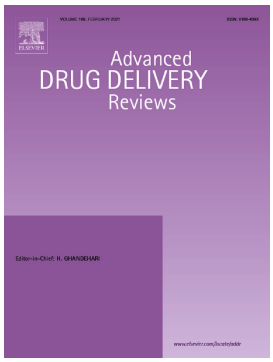
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Advanced Oral Therapies for Colorectal Cancer via Nanomedicine and Microbiota Modulation

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Abstract

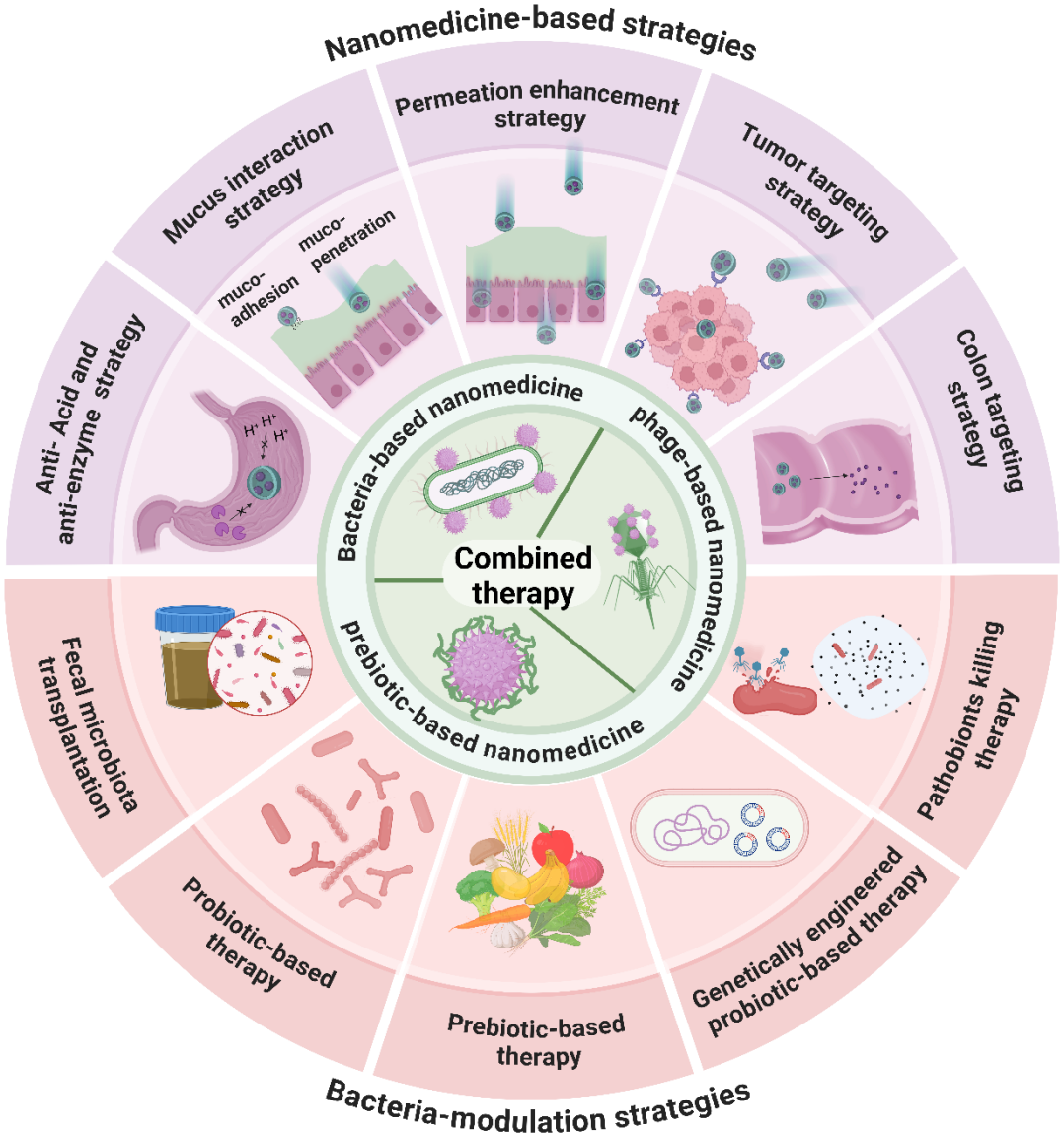
Colorectal cancer (CRC) is the third most common malignancy and the second most lethal cancer worldwide and has become a significant burden on global healthcare. The primary treatments for CRC include surgery, chemotherapy, and targeted therapy. However, these approaches face several limitations, such as invasive administration, poor patient compliance, systemic toxicity, drug resistance, and recurrence. Thus, alternative therapeutic strategies to overcome these challenges are urgently needed. Oral administration represents a promising approach for CRC treatment because of its noninvasive nature, safety, ease of self-administration, and high patient compliance. Moreover, when applied in CRC treatment, oral therapies can potentially increase therapeutic efficacy and reduce systemic toxicity via local treatment. Furthermore, the unique pathophysiological features of CRC, such as the overexpression of certain receptors and dysbiosis, present valuable opportunities for the development of advanced oral therapies. This review focuses on cutting-edge oral therapies for CRC, including nanomedicine-based and bacteria-modulating strategies. These approaches show significant promise for improving therapeutic outcomes and can be combined with existing treatment modalities to improve efficacy and minimize adverse effects. Additionally, we provide critical perspectives on the challenges and future perspectives of these strategies for CRC management. This review aims to provide novel insights into the design and advancement of oral therapies, paving the way for more effective and patient-friendly CRC treatments.

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Keywords

Colorectal cancer; Oral administration; Nanomedicine; Gut microbiota; Advanced therapy

Graphical abstract



Advanced oral therapies for CRC treatment

1. Introduction

According to the Global Cancer Statistics Report of 2022, colorectal cancer (CRC) ranks as the third most prevalent malignancy and the second leading contributor to cancer-related mortality worldwide [1-3]. By 2030, the global burden of CRC is projected to increase by 60%, with more than 2.2 million new cases and 1.1 million cancer-related deaths [4]. Current therapies for CRC include surgery, chemotherapy, radiotherapy, and targeted therapy [5, 6]. Other emerging strategies, such as phototherapy and gene therapy, have shown great potential in preclinical and early clinical stages [7-10]. While these therapeutic modalities have demonstrated efficacy, most are invasive, as most drugs for CRC treatment are administered through intravenous injections [6]. Such invasive administration routes result in multiple drawbacks, including (1) the need for professional administration; (2) pain, irritation, and bruising associated with injections, leading to low patient compliance; (3) side effects linked to intravenous injections, such as blood clots and infection; and (4) systemic drug exposure, resulting in off-target toxicity [11]. These limitations not only affect patient compliance and quality of life but also restrict the clinical potential of these therapeutic strategies. Furthermore, drug resistance and treatment insensitivity in CRC patients highlight the need for effective alternative therapeutics.

Oral drug delivery has emerged as a preferred route of administration because of its noninvasive nature, high patient compliance, and ease of use [12]. Furthermore, oral administration is particularly attractive for the local treatment of gastrointestinal (GI) tract diseases, such as CRC, as it can potentially increase local drug accumulation and minimize systemic exposure [13]. Additionally, the pathophysiological features of CRC provide unique opportunities for advanced oral therapies. An extended retention time in the colon allows prolonged drug absorption following oral administration [14]. Reduced proteolytic activity in the colon facilitates the delivery of peptides and proteins [14]. Increased intestinal permeability due to defects in the mucus layer and epithelial barrier promotes the accumulation of certain drugs at tumor sites [15]. Furthermore, CRC-associated genetic mutations and changes in the tumor microenvironment (TME) represent specific treatment targets. Together, these factors support the rationale and potential of oral therapies for CRC [16].

However, oral delivery of drugs remains a formidable challenge for many therapeutic agents, particularly hydrophobic small molecules and macromolecular biologics. This is primarily due to a series of physiological barriers within the GI tract [17-19], such as degradation by the harsh acidic environment and digestive enzymes, entrapment and cleavage by the thick and viscous mucus barrier, and insufficient permeability across the intestinal epithelium [20]. Advanced drug delivery systems, such as nanomedicine-based oral therapies, have shown great promise for oral delivery. Nanomedicines, characterized by their small size, large surface area, and versatile functionalization, enable fine-tuned biological interactions through physicochemical modulation or surface engineering [18]. Various nanomedicine-based oral therapies, such as lipid-based nanocarriers, polymer-based nanocarriers, and inorganic nanocarriers, have been developed for the oral delivery of small-molecule drugs and biomacromolecules for CRC treatment and have exhibited promising efficacy [21]. Furthermore, strategies such as acid and/or enzyme inhibition, colon and/or tumor targeting, mucus adhesion or penetration, and permeation enhancement have been employed to optimize nanomedicine-based oral delivery for CRC treatment [21].

The colon harbors an extensive bacterial population, estimated at $10^{11–12}$ colony-forming units per gram (CFU/g), accounting for more than 70% of all microbes found in the human body [22]. CRC exhibits a unique association with the gut microbiota and its metabolites, considering the close local interactions in the colon. Research conducted over the past decade has highlighted the dysbiosis that occurs during colorectal tumorigenesis, chemotherapy, and immunotherapy, providing a rationale for modifying the gut microbiota to treat CRC or enhancing the response to chemotherapy and immunotherapy [23]. Moreover, the oral administration of bacteria-based therapies offers advantages for localized treatment and colonization and has great potential for CRC therapy. Examples of promising bacterial modulation-based advanced oral therapeutics include fecal microbiota transplantation, probiotic and/or prebiotic delivery, genetically engineered probiotics, and pathobiont-killing therapies.

In this review, we first summarize the pathophysiological features of CRC and the potential therapeutic targets they present. We then outline the current standard of care, clinically established CRC therapies and emerging CRC therapies, which are administered intravenously. By emphasizing the distinct advantages of oral administration in CRC treatment, we review advanced oral therapies in the context of significant challenges within the GI tract and the TME for CRC,

with a focus on nanomedicine-based strategies, bacterial modulation-based therapies and their combinations, to provide an in-depth discussion of representative approaches in the literature. This review aims to provide novel insights into the development of advanced oral therapies for CRC, emphasizing enhanced local treatment, reduced systemic and injection-associated side effects, and improved patient compliance.

2. Pathophysiological features of CRC

The pathophysiological features of CRC not only play important roles in cancer pathogenesis and progression but also provide multiple opportunities for advanced oral therapies (Figure 1). CRC originates in the epithelial cells lining the colon or rectum through spontaneous mutations, exposure to environmental mutagens, or inflammation-induced (epi-)genetic changes [24, 25]. The mutation and clonal expansion of these cells (polyps) drive proliferation, leading to the formation and progression of malignant tumors. Further mutations and alterations in the TME enable metastasis [15]. Genetic and epigenetic alterations in CRC have been extensively studied [26, 27], providing potential biomarkers for predicting treatment outcomes and prognosis or identifying therapeutic targets. A recent study comprehensively analyzed the genomic landscape of 2023 individuals with CRC, offering a valuable reference for future investigations [16]. Representative mutations relevant to oral advanced therapies include the presentation of receptors (e.g., epidermal growth factor receptor, EGFR) for targeted drug delivery, increased accumulation of certain enzymes (e.g., matrix metalloproteinases) for enzyme-responsive drug release, and the expression of protumor molecules (e.g., vascular endothelial growth factor, VEGF) for targeted inhibition of tumor proliferation and metastasis [28-30].

Colon mucus is a dilute, aqueous, and viscoelastic secretion produced by goblet cells, forming two distinct layers, the inner and outer layers, with a total thickness of approximately 107–155 μm. The inner mucus layer is dense, tightly attached to the epithelium, and impenetrable to bacteria because of its small pore size (~0.5 μm) and antimicrobial peptides. In contrast, the outer mucus layer is unattached and loose, with larger pores that allow penetration by bacteria and particles up to 0.5 μm in diameter. These mucus layers serve as crucial barriers, protecting the underlying epithelium from the luminal contents through continuous renewal (~1–2 hours). In addition, high levels of sialic acid and sulfate impart an overall negative charge [31, 32]. In individuals with CRC, the

mucus barrier is often defective because of goblet cell loss [33, 34]. However, the specific changes in mucus renewal time, thickness, rigidity, and physicochemical properties remain unexplored. Increased epithelial permeability in CRC, caused by decreases in the numbers of enterocytes and tight junctions, further compromises mucosal barrier function. These defects facilitate the invasion of bacteria and pathogen-associated molecular patterns into the gut mucosa, leading to dysbiosis and promoting CRC development [33-35].

The colonic microbiota plays critical roles in maintaining homeostasis, regulating immune responses, and modulating treatment outcomes, particularly in CRC, because of its direct local interactions with tumor tissues. Microbiota dysbiosis and reduced bacterial diversity are hallmarks of CRC and worsen during chemotherapy and radiotherapy [23, 35]. CRC is associated with the enrichment of specific bacteria (e.g., *Bacteroides fragilis* and *Fusobacterium nucleatum*) [36, 37] and decreased abundance of other bacteria (e.g., *Faecalibacterium prausnitzii*, *Barnesiella intestinihominis*, *Alistipes finegoldii*, *Bacteroides eggerthii*, and *Eubacterium siraeum*) and their metabolites, such as short-chain fatty acids (SCFAs) [38-40]. Moreover, an association between the use of oral antibiotics, particularly those with antianaerobic activity, and an increased risk of colon cancer, especially in the proximal colon, has been reported [41]. These findings underscore the critical role of the microbiota in CRC progression.

