The effects of a modified Mediterranean diet on gut microbiota and clinical outcomes in patients with metastatic colorectal cancer undergoing first-line chemotherapy with either anti-EGFR or anti-VEGF agent: protocol for a randomized pilot study

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Abstract

Background: the gut microbiota is attracting increasing interest as an initiator of colorectal cancer. It has been observed that microbial imbalance in the gut and in cancer tissue is facilitated by a Western type diet, rich in meat, sugars, and refined grains, while a Mediterranean diet, rich in low saturated fat and fibers, promotes gut eubiosis, and results in reduced risk of developing colorectal cancer.

Objective: to analyze and compare the gut microbiota of patients with metastatic colorectal cancer undergoing first-line chemotherapy +/- a biological agent (anti-EGFR or anti-VEGF), and receiving either a free standard Western diet, or a modified Mediterranean diet.

Methods: this is a pilot non-drug, interventional prospective, randomized, controlled, single-center, open-label trial. Patients (n=40) will be randomized 1:1 to either a modified Mediterranean diet or a free Western type diet. Blood and fecal samples will be collected at baseline and control visits, for metagenomic and metabolomic analysis. The primary endpoint is the Firmicutes/Bacteroidetes ratio after completion of the third cycle of first-line chemotherapy (time T1). Secondary endpoints are: a) the percentage of patients experiencing gastrointestinal side effects at T1; b) the percentage of patients experiencing grade 3/4 gastrointestinal side effects at T1; c) changes in the Firmicutes/Bacteroidetes ratio, overall microbiome composition, and metabolome at T1, and after the sixth chemotherapy cycle (T2) versus baseline.

Results: this pilot trial has received ethics approval on July 24th 2024. By January 2025, 7 participants have been recruited. The

study will conclude with the visit at T2 for the last enrolled patient. Results are expected to be published in October 2028.

Conclusions: this study has the potential to provide critical insights into the role of diet in modifying the gut microbiota, diminishing chemotherapy-related side effects, and possibly enhancing the therapeutic efficacy in metastatic colorectal cancer. Additionally, data may pave the way for future research in immunotherapy, potentially influencing both clinical practice and public health strategies. Clinical Trial: Clinicaltrial.gov NCT06794931 (https://clinicaltrials.gov/search?term=NCT06794931)

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Original article

The effects of a modified Mediterranean diet on gut microbiota and clinical outcomes in patients with metastatic colorectal cancer undergoing first-line chemotherapy with either anti-EGFR or anti-VEGF agent: protocol for a randomized pilot study

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Abstract

Background: the gut microbiota is attracting increasing interest as an initiator of colorectal cancer. It has been observed that microbial imbalance in the gut and in cancer tissue is facilitated by a Western type diet, rich in meat, sugars, and refined grains, while a Mediterranean diet, rich in low saturated fat and fibers, promotes gut eubiosis, and results in reduced risk of developing colorectal cancer.

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Conclusions: this study has the potential to provide critical insights into the role of diet in modifying the gut microbiota, diminishing chemotherapy-related side effects, and possibly enhancing the therapeutic efficacy in metastatic colorectal cancer. Additionally, data may pave the way for future research in immunotherapy, potentially influencing both clinical practice and public health strategies.

Trial registration: Clinicaltrial.gov NCT06794931 (https://clinicaltrials.gov/search?term=NCT06794931)

Keywords: Colorectal carcinoma; Modified Mediterranean Diet; gut microbiota; metagenomics; metabolome; pilot study

Introduction

Colorectal carcinoma (CRC) is one of the most frequent and deadly malignant tumors, globally [1]. The overall incidence and mortality rates are increasing, probably due to population aging, and increasing incidence of CRC risk factors associated with socioeconomic development [1]. Additionally, a relevant rise in early-onset CRC diagnosis was observed in the last three decades [2].

