang X, Zhang J, et al. Rationale and design of a prospective, multicenter, phase II clinical trial of safety and efficacy evaluation of long course neoadjuvant chemoradiotherapy plus tislelizumab followed by total mesorectal excision for locally advanced rectal cancer (NCRT-PD1-LARC trial). *BMC Cancer* 2022; 22: 462.
107. Louis P and Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* 2009; 294: 1–8.
108. Machiels K, Joossens M, Sabino J, et al. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut* 2014; 63: 1275–1283.
109. Leonard MM, Valitutti F, Karathia H, et al. Microbiome signatures of progression toward celiac disease onset in at-risk children in a longitudinal prospective cohort study. *Proc Natl Acad Sci U S A* 2021; 118: e2020322118.
110. Uchiyama S, Sun J, Fukahori K, et al. Dual actions of group B *Streptococcus* capsular sialic acid provide resistance to platelet-mediated antimicrobial killing. *Proc Natl Acad Sci U S A* 2019; 116: 7465–7470.