REVIEW
Turk J Surg

Re-arming checkpoint blockade in MSS colorectal cancer: A precision-microbiome playbook from mechanisms to clinic

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ABSTRACT

Immune checkpoint blockade transforms outcomes for the 15% of colorectal cancers (CRCs) with mismatch-repair deficiency; yet most tumours remain refractory. Beneficial gut microbes can change this. *Akkermansia muciniphila*, *Bacteroides fragilis,* and short-chain fatty acid producers prime dendritic cells to produce interleukin (IL)-12, polarise Th1 cells, and reinvigorate $CD8^+$ T-cells. Antibiotics, Western-style diets, and *Fusobacterium nucleatum* foster myeloid suppression and β-catenin- or IL-17-mediated signalling, which blunt checkpoint activity. Multi-omics analyses link biosynthetic genes for inosine, riboflavin, and folate to durable clinical benefit. Faecal microbiota transplantation from responders has produced objective regressions in otherwise refractory microsatellite-stable disease. This narrative review maps CRC–microbiota–immune crosstalk, evaluates biomarkers and interventions, and proposes a CRC-specific, three-tiered clinical algorithm. We outline standards for trial design and manufacturing processes to facilitate the translation of microbiota-guided therapy into routine practice.

Keywords: MSS CRC, fecal microbiota transplantation, live biotherapeutic products, short-chain fatty acids, microbiome biomarkers, qPCR panel, antibiotic stewardship, inosine, riboflavin pathway

INTRODUCTION

Colorectal cancer (CRC) remains the second leading cause of cancer-related mortality worldwide despite incremental advances in screening and systemic therapy (1). The advent of immune-checkpoint blockade (ICB) targeting programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) has delivered durable responses in mismatch-repair-deficient (dMMR) tumours; however, fewer than 15% of CRCs exhibit dMMR, and response rates in the microsatellite-stable (MSS) majority remain below 10% (2). Understanding and overcoming this resistance constitute a pressing clinical need.

Over the past decade, the gut microbiota has emerged as a pivotal modulator of anticancer immunity. Landmark murine experiments showed that broad-spectrum antibiotics abrogate, while specific commensals restore, the efficacy of anti-PD-1 therapy (3). Subsequent work delineated mechanistic pathways: Short-chain fatty acids enhancing dendritic cell interleukin (IL)-12 production (4), microbial inosine acting on A_2A receptors to potentiate Th1 polarisation (5), and bacterial outer membrane vesicles amplifying STING signalling in tumour-infiltrating myeloid cells (6). Crucially, these effects are not generic across tumour types; colorectal models display unique dependencies on intraluminal taxa that modulate the Wnt-β-catenin and IL-17 networks (7).

Clinical investigations corroborate these findings. In prospective cohorts, higher alpha diversity and enrichment of *Bacteroides* spp. are associated with objective response and prolonged progression-free (PFS) survival with anti-PD-1 therapy (8). Multi-omics profiling links microbial gene clusters for tryptophan metabolism and vitamin B biosynthesis to heightened $CD8^+$ T-cell infiltration (9), while antibiotic exposure within 60 days of ICB initiation independently predicts poorer outcomes (10). Importantly, early-phase trials of faecal-microbiota transplantation (FMT) from

Cite this article as: Alsheikh ZSA, Qingsong T, Qinjie L, Youkun C. Re-arming checkpoint blockade in MSS colorectal cancer: aprecision-microbiome playbook from mechanisms to clinic. *Turk J Surg*. [Epub Ahead of Print]

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Received: 06.08.2025
Accepted: 07.01.2026
Epub: 08.05.2026

DOI: 10.47717/turkjsurg.2026.2025-8-3

Available at **www.turkjsurg.com**

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ICB responders into refractory metastatic CRC patients have reported partial responses and disease stabilisation without grade ≥3 toxicity (11).

Nevertheless, the field is rife with contradictions. Some studies fail to reproduce predictive taxa across geographic cohorts (12), and dietary fibre interventions that enhance ICB efficacy in melanoma yield neutral effects in CRC models (13). Disparities likely arise from tumour-intrinsic immunogenicity, host genetics, cancer stage, and methodological heterogeneity in microbial sampling and bioinformatics pipelines. These discrepancies underscore the necessity for CRC-specific standardised research approaches.

Against this backdrop, we undertake a comprehensive narrative synthesis of the literature published from January 2019 to July 2025, restricted to PubMed-indexed sources to ensure rigorous curation. Our objectives are fourfold:

1. Map mechanistic crosstalk between gut microbes, epithelial/immune compartments and tumour genomics that influences ICB outcomes (5,7,14),

2. Critically appraise biomarker studies delineating microbial signatures of response and resistance (8,10,15),

3. Evaluate interventional strategies—dietary modulation, pre-, pro-, and post-biotics, FMT, defined bacterial consortia, and targeted antibiotics—that aim to "re-arm" checkpoint blockade (11,16-18),

4. Identify controversies and future priorities, including causal inference challenges, regulatory hurdles for live biotherapeutics, and equitable access in low-resource settings.

Consistent with the Turkish Journal of Surgery's directive that review articles encompass "Clinical and Research Consequences" before concluding, the subsequent sections translate mechanistic insights into clinical algorithms and considerations for trial design. By weaving bench and bedside evidence into a coherent translational narrative, we aspire to furnish surgeons, oncologists, and microbiome scientists with actionable knowledge that can accelerate the integration of microbiota-guided strategies into routine CRC care.

Current Landscape of ICB in CRC

Immune checkpoint inhibitors (ICIs) have redefined management of the approximately 15% of metastatic colorectal cancers that harbour mismatch-repair deficiency (dMMR) or high microsatellite instability (MSI-H). Pembrolizumab monotherapy is a first-line standard of care, having demonstrated superior PFS survival compared with chemotherapy in KEYNOTE-177 (2). Nivolumab, alone or in combination with low-dose ipilimumab, provides durable responses in previously treated patients with dMMR/MSI-H mCRC, with 5-year overall survival exceeding 60% (1). These successes have prompted exploration of earlier-stage settings: For example, neoadjuvant short-course nivolumab plus ipilimumab achieved pathological complete response in 95% of dMMR rectal cancers, enabling organ preservation strategies (2).