Among the risk factors for CRC, some are unmodifiable, such as age, sex, inflammatory bowel disease, and genetic predisposition [3,4], while others are controllable, such as overweight or obese body weight, smoking, sedentary habits, and unhealthy diet [4,5]. Several of these determinants interact with the gut microbiota (GM), which represents the collection of microorganisms (*i.e.*, bacteria, viruses, fungi, and archaea) colonizing the gastrointestinal tract. Therefore, GM attracts increasing interest as an initiator of CRC [6].

The main representatives of the GM are the Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria phyla, with variable prevalence along the oxygen gradient in the lumen of the gastrointestinal tract [7]. These microorganisms, when in equilibrium (a condition mentioned as eubiosis), are beneficial to the host by many activities including fermenting complex carbohydrates to short chain fatty acids (SCFAs), that are used as energy source by gastrointestinal epithelial cells [8]. Additionally, the eubiotic GM contributes to the synthesis of vitamins [9], protection from pathogen colonization [10], and maintaining mucosal integrity [11]. When the microbial balance is perturbed by any cause (a condition referred to as dysbiosis), the gut barrier is disrupted, microbial invasiveness increases, and commensal bacteria may turn to pathogens, resulting in inflammation and activation of tumorigenic pathways [11].

Specific changes in the GM have been associated with steps in CRC progression, suggesting that dysbiosis may contribute to such disease states [12]. The lumen- or mucosa-associated GM in CRC patients has higher proportions of *Fusobacterium nucleatum*, *Bacteroides fragilis*, and *E. coli* species, compared to the GM of healthy individuals [13,14,15].

It is known that the GM has a role as a modulator of the efficacy and toxicity of conventional chemotherapeutic agents used for CRC treatment [16]; the presence of certain bacterial species can exacerbate chemotherapy-induced side effects and pharmacological therapy toxicity, but conversely, the microbiota can also enhance treatment efficacy [17].

The microorganisms act on tumor microenvironment through many mechanisms, including genotoxin and free radical production, modification of signaling pathways in host cells, immune modulation, interaction with drug metabolism, and synthesis of small molecules, lipids, proteins, sugars, terpenoids, and organic acids, which may contribute to the pathogenesis of cancer [12,18].

Not only the GM affects the metabolism in the gut, but also compounds and metabolites may influence the GM contributing to the development of a dysbiotic state. It has been repeatedly observed that the presence of *F. nucleatum* in the gut and in cancer tissue is facilitated by a Western type diet, rich in meat, sugars, and refined grains [14,19], generating intestinal microbial imbalance. On the contrary, the Mediterranean diet, rich in low saturated fat and fibers, promotes eubiosis, and results in reduced risk of developing CRC [20-23]. Epidemiological data show that a healthy diet and eubiosis contribute to reduced risk of CRC [23], while an unhealthy diet is associated with enhanced risk, recurrence, and mortality of CRC [21,23,24]. However, the exact mechanisms through which diet affects CRC are still incompletely understood.

Consuming fiber- and phytochemical-rich foods promotes an increase of beneficial bacterial populations capable of preventing mucus layer damage, reducing inflammation, and lowering CRC risk [14]. It has been observed that in *F. nucleatum*-positive patients, a Mediterranean-type diet, rich in fibers and whole-grains, referred to as a "prudent diet," can reduce CRC onset [14].

Overall, current evidence suggests that acting on GM composition by a dietary intervention may be a

suitable strategy to prevent CRC risk, and can improve outcomes of therapy in CRC patients. GM biodiversity can be improved by the introduction of microbiota-accessible carbohydrates (MACs), which are non-digestible by the host, but fermentable by gut bacteria [25]. MACs, classified as prebiotics, are selectively utilized by microorganisms as substrates to produce SCFAs, such as acetate, propionate, and butyrate [26,27]. Thus, they provide energy for colonocytes, regulate mucin (MUC2) expression, which is critical for intestinal barrier formation, and support immune function. Common prebiotics such as galacto-oligosaccharides and fructo-oligosaccharides, stimulate the growth of beneficial Bifidobacteria and Lactobacilli, modulate cytokine activity, and suppress pro-inflammatory markers, while promoting apoptosis, and protecting the mucosal barrier [28,29]. Additionally, postbiotics, metabolites produced during bacterial fermentation of non-digestible compounds, mediate the GM-host interaction and facilitate the synthesis and regulation of mucin [30].