Moreover, CRC progression is strongly influenced by the interactions of cancer cells with the TME. The features of the TME provide additional targets for advanced oral therapies: (1) an acidic microenvironment (pH ~6.5) resulting from the high glycolytic rate of tumor cells; (2) hypoxia due to rapid tumor growth depleting the oxygen supply [42]; (3) increased reactive oxygen species (ROS) generated by glycolysis; (4) abundant infiltration of stromal cells (e.g., fibroblasts) and extracellular matrix components; and (5) increased infiltration of immunosuppressive cells (e.g., tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs)) and molecules (e.g., hypoxia-inducible factor 1-alpha (HIF-1α)) [43, 44].

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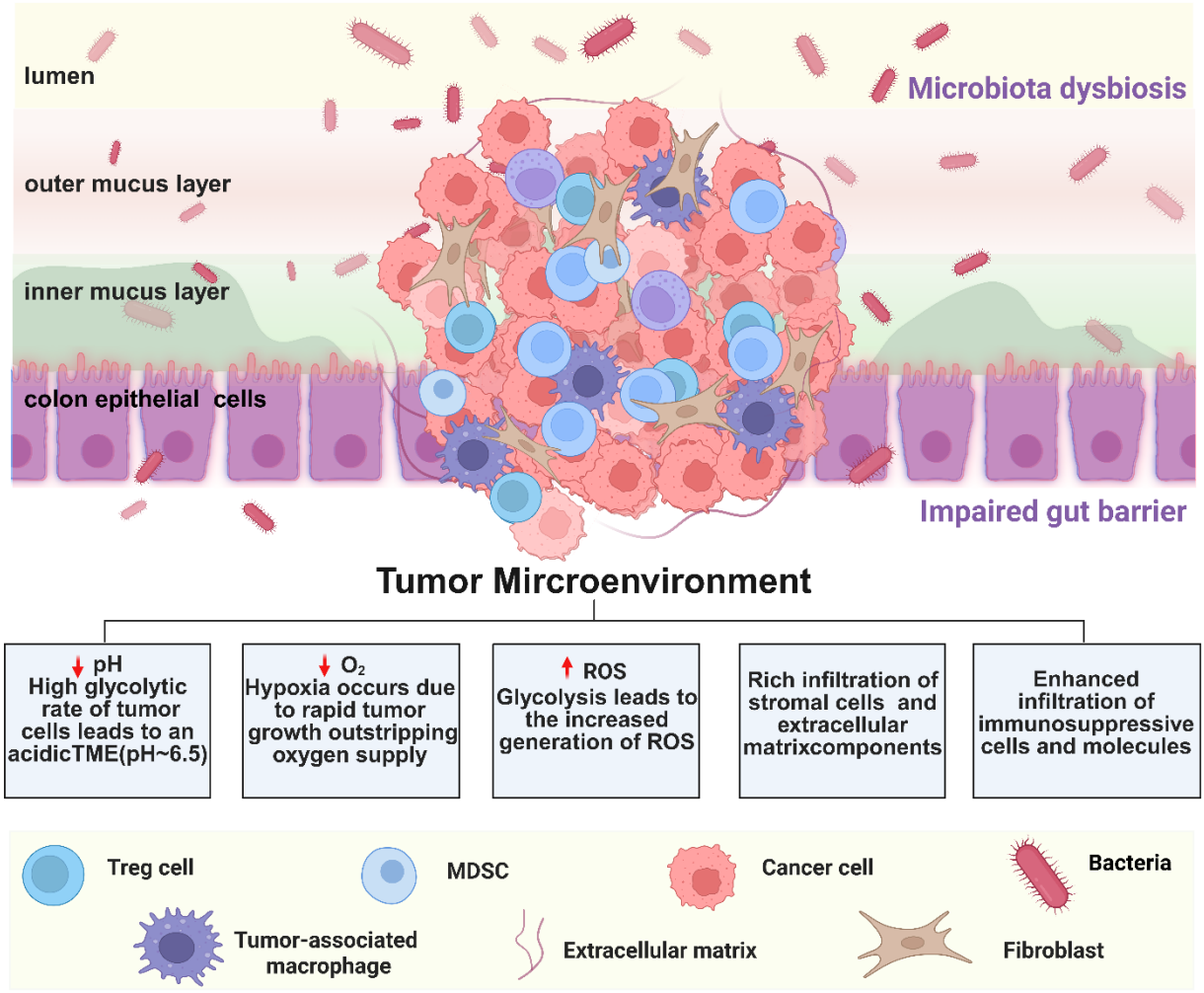


Figure 1. The key pathophysiological features of CRC-related genetic alterations include increased intestinal permeability, microbial dysbiosis, and remodeling of the TME. Created with BioRender.com.

3. Current CRC treatments and challenges

3.1 Standard of care

According to the European Society for Medical Oncology (ESMO) and American Society of Clinical Oncology (ASCO) guidelines, the standards of care for CRC depend on the stage and molecular characteristics of the disease [45, 46]. For localized CRC, typical treatment involves surgery with neoadjuvant and/or adjuvant chemotherapy, especially for patients with stage III

tumors [45, 46], and a combination of fluoropyrimidine (such as 5-fluorouracil (5-FU) or capecitabine) and oxaliplatin (leucovorin–5-FU–oxaliplatin (FOLFOX) or capecitabine–oxaliplatin (CAPOX)) is commonly used [45, 46]. Treatment for metastatic CRC is more tailored. First-line treatment involves combination chemotherapy (FOLFOX and leucovorin–5-FU–irinotecan (FOLFIRI)) with targeted therapies such as an anti-VEGF antibody (bevacizumab) or anti-EGFR antibody (e.g., panitumumab), particularly in patients with rat sarcoma (RAS) wild-type tumors. Immunotherapies, such as pembrolizumab, are the first-line treatments for patients with microsatellite instability-high (MSI-H) tumors. Treatment approaches are continually evolving, with the latest guidelines emphasizing precision medicine, immunotherapy, and targeted therapies, especially for patients in advanced stages and with specific molecular subtypes [47].

3.2 Clinically approved therapeutics for CRC

Clinically approved therapeutics for CRC include primarily small-molecule drugs and biologics (Table 1). Chemotherapies, predominantly small-molecule drugs such as 5-FU, oxaliplatin, and irinotecan, play pivotal roles in the clinical management of CRC patients [48]. These therapies are cost effective and have been employed for decades, resulting in significantly improved five-year and overall survival rates in CRC patients. However, chemotherapy is associated with severe side effects stemming from off-target effects. The mechanism of action of most chemotherapeutic agents involves the inhibition of DNA synthesis in rapidly dividing cells, but these agents cannot distinguish between cancer cells and healthy cells [49]. Common side effects include fatigue, hair loss, vomiting, loss of appetite, nausea, and neurotoxicity [49]. In more severe cases, these treatments can lead to infection, organ damage, or significant bleeding [50]. These adverse effects not only diminish patient quality of life but also restrict the use of chemotherapeutic agents because of the limited dosages that can be administered safely while achieving therapeutic efficacy. Targeted therapies, such as monoclonal antibodies, have transformed CRC treatment in recent years. Antibodies such as bevacizumab, cetuximab, and pembrolizumab have been approved as first-line treatments for specific individuals with CRC [43].

3.3 Emerging therapies under investigation for CRC

In addition to established and clinically approved treatments, several novel strategies for CRC are being explored at the preclinical and early clinical stages, notably phototherapy and gene therapy.

Phototherapy, including photodynamic therapy (PDT) and photothermal therapy (PTT), has attracted considerable attention for cancer treatment, especially in combination with other modalities such as chemotherapy [51]. PDT, administered intravenously, is approved for malignant cancers such as esophageal, lung, and prostate cancers, where light activation of photosensitizers generates ROS to induce tumor cell death [52]. Although not yet approved for CRC, endoscopic access to the colorectum makes phototherapy an attractive option for local or palliative treatment [7]. Ongoing clinical studies have shown encouraging results in phototherapy for CRC treatment [7, 53, 54]. For example, in a retrospective study of 52 patients with stage III–IV CRC, PDT achieved a response rate of 44.4% compared with a disease control rate of 88.9%, while it also increased the infiltration of immune cells, including T cells, B cells, and macrophages, in the TME without severe adverse effects associated with PDT [8]. PTT leverages heat generated by photothermal materials under light irradiation to induce thermal erosion and cancer cell death [55, 56]. While photothermal nanomaterials have shown promising efficacy in preclinical models of CRC, clinical investigations remain limited [17, 57]. The major challenges associated with phototherapy in CRC treatment include poor tissue penetration of light, hypoxia within the TME, which limits ROS production, and poor tumor targeting by photosensitizers [55]. Future advances in optimized light sources, hypoxia alleviation and oxygen supply in the TME, tumor-specific photosensitizers, and combinations with chemotherapy or immunotherapy hold great promise for improving the clinical outcomes of phototherapy [55].

Gene therapies predominantly rely on the intravenous administration of nucleic acid-based therapeutics, such as DNA, mRNA, and siRNA, to deliver them directly into the systemic circulation or target tissues [58-60]. With the advancement of gene delivery systems and gene editing technologies, gene therapy is emerging as a promising modality for treating CRC [61-63]. Currently, many strategies are under investigation in preclinical and early clinical studies, including the silencing of oncogenic genes, such as *KRAS* and *APC* [64]; the delivery of therapeutic genes, such as *IL-12* or *IL-15*, to stimulate antitumor immunity [9, 65]; and cell-based gene therapies that involve engineering immune cells to enhance tumor clearance [10, 66]. Recent advances in genome sequencing have expanded the repertoire of CRC-specific targets [16] and nanotechnology represents an alternative to viral vectors, enabling more precise, immune-evasive, and tumor-targeted delivery systems [67]. Cell-based gene therapies, most notably chimeric antigen receptor (CAR) T-cell therapy, have transformed the treatment of hematologic malignancies but remain

largely ineffective in solid tumors including CRC [68]. The main barriers include tumor heterogeneity, the immunosuppressive TME, and physical obstacles to T-cell infiltration [9, 69]. Innovative strategies such as dual-targeting and logic-gated CAR constructs to improve tumor specificity (NCT05736731) and the secretion of immune-stimulating cytokines to remodel the TME and resist exhaustion [70] are under preclinical and early clinical evaluation. Furthermore, large-scale randomized clinical trials and the development of scalable manufacturing pipelines would advance the clinical application of gene therapies [71].

3.4 Challenges for established CRC treatment

Most of the treatments mentioned are administered by intravenous injection (Table 1). Intravenous administration, which requires professional medical management and repeated hospital visits, compromises patient compliance and places a significant burden on healthcare resources. This is particularly challenging for CRC patients, who often require long-term, multicycle therapy because of the high risk of recurrence. Furthermore, repeated injections can lead to complications such as hypersensitivity reactions. Systemic anticancer drug exposure often causes severe off-target effects, exacerbating patient morbidity [72, 73]. These limitations underscore the need for alternative, patient-friendly strategies that can provide targeted drug delivery to disease sites, enhance treatment accessibility, and ultimately improve patient outcomes.

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Table 1. Clinically approved therapeutics for CRC.

Drug type	Drug molecule	Mechanism(s) of action	Administration route	Advantages	Limitations	References
Small molecule drugs	5-FU	Inhibits thymidylate synthase, disrupts DNA synthesis	i.v.	Cornerstone of chemotherapy; effective in combination regimens (e.g., FOLFOX and FOLFIRI)	High toxicity; side effects include diarrhea, mucositis, and myelosuppression	[74]
	Oxaliplatin	Platinum-based DNA crosslinking agent	i.v.	Effective in combination with fluoropyrimidines (e.g., FOLFOX); increases DNA damage	Neurotoxicity, especially cumulative peripheral neuropathy	[75]
	Irinotecan	Topoisomerase I inhibitor that leads to DNA damage	i.v.	Effective against metastatic CRC in combination regimens (e.g., FOLFIRI)	Severe diarrhea and neutropenia	[76]
	Leucovorin	Enhances the inhibition of thymidylate synthase induced by 5-FU	i.v. or p.o.	Increases the efficacy of 5-FU in regimens like FOLFOX and FOLFIRI; reduces the toxicity of high-dose methotrexate in other cancers	May increase 5-FU-related toxicities, such as GI issues	[77]
	Capecitabine	Oral prodrug of 5-FU	p.o.	Convenience of oral administration; fewer infusion-related side effects	Similar toxicity profile to 5-FU; hand-foot syndrome may occur	[78]

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	Regorafenib	Multi-kinase inhibitor targeting VEGFR, FGFR, etc.	p.o.	Option for chemotherapy-resistant metastatic CRC	High toxicity; common side effects include fatigue, hand-foot syndrome, and hypertension	[79]
	Trifluridine/Tipiracil	Incorporates into DNA, inhibiting its synthesis; protects trifluridine from degradation	p.o.	Effective against heavily pretreated metastatic CRC	Commonly causes neutropenia, fatigue, and GI toxicity	[80]
Biologics	Bevacizumab	VEGF inhibitor; inhibits angiogenesis	i.v.	Extends the survival of patients with metastatic CRC when combined with chemotherapy	Risks of hypertension, bleeding, and thromboembolism	[81]
	Cetuximab	EGFR inhibitor; prevents tumor cell proliferation	i.v.	Effective against RAS wild-type CRC; enhances the response in combination with chemotherapy	Limited to patients with RAS wild-type tumors; causes rash, diarrhea, and hypomagnesemia	[82]
	Panitumumab	EGFR inhibitor; similar to cetuximab	i.v.	Fully human monoclonal antibody; reduces the risk of infusion reactions	Same limitations as cetuximab; less effective against RAS-mutant tumors	[83]
	Pembrolizumab	PD-1 inhibitor; increases the immune	i.v.	Effective against MSI-H/dMMR tumors; long-lasting responses	High immune-related toxicity, including colitis and endocrinopathies;	[84]

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		response to cancer cells			ineffective against pMMR CRC	
	Nivolumab	PD-1 inhibitor; increases the immune response	i.v.	Similar benefits as pembrolizumab	Limited to specific CRC subtypes	[85]
	Ipilimumab	CTLA-4 inhibitor, enhances T-cell activation	i.v.	Effective in combination with nivolumab for some CRC patients	High immune-related toxicity, including colitis and endocrinopathies	[86]

5-FU, 5-fluorouracil; i.v., intravenous injection; p.o., oral administration; FOLFOX, leucovorin–5-fluorouracil–oxaliplatin; FOLFIRI, leucovorin–5-fluorouracil–irinotecan; VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; EGFR, epidermal growth factor receptor; MSI-H/dMMR, microsatellite instability high/mismatch repair-deficient; pMMR, mismatch repair-proficient.