By contrast, patients with MSS tumours—comprising 85% of CRC—derive minimal benefit, with objective response rates <10% despite combination regimens that add chemotherapy, radiotherapy, VEGF or EGFR blockade (2). Biomarker-driven efforts reveal that an "inflamed" tumour microenvironment (TME), characterised by high $CD8^+$ T-cell density and interferon-γ signatures, predicts ICI response regardless of MSI status; yet such phenotypes are rare in *de novo* MSS disease (8). Accordingly, current research pivots toward strategies that convert "cold" MSS lesions into "hot" lesions, including oncolytic viruses, personalised vaccines, KRAS G12C inhibitors, and—most prominently—gut-microbiota modulation (10).

Antibiotic exposure around the time of ICI initiation is independently associated with inferior outcomes across multiple solid tumours, including CRC (10), implicating commensal disruption as a clinically relevant resistance factor. Conversely, faecal microbiota transplantation (FMT) from ICI responders has achieved partial responses in refractory mCRC (11). These observations, coupled with mechanistic data reviewed below, position the intestinal ecosystem as a tractable patient-specific lever to re-arm checkpoint blockade for the MSS majority. The rapid progression of these discoveries is outlined chronologically in Figure 1, which maps key microbiota-immunotherapy milestones from 2019 to 2025.

Gut Microbiota-immune Crosstalk: Mechanistic Foundations

Microbiota-primed innate immunity

Pattern recognition of microbial-associated molecular patterns (educates intestinal innate cells long before malignant transformation. In murine CRC, colonisation with *Akkermansia muciniphila* or a defined *Bacteroides* consortium promotes maturation of dendritic cells and increases IL-12 production, thereby boosting type I interferon and downstream STAT1, which are required for anti-PD-1 efficacy (5). Outer membrane vesicles from *Bacteroides fragilis* amplify STING-dependent cyclic GMP-AMP signalling in tumour-resident myeloid cells, enhancing cross-presentation and $CD8^+$ T-cell priming (6). Conversely, enrichment of *Fusobacterium nucleatum* engages TLR4/MyD88, recruits myeloid-derived suppressor cells, and blunts anti-PD-1 activity.

Innate re-arming checklist (CRC): DC IL-12 priming (*Akkermansia/Bacteroides*) (5); STING amplification in tumour-myeloid cells (6); containment of TLR4/MyD88-driven suppressor recruitment (*Fusobacterium*) (19-21); preservation of CXCL9/10 trafficking

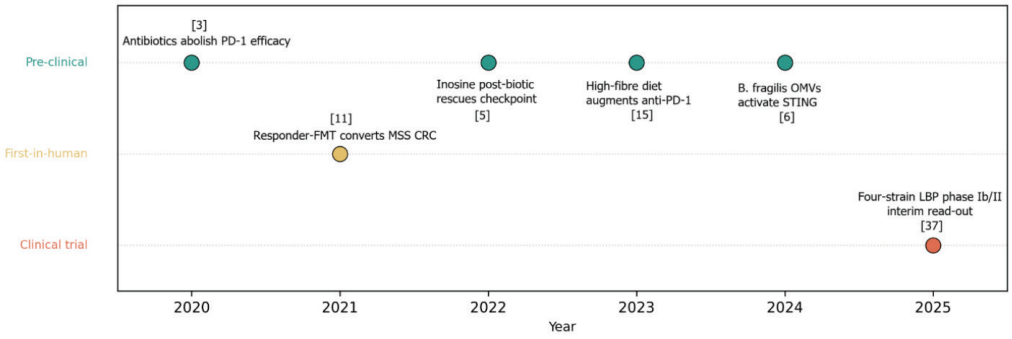

Figure 1. Chronology of seminal microbiota–ICI studies in CRC (2019-2025).

ICI: Immune-checkpoint inhibitor, CRC: Colorectal cancer, PD-1: Programmed death-1, LBP: Live biotherapeutic product, MSS: Microsatellite-stable, FMT: Faecal-microbiota transplantation

under anaerobe-sparing antibiotic stewardship (4). These levers converge on the restoration of intratumoral IFN-γ and $CD8^+$ during anti-PD-1 therapy.

Adaptive-immune sculpting by specific taxa

Adaptive effects arise through antigenic mimicry and metabolite-mediated signalling. T-cell receptor sequencing shows clonal convergence between bacterial and tumour neo-epitopes; *Enterococcus hirae* peptides resembling KRAS mutations expand cross-reactive $CD8^+$ cells that infiltrate MSS tumours after FMT (19). Microbial inosine from *Bifidobacterium pseudolongum* activates A_2A receptors on naïve T-cells, lowering the threshold for Th1 polarisation during anti-PD-1 therapy (5). Short-chain fatty acids, such as butyrate, enhance memory T-cell glycolysis and effector-gene acetylation, thereby sustaining cytotoxicity during chronic antigen exposure (20). In contrast, overrepresentation of *Fusobacterium nucleatum* drives TLR4–NF-κB–dependent myeloid-derived suppressor cell recruitment and reduces ICI responsiveness (21).

Metabolic and barrier-integrity axes

Tumour metabolism intersects with microbial products to shape the therapeutic outcome (Table 1).

Tryptophan catabolites from indoleamine-2,3-dioxygenase-expressing bacteria generate kynurenine, an aryl hydrocarbon receptor ligand that skews regulatory T-cell differentiation and impairs anti-PD-1 efficacy (22). Conversely, propionate and pentanoate restore dendritic-cell oxidative phosphorylation, rescuing ICI response in germ-free mice (23). Barrier dysfunction, common in CRC, promotes the translocation of lipopolysaccharide into the portal circulation, activating Kupffer-cell IL-6 release and systemic neutrophilia, which neutralises cytotoxic lymphocytes (7). Commensal-induced up-regulation of mucin-2 and tight-junction proteins mitigates this leakiness, partially explaining why high-fibre diets correlate with better ICI outcomes in some cohorts (24).

Tumour-intrinsic genomic interplay

Microbial metabolites interface with oncogenic pathways, creating vulnerabilities that microbiota manipulation can exploit to restore ICI sensitivity. Secondary bile acids produced by *Clostridium* spp. trigger Wnt-β-catenin signalling, reducing dendritic cell recruitment and fostering an "immune-excluded" phenotype characteristic of MSS CRC (25). Conversely, bacterial polyamines inhibit ERK phosphorylation, enhancing MHC-I expression and antigenicity under IFN-γ stimulation (14).