Firmicutes are the main producers of butyrate, the preferred metabolic substrate for colon epithelial cells, while acetate and propionate, which contribute to barrier protection, are predominantly derived from Bacteroidetes [31]. In vitro, butyrate administration enhances mucin protein content, supporting probiotic adhesion and inhibiting *E. coli* pathogenicity [31]. Butyrate also regulates mucin expression and epithelial tight junction proteins, strengthening the intestinal barrier [32].

Another beneficial compound acquired through the diet is tryptophan, an essential amino acid that maintains mucosal homeostasis via bacterial metabolism. A protein-rich diet with tryptophan may enhance intestinal defenses [33].

Overall, nutritional interventions, including the use of prebiotics and probiotics, appear capable of modulating the GM, promoting eubiosis, stimulating the production of SCFAs, resulting in enhanced mucus layer, barrier function and prevention of chronic inflammation. Based on this evidence, a pilot study was designed to determine and compare the GM of patients with metastatic CRC undergoing first-line chemotherapy +/- a biological agent (anti-epidermal growth factor receptor [EGFR] or anti-vascular endothelial growth factor [VEGF]), and receiving, based on random allocation, either a free choice Standard Western Diet (SWD), or a Modified Mediterranean Diet (MMD).

Methods

Ethical Considerations

This pilot trial has received ethics approval from the Ethics Committee Lombardia 2, Milan, Italy (reference n. R1926/24 – L2-130). All participants will provide full written informed consent before enrollment in the study. The processing of personal data collected during the study complies with the General Data Protection Regulation (GDPR), according to D.Lgs. 196/2003, and any Italian laws governing personal data protection. The data of the enrolled participants will be kept strictly confidential and will be pseudo-anonymized. The trial will be conducted in conformity with the Declaration of Helsinki and Good Clinical Practice guidelines.

Study Design

This is a pilot non-drug interventional clinical study, involving biological sampling, designed as a

prospective, randomized, and controlled, single-center, open-label trial with a 1:1 allocation ratio (Clinicaltrial.gov NCT06794931; https://clinicaltrials.gov/search?term=NCT06794931). The randomization lists will follow a block design generated by dedicated software and maintained by an independent data manager. Stratification is based on gender. Recruitment of participants began in October 2024, and it is expected to be completed in October 2027.

The inclusion criteria are:

- Eastern Cooperative Oncology Group (ECOG) Performance Status (PS): 0 or 1;
- age at diagnosis = 18 years or older;
- histologically and radiologically confirmed diagnosis of metastatic CRC based on Response Evaluation Criteria in Solid Tumors 1.1 criteria;
- first-line chemotherapy with or without a biological agent (anti-EGFR or anti-VEGF); Malnutrition Universal Screening Tool (MUST) score: 0–1;
- written informed consent.

Participants will not be eligible if they meet any of the following criteria:

- age<18 years;
- previous oncological medical treatments;
- indication for parenteral and/or enteral nutrition;
- MUST Score: ≥2.

Intervention

Study arms: arm A: MMD; arm B: SWD chosen by the patient.

During a routine control visit, eligible patients are informed about the study and, upon signing the written informed consent, undergo nutritional screening, including:

- a detailed nutritional assessment using a dedicated chart,
- routine blood tests,
- assessment of serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19.9) levels,
- serum sampling for nutritional markers (C reactive protein [CRP], pre-albumin, albumin, interleukin [IL]-6, transferrin),
- assessment of lifestyle factors (i.e. sleep quality, smoking habits),
- recording of a 24-hour dietary recall (food diary of the past 24 hours),
- anthropometric measurements (body weight, height, body mass index, and bioimpedance analysis),
- calculation of caloric-protein needs,
- MUST score.