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4. Oral route: Noninvasive and local drug delivery to treat CRC

Oral administration is widely regarded as the preferred route for drug delivery because of its safety, painless nature, and convenience [87]. The oral route overcomes the drawbacks associated with intravenous administration, significantly improving patient compliance. In the context of CRC, oral administration has additional advantages. The unique pathophysiology of CRC, as described in Section 2, provides promising opportunities for local and targeted treatment via oral delivery, with potential benefits such as increased local bioavailability, enhanced antitumor efficacy at the lesion site, and reduced systemic exposure.

Moreover, the association between diet and CRC has been extensively studied [88]. The World Cancer Research Fund/American Institute for Cancer Research highlights that consuming whole grains, dietary fiber, and dairy products can lower CRC risk [89]. Emerging evidence implicates the gut microbiota as a key mediator of the relationship between diet and cancer, particularly in CRC, given the close local interaction between the gut microbiota and colon tumors [23]. Dietary components, such as protein, fat, fiber, and vitamins, can influence the composition and function of the gut microbiota [23]. For example, dietary fiber serves as a primary energy source for the microbiota in the cecum and colon, generating crucial metabolites such as SCFAs. These metabolites stimulate intestinal mucus secretion, regulate immune responses, and suppress inflammation and carcinogenesis, thereby contributing to gut health [90]. Whole grains, a rich source of soluble and insoluble fibers, have been shown to reduce CRC risk by approximately 20% when three servings (90 g) are consumed per day, with a further reduction in risk observed with greater intake [91]. Moreover, dietary fiber intake is positively associated with improved responses to immune checkpoint inhibitors and a reduced incidence of immune-related adverse events in cancer patients [92]. These findings underscore the potential for CRC prevention and treatment through modulation of the gut microbiota via the oral route. Although no microbiota-based therapies have yet been approved for CRC specifically, advanced oral therapies could play a promising role in their clinical development, enabling colonization in the colon or the local delivery of drugs for microbiota modulation after oral administration.

Despite the advantages of the oral route for CRC treatment, current CRC therapeutics are predominantly administered invasively via intravenous injection, and only a limited number of

small-molecule drugs, such as capecitabine, are available for oral administration (Table 1) [61]. To date, no biologic formulation has been approved for oral delivery in CRC treatment. The unmet need for oral therapeutics can largely be attributed to the complex and harsh conditions of the GI tract, as well as the physicochemical properties of many drugs. The heterogeneity of the GI tract presents significant challenges for oral delivery. The pH gradient ranging from 0.4–4.0 in the stomach to 5.0–7.0 in the small intestine and 6.4–7.0 in the colon requires therapeutics to remain stable across a broad range of pH value [93]. The physiological complexity of each GI tract segment further complicates oral delivery. Gastric fluid contains digestive enzymes such as pepsin, which compromise protein stability; small intestinal fluids contain trypsin, chymotrypsin, lipases, and bile salts, which degrade proteins and lipophilic drugs; and colonic fluids are highly rich in diverse microbiota [93]. Retention times differ across segments, with approximately 2 h in the stomach, 6 h in the small intestine, and up to 24 h in the colon [87]. While the long residence time of the colon provides opportunities for local CRC therapy, oral formulations must first withstand the acidic gastric environment and enzymatic degradation in the small intestine. These factors collectively affect drug absorption and release kinetics. In addition, CRC is frequently associated with a chronic inflammatory microenvironment, resulting from inflammation-associated tumorigenesis, tumor elicited inflammation, and therapy-induced inflammation [15]. Chronic inflammation leads to breakdown of the epithelial barrier, increased ROS levels, and elevated levels of proinflammatory cytokines and chemokines [15, 94, 95]. These factors can profoundly affect the stability, release, and efficacy of oral therapeutics. Furthermore, the intrinsic physicochemical properties of different drugs must be considered. With respect to small molecules, particularly hydrophobic compounds, instability in the GI tract often leads to rapid degradation, poor bioavailability, suboptimal therapeutic outcomes, and dose-dependent toxicity [14]. For biologics, the challenges are even more pronounced, including degradation by gastric acid and digestive enzymes (e.g., pepsin, trypsin, and chymotrypsin), entrapment or cleavage by mucus, and inherently low permeability across the intestinal epithelium. These hurdles result in oral bioavailability typically less than 1% [62]. Even clinically approved oral agents for CRC remain limited by efficacy and safety concerns. For example, capecitabine, an oral prodrug of 5-FU, has a short plasma half-life (0.55–0.89 h) and nonselective cytotoxicity, leading to adverse effects such as diarrhea and abdominal pain [63].

Taken together, these challenges underscore the need for advanced oral delivery systems with enhanced colon-targeting and tumor-specific capabilities, which hold promise for improving efficacy while minimizing systemic toxicity.

5. Advanced oral therapies for CRC treatment

5.1 Targeted Drug Delivery: Nanomedicine-based therapies

Given the limitations of conventional drugs in CRC treatment—including poor specificity, systemic toxicity, and suboptimal efficacy—nanomedicines have attracted considerable research interest for direct drug delivery because of their excellent cargo protection, precise region- and cell-specific targeting, efficient cellular uptake, and controllable drug release. Some nanomedicines, such as Doxil® and Nanoxel®, have already been approved for clinical use in cancer patients via intravenous injections [96]. Tailored physicochemical modifications and surface engineering of nanoparticles for oral delivery hold significant promise for the development of advanced therapies in CRC treatment. To date, various nanomedicines have been explored in this context, including lipid-based nanomedicines (e.g., solid lipid nanoparticles, nanostructured lipid carriers, lipid nanocapsules, nanoemulsions, self-nanoemulsifying drug delivery systems, and liposomes) [97], polymer-based nanomedicines (e.g., polymeric nanoparticles, polymersomes, polymeric micelles, and dendrimers) [98], lipid–polymer hybrid nanomedicines (LPNs; e.g., monolithic matrix LPNs and core–shell LPNs) [99], and scaffolds (e.g., nanofibers, hydrogels, and films) [100] (Figure 2A). Considering the heterogeneity of the GI tract, multiple strategies, including anti-acid, anti-enzyme, mucus interaction, permeation enhancement, and tumor targeting strategies, have been developed to facilitate nanomedicine-based oral drug delivery (Figure 2B). In this section, we review these strategies and their potential to overcome pathophysiological barriers, thereby advancing nanomedicine-based oral therapies for CRC treatment.

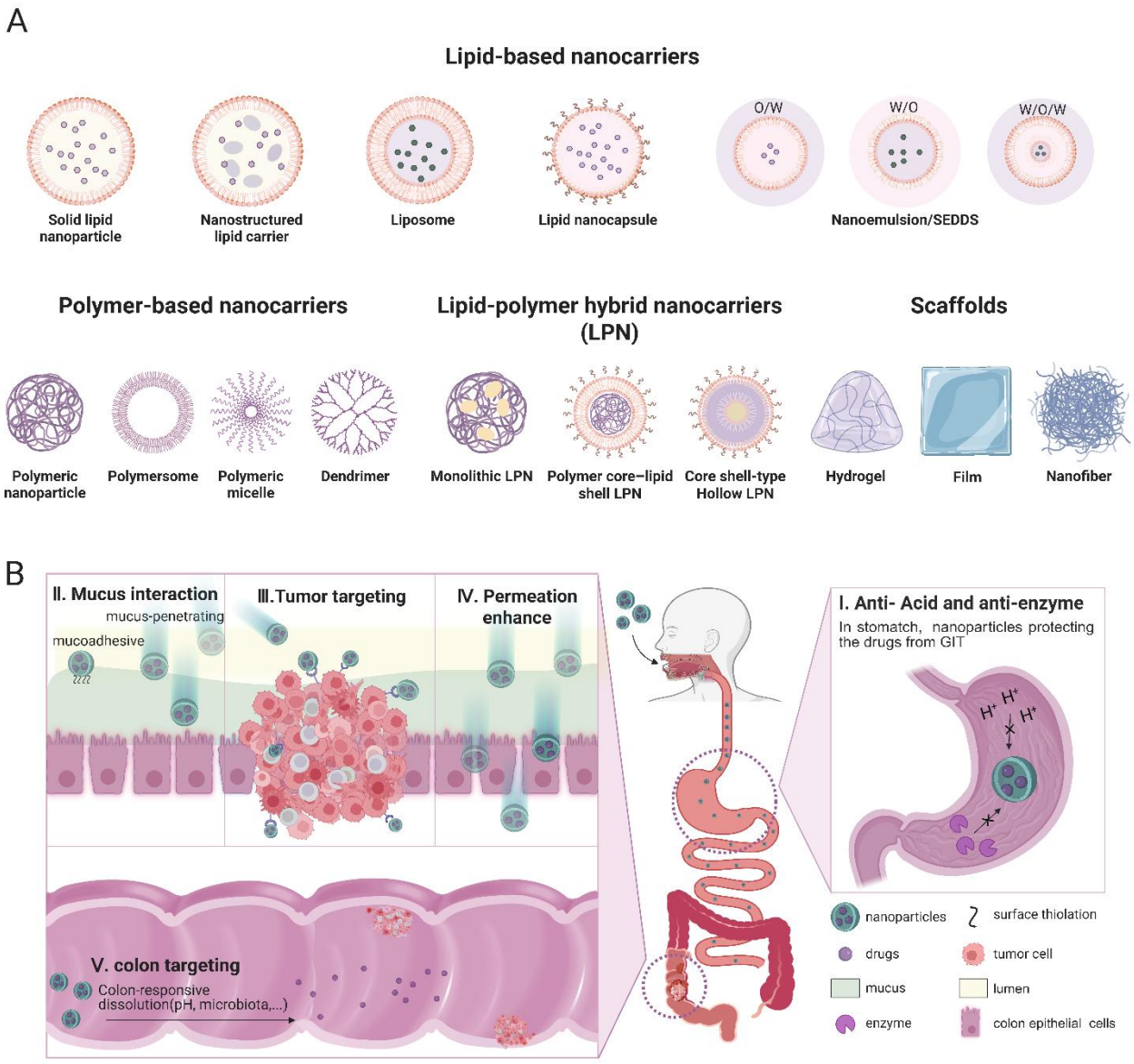


Figure 2. Advanced nanomedicine-based oral therapies for CRC treatment. (A) Representative nanomedicines employed in advanced oral therapies for CRC treatment. (B) Strategies to facilitate the in vivo biological interactions of nanomedicines after oral administration. Created with BioRender.com.

5.1.1 Anti-acid and anti-enzyme strategies

Nanomedicine offers a promising platform for improving drug stability through inherent antiacid and antienzyme properties or via the incorporation of acid- and enzyme-resistant excipients and coatings, providing protection during GI transit following oral administration.

pH-sensitive polymers and proximal gut–indigestible polymers have been utilized in this context. pH-dependent strategies leverage the pH gradient along the GI tract (stomach: pH 0.4–4.0; small intestine: pH 5.0–7.0; and colon: pH 6.4–7.0) [101]. pH-sensitive methacrylic acid copolymers, such as the pH-sensitive polymer Eudragit S100, which remains unionized at pH > 7, and Eudragit L100, which is ionized at pH > 6 [102], have been utilized for anti-acid strategies to protect the formulation against gastric acid. Eudragit S100 has been widely employed for colon-targeted drug delivery and combining Eudragit S100 and Eudragit L100 at specific ratios allows for precise release profiles tailored to the desired pH range. Within the realm of advanced oral nanomedicine-based therapies, delivery systems can be coated with pH-sensitive polymers [103] or lyophilized and encapsulated in enteric capsules made of such polymers [104, 105]. Narayan et al. developed double-layer coated mesoporous silica nanoparticles (MSNs) with an inner layer of a chitosan–glucuronic acid conjugate and an outer layer of Eudragit S100 for oral capecitabine delivery. Oral administration of the colon-targeting nanosystem significantly reduced the number of tumors in an orthotopic rat CRC model [106]. Other pH-sensitive polymers, such as cellulose derivatives, have been developed as well. For example, Yusefi et al. synthesized cellulose nanocrystals for delivery of the colon-targeting drug 5-FU [107]. Compared with pH 1.2 and pH 4.2 media, cellulose nanocrystals showed increased drug release in pH 7.4 media [107].