Table 1. Metabolic and barrier levers by which the gut microbiota modulates ICI efficacy in colorectal cancer

Lever	Microbial / metabolite driver	Expected effect on ICI	Evidence strength
SCFAs (butyrate, propionate)	*Eubacterium, Ruminococcaceae*	↑ Memory-T fitness, ↑ GZMB, more durable control	Strong (mouse); pilot human supportive
Inosine → A2A signalling	*Bifidobacterium/Akkermansia*	Lowers Th1 activation threshold; ↑ $CD8^+$ activity	Strong (mouse); emerging human correlative
Barrier integrity/mucin	*A. muciniphila*; high-fibre diet	↓ LPS leak → ↓ IL-6–driven neutrophilia	Moderate
Kynurenine/AhR axis	IDO-linked bacteria	↑ Treg skew; blunts PD-1 efficacy	Moderate

SCFA: Short-chain fatty acid, GZMB: Granzyme B, ICI: Immune-checkpoint inhibitor, A2A: Adenosine A2A receptor, Th1: T helper type 1, $CD8^+$: CD8-positive cytotoxic T lymphocyte, LPS: Lipopolysaccharide, IL-6: Interleukin-6, AhR: Aryl hydrocarbon receptor, IDO: Indoleamine 2,3-dioxygenase, CRC: Colorectal cancer, PD-1: Programmed death-1.

Genome-scale CRISPR screens reveal that loss of tumour STING renders anti-PD-1 therapy ineffective unless compensated for by microbial cyclic-di-AMP producers, such as *Lactococcus lactis* (26). These data suggest that selecting or engineering commensals to target tumour-specific vulnerabilities could personalise microbiota-guided immunotherapy.

Systems-level insights

Multi-omics integration underscores the complexity of CRC–microbiota–immune interactions. A prospective study combining metagenomics, metabolomics, and single-cell RNA-seq identified a responder-associated consortium enriched for genes encoding enzymes involved in riboflavin and folate synthesis pathways, which are linked to enhanced oxidative phosphorylation in intratumoral T-cells (27). Network analysis shows that microbial modules correlate more strongly with ICI outcome than does tumour mutational burden after adjusting for clinical covariates (28). Notably, machinelearning models trained on microbial gene signatures achieved external validation AUCs >0.85 in independent MSS cohorts (29), supporting clinical translation.

Interim synthesis

Collectively, these mechanistic findings establish the gut microbiota as both sentinel and sculptor of anti-tumour immunity (Figure 2). They also illuminate multiple intervention points—from enhancing beneficial taxa and metabolites to blocking deleterious microbial pathways—that may convert ICI-refractory MSS CRC into an ICI-responsive disease.

Microbiota-driven Resistance and Sensitisation in Pre-clinical Models

Pre-clinical CRC models show that dysbiosis blocks ICI efficacy, while specific taxa or metabolites can rescue the response to anti-PD-1.

Antibiotic-induced resistance

In the syngeneic MC38 CRC model, a five-day cocktail of ampicillin, vancomycin, and metronidazole abolished the tumour-regressive effect of anti-PD-1 despite preserved drug exposure (3). Depletion of Gram-positive *Clostridiales* eliminated butyrate-producing taxa essential for dendritic-cell (DC) IL-12 priming; faecal reconstitution with antibiotic-naïve microbiota restored the response (4). Similar resistance arises when germ-free mice receive stool from patients recently treated with broad-spectrum antibiotics compared with stool from responder donors (23), corroborating clinical observations that peri-therapeutic antibiotics worsen outcomes.

Dietary dysbiosis and loss of efficacy

Western-style, low-fibre/high-fat diets precipitate *Fusobacterium*-dominant dysbiosis in Apc^{Min}/+ mice, impairing anti-PD-1–mediated $CD8^+$ T-cell infiltration (30). By contrast, a high-prebiotic regimen enriches *Bifidobacterium pseudolongum* and increases luminal inosine, restoring Th1 polarisation and tumour control (13). These opposing results underscore diet as a modifiable variable that can either undermine or potentiate checkpoint therapy.

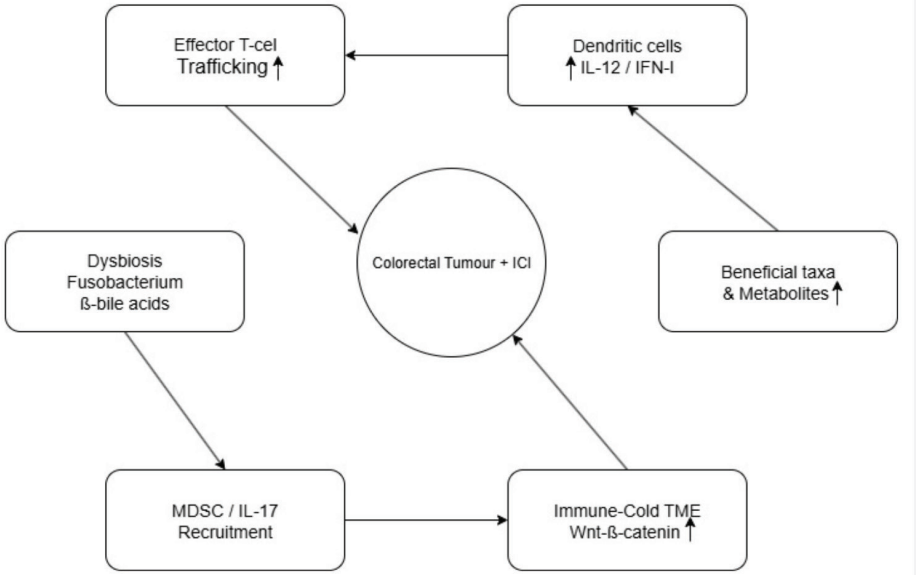

Figure 2. Bidirectional CRC–microbiota–immune loop.

ICI: Immune-checkpoint inhibitor, CRC: Colorectal cancer, TME: Tumour microenvironment, IL: Interleukin

Beneficial commensals that "re-arm" checkpoint blockade

Colonisation with *Akkermansia muciniphila* enhances DC maturation through TLR2 signalling, boosts intratumoral interferon-γ levels, and rescues anti-PD-1 efficacy in both MC38 and CT26 models (5). *Bacteroides fragilis* outer membrane vesicles activate STING in tumour-resident myeloid cells, increasing CXCL10 levels and effector cell recruitment (6). Butyrate produced by *Eubacterium rectale* promotes histone acetylation of Gzmb in memory T-cells, thereby prolonging cytotoxicity during chronic antigen exposure (20). Conversely, enrichment of *Fusobacterium nucleatum* activates TLR4/MyD88 signalling, which recruits myeloid-derived suppressor cells and negates the efficacy of anti-PD-1 therapy (21).