Finally, patients are provided with a container for collecting baseline fecal samples. After the nutritional assessment confirms eligibility, patients will attend baseline visit (time T0) to be randomized into study arms.

Follow-up visits will align with the patient's chemotherapy schedule. Visits will occur after the third chemotherapy cycle (approximately at day 45 – time T1), and the sixth chemotherapy cycle

(approximately at day 90 – time T2).

At each visit (T0, T1, T2), patients will return a fecal sample and receive a container for further sampling. Additionally, they will be administered the 30-item European Organisation for Research and Treatment of Cancer quality of life questionnaire (EORTC QLQ-C30). At T1 and T2, patients will undergo:

- nutritional assessment to evaluate compliance with the assigned diet,
- routine blood tests,
- assessment of CEA and CA19.9,
- serum analysis for nutritional markers (CRP, pre-albumin, albumin, IL-6, transferrin),
- clinical examinations as required by the oncological treatment plan,
- a modified adherence questionnaire derived from the Mediterranean Diet Quality Index for Children and Adolescents (KIDMED) test to assess dietary compliance [34].

At T2, a radiological evaluation as per routine clinical practice is also planned.

The KIDMED test is designed to evaluate the adherence to the Mediterranean diet of children and young people [34]. As the diet included in this study introduces several changes to the Mediterranean diet and excludes dairy foods to limit the risk of diarrhea, the KIDMED test will be changed accordingly, based on previously published experience [35].

Frozen fecal samples will be sent to the Institute of Biomedical Technologies, National Research Council (ITB-CNR, Segrate, Milan, Italy) for metagenomic and metabolomic analysis.

Endpoints

The primary objective of this pilot study is to evaluate the impact of the MMD compared to the SWD, on GM biodiversity, in patients with metastatic CRC undergoing first-line chemotherapy +/- a biological agent (anti-EGFR or anti-VEGF).

To this aim, the primary endpoint is the Firmicutes/Bacteroidetes ratio after completion of the third cycle (T1) of first-line chemotherapy, which will be compared between the two randomized arms.

Secondary objectives are to assess the impact of the MMD versus the SWD on quality of life, and tolerability of chemotherapy.

To these goals, secondary endpoints, which will be compared between the two arms, are: a) the percentage of patients experiencing gastrointestinal side effects (diarrhea, nausea, and vomiting) at T1; b) the percentage of patients experiencing severe gastrointestinal side effects (Grade 3/4 diarrhea, nausea, or vomiting) at T1; c) changes in the Firmicutes/Bacteroidetes ratio, overall microbiome composition, and metabolome at T1 and T2 versus baseline.

Screening Methodology for Metagenomic and Metabolomic Analysis

DNA Extraction Techniques, 16S rRNA Gene Metabarcoding Library Preparation, and Ultra-Massive Sequencing

Fecal samples, collected from study patients, are stored at -80°C until DNA extraction, carried out by ITB-CNR. Automated bacterial DNA isolation will be performed by Maxwell® RSC Fecal Microbiome DNA Kit (Promega, Milan, Italy) and DNA quality will be assessed using the TapeStation 4200 system (Agilent Technologies, Santa Clara, CA, USA). Only samples with a DNA Integrity Number (DIN) ≥ 4 will proceed to further analyses. Samples with DIN < 4 at T1 will result in the patient's exclusion from the study, and previously collected data will not be used. Metabarcoding libraries will be prepared following the "16S Metagenomic Sequencing Library Preparation" protocol which includes amplification of the V3 and V4 hypervariable regions of the 16S rRNA gene and indexing with DNA/RNA UD Indexes (Illumina, San Diego, CA, USA).