Nondigestible polymers in the proximal gut that are selectively metabolized by the colon microbiota, such as polysaccharides, have also been harnessed for colon-targeted nanomedicine applications [108]. Examples of naturally occurring polysaccharides employed for this purpose include pectin [109], starch [110], alginate [111], chitosan [112], dextran [113], chondroitin sulfate [114], and inulin [115]. These polysaccharides exhibit a wide range of molecular weights and chemically active groups, allowing for tailored nanomedicine designs. They can be used to form nanoparticles, or to encapsulate nanoparticles within polysaccharide-based microparticles or hydrogels [116], enabling microbiota-responsive dissolution and targeted drug release upon reaching the colon. Kang et al. developed doxorubicin- and metformin-loaded guar gum microparticles by emulsification via the cumulative solidification method. The results revealed the restricted release of metformin and doxorubicin in simulated gastric and intestinal fluid and increased release in simulated colonic medium with a 4% rat cecal content, confirming enzymatic degradation of the guar gum microparticles. An in vivo study revealed that the tumor inhibition rate of this microparticle system was 78% greater than that of free drugs [117]. Additionally, certain

polysaccharides, such as inulin and dextran, also act as prebiotics, conferring additional benefits [118]. This feature is particularly desirable in CRC treatment owing to the dysbiosis present in the disease. Prebiotic-based oral advanced therapies are discussed in Section 5.2.4.

5.1.2 Mucus interaction strategies

The thick and viscous colonic mucus layer in the GI tract poses a substantial barrier to nanomedicine-based oral therapies for CRC. It not only prevents interactions between nanomedicines and the underlying epithelium but also subjects nanomedicines to continuous clearance because of mucus renewal. For oral CRC treatment, overcoming the mucus barrier of the colon is particularly challenging. Thus, mucoadhesion and mucopenetration strategies have been exploited to address this issue.

In terms of mucoadhesion, nanomedicines can bind to mucus through noncovalent or covalent interactions, leading to enhanced interactions with the mucus and a prolonged residence time within the mucus layer, thereby favoring reduced nanomedicine clearance [119]. Noncovalent interactions include mainly electrostatic interactions and hydrogen bonds [119]. Positively charged nanomedicines can achieve mucoadhesive interactions because of the negative charge of mucus. In addition, nanomedicines can be assembled from or coated with mucoadhesive polymers, such as chitosan [120], alginate [121], or cellulose [122], to facilitate mucoadhesion. A study by Han et al. demonstrated the efficacy of a mucoadhesive nanomedicine comprising self-assembling nanoparticles composed of chitosan-conjugated curcumin and 7-ethyl-10-hydroxycamptothecin (SN38), achieving a tumor growth inhibition rate of 94.9% in an azoxymethane (AOM)- and dextran sulfate sodium (DSS)-induced colitis-associated cancer (CAC) murine model after oral administration [123]. Covalent interactions, on the other hand, involve the formation of disulfide bonds between thiolated groups on nanomedicines and active sulfhydryl groups in the cysteine-rich mucin layer, which are stronger than conventional noncovalent interactions [124]. Zhao et al. developed α-lactalbumin peptosomes coloaded with a microRNA-31 inhibitor and curcumin encapsulated in thiolated TEMPO-oxidized Konjac glucomannan microspheres to enable covalent mucoadhesion [124]. Although this system demonstrated effective colon distribution following oral administration, rectal administration yielded superior therapeutic outcomes in an AOM/DSS-induced CAC model [124]. This difference could be attributed to the trapping of nanomedicines

within the thick and highly digestive mucus layer in the stomach and small intestine [125]. Preventing gastric and small intestine mucus adhesion when delivering mucoadhesive nanomedicines intended for colon targeting is critical to overcome this challenge. By coating nanomedicines with colon-targeting polymers, such as Eudragit S100, premature adhesion to the mucus present in the stomach and small intestine can be avoided, enabling a selective interaction with the colonic mucus.

The primary objective of mucopenetration is to facilitate rapid passage through the mucus layer to enable effective interaction with the underlying epithelium. Two primary approaches—passive and active mucus penetration—have been explored to achieve this goal. Passive mucopenetration focuses on minimizing interactions between the nanomedicine surface and the mucus layer. Smaller nanomedicines generally exhibit better mucus penetration, whereas larger particles are more likely to become entrapped. Additionally, hydrophilic and neutral surfaces have been shown to facilitate passage through the mucus barrier [126]. Surface modifications, such as the incorporation of polymers with mucopenetration-enhancing properties, such as polyethylene glycol (PEG) [127] and poloxamer [128], have proven effective at improving mucus penetration. Notably, the chain length and modification density of PEG significantly influence its interaction with the mucus. For example, compared with their longer counterparts, shorter PEG chains exhibit superior penetration [129]. The key physicochemical properties of PEG coatings and PEGylation strategies to achieve muco-inert PEG coatings on nanomedicines have been thoroughly discussed in the literature [127, 130]. Zwitterionic nanomedicines, inspired by virus capsids with balanced positive and negative surface charges, represent another strategy for efficient mucus penetration [126]. Active mucopenetration utilizes external or internal forces, such as nanomotors or nanobots driven by external stimuli such as near-infrared (NIR) light [131], ultrasound [132], magnetic fields [133], or self-promoting gases [134], to propel nanomedicines through the mucus. Zu et al. developed a nanoplatform that combines silk fibroin-based nanoparticles with sonosensitizers and immunoadjuvants [135]. By coating the system with a hybrid membrane of plant-extracted lipids and Pluronic F127 and applying ultrasound irradiation, they achieved enhanced mucus and tumor penetration. This approach facilitated immunotherapy in a CAC murine model after oral administration, resulting in fewer colon tumors and increased infiltration of $CD4^+$ and $CD8^+$ cells [135] (Figure 3). In a follow-up study, they employed a similar system modified with Pluronic F127 for magnetic field-driven mucus penetration [136]. In this study, gingerol-loaded magnetic

mesoporous silicon nanoparticles functionalized with lipids extracted from mulberry leaves improved mucus and tumor penetration and therapeutic outcomes after oral administration [136]. While external stimuli such as ultrasound and magnetic fields show promise, their reliance on specialized equipment and precise control pose significant challenges for clinical translation. Internal stimulation-based nanomotors present a compelling alternative. Serra-Casablancas et al. designed catalase-bound MSNs capable of decomposing hydrogen peroxide (H_2O_2) into water and oxygen to drive self-propulsion. Compared with passive nanoparticles, enhanced Brownian motion facilitated a 60-fold improvement in mucus penetration in both in vitro and ex vivo models [134]. This approach is particularly appealing in the context of CRC, where increased H_2O_2 levels in the colonic mucus provide a disease-specific advantage for propulsion [137]. Furthermore, nanomedicines with mucolytic properties have been developed [126]. Functionalization with disulfide bond cleavage agents, such as N-acetylcysteine, or mucolytic enzymes, such as papain or trypsin, enables these systems to degrade mucus, thereby facilitating penetration [126]. Steiger et al. employed a mucin-specific protease, mucinase, and a phospholipase to increase mucus diffusivity and epithelial cell membrane permeability for oral peptide delivery. Enteric capsules containing both enzymes and the peptide drug desmopressin acetate (DDAVP; MW = 1069 Da) achieved a relative bioavailability of 155% compared with that of the drug alone after oral administration in a canine model [138]. However, this strategy raises safety concerns, as disruption of the protective mucus barrier may increase the diffusion of pathogens and bacteria, potentially leading to infection in the underlying epithelium [139].

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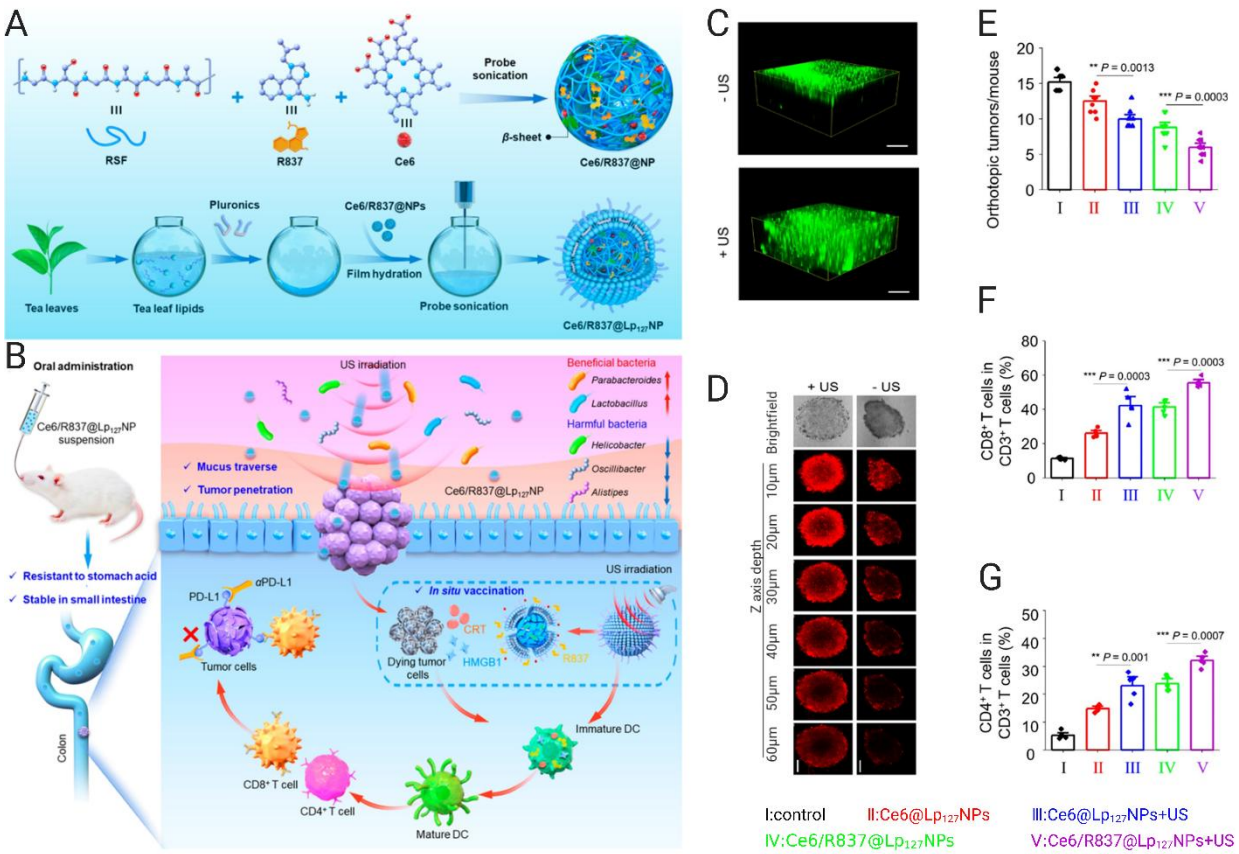


Figure 3. Mucus- and tumor-penetrating nanoparticles as oral sonosensitizers and immunoadjuvants to treat CRC. (A) Schemes of the methods used to prepare silk fibroin-based dual sonosensitizer (chlorin e6 (Ce6))- and immunoadjuvant (imiquimod, R837)-loaded nanoparticles as the core surface coated with plant-extracted lipids and Pluronic F127 (p127). The nanoparticles were named Ce6/R837@Lp127NPs. (B) Schematic of colonic mucus penetration, tumor accumulation, in situ vaccination, and activation of the antitumor immune response by Ce6/R837@Lp127NPs for CRC treatment. (C) Three-dimensional fluorescence images showing the penetration profiles of the NPs in the simulated mucinous hydrogel with or without ultrasound irradiation. (D) Penetration profiles of the NPs into 3D tumor spheroids with or without ultrasound irradiation. (E) Number of orthotopic tumors from AOM/DSS-induced orthotopic CRC models after different treatments. (F) Percentages of $CD8^+$ T cells and (G) $CD4^+$ T cells among $CD3^+$ cells in the spleen at the end of the experiments. Reproduced with permission from ref. [135].

5.1.3 Permeation enhancement strategies

Despite the small increase in intestinal permeability and reduced mucus levels in CRC tissues, gut leakage remains relatively limited, particularly in the context of solid tumor tissue [140]. The epithelial layer, comprising epithelial cells tightly bound via tight junctions with a gap of approximately 2 nm, forms a formidable barrier limiting drug entry [141]. Drugs penetrate this barrier through two primary routes: the transcellular route, involving endocytosis, transcytosis, and exocytosis, and the paracellular route, involving the traversal of tight junctions. The ability of drugs to permeate this barrier is influenced predominantly by their size, water solubility, and polarity [142]. Lipophilicity is positively associated with transcellular permeation because of interactions with the phospholipid bilayer of cell membranes [143]. However, excessive lipophilicity constrains oral delivery and therapeutic dosing, as lipophilic drugs are rapidly metabolized in and cleared from the GI tract. Polarity, typically quantified by the polar surface area or the number of hydrogen bond donors and acceptors, is negatively correlated with permeability; thus, the penetration of small, hydrophilic molecules is limited. In terms of size, small molecules (<10 nm) can pass through tight junctions, whereas larger molecules, particularly biomacromolecules, face significant challenges [144]. In the context of nanomedicine-based advanced therapies, two strategies have been employed to increase drug permeation: (1) the use of permeation enhancers (PEs) to facilitate paracellular or transcellular transport and (2) the surface modification of nanomedicines with receptor or transporter ligands to enable transcellular transport.