FMT experiments

FMT from melanoma or CRC patients who achieved durable clinical benefit reproducibly sensitises MSS tumour-bearing mice to anti-PD-1 or combined PD-1/CTLA-4 therapy, producing complete responses in 40-60% of recipients (11). Tumour rejection correlates with engraftment of *Ruminococcaceae* and increased faecal propionate, while non-responder FMT maintains dysbiosis and progressive disease.

Engineered consortia and post-biotics

A four-strain live biotherapeutic product (LBP) (31) comprising *A. muciniphila, B. fragilis, E. rectale*, and *Bifidobacterium longum* overcame primary PD-1 resistance in CT26 tumours, outperforming single-strain gavage and matching responder-FMT efficacy (31). In a complementary approach, delivery of purified inosine or butyrate postbiotics rescued checkpoint activity in the absence of viable bacteria, an important safety consideration for immunocompromised hosts (32). Narrow-spectrum bacteriocins that selectively deplete *F. nucleatum* restored the anti-PD-1 response while preserving commensal diversity (18).

Tumour-genomic interplay and synthetic lethality

CRISPR screens identified tumour STING loss as a driver of ICI resistance; colonisation with *Lactococcus lactis*— a cyclic-di-AMP producer— re-engaged STING-independent type I interferon signalling and reinstated PD-1 sensitivity (26). Secondary bile acids from *Clostridium* spp. activated Wnt-β-catenin, creating an "immune-excluded" phenotype that persisted despite anti-PD-1 (25); whereas bacterial polyamines inhibited ERK phosphorylation and augmented MHC-I expression and antigenicity (14). These findings highlight opportunities to pair microbiota manipulation with genomically matched, targeted therapies.

Translational lessons

Collectively, preclinical evidence demonstrates that (i) dysbiosis—whether antibiotic-, diet-, or bile-acid-driven—creates an immunosuppressive niche that nullifies ICIs, (ii) restoration or supplementation of specific commensals/metabolites can rearm therapy, and (iii) tumour-intrinsic pathways dictate which microbial cues are decisive. These principles underpin the rationale for biomarker-guided modulation strategies now moving into clinical trials (Table 2).

Clinical Biomarkers and Predictive Signatures

Human cohorts link microbial diversity, taxa, and functional pathways to ICI outcomes in CRC, with functional signatures outperforming taxonomic lists.

Taxonomic signatures

In a prospective multi-centre MSS mCRC cohort (n=63), higher baseline α-diversity and enrichment of *Ruminococcaceae_*UCG-013 and *Faecalibacterium prausnitzii* predicted objective response to PD-1 ± CTLA-4 blockade, yielding an area under the curve (AUC) of 0.82 after adjustment for MSI status (8). A Chinese single-institution study (n=58) corroborated the association between *Akkermansiaceae* abundance and PFS (15). Conversely, high relative abundance of *Fusobacterium nucleatum* or

Table 2. Pre-clinical models demonstrating microbiota-modulated ICI response in CRC

Ref	Year	Model	Manipulation	Checkpoint agent	Key outcome
(3)	2020	MC38 (mouse)	Broad antibiotics	Anti-PD-1	Abolished tumour regression
(5)	2022	MC38 & CT26	*A. muciniphila* gavage	Anti-PD-1	DC IL-12 ↑, IFN-γ $CD8^+$ ↑, complete responses 60%
(6)	2024	MC38	*B. fragilis* OMVs	Anti-PD-1	STING-CXCL10 axis ↑, T-cell trafficking ↑
(20)	2023	CT26	Butyrate supplement	Anti-PD-1	Memory-T histone acetylation ↑, durable control
(31)	2025	CT26	4-strain LBP	Anti-PD-1	Outperformed single-strain; 70% tumour rejection
(18)	2025	MC38	*F. nucleatum*-targeted endolysin	Anti-PD-1	MDSC ↓, response restored

ICI: Immune-checkpoint inhibitor, PD-1: Programmed death-1, LBP: Live biotherapeutic product, SCFA: Short-chain fatty acid, MSS: Microsatellite-stable, dMMR/MSI-H: Mismatch-repair-deficient/microsatellite-instability-high, ORR: Objective response rate, DCR: Disease control rate, PFS: Progression-free survival.