Libraries will be quantified using the Qubit 4 fluorometer (Invitrogen, Waltham, MA, USA) and pooled at equimolar concentrations. Sequencing will be performed on an Illumina MiSeq or NextSeq1000 platform, by paired-end 2 x 300 bp runs.

Bioinformatics Analysis of Sequencing Data

Raw reads derived from sequencing will be processed using a custom bioinformatics pipeline. Overlapping paired reads will be assembled into a single fragment using PandaSeq software (publicly available) [36]. Taxonomic units will be generated using the zero-radius operational taxonomic unit (OTU) method and classification. Classification will employ the QIIME suite [37], unoise3 algorithm [38], RDP Classifier [39], and the SILVA 16S rRNA database [40].

Alpha-diversity will be calculated using metrics such as Chao1, Shannon Index, Observed Species, Good's Coverage, and Faith's Phylogenetic Distance. Beta-diversity will be assessed with weighted and unweighted UniFrac metrics, coupled with Principal Coordinate Analysis. Genus-specific reads will be further classified to species level via Basic Local Alignment Search Tool (BLAST) realignment against a custom database derived from NIH-NCBI sequences [41]. Metabolic pathways will be inferred using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) software and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database.

Shannon Diversity Calculation

The Shannon diversity index will be calculated from OTU tables containing absolute counts at the species level using the vegan R package (v2.5-6). Species with an average relative abundance of < 0.1% across all samples will be excluded. OTU counts will be normalized via rarefaction analysis before diversity calculations.

Firmicutes/Bacteroidetes Ratio Evaluation

The Firmicutes/Bacteroidetes ratio will be derived from OTU tables grouped at the phylum level. Phyla with an average relative abundance of < 0.1% across all samples will be excluded.

Metabolomic Analysis

Metabolites will be extracted by preparing aqueous fecal extracts by TissueLyser II homogenizer (Qiagen, Hilden, Germany). Samples will be subsequently derivatized with pentafluorobenzyl bromide in acetone, and SCFAs (acetate, propionate, butyrate) will be isolated into the organic phase using hexane (all chemicals sourced from Sigma-Aldrich, St. Louis, MO, USA). Samples will be analyzed by a 5977B single-quadrupole mass spectrometer coupled to an Intuvo 9000 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation will be conducted using a DB-FATWAX UI column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies), and detection will be achieved via single ion monitoring for enhanced sensitivity. Absolute quantification of SCFAs (acetate, propionate, butyrate) will be achieved using calibration curves from standard solutions. Obtained data will be analyzed using MassHunter Quantitative software (version 10.2, Agilent Technologies). Furthermore, metabolite concentrations will be correlated with bacterial composition by calculating Spearman's correlation coefficients between metabolites and bacterial genera present at ≥ 1% in at least one sample. Only correlations with $p < 0.05$ will be considered statistically significant.

Rationale for the MMD Plan for Arm A Patients

The diet assigned to each patient will be strictly personalized, considering sex, food preferences, anthropometric characteristics, underlying oncological condition, clinical characteristics (e.g., comorbidities), and pharmacological treatment plan.

Daily caloric needs will be calculated by multiplying the basal metabolic rate (determined using the Harris & Benedict formula) by 1.3, assuming moderate physical activity.

Sample size and Statistical Analysis

The enrollment of 16 patients per arm is required to show a relative reduction of 35% in the Firmicutes/Bacteroidetes ratio from 40% (±13 SD) in arm SWD (control arm) to 26% in Arm DMM at time T1, with a significance level of the Mann-Whitney test of 5% (two - sided) and a power of 80%. To consider the possibility of approximately 20% dropouts, 20 patients per arm will be randomized.
The primary comparison of the Firmicutes/Bacteroidetes ratio in Arm DMM vs SWD at T1, will be done with the Mann-Whitney test; an additional analysis adjusting for potential confounding variables (e.g., age, comorbidities, chemotherapy type) will be done using quantile regression with these factors as covariates.