Various PEs that modulate paracellular and/or transcellular permeability have been extensively developed [145]. Surfactants [146] and bile salts [147] are among the most common classes of PEs. Their mechanisms of action and effects on drug delivery have been comprehensively described in the literature [148, 149]. In addition, PEs hold particular promise for the oral delivery of lipophilic drugs, as they not only increase their solubility by forming micelles but also improve their permeability [150, 151]. For example, Sze et al. encapsulated paclitaxel, a poorly soluble chemotherapeutic agent, within poly (ethylene glycol)-block-poly (ε-caprolactone) (PEG-b-PCL) micelles, significantly increasing its solubility compared with that of the free drug. The micelles showed improvements in uptake and the apparent permeability coefficient in MDCK-MDR1 cell monolayer models. Furthermore, in vivo studies showed that PEG-b-PCL micelles effectively increased the oral bioavailability of paclitaxel in rats [152]. Similarly, Almeida et al. encapsulated

camptothecin, another poorly soluble drug, within oleic acid-chitosan-PEG (OA-CS-PEG) micelles. This formulation increased the solubility and intestinal permeability of camptothecin in Caco-2 monolayers and 3D models, which included Caco-2 cells, HT29-MTX mucus-producing cells, and HPMEC-St1.6R cells. The oral administration of these micelles reduced tumor progression in a CAC murine model [153]. While PE–micellar systems show considerable promise for the oral delivery of poorly soluble drugs, maintaining micelle stability in vivo after oral administration remains a challenge. The large fluid volume of the GI tract can dilute polymer concentrations below the critical aggregation concentration, leading to micelle disassembly [145]. Stability testing can be conducted in vitro using simulated GI fluids by assessing the particle size and number after incubation, or in vivo using Förster resonance energy transfer (FRET) experiments [154].

PEs have also been widely applied for the systemic delivery of proteins and peptides via oral administration [145]. Recently, their use has been extended to include larger biomacromolecules such as antibodies for CRC treatment. Fluorinated cationic polymers can greatly enhance the cross-membrane and transmucosal penetration properties of nanoparticles [155]. Zhu et al. developed fluorocarbon-modified chitosan (FCS), which was mixed with proteins to form nanoparticles and subsequently lyophilized with mannitol for encapsulation into enteric capsules [104]. FCS facilitated transmucosal protein delivery by inducing the transient rearrangement of tight junction proteins in intestinal epithelial cells. Notably, the oral administration of FCS-assembled anti-PD-1 antibodies, alone or in combination with anti-cytotoxic T-lymphocyte antigen-4 (anti-CTLA-4) antibodies, achieved therapeutic responses comparable to those of intravenous injection in subcutaneous CRC murine models at a fivefold higher oral dose than the intravenous dose, demonstrating its capability for systemic delivery [104]. They further changed the model antibody to an anti-PD-L1 antibody and studied the efficacy of the resulting therapeutic in an orthotopic CRC murine model to evaluate local treatment after oral administration. Compared with the intravenous injection of anti-PD-L1, the oral administration of FCS/anti-PD-L1 achieved comparable therapeutic efficacy and tended to reduce systemic immune-related adverse effects (Figure 4) [105].

Strategies targeting epithelial receptors (e.g., vitamin B receptors and transferrin receptors) or transporters (e.g., solute carrier transporters and peptide transporters 1 and 2) for transcellular delivery have been extensively explored for systemic peptide delivery via the oral route and have

shown promising bioavailability. However, these approaches are seldom applied for localized CRC treatment via oral delivery, and future research focusing on this targeting approach could be promising.

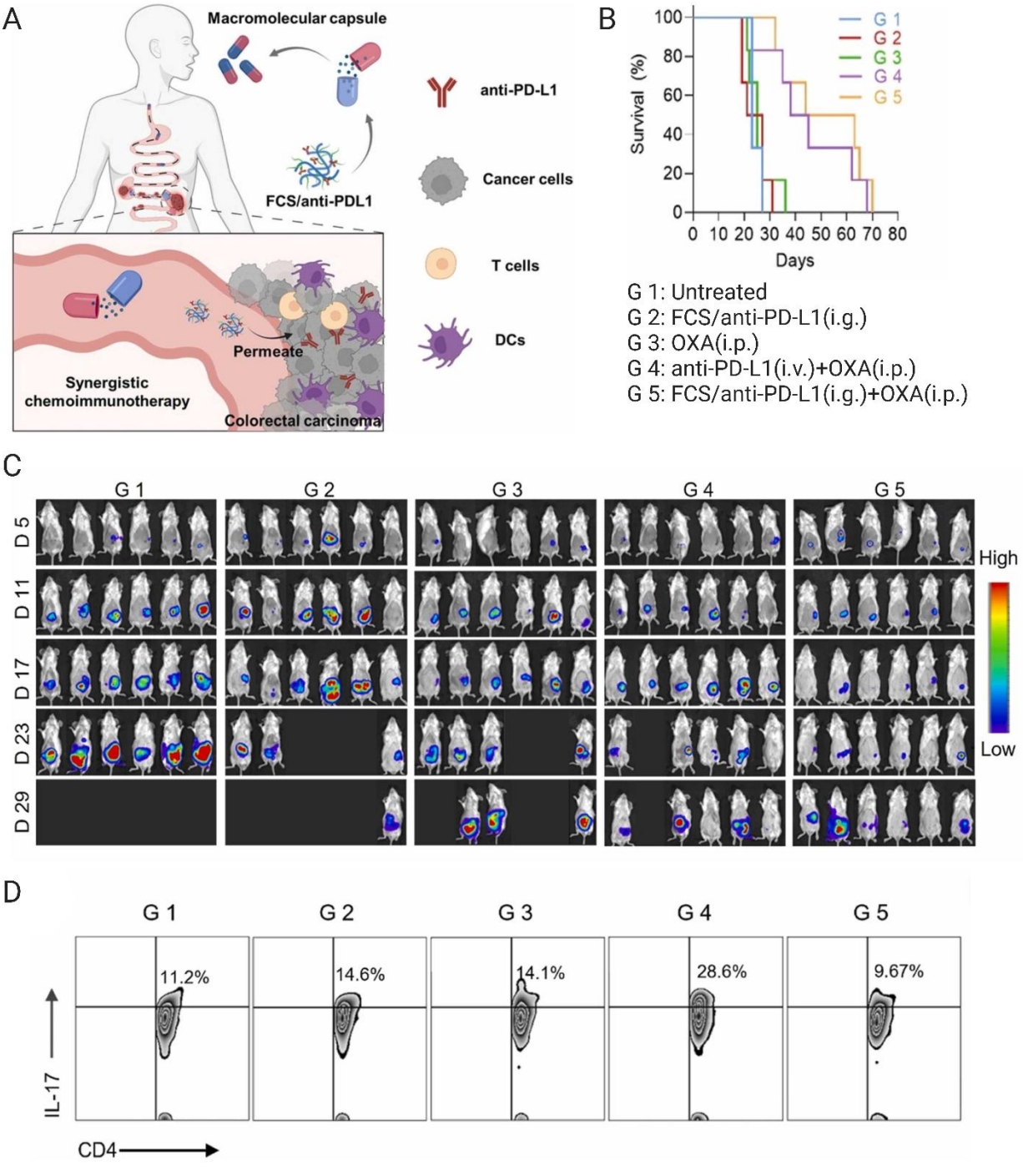


Figure 4. Permeation enhancement of FCS for oral anti-PD-L1 delivery in local CRC therapy. (A) Schematic of CRC therapy with FCS/anti-PD-L1 capsules. (B) Cumulative modality-free survival rates of mice in the different groups. (C) Bioluminescence imaging of orthotopic colorectal tumors from mice after different treatments. (D) Representative flow cytometry plots of IL-17^+ $CD4^+$ cells in the lymph nodes after various treatments. Reproduced with permission from ref. [105].

5.1.4 Protein corona interaction strategies

The protein corona is a dynamic layer of proteins and other biomolecules that adsorb onto nanoparticles upon contact with biological fluids, primarily through electrostatic and hydrophobic interactions [156]. In oral delivery, the complex GI environment strongly influences protein corona formation. For example, gastric fluids can generate pepsin- or gastric lipase-dominated coronas; small intestinal fluids may yield coronas enriched with pancreatic lipase, trypsin, chymotrypsin, and bile salts, whereas in the colon, where proteolytic enzymes are scarce but mucus is abundant, mucin-enriched coronas predominate [157]. In addition, the physicochemical properties of nanoparticles, including size, surface charge, and material composition, profoundly affect both the type and structure of adsorbed proteins [158].

Protein corona formation can alter nanoparticle characteristics, masking surface ligands, changing physicochemical properties, modulating drug release, and ultimately affecting biodistribution and therapeutic efficacy [159]. Researchers have developed strategies to avoid or utilize protein corona interactions with nanoparticles to facilitate oral drug delivery, but this research is limited in the context of CRC treatments. Learning from these strategies could facilitate advances in oral therapies for CRC. Strategies to mitigate these interactions include stealth coatings, such as PEGylation, which reduce nonspecific protein adsorption while preserving surface functionality [159, 160]. However, other modifications have emerged in recent years. For example, Wang et al. engineered MSNs decorated with a small cyclic FcRn-binding peptide (FcBP) to avoid protein corona adsorption [161]. Compared with IgG Fc–decorated MSNs, FcBP functionalization not only diminished protein adsorption and mucus interactions but also enhanced intestinal transport [161]. Given the thick mucus layer in the colon, such corona avoidance strategies are particularly relevant for CRC therapy. In contrast, deliberate manipulation of the protein corona can transform it from a barrier into a tool. Yao et al. demonstrated that transferrin-modified PLGA nanoparticles, when

precoated with mucin as an active protein corona, absorbed proteins involved in endoplasmic reticulum–Golgi trafficking, thereby enhancing intracellular transport and exocytosis [162]. This active corona differed from the passive coronas formed in mucus-secreting cells, which reduced endocytosis [162]. This research inspired the development of oral therapeutics for CRC by leveraging engineered protein coronas as functional tools to overcome mucus and epithelial barriers.

Notably, the protein corona composition is not static but highly context dependent. Wu et al. reported that intestinal protein coronas formed under disease conditions such as colitis or diabetes differ significantly from those formed under healthy conditions, with disease-derived coronas enriched in proteins that facilitate transcytosis across the epithelium [163]. However, systematic studies on CRC-specific intestinal coronas remain limited. Moreover, protein coronas exhibit high interindividual variability [164], underscoring the need for more research into personalized coronas and their implications for nanomedicine.

5.1.5 Tumor-targeting strategies

The pathological characteristics of CRC include multiple targets for the development of oral nanomedicine-based treatments, including overexpressed receptors, tumor-associated antigens, and a distinct TME. These characteristics have been leveraged to develop strategies for tumor-targeted delivery and stimulus-responsive drug release via the oral route. This section explores these targets and their strategic application in oral nanomedicine for CRC treatment (Table 2).

5.1.5.1 Receptor- and antigen-targeting strategies

The pathological landscape of CRC tumor tissue provides molecular targets for active targeting after oral administration, particularly owing to the increased expression of specific receptors and antigens, including CD44, the mannose receptor, and the folate transporter [165]. Nanomedicines can be functionalized with targeting ligands, such as antibodies, peptides, carbohydrates, or small molecules, enabling selective binding to tumor-specific or tumor-overexpressing antigens and receptors on the cell surface or within the TME, leading to increased CRC tissue retention and enhanced tumor cell internalization [166]. For example, hyaluronic acid (HA), a well-known CD44-targeting ligand, is highly biocompatible and has additional therapeutic potential because of its interaction with Toll-like receptors[167-169]. Hou et al. developed paclitaxel-loaded polylactic

acid–polyethyleneimine nanoparticles coated with an outer layer of inulin and an inner layer of HA. The inulin shell was selectively degraded by colon-specific bacteria, exposing HA for the active targeting of tumor tissues. This nanomedicine exhibited extended in vivo retention (up to 24 h) and substantial tumor accumulation, achieving notable antitumor efficacy in an orthotopic CRC murine model following oral administration [170]. Epithelial cell adhesion molecule (EpCAM) plays a vital role in increasing the aggressiveness of cancer proliferation through multiple signaling pathways, including those associated with cell adhesion, migration, proliferation, cycle metabolism, and metastasis [171]. Liu et al. conjugated the anticancer protein fragmented human lactoferrin to an EpCAM-targeted ligand, ankyrin repeat protein, for targeted delivery to CRC tissues [172]. Compared with unmodified proteins, EpCAM-targeted proteins exhibited more than 1000-fold greater anticancer activity in the human CRC cell line HT-29. In addition, compared with the oral administration of unmodified proteins, the oral administration of targeted proteins increased antitumor efficacy in orthotopic HT-29 CRC murine models [172].

Gene therapies have attracted significant interest for the development of precise CRC therapies [173]. While oncogene silencing via nanocarriers has shown promise for intravenous applications [174, 175], translating these strategies to oral delivery remains challenging. This is primarily due to the harsh GI environment, where nucleic acids undergo enzymatic degradation and exhibit poor mucosal permeability, limiting their therapeutic efficacy [176]. CD98 expression is increased in human colonic biopsy samples from patients with CRC [177]. Xiao et al. formulated CD98 siRNA (siCD98) and camptothecin-loaded CD98 Fab-functionalized poly(lactic-co-glycolic) acid (PLGA) nanoparticles embedded in a chitosan/alginate hydrogel. This system enabled tumor-specific CD98 targeting and silencing, and oral administration achieved greater therapeutic efficacy than that of nonfunctionalized siCD98/CPT nanoparticles in an orthotopic CRC murine model [177]. Lipid nanoparticles have garnered considerable attention for gene delivery, especially following the success of the Pfizer–BioNTech COVID-19 mRNA vaccine. However, their application in oral gene delivery remains underexplored. Ball et al. investigated the oral delivery of siRNA-loaded lipid nanoparticles and observed that although the nanoparticles were retained in the GI tract for more than 8 h and were internalized by epithelial cells in the small intestine and colon, gene silencing was not achieved [178]. These findings suggest that lipid nanoparticles require protection from the harsh conditions of the stomach and upper intestine to enable effective siRNA delivery into intestinal epithelial cells.