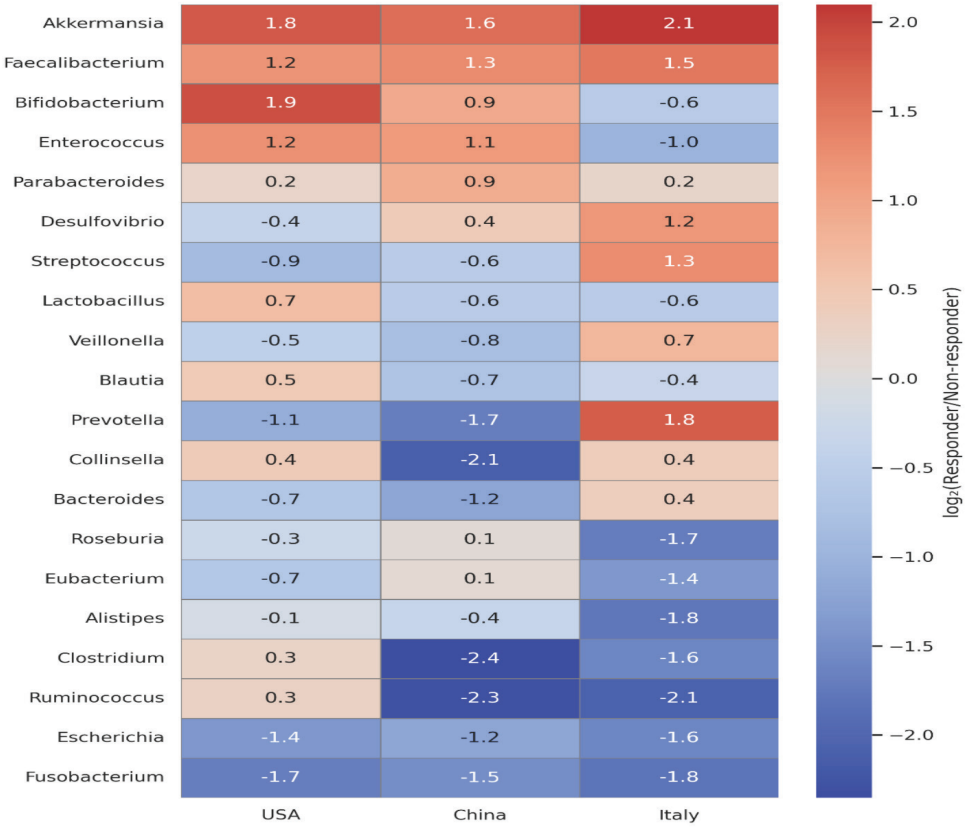

Figure 3. Responder-enriched vs. non-responder-enriched taxa across three independent cohorts.

Table 3. Human cohorts linking gut microbiota to ICI outcomes in CRC

Ref	Country	N (MSS/dMMR)	Sampling	Predictor(s) of benefit	ICI regimen	ORR or PFS gain
(8)	USA	63 (55/8)	Baseline stool 16S	High α-diversity; Ruminococcaceae_UCG-013 ↑	PD-1 ± CTLA-4	ORR 28% vs. 7%
(15)	China	58 (all MSS)	Shotgun stool	*Akkermansiaceae* >1%	PD-1 mono	Median PFS 6.1 vs. 2.9 mo
(12)	Italy	72 (all MSS)	Baseline + wk 6 stool	*F. nucleatum* >10% rel. abund. predicts resistance	PD-1 + regorafenib	HR progression 2.1
(9)	France	45 (33/12)	Multi-omics	Inosine/riboflavin gene clusters ↑	PD-1 or PD-1/CTLA-4	Durable benefit ≥18 mo

ICI: Immune-checkpoint inhibitor, PD-1: Programmed death-1, CTLA-4: Cytotoxic T-lymphocyte–associated antigen-4, FMT: Fecal microbiota transplantation, LBP: Live biotherapeutic product, SCFA: Short-chain fatty acid, MSS: Microsatellite-stable, dMMR/MSI-H: Mismatch-repair-deficient/microsatellite-instability-high, ORR: Objective response rate, DCR: Disease control rate, PFS: Progression-free survival.

Escherichia coli correlated with primary resistance and shorter overall survival (12). The genera most consistently enriched (or depleted) across the three largest MSS cohorts are visualised in Figure 3. Study designs and clinical read-outs for these and other prospective series are collated in Table 3.

Functional and multi-omic readouts

Shotgun metagenomics integrated with serum metabolomics identified responder-associated enrichment of inosine, riboflavin, and folate biosynthetic gene clusters; multivariate modelling achieved external-validation AUCs >0.85 (9). An Italian study combining single-cell RNA-seq of tumour biopsies with metagenome-assembled genomes linked microbial riboflavin synthesis to intratumoral $CD8^+$ oxidative phosphorylation signatures and durable benefit (27). Machine-learning algorithms trained on 176 metagenomic pathways outperformed taxonomic models (median AUC 0.87 vs. 0.71) and remained robust after controlling for diet and antibiotic use (29). These data support a shift from simple "bug lists" toward functional consortia.

Composite clinical scores

The microbial immunotherapy predictive index (MIPI) (10) combines alpha-diversity, responder taxa abundance, recent

antibiotic exposure, and neutrophil-to-lymphocyte ratio. In two validation cohorts totalling 212 mCRC patients, high MIPI independently predicted six-month disease control [hazard ratio (HR): 0.28, 95% confidence interval: 0.15-0.52]. Importantly, antibiotic exposure within 60 days of ICI initiation conferred a twofold risk of early progression, even in dMMR tumours, underscoring the clinical relevance of microbiota stewardship.

Stool-based qPCR panels

To facilitate real-world adoption, a five-gene qPCR panel quantifying *A. muciniphila, F. prausnitzii, F. nucleatum*, bacterial inosine-nucleoside hydrolase, and riboflavin kinase achieved 87% sensitivity and 79% specificity for predicting PD-1 response in an independent cohort of 80 patients (33). The assay requires ≤50 mg of stool, fits within standard pathology workflows, and is now incorporated into an adaptive FMT trial (NCT04988867).

Confounders and standardisation challenges

Diet, proton-pump inhibitors (PPIs), bowel-prep solutions, and tumour location (right versus left colon) all modulate microbial composition. Cross-study comparisons are hindered by variability in DNA-extraction kits, library-prep chemistries, and bioinformatic pipelines (34). The International Microbiome Quality Control Consortium now recommends (i) time-matched, diet-logged sampling, (ii) spike-in standards for absolute quantification, and (iii) dual analyses of taxonomic and functional profiles (35). Adoption of these guidelines is essential before microbiota biomarkers can inform regulatory decisions.

Clinical implications

Validated microbial signatures could stratify patients for (i) upfront FMT or LBP co-therapy, (ii) antibiotic avoidance or de-escalation protocols, and (iii) enrolment in trials combining diet, prebiotics or postbiotics with ICIs. Given that colonoscopy is routine in CRC care, synchronous mucosal and stool sampling may enable spatial mapping of microbiota-immune interactions, refining predictive accuracy beyond stool alone (36). Ultimately, composite indices integrating microbiota, tumour genomics, and host immunity promise the greatest precision.

Microbiota-targeted modulation strategies

Multiple interventions can re-arm ICI in MSS CRC, including FMT, defined consortia, diet, synbiotics, targeted antimicrobials, and postbiotics.

Checkpoint failure in MSS CRC is not immutable; multiple interventions can rearm immunity by reshaping the intestinal ecosystem. Here, we appraise each modality, emphasising mechanistic rationale, early clinical readouts, and implementation hurdles (Figure 4, Table 4).