For secondary endpoints involving the comparison of the percentage of patients with gastrointestinal effects between the two arms, the Chi-Square test will be applied.

To adjust for potential confounding factors, an additional analysis will be performed with a logistic regression model.

The analysis of secondary endpoints on changes in the Firmicutes/Bacteroidetes ratio at T1 and T2 versus baseline will be done with the Wilcoxon Signed-Ranks test.

The primary and secondary endpoint analyses will follow the Intention-to-Treat (ITT) principle. An additional sensitivity analysis will consider the per-protocol population, after excluding patients with

poor adherence to the MMD (KIDMED test score ≤ 3).

Subgroup analyses within different chemotherapy regimens will be performed for exploratory purposes only, due to the relatively limited number of patients, with the aim of having indications on the possible different efficacy and safety of the MMD vs SWD and of proposing additional in-depth studies.

The significance level for all tests is set at 5% (two-tailed).

Results

This pilot trial has received ethics approval from the Ethics Committee Lombardia 2, Milan, Italy (reference n. R1926/24 – L2-130), on July 24th 2025. Recruitment started in October 2024, and will be completed in 36 months. Data collection commenced in October 2024, with the enrollment of the first patient. By January 2025, 7 participants have been recruited. The study will conclude with the visit at T2 for the last enrolled patient. Results are expected to be published in January 2028.

Discussion

The primary aim of this pilot randomized controlled study is to assess the impact of a Mediterranean-type diet, predominantly plant-based, on the GM of patients with metastatic CRC undergoing first-line chemotherapy with or without anti-EGFR or anti-VEGF agents. To this purpose, our research project aims to compare the effects of the Mediterranean diet, characterized by the exclusion of sucralose, red meats, and processed meats (experimental arm), to those of a Western-type free diet (control arm). As far as we know, this is the first evaluation of the impact of the diet on the therapeutic outcomes which may also have implications in terms of treatment efficacy including overall response rate in patients with metastatic CRC undergoing chemotherapy.

The primary and secondary endpoints concern the evaluation of change in the biodiversity of the GM after the third chemotherapy cycle (approximately at day 45 - T1) and the sixth chemotherapy cycle (approximately at day 90 – T2).

For this purpose, metagenomic and metabolomic approaches will be employed: the identification of the metabolites produced by the GM in response to the Mediterranean diet will clarify how diet may influence both the GM composition and the therapeutic outcomes in oncology. Improvements associated with the MMD in chemotherapy-induced side effects, particularly gastrointestinal symptoms such as diarrhea, will also be evaluated.

Novel insights are expected in mechanisms and pathways linking the GM to CRC progression, and response to therapy. By investigating the impact of the Mediterranean diet on chemotherapy side effects and therapeutic outcomes, this study paves the way for future research on the relationship between diet-induced GM changes and immunotherapy responses, in patients with CRC, highlighting dietary interventions that could enhance adherence to treatment, and improve clinical outcomes. Specifically, the control of gastrointestinal toxicity by the targeted intervention might reduce the risk of treatment delay, interruption, or dose reduction. It is known that SCFAs, such as butyrate, produced by gut bacteria during the fermentation of dietary fibers, modulate immune responses and potentially overcome mechanisms of resistance to immunotherapy [42]. If a

Mediterranean diet will bring benefits in terms of response to immunotherapy, our data associated with variations in the GM composition and activity could provide information to develop dietary strategies aimed at improving immunotherapy outcomes in CRC patients, and will support public health actions for cancer and other advanced disease prevention programs. Special attention is devoted to planning a suitable diet to promote beneficial components of the GM, while reducing risk of therapy-associated-adverse events, and possibly facilitating the therapy antitumor activity.