Furthermore, with advancements in biotechnology, researchers have found that embedding nanoparticles within the membranes of cells or microbes endows the materials with promising tumor-targeting properties. For example, Wang et al. leveraged the specific immunoglobulin G binding ability of *Staphylococcus aureus*, which is present at high levels in CRC tissues, to camouflage platinum-sprayed MSNs with the bacterial membrane. This innovative nanomedicine achieved a 4.3-fold increase in CRC-specific intestinal accumulation and a tumor inhibition rate of 99.1% in mice bearing orthotopic CRC tumors following oral administration [179]. Similarly, Shen et al. developed miR21-loaded ZnO nanoparticles coated with yeast shells derived from *Saccharomyces cerevisiae*. These nanoparticles targeted Dectin-1 on macrophages and effectively suppressed tumor growth in $Apc^{Min/+}$ and in situ tumor-bearing mouse models, with fewer and smaller polyps observed in the colon. This system exhibited the ability to target tumors, effective tumor growth inhibition, and low toxicity to nontumor cells (Figure 5) [180].

Despite advances in targeted nanomedicine, many receptors and antigens that have been successfully utilized for intravenous delivery, such as carcinoembryonic antigen and EGFR [181], remain underexplored for oral delivery. Extending these targeting strategies to oral nanomedicines holds great promise for CRC therapy. Nanotechnology has enabled the surface functionalization of nanoparticles with targeting ligands, including antibodies, to increase the tumor-specific delivery of therapeutic payloads. Integrating this approach with colon-targeting and mucus-penetrating strategies, as discussed above, may further improve the precision and efficacy of advanced oral therapies for CRC treatment.

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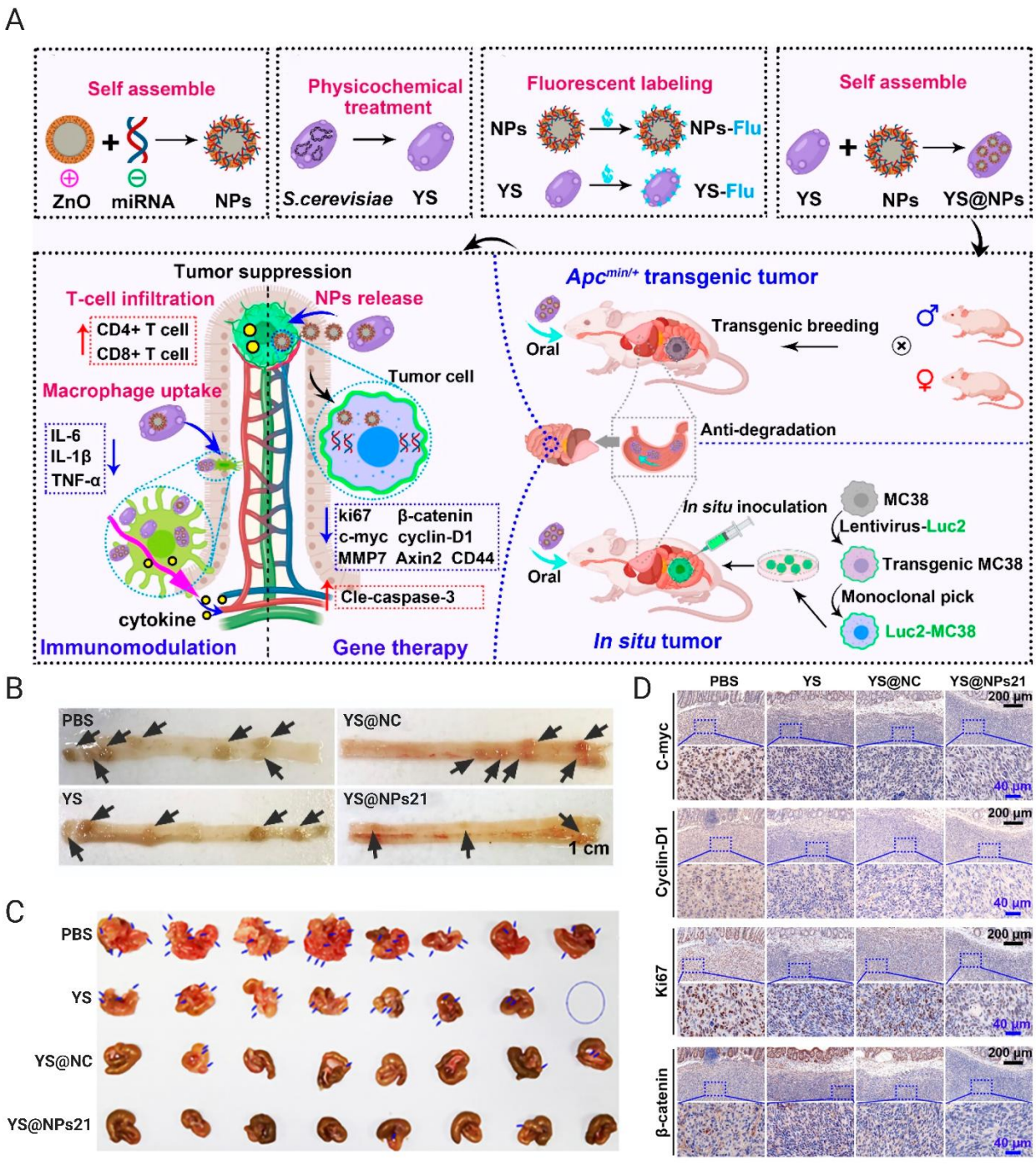


Figure 5. Tumor-targeting nanoparticles for oral miRNA delivery in CRC treatment. (A) Schematic of the strategy used to generate a probiotic yeast-mediated oral gene delivery system for CRC therapy. (B) The size and number of gut polyps in *Apc*$^{Min/+}$ mice from different groups. (C) Anatomical images of the cecum (the site of tumor cell inoculation) in orthotopic tumor-bearing model mice from different treatment groups. (D) Immunohistochemical staining of tumor tissue

(from the cecum) was performed to detect the expression of the proliferation-related proteins C-myc, Cyclin-D1, Ki67, and β-catenin. Reproduced with permission from [180].

5.1.5.2 Drug release mediated by stimuli in the TME

The TME is characterized by specific features, such as hypoxia, elevated ROS levels, an acidic pH, and increased infiltration of several enzymes [182], which present unique opportunities for the development of advanced oral nanomedicines for CRC treatment. TME-responsive nanomedicines offer the possibility to release drugs specifically in the TME, thereby improving the therapeutic index.

Some enzymes, including matrix metalloproteinase (MMP) and hyaluronidase (HAase), which have been leveraged in enzyme-sensitive drug release strategies, are upregulated within the TME. For example, Qin et al. developed a chitosan nanoparticle system loaded with 5-FU, which was crosslinked with HA and assembled with *Saccharomyces cerevisiae. Saccharomyces cerevisiae* modification provided tumor-targeting capabilities, while HA crosslinking facilitated enzyme-triggered release by HAase within the TME. Oral administration of this formulation effectively inhibited tumor growth and prolonged the survival of orthotopic CRC model mice [183]. The hypoxic TME has been widely exploited for targeted drug delivery. Anaerobic bacteria, in particular, have shown intrinsic tumor-targeting capabilities because of their natural tropism for hypoxic regions. For example, Fan et al. employed the anaerobic bacterium *Veillonella atypica* to deliver $BaTiO_3$ nanocubes via a surface click chemistry strategy [184]. This nanosystem effectively modulated the immunosuppressive TME by increasing dendritic cell (DC) maturation, promoting macrophage M1 polarization, increasing effector T-cell infiltration, and reducing regulatory T-cell populations. Following oral administration in an orthotopic CRC murine model, this approach enabled synergistic catalytic therapy and immunotherapy [184]. pH-responsive drug release systems, which exploit the difference between normal tissue pH (~7.4) and the acidic TME (~6.5), have been utilized in intravenous antitumor nanomedicines. However, such strategies are less applicable to oral formulations, as complex changes in the pH of the GI tract could lead to premature drug release. Future research could focus on designing oral nanomedicines for precise colon targeting and TME pH responsiveness. Similarly, ROS-responsive strategies, which have been widely employed in intravenous anticancer therapies because of the elevated ROS levels in

the TME [185-188], remain underexplored in the context of oral nanomedicines for CRC. However, such strategies have shown considerable promise in the development of oral nanomedicines for the treatment of inflammatory bowel disease (IBD) [189-192]. By adapting these strategies, the development of ROS-responsive oral nanomedicines for CRC could be a promising avenue for further exploration.

As previously discussed, certain receptors, antigens, and enzymes that are overexpressed have been harnessed for tumor targeting. However, in the context of oral nanomedicines for CRC, accounting for the expression of some of these targets in the upper GI tract is critical. A combination of receptor-targeting and colon-targeting strategies could be employed to achieve colon-specific targeting followed by selective tumor targeting. For example, coating nanoparticles with colon-targeting polymers or encapsulating nanoparticles in enteric capsules can help prevent premature targeting and release. Lu et al. developed hierarchically targeted nanocarriers to deliver cisplatin/superparamagnetic iron oxide nanoparticles for oral CRC treatment. These nanocarriers were coated with folic acid (FA) and a dextran shell in a layer-by-layer fashion, which prevented FA-mediated uptake by enterocytes in the small intestine, allowing efficient targeted delivery to the tumor [193]. When they reach the colon, the dextranase-degraded dextran- and FA-modified nanoparticles are exposed to facilitate FA receptor-mediated tumor targeting and internalization. This nanosystem significantly inhibited tumor growth and suppressed metastatic peritoneal carcinomatosis in mice bearing orthotopic colon tumors (Figure 6) [193].

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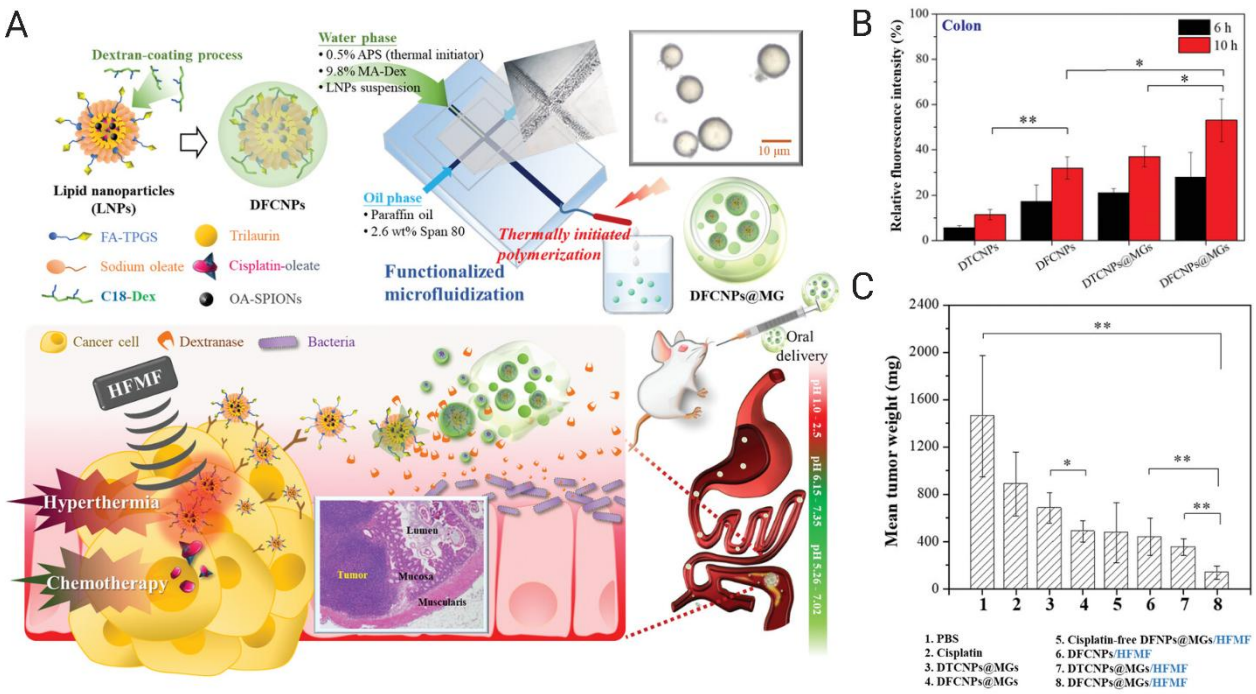


Figure 6. Hierarchically targeted dextran/FA nanocarriers of oral cisplatin/superparamagnetic iron oxide nanoparticles for CRC treatment. (A) Schematic of hierarchically targeted microgels for combined local chemotherapy/thermotherapy for CRC via oral administration. (B) Relative fluorescence intensity of Cy5.5-labeled LNPs and LNPs@MGs in the colons of mice bearing in situ CT26 tumors at 6 and 10 h after oral gavage. (C) Mean tumor masses of tumor-bearing mice in different treatment groups 14 days after the first treatment. Reproduced with permission from ref.[193].

Table 2. Tumor-targeting oral nanomedicine strategies for CRC treatment.