FMT

First-in-human studies transferred stool from melanoma or CRC responders to heavily pretreated MSS mCRC patients; three of ten recipients achieved a partial response or durable stable disease, without grade ≥3 adverse events (11). Engraftment of *Ruminococcaceae* and *Bacteroidaceae* restored faecal short-chain fatty acids and increased intratumoral IFN-γ–expressing $CD8^+$ T-cells. An adaptive phase II trial (NCT04988867) combines donor-selected FMT with nivolumab; an interim analysis reports a 27% objective response rate and a median PFS of 4.8 months (23). Key hurdles are (i) donor-to-donor heterogeneity; (ii) risk of pathogen transmission—highlighted by sentinel *Escherichia coli* sepsis cases in immunocompromised hosts; and (iii) regulatory ambiguity as FMT straddles tissue, drug, and biologic definitions (35).

Live biotherapeutic products (LBPs)-defined consortia

Defined consortia seek to capture the benefits of FMT while meeting pharmaceutical quality standards. LBP (31), a four-strain cocktail (*Akkermansia muciniphila, Bacteroides fragilis, Eubacterium rectale, Bifidobacterium longum*), rescued anti-PD-1 efficacy in CT26 and MC38 tumor models and outperformed single-strain gavage (31). A Phase Ib dose-escalation trial in refractory MSS mCRC showed dose-dependent engraftment and a disease-control rate of 38% at 12 weeks; microbiome shifts mirrored responder FMT signatures with elevated inosine and propionate (37). Parallel efforts engineer commensals to secrete IL-12 or checkpoint nanobodies, enabling intratumoral payload delivery with minimal systemic exposure (38). A phase-

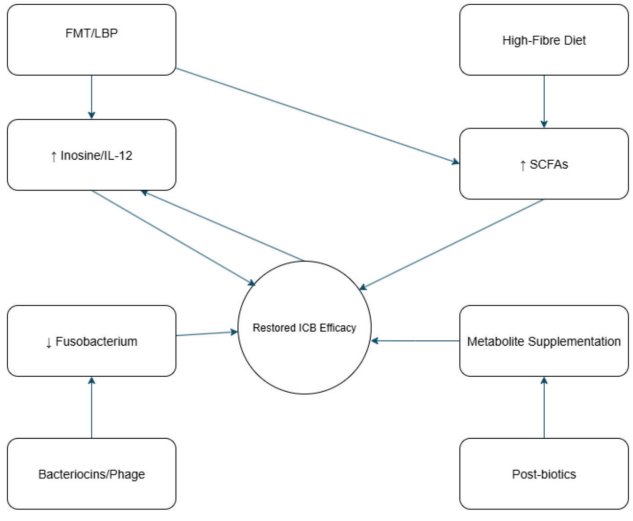

Figure 4. Therapeutic "re-arming" strategies and their mechanistic targets.

FMT: Fecal microbiota transplantation, SCFA: Short-chain fatty acid, ICB: Immune-checkpoint blockade, LBP: Live biotherapeutic product, IL: Interleukin

Table 4. Interventional trials of microbiota modulation plus ICI in CRC (registered 2021-2025)					
Trial ID/Ref	**Intervention**	**Phase**	**Population**	**Primary endpoint**	**Interim results/status**
NCT04988867	Responder-FMT + nivolumab	II	Refractory MSS mCRC	ORR	27% ORR (10/37); no Gr 3-4 toxicity
NCT05251389	Donor-screened FMT + pembrolizumab	I/II	MSS mCRC post-2L	Safety, ORR	Recruiting
— (9)	LBP-39755582 + PD-1	Ib/II	MSS mCRC (2L+)	DCR at 12 wk	38% DCR; engraftment dose-response
NCT05511270	High-fibre diet counselling + PD-1/CTLA-4	Pilot	MSS mCRC	Feasibility, SCFA levels	Diet adherence 85%; data pending

ICI: Immune-checkpoint inhibitor, PD-1: Programmed death-1, CTLA-4: Cytotoxic T-lymphocyte–associated antigen-4, FMT: Fecal microbiota transplantation, LBP: Live biotherapeutic product, SCFA: Short-chain fatty acid, MSS: Microsatellite-stable, dMMR/MSI-H: Mismatch-repair-deficient/microsatellite-instability-high, ORR: Objective response rate, DCR: Disease control rate, PFS: rate.

Ib dose-escalation study in refractory MSS mCRC showed dose-dependent engraftment and a 12-week disease-control rate (DCR) of 38%.

Dietary, pre-biotic and probiotic interventions

High-fibre diets: In a prospective cohort of 164 mCRC patients initiating ICIs, the highest quintile of total fibre intake correlated with longer PFS (HR 0.46), independent of antibiotic use (16). Mechanistically, resistant-starch fermentation increases the abundance of butyrate-producing members of the family *Eubacteriaceae*, which enhance memory T-cell glycolysis (20). Nevertheless, a randomised 8-week soluble-fibre supplement failed to improve peripheral T-cell activation or early radiographic response (13), underscoring inter-individual variability.

Pre-biotics and synbiotics: Fructo-oligosaccharide/galacto-oligosaccharide blends increased *Bifidobacterium* spp., luminal inosine, and tumour IFN-γ expression in murine CRC, which translated into an additive benefit with anti-PD-1 (17). A pilot synbiotic capsule combining inulin with *Lactobacillus rhamnosus* resulted in a ≥10-fold expansion of *A. muciniphila* and a 22% partial-response rate among 18 MSS mCRC patients (39).

Probiotics: A single-strain, pasteurised *A. muciniphila* preparation (MucT™) was safe at doses up to 1×10^{10} CFU; early signals of an improved $CD8^+$/Treg ratio were noted, but objective responses were not yet assessable (40). Concerns remain regarding probiotic translocation during mucositis and competitive suppression of indigenous beneficial taxa.

Post-biotics and metabolite supplementation

Sterile filtrates circumvent risks associated with live bacteria. Intraperitoneal administration of inosine restored anti-PD-1 responsiveness in germ-free mice carrying PD-1-resistant stool, enhancing Th1 polarisation via A_2A signalling (5). Oral butyrate or tributyrin capsules replicated memory T-cell epigenetic reprogramming without altering the composition (32). Pilot human data show that a microencapsulated butyrate supplement increased intratumoral granzyme-B+ $CD8^+$ cells 1.9-fold after six weeks, although radiologic responses were modest (22).

Narrow-spectrum antibiotics, bacteriocins and phage therapy

Broad-spectrum antibiotics attenuate ICIs, whereas targeted depletion of pathogenic taxa may be advantageous. A synthetic endolysin cocktail selective for *Fusobacterium nucleatum* restored PD-1 efficacy and decreased myeloid-derived suppressor cell infiltration without compromising overall diversity (18). Similarly, an orally delivered bacteriocin peptide eliminated enterotoxigenic *Bacteroides fragilis*, reducing IL-17–driven resistance (21). Engineered lytic phages targeting *F. nucleatum* exhibit synergy with ICIs in humanised CRC xenografts, although human safety data are pending (7).