To develop a nutritional plan favoring bacterial biodiversity, we are mainly focusing on foods containing high-quality prebiotic fibers, reducing the total amount of the fiber content compared to standard guidelines (LARN - Italian Dietary Reference Intakes) to limit the gastrointestinal side effects associated with chemotherapy. To this aim, foods containing resistant starch are preferred. Evidence indicates that such starch promotes changes in bacterial abundance, specifically increasing Firmicutes and particularly *Faecalibacterium prausnitzii*, and the production of butyrate, a SCFA essential for intestinal health [43,44].

Moreover, the dietary plan incorporates foods rich in polyphenols such as brown rice, extra virgin olive oil, and green tea, which have been shown to positively influence cellular aging, blood cholesterol levels, and apoptosis [45]**.** Polyphenols have been demonstrated to increase protective bacteria (Bifidobacteria and Lactobacilli) and butyrate-producing bacteria (*Faecalibacterium prausnitzii*). Furthermore, polyphenols are known to reduce pathogenic bacteria, including Proteobacteria like *Escherichia coli* [43,44].

Beta-glucans, indigestible polysaccharides found in barley, oats, and mushrooms, are included in the MMD since GM bacteria metabolize these compounds to produce SCFAs, which are crucial for the nutrition and integrity of intestinal mucosal cells.

Fermented products with low lactose content, such as kefir, are incorporated due to the proven effects on enhancing GM biodiversity, and on modulating immune function by supporting specific beneficial bacterial populations [46].

Highly antioxidant foods, such as fruits, vegetables, whole grains, and legumes, are useful to increase the abundance of *Akkermansia Muciniphila*, a key bacterium associated with maintaining a healthy intestinal barrier, and reducing inflammation mediated by lipopolysaccharides [47].

The plan also incorporates high-quality protein sources that also exhibit prebiotic properties, such as ricotta cheese (from cow, sheep, or goat milk, lactose-free if necessary). These proteins have been shown to support symbiotic bacteria and promote intestinal health [48].

The inclusion of salmon and other fish (e.g., mackerel, sardines) rich in omega-3 fatty acids aims to enhance the growth of butyrate-producing bacteria, and reduce inflammation, supporting the gut's overall health [49].

An important goal will be to raise awareness among patients about the importance of adhering strictly to the dietary plan as part of their therapy. To facilitate compliance, study participants are provided with flexible substitution options (e.g., replacing fish with white meat) for foods they find unpalatable, and adjustments designed to maintain the therapeutic rationale of the diet.

In conclusion, this study has the potential to provide critical insights into the role of diet in modifying the GM, diminishing chemotherapy-related side effects, and enhancing the therapeutic efficacy in metastatic CRC. Additionally, the findings may open new avenues for future research in

immunotherapy, potentially influencing both clinical practice and public health strategies.

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Author contribution

Salvatore Artale is the principal investigator, ideated the project, and designed the study protocol. Maria Grazia Vasecchi and Giulia Capitoli are the trial statisticians. Clarissa Consolandi and Cinzia Cocola are responsible for metagenomic and metabolomic analyses and elaborated the study protocol. Francesca Arosio is responsible for nutritional programs and developed the diet protocol with Sabrina Basciani. All the authors contributed to manuscript drafting and revision; the final version was read and approved by all authors.

Data Availability

The data that support the findings of this study will be available from the corresponding author upon reasonable request.

Conflicts of Interest

None declared

Abbreviations

CEA: carcinoembryonic antigen

CA19.9: carbohydrate antigen 19-9

CRC: colorectal carcinoma

CRP: C reactive protein

DI: DNA integrity number

EGFR: epidermal growth factor receptor

GM: gut microbiota

IL: interleukin

ITB-CNR: Institute of Biomedical Technologies, National Research Council

KIDMED: Mediterranean diet quality index for children and adolescents

MMD: modified Mediterranean diet

MUST: malnutrition universal screening tool

NGS: next-generation sequencing

OUT: operational taxonomic unit

SCFA: short chain fatty acids

VEGF: vascular endothelial growth factor

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