Strategy	Target	Ligand	Formulation	Effect(s)	References
Receptor and antigen targeting	CD44	HA; facilitates the internalization of NPs by tumor cells via CD44-mediated cellular uptake	Paclitaxel loaded polylactic acid-polyethyleneimine nanoparticles with a double outer layer of inulin and an inner layer of HA.	Remained in vivo for 24 h, accumulated at the tumor site, and achieved effective anti-tumor efficacy in an orthotopic CRC murine model	[170]
	CD98	CD98 Fab; facilitates the internalization of nanomedicines by tumor cells via CD98-mediated cellular uptake	siCD98- and camptothecin-coloaded CD98 Fab-functionalized PLGA nanoparticles embedded in a chitosan/alginate hydrogel	Enhanced antitumor efficacy in an orthotopic CRC murine model	[177]
	Folate receptor	FA; facilitates the internalization of NPs by tumor cells via folate receptor-mediated cellular uptake	Cisplatin/superparamagnetic iron oxide nanoparticle (SPION)-loaded trilaurin-based lipid nanoparticles coated with FA and a dextran shell in a layer-by-layer manner	Inhibited tumor growth and suppressed metastatic peritoneal carcinomatosis in orthotopic CRC-bearing mice	[193]
			5-FU- and sodium butyrate-coloaded β-lactoglobulin nanoparticles functionalized with FA	Remarkable toxicity to both HCT116 and Caco-2 cells compared with the free drugs	[194]
	EpCAM	Ankyrin repeat protein; facilitates the internalization of NPs by	Ankyrin repeat protein-tagged human lactoferrin fragment -conjugate	Increased antitumor efficacy compared with nontargeted proteins in orthotopic and	[172]

		tumor cells via EpCAM-mediated cellular uptake		subcutaneous CRC murine models	
	Galectin-3 receptors	Citrus pectin; facilitates the internalization of NPs by tumor cells via galectin-3 receptor-mediated cellular uptake	5-FU-loaded citrus pectin nanoparticles coated with Eudragit S100	Increased colon retention and decreased systemic absorption	[195]
	Dectin 1	Yeast shell from *Saccharomyces cerevisiae*; facilitates the phagocytosis of NPs by macrophages via Dectin-1-mediated cellular uptake	miR21-loaded ZnO NPs assembled with the yeast shell from *Saccharomyces cerevisiae*	Effectively suppressed tumor growth in both $Apc^{Min/+}$ mice and mice bearing in situ tumors	[180]
	IgG	*Staphylococcus aureus* membrane; facilitates colonization in the intestinal tract via IgG recognition	Asymmetric platinum-sprayed mesoporous silica camouflaged within *Staphylococcus aureus* membranes	4.3-Fold greater biological chemotactic anchoring of CRC-related intestine and tumor inhibition rate of 99.1% in mice bearing orthotopic CRC tumors	[179]
TME responsive	HAase	HAase degrades the HA crosslinked NPs to facilitate drug release	5-FU loaded chitosan nanoparticles crosslinked with HA and assembled with *Saccharomyces cerevisiae*	Effectively inhibited tumor growth and prolonged mouse survival in an orthotopic CRC model	[183]

	Hypoxia	The anaerobic bacterium *Veillonella atypica* targets the hypoxic TME	BaTiO3 nanocubes conjugated to *Veillonella atypica* surface via click chemistry	Enhanced synergetic catalysis and immunotherapy in an orthotopic CRC model	[184]

HA, hyaluronic acid; FA, folic acid; EpCAM, epithelial cell adhesion molecule; TME, tumor microenvironment.

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As illustrated in Table 3, all developed oral nanomedicine-based therapies have exhibited considerable promise at the preclinical stage for the treatment of CRC. Nevertheless, each of these strategies falls short in addressing the full spectrum of clinical requirements for CRC and is associated with certain limitations. Owing to the complexity of the GI environment and the anatomical localization of tumors, these approaches are generally not suitable for use as monotherapies. Instead, combination strategies can substantially enhance the therapeutic efficacy of orally administered targeted nanomedicines for CRC. Furthermore, the clinical translation of nanomedicine-based interventions for CRC continues to face multiple challenges and safety concerns, which primarily include the following: (1) biological barriers: the increased oral bioavailability still does not meet clinical needs [131, 196]; (2) patient variability and manufacturing hurdles: considerable interpatient heterogeneity complicates the prediction of treatment response, while ensuring consistent quality control of complex nanoformulations remains technically challenging and costly [197-199]; and (3) safety considerations: potential toxicity of carrier materials, risks of immunogenicity, and possible disruption of gut microbiota equilibrium [200-202]. Addressing these critical issues must be prioritized in future research endeavors.

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Table 3. Summary of nanomedicine-based oral strategies for CRC.

Delivery strategy	In vivo performance	Advantages	Limitations
Anti-acid and anti-enzyme strategies	Enhanced cargo delivery to the colon and increased antitumor efficacy compared to nonmodified delivery systems, reaching a 78% tumor inhibition rate in one study [117]	-Good safety profiles. Eudragit polymers (e.g., Eudragit S100 and L100) are approved by FDA. Many polysaccharides (e.g., inulin and some alginate derivatives) are Generally Recognized As Safe (GRAS) for oral use.	-Limited efficacy. Need to combine with other strategies to improve the efficacy.
Mucus interaction strategies	Engineered mucus interactions avoid mucus cleavage and contributes to enhanced tumor killing efficacy, reaching a 94.9% tumor inhibition rate in one study [123]	-Overcome the mucus barrier. -PEG, a well-established mucus-penetrating polymer, is recognized as safe and widely accepted as an excipient in FDA-approved products.	-Complex engineering may hinder large-scale manufacturing and clinical translation. -Mucolytic strategies raise potential safety concerns.
Permeation enhancement strategies	Enhanced transmucosal delivery. Demonstrated improved antibody transport, with oral administration achieving comparable antitumor efficacy to intravenous injection in one study [203].	-Overcome the epithelium barrier. -Showed promising efficacy in many preclinical studies, even for large molecular biologics, such as antibodies [104, 203].	-Need high doses and precise colocalization with the cargo at the target site. -Increased intestinal permeability may pose risks to

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			the fragile CRC gut and lead to translocation of microbiota and their metabolites into the mucosa.
Protein corona interaction strategies	Exploiting the protein corona interaction can facilitate mucus penetration and intestinal transport.	-Potential ability to overcome the mucus and epithelium barrier.	-Limited understanding of its application in CRC. -Highly context-dependent and disease-specific with limited knowledge of its change in CRC context.
Tumor targeting strategies	Enhanced tumor targeting delivery, reaching a TIR of 99.1% in one study [179].	-Many targeted ligands have been developed and have shown promising effects for oral delivery. -Enhanced efficacy and decreased side effects through tumor targeting. -Promising results for many kinds of drug delivery, even for siRNA [177] and miRNA [180] delivery.	-Complex modification of nanoparticles posing challenges for large-scale manufacturing and clinical translation.

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5.2 Indirect Modulation: Bacteria-mediated strategies

The colon harbors a vast population of gut microbes that play pivotal roles in maintaining colonic homeostasis [204]. In the past decade, extensive research has highlighted the role of microbiota dysbiosis in CRC pathogenesis [205]. Dysbiosis profoundly influences CRC development and progression by promoting chronic inflammation, altering immune responses, and producing harmful metabolites. This imbalance can lead to DNA damage, genomic instability, and ultimately, tumor formation [205-207]. Furthermore, emerging studies have emphasized the importance of modulating the gut microbiota as a novel CRC treatment strategy [208]. Such modulation not only enhances the efficacy of chemotherapy and immunotherapy but also mitigates their toxicity, thereby improving therapeutic outcomes by increasing drug effectiveness and safety. In this section, we review the potential of bacteria-based approaches as advanced oral therapies for CRC treatment, with a focus on fecal microbiota transplantation, probiotic-based therapies, genetically engineered probiotics, prebiotic-based therapies, and pathobiont killing therapies (Figure 7).

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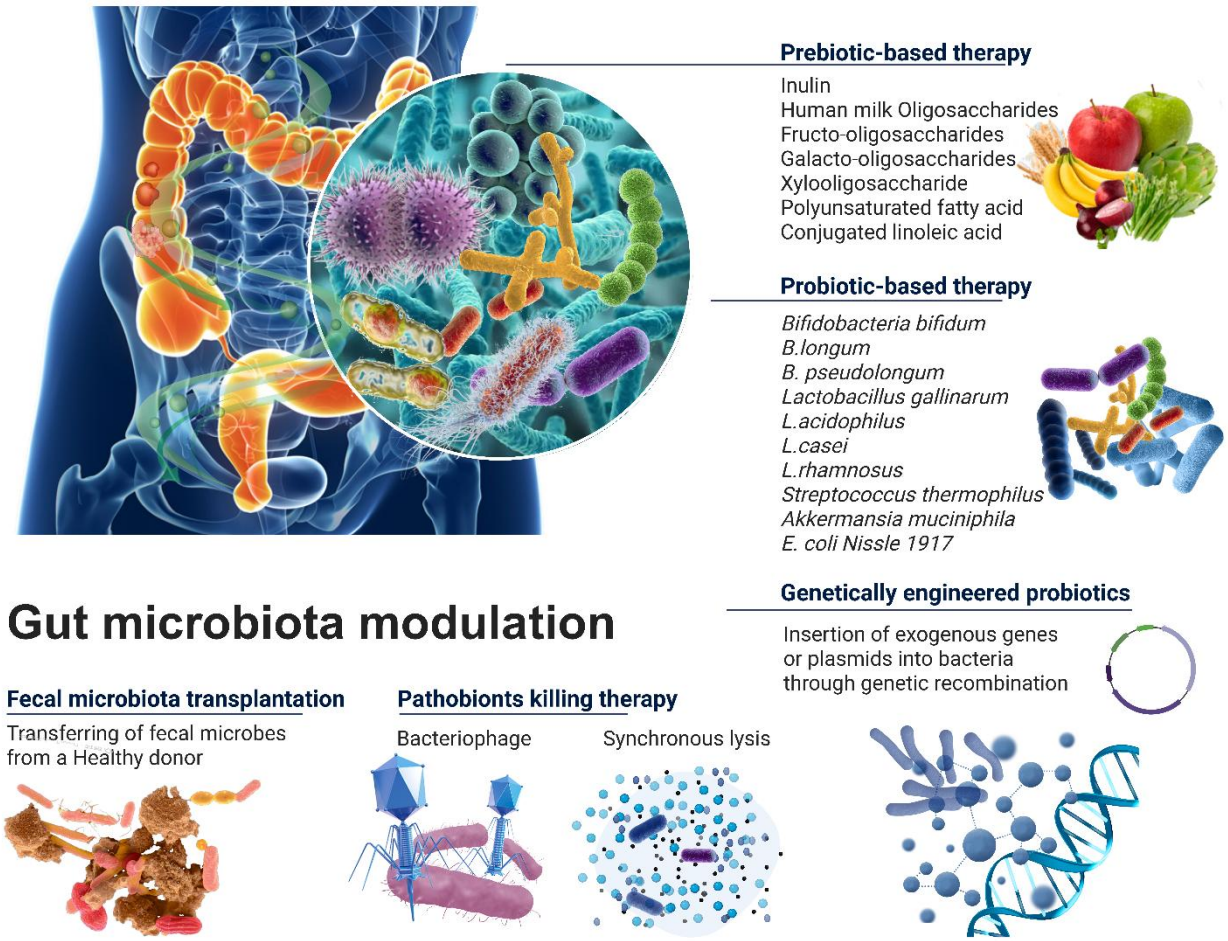


Figure 7. Bacterial modulation-based approaches, such as fecal microbiota transplantation, probiotic-based therapies, genetically engineered probiotics, prebiotic-based therapies, and pathobiont-killing therapies, as advanced oral therapies for CRC. Created with BioRender.com.

5.2.1 Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) was among the earliest strategies for modulating the gut microbiota. FMT involves transferring microbiota from the feces of healthy donors to the colon of recipients, with the aim of introducing or re-establishing a stable microbial community [209]. Recent evidence supports the potential of oral capsules as an efficient method for FMT, as documented in clinical and preclinical studies (ChiCTR2100046768 and NCT04729322) [209]. These advancements pave the way for FMT-based oral therapies. FMT has been approved for treating *Clostridium difficile* infection, which is driven by GI dysbiosis and *Clostridium difficile*

overgrowth. Currently, FMT is undergoing extensive preclinical and clinical evaluation for applications in CRC [210, 211]. FMT has been shown to exert direct antitumor effects in CRC by reversing intestinal microbial dysbiosis to enhance anticancer immune responses [212]. FMT has also shown promise in mitigating chemotherapy-induced toxicity in orthotopic CRC murine models via a mechanism mediated by the TLR–MyD88–NF-κB signaling pathway [213].

Moreover, FMT can be applied to modulate the immune system and enhance the response to immunotherapies. Recent studies have highlighted its role in improving outcomes when combined with immune checkpoint inhibitors. Preclinical data show that FMT to germ-free animals can recapitulate the responses to immune checkpoint inhibitors observed in human donors [214-216]. Trials testing similar approaches in patients with CRC are currently ongoing (NCT04729322 and NCT04130763). Notably, Baruch et al. conducted a single-center clinical trial involving 15 patients with metastatic, anti-PD-1-refractory, MSI-H cancers. By combining oral FMT capsules with anti-PD-1 therapy, this study demonstrated enhanced efficacy in this population of patients (NCT04729322). While immune checkpoint inhibitors have revolutionized CRC treatment, their clinical use remains limited to MSI-H CRC patients, who account for only 15% of all individuals with CRC. Microsatellite-stable (MSS) CRC patients respond poorly to PD-1 inhibitors, creating an unmet therapeutic need. Zhao et al. addressed this issue by conducting a phase II trial evaluating a combination of oral FMT capsules, the anti-PD-1 antibody tislelizumab, and fruquintinib in 20 patients with refractory MSS metastatic CRC. This study reported improved survival outcomes and manageable safety profiles, suggesting a promising new treatment option for this patient population with a low response to immunotherapy (ChiCTR2100046768).

While FMT offers the convenience of a broad-spectrum approach, it also presents challenges, including immunogenicity, nonspecificity, and an incomplete understanding of the underlying molecular mechanisms. Identifying the specific bacterial strains and the metabolites responsible for the therapeutic effects is an area of intense interest. Further subclassification and analysis of the microbiota derived from CRC patient feces could elucidate the roles of individual microbial strains and metabolites in CRC pathogenesis and treatment, advancing the development of microbiota-targeted therapies.