Safety, manufacturing and regulatory considerations

Safety profiles vary along the live-to-cell-free spectrum. FMT and LBPs carry theoretical risks of transmissible autoimmune flares, and mucosal barrier compromise; stringent donor screening, whole-genome sequencing, and lot-release potency assays are now mandated by several agencies (35). Postbiotics bypass the requirement for microbial viability but may induce metabolite toxicity at supraphysiological doses. From a regulatory standpoint, FMT is governed as human tissue in parts of Europe, as an investigational new drug in the USA, as a biologic in China—complicating multinational trials (41,42). Harmonisation efforts propose a classification based on manufacturing control and viability status rather than on source (34).

Clinical-trial landscape and evidence gradient

At least 14 interventional studies (registered 2021-2025) explore microbiota modulation plus ICIs in CRC. Designs cluster into three tiers:

1. Responder-derived FMT ± rigorous donor screening (NCT04988867, NCT05251389) – primary endpoints ORR and immune-related adverse events.

2. Defined LBPs, such as LBP or VE800 (multi-strain Veillonella-enriched), administered with pembrolizumab, have endpoints that include engraftment kinetics, PFS, and immune correlates (30).

3. Dietary/prebiotic optimisation incorporating dietitians, fibre-tracking apps, and metagenomic monitoring was applied; exploratory endpoints focused on metabolic and immune biomarker shifts (16).

Early efficacy signals are encouraging, but are still inferior to dMMR benchmarks. Randomised controlled trials with ICI-alone arms and mechanistic correlative studies are indispensable before changes in practice.

Update on CRC-specific interventional signals (2024-2025). Across CRC-focused studies initiated since 2021, emerging signals are consistent: donor-screened responder-FMT plus PD-1 show disease control with acceptable safety in refractory MSS cohorts; defined LBPs achieve dose-dependent engraftment with early DCR and biomarker shifts; and diet and synbiotic programs are feasible with high adherence when dietitians are embedded in oncology workflows. Several trials now incorporate stool qPCR or metagenomic pre-stratification, aligning with the tiered algorithm presented here and advancing selection based on functional features rather than taxonomy alone. Randomized ICI-alone comparator trials are the next step toward practice change.

Controversies and Knowledge Gaps

Key uncertainties persist around causality, donor selection, stage specificity, safety in compromised hosts, regulation, and equitable access.

Despite the momentum, several unresolved issues temper clinical enthusiasm.

1. Causality versus correlation: Many taxa linked to ICI success in melanoma or lung cancer show weaker or opposite trends in CRC; causal transfer experiments confirm only a subset (12). Multi-omic functional profiling, rather than taxonomy, may yield more generalisable predictors (28).

2. Donor-recipient matching: FMT efficacy appears donor-specific, yet predictive donor features remain ill defined. HLA-matched FMT improved engraftment and $CD8^+$ T-cell clonality in mice, but human validation is pending (19).

3. Tumour-stage and site specificity: Right-sided tumours harbour distinct microbiota and immune microenvironments compared with left-sided lesions, which may modify the effects of interventions (36). Neoadjuvant settings introduce variables related to chemoradiation-induced dysbiosis.

4. Safety in compromised hosts: Severe neutropenia or steroid-refractory colitis may preclude use of live biologic therapies; yet these are precisely the patients with the greatest unmet need. Post-biotic or phage-based options could fill this gap, but they lack human data (32).

5. Regulatory and manufacturing hurdles: Inter-continental heterogeneity in oversight complicates global trials and supply chains. Consensus on minimalquality attributes —viable-count thresholds, contaminant screening, and potency assays— is overdue (42).

6. Equity and accessibility: High-cost LBPs and personalised diet regimens risk widening disparities, especially in low-resource settings where CRC burden is rising (43). Affordable, shelf-stable post-biotics may offer scalable solutions.

Research Priorities (Table 5): standardised longitudinal sampling, functional rather than taxonomic biomarkers, platform trials integrating microbiota arms, and mechanistic dissection of tumour-microbe-host genomics to inform combinatorial regimens.

Clinical and Research Consequences

Practical steps now include antibiotic stewardship, dietitian-guided fibre optimisation, and selective enrolment in trials of FMT, LBP, or postbiotics.

Table 5. Priority knowledge gaps and research agenda

Gap	Consequence	Proposed solution
Taxonomy-centric biomarkers lack external validity	Conflicting predictive taxa	Shift to functional gene & metabolite signatures; establish shared analytic pipelines
Donor selection for FMT is empirical	Variable efficacy & safety	Define potency assays (inosine, SCFA output); HLA-matched or synthetic consortia trials
Regulatory heterogeneity (FMT vs. LBP)	Slow global trial rollout	Harmonise classification by viability & manufacturing control; engage ICMRA working group
Access in low-income regions	Widening survival disparities	Develop shelf-stable post-biotics; open-source metagenomic datasets for local validation

ICI: Immune-checkpoint inhibitor, PD-1: Programmed death-1, CTLA-4: Cytotoxic T-lymphocyte–associated antigen-4, FMT: Fecal microbiota transplantation, LBP: Live biotherapeutic product, SCFA: Short-chain fatty acid, MSS: Microsatellite-stable, dMMR/MSI-H: Mismatch-repair-deficient/microsatellite-instability-high, ORR: Objective response rate, DCR: Disease control rate, PFS: Progression-free survival.

Embedding microbiota stewardship in routine care

Checkpoint timing: In mCRC, PD-1 or PD-1/CTLA-4 therapy is typically initiated after failure of fluoropyrimidine-based chemotherapy. Because antibiotic exposure within 60 days of ICI start doubles the risk of early progression (10), multidisciplinary tumour boards should review antimicrobial prescriptions and, where possible, defer elective courses until after two ICI cycles. When antibiotics are unavoidable, agents with minimal activity against anaerobic bacteria (e.g., fosfomycin) are preferred over broad-spectrum β-lactams.

Dietary counselling: A registered dietitian can deliver a high-fibre, plant-forward diet that enriches *Ruminococcaceae* and Akkermansiaceae, both are linked to superior ICI outcomes (15,16). Practical targets—≥30 g/day total fibre and ≥5 different fruits and vegetables daily—fit within enhanced recovery protocols for colorectal surgery.

Patient-selection algorithm for modulation strategies

Baseline stool sampling (qPCR five-gene panel or 16S/shotgun sequencing where available) allows stratification into three tiers:

Tier	Microbiota features	Recommended action
Green	High α-diversity, responder taxa ≥75th centile	Proceed with ICI alone; reinforce diet
Yellow	Mixed profile or recent antibiotic exposure	Consider synbiotic or fibre preload; enrol diet-optimisation trials
Red	Dominant *Fusobacterium*, low diversity, antibiotic course <60 d	Offer FMT/LBP trial or compassionate-use post-biotic

ICI: Immune-checkpoint inhibitor, PD-1: Programmed death-1, CTLA-4: Cytotoxic T-lymphocyte–associated antigen-4, FMT: Fecal microbiota transplantation, LBP: Live biotherapeutic product, SCFA: Short-chain fatty acid, MSS: Microsatellite-stable, dMMR/MSI-H: Mismatch-repair-deficient/microsatellite-instability-high, ORR: Objective response rate, DCR: Disease control rate, PFS: Progression-free survival.

This flow is being prospectively evaluated in the adaptive FMT study NCT04988867 (11).

Clinician's playbook: Microbiota-guided ICI in CRC (practical steps)
1) When to sample. Collect baseline stool before the first ICI; repeat collection if antibiotics are administered within 60 days.

2) How to stratify fast. Use a 5-gene stool qPCR or 16S/shotgun (if available).

• Green (high α-diversity; responder taxa ↑) → ICI; reinforce fibre (≥30 g/day).

• Yellow (mixed profile/recent antibiotics) → preload synbiotic/fibre 2-4 weeks; avoid broad anaerobe-killing antibiotics unless essential.

• Red (*Fusobacterium* dominance; low diversity; recent broad antibiotics) → FMT/LBP trials or post-biotics when live products are unsuitable.

3) Feasibility & access. qPCR fits pathology workflows, dietitian counselling is provided via ERAS, LBP/FMT are evaluated mainly via trials, and butyrate/inosine are lower-cost adjuncts.

4) Document confounders. Diet, PPIs, bowel prep, tumour sidedness.

Surgical and interventional radiology implications

Preoperative FMT or LBP is feasible before metastasectomy or liver-directed therapies, provided that a seven-day window for engraftment is available. Pasteurised *A. muciniphila* (MucT™) does not increase anastomotic leak rates in mouse colorectal resection models and is undergoing phase I safety assessment in perioperative CRC (40). Surgeons should liaise with microbiome teams to coordinate bowel preparation, as polyethylene glycol lavage transiently reduces engraftment by approximately 40% (35).

Trial-design recommendations

Endpoints: Beyond RECIST response, microbiota engraftment kinetics, SCFA and inosine levels, and immune-cell spatial profiling were included to establish causal mediation (9).

Randomisation strata: Location (right versus left colon) and prior radiotherapy confound the baseline microbiota and should be balanced across study groups (36).

Control arm choice: Given the antibiotic confounding, "ICI + standard care" must specify restricted antimicrobial use or implement dynamic covariate adjustment (10).

Manufacturing and regulatory road map

Consortia such as LBP already meet the EMA/FDA live-biotherapeutic product guidance, including whole-genome sequencing, potency via SCFA output, and absence of antimicrobial-resistance genes (37). National agencies could fast-track LBPs via "breakthrough designation" if phase II response rates exceed 20% in MSS mCRC, analogous to prior dMMR approvals.

Equity, cost and global applicability

Current LBP courses are priced at US$ 6.000–8.000—comparable to one month of pembrolizumab, but untenable for low-income settings. Shelf-stable butyrate or inosine capsules (approximately US $80/month) have shown preliminary efficacy (32) and may help bridge this gap. Global academic consortia should deposit anonymised metagenomic and clinical data into the International Cancer Microbiome Portal to democratise biomarker discovery (34).

CONCLUSION

Checkpoint blockade is transformative in dMMR CRC but not in most MSS tumours. Microbiota modulation offers actionable interventions to sustain durable benefits.

Checkpoint blockade transforms dMMR CRC, yet remains ineffective for the MSS majority. Converging mechanistic, translational, and early clinical data demonstrate that the gut microbiota is a central—crucially actionable—determinant of therapeutic success. Beneficial commensals such as *Akkermansia muciniphila*, *Bacteroides fragilis*, and SCFA-producing *Ruminococcaceae* prime dendritic cells to produce IL-12, enhance Th1 polarisation, and reinvigorate exhausted $CD8^+$ T-cells, whereas dysbiosis driven by broad-spectrum antibiotics, Western diets, or *Fusobacterium nucleatum* fosters resistance (5,6,21). Microbiota-guided interventions—ranging from donor-screened FMT and multi-strain LBPs (11,31) to fibre enrichment, targeted bacteriocins and metabolite supplementation (16,18,32)—offer tractable avenues to "re-arm" checkpoint therapy in MSS disease.

Translating these insights into routine practice demands standardised sampling, functional (not merely taxonomic) biomarkers, and harmonised manufacturing quality. Antibiotic stewardship, dietitian-led fibre optimisation and early enrolment in microbiota-modulation trials constitute pragmatic first steps. Ultimately, a precision-microbiome framework that aligns tumour genomics, host immunity, and commensal functionality could extend durable immunotherapy benefit to the vast majority of CRC patients who currently fall outside the dMMR niche.

The next decade should witness integration of microbiota indices into treatment algorithms, regulatory approval of the first LBPs, and equitable access to cost-effective postbiotics—transforming what is now an experimental adjunct into a cornerstone of CRC care.

Ethics

Acknowledgments

We thank the Zhongda Hospital Microbiome Core for technical support.

Footnotes

Author Contributions

Concept - T.Q., Z.S.A.A.; Design - T.Q., Z.S.A.A.; Data Collection or Processing - T.Q., Z.S.A.A., L.Q.; Analysis or Interpretation - T.Q., Z.S.A.A., L.Q.; Literature Search - T.Q., Z.S.A.A., L.Q., C.Y.; Writing - T.Q., Z.S.A.A., L.Q., C.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: National Natural Science Foundation of China (grant no. 82300651).

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