5.2.2 Probiotic-based therapies

Probiotics, particularly bacterial strains from the genera *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, and *Escherichia*, have been extensively reported to confer diverse benefits to the host [217]. These benefits include restoring gut homeostasis, modulating immune responses, producing anti-inflammatory or anticarcinogenic compounds such as SCFAs, and inhibiting the growth of pathogenic or harmful bacteria [218]. Hallmarks of CRC-associated dysbiosis are the enrichment of CRC-promoting bacteria and the depletion of e.g., *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* [219]. The oral administration of these probiotics has shown great promise in CRC prevention and treatment. *Lactobacillus* and *Bifidobacterium* are known to increase immune resistance to intestinal infections, stabilize the intestinal mucosal barrier, and prevent colon cancer [220]. For example, the daily oral administration of microencapsulated *Lactobacillus acidophilus* has been demonstrated to be an effective strategy for enhancing survival in the harsh GI environment and significantly reducing the incidence, multiplicity, and size of tumors in $Apc^{Min/+}$ mice [221]. Similarly, daily gavage with *Lactobacillus gallinarum* has been shown to inhibit intestinal tumor development in $Apc^{Min/+}$ mice and CAC model mice through the production of protective metabolites such as indole-3-lactic acid (ILA), which promote apoptosis in CRC cells. Additionally, *Lactobacillus gallinarum* administration increases the abundance of beneficial bacteria, including *Lactobacillus helveticus* and *Lactobacillus reuteri* [222]. *Bifidobacterium longum* is an anaerobic, gram-positive bacterium. Under conditions of gut dysbiosis, exogenous supplementation with *Bifidobacterium longum* can lead to rapid colonization of the intestine, enhanced barrier function, and increased tumor-targeting ability. *Bifidobacterium longum* demonstrates considerable antitumor activity by selectively inhibiting angiogenesis and hypoxic tumor growth. The oral administration of *Bifidobacterium longum* leads to reductions in the tumor formation rate, number, and size. Furthermore, *Bifidobacterium longum* increases the abundance of beneficial bacteria such as *Lactobacillus*, *Bifidobacterium*, *Alistipes*, and *Parabacteroides* while reducing the abundance of pathogenic bacteria, including *Desulfovibrio*, *Helicobacter*, and *Enterorhabdus* [223]. Similarly, the efficacy of *Bifidobacterium bifidum* in reducing the incidence of colon tumors in CAC murine models has been shown, although its effectiveness (27% reduction) was lower than that of *Lactobacillus acidophilus* (57% reduction) [224]. In addition, other probiotics have shown promising effects. *Streptococcus thermophilus*, for example, significantly reduced tumor development in $Apc^{Min/+}$ and CAC mice owing to its production of β-galactosidase,

an enzyme that inhibits the Warburg effect [225]. Additionally, *Akkermansia muciniphila* has been shown to suppress colon tumor development by promoting the accumulation of M1-like macrophages in $Apc^{Min/+}$ mice in an NLRP3-dependent manner [226].

The evidence discussed above underscores the potential of oral probiotics as promising advanced therapies for CRC. Notably, probiotics not only exhibit intrinsic antitumor effects but also regulate the composition and abundance of the intestinal microbiota. Their incorporation as adjunct therapies alongside conventional treatments has shown great promise. As mentioned above, FMT has the potential to increase the responsiveness of CRC patients to immunotherapy, albeit through mechanisms that remain incompletely understood. Recently, researchers have shifted their focus toward identifying specific probiotic strains responsible for this immunomodulatory synergy. Mager et al. reported that oral gavage with *Bifidobacterium pseudolongum*, *Lactobacillus johnsonii*, and *Olsenella* species significantly increased the efficacy of anti-CTLA-4 treatment [227]. Further investigation revealed that the metabolite inosine produced by *Bifidobacterium pseudolongum* could potentiate the differentiation of TH1 cells, and this differentiation promoted the infiltration of IFN-γ^+ $CD4^+$ and IFN-γ^+ $CD8^+$ T cells, effectively inhibiting tumor progression when combined with anti-CTLA-4 therapy [227]. Similarly, Tanoue et al. isolated 11 low-abundance bacterial strains from the feces of healthy individuals, seven of which belong to the phylum *Bacteroidetes*. When these strains cocolonized the gut of mice after oral gavage, they induced IFN-γ^+ $CD8^+$ T cells with cytotoxic functionality. Such immune activation enhances resistance to pathogenic infection and significantly increases the antitumor efficacy of immune checkpoint inhibitors [228]. In addition, the oral administration of *Lactobacillus gallinarum* increased the efficacy of anti-PD-1 antibody therapy in a CAC mouse model. This effect was achieved by reducing the infiltration of $Foxp3^+$ $CD25^+$ Tregs in tumors and enhancing the effector functions of $CD8^+$ T cells, contributing to more robust antitumor immune responses (Figure 8) [229].

In addition, gut microbiota dysbiosis is a critical factor contributing to the side effects of chemotherapy in patients with CRC [230]. Probiotic-based therapies represent a promising strategy to regulate the gut microbiota, potentially alleviating the severe side effects of these therapeutic modalities. The oral administration of 20Bi® (Eurofarma, Brazil), a commercially available probiotic mixture, was shown to increase the efficacy and safety of 5-FU in a rat model of CRC induced by 1,2-dimethylhydrazine [231]. Similarly, the probiotic drug BIO-THREE has been

shown to mitigate intestinal damage caused by oxaliplatin in both preclinical mouse models and human patients. This effect was mediated in part by restoring the abundance of beneficial gut bacteria such as *Bacteroides* and *Prevotella* [232]. In addition to mitigating chemotherapy-induced toxicity, specific probiotic strains have been shown to alleviate related complications. For example, oral supplementation with *Lactobacillus casei* variety *rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* alleviated chemotherapy-related diarrhea in mouse models by decreasing the mRNA expression of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 [233]. Furthermore, a randomized, single-blind, placebo-controlled prospective study demonstrated that the postoperative administration of probiotics to CRC patients attenuated the GI complications and gut microbiota dysbiosis induced by chemotherapy (ChiCTR2000040916). This intervention promoted the production of SCFAs, particularly increasing the levels of acetate, butyrate, and propionate, which are beneficial for gut health and recovery [234].

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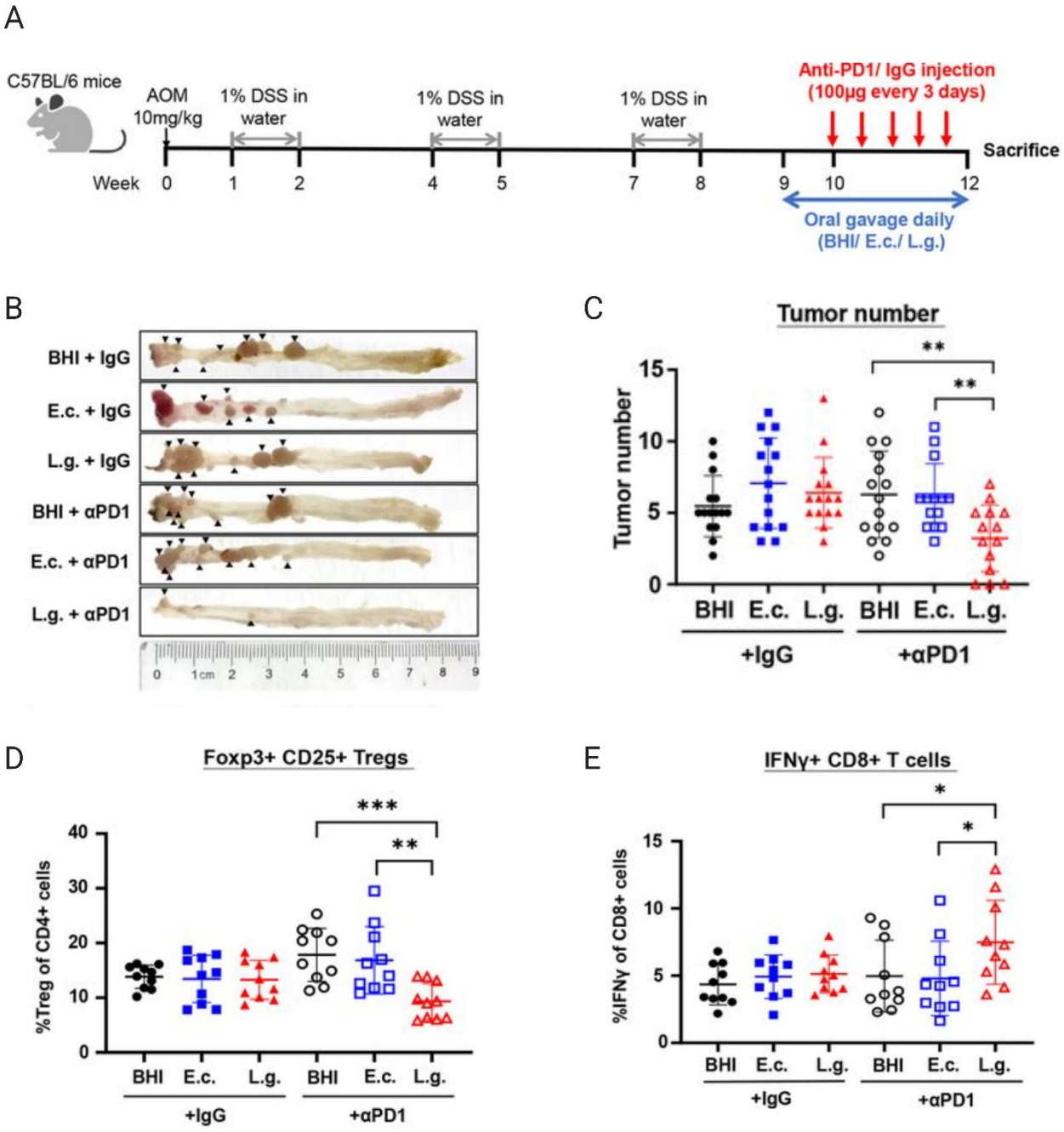


Figure 8. Oral *Lactobacillus gallinarum* increases the efficacy of anti-PD1 therapy in CRC. (A) Schematic of how oral *Lactobacillus gallinarum* improves the efficacy of anti-PD1 injection therapy in AOM/DSS-induced CRC. (B) Representative images of the colon tissues of AOM/DSS-induced CRC mice receiving different treatments. (C) Tumor numbers in AOM/DSS-induced CRC mice after different therapies. (D) Percentages of $Foxp3^+$ $CD25^+$ Tregs in colon tumor tissues

after different treatments. (E) Percentages of IFN-γ^+ $CD8^+$ T cells in colon tumor tissues after different treatments. Reproduced with permission from ref. [229].

5.2.3 Genetically engineered probiotic-based therapies

Engineered probiotics with enhanced functionality represent a safe and effective strategy to combine the therapeutic benefits of probiotics with the delivery of adjunct therapies. Common engineered bacteria include *Salmonella*, *Escherichia coli*, and *Lactobacillus*. Through genetic recombination techniques, these bacteria can be modified to carry therapeutic genes or plasmids with specific functions. These genes may encode tumor-specific antigens, immunostimulatory factors, or cytotoxic proteins, thereby contributing to tumor treatment [235]. For example, Zhang et al. developed engineered bacteria expressing the antigen AH1-A5 as a tumor nanovaccine, encapsulated within sodium alginate/chitosan hydrogels for spatiotemporal protection during GI transit. The oral administration of this engineered bacterial hydrogel facilitated the release of antigen peptides, activating $CD4^+$ and $CD8^+$ T cells in mesenteric lymph nodes and significantly increasing antitumor efficacy in a subcutaneous CRC model [236]. Similarly, Griffin et al. engineered a *Lactobacillus lactis* strain to express SagA, a stimulatory molecule that enhances signaling through the innate immune sensor protein NOD2. The oral administration of this SagA-engineered probiotic improved the anti-PD-L1 antitumor response in a subcutaneous CRC murine model [237]. Engineered bacteria have also been used to reshape the metabolic profile of the TME to facilitate immunotherapy. For instance, *Escherichia coli* Nissle 1917 (EcN) was genetically modified to convert ammonia, a metabolite overproduced in the TME, to L-arginine. The oral administration of this engineered strain increased intratumoral arginine availability and the numbers of tumor-infiltrating T cells and exerted synergistic effects when combined with PD-L1-blocking antibodies in a subcutaneous CRC mouse model [238]. Additionally, engineered bacteria can express therapeutic proteins, including immune checkpoint inhibitors. Gurbatri et al. constructed *Escherichia coli* strains capable of producing anti-PD-L1 nanobodies, anti-CTLA4 nanobodies, and granulocyte–macrophage colony-stimulating factor (GM-CSF). Oral administration to $Apc^{Min/+}$ mice led to the selective and robust colonization of adenomas, which resulted in a 50% reduction in the tumor burden and induced immune-mediated tumor cell death (Figure 9) [239].

Genetically engineered probiotics represent a promising strategy for CRC therapy by combining the therapeutic efficacy of probiotics and engineered therapeutic gene expression. However, several challenges remain, including ensuring probiotic stability during GI transit, achieving precise control of gene expression, optimizing colonization efficiency, and maintaining biosafety. For instance, the encapsulation of engineered *Escherichia coli* within a polydopamine-based system can effectively protect the bacteria from gastric acid degradation, significantly increasing their survival rate, and prolonging their retention time in the intestinal tract [240]. Future progress will rely on interdisciplinary integration, leveraging pathological insights to identify novel targets, formulation innovations to improve oral delivery, and advances in synthetic biology to fine-tune gene editing and expression. Taken together, these developments can drive the evolution of precision, oral, genetically engineered, probiotic-based therapies for CRC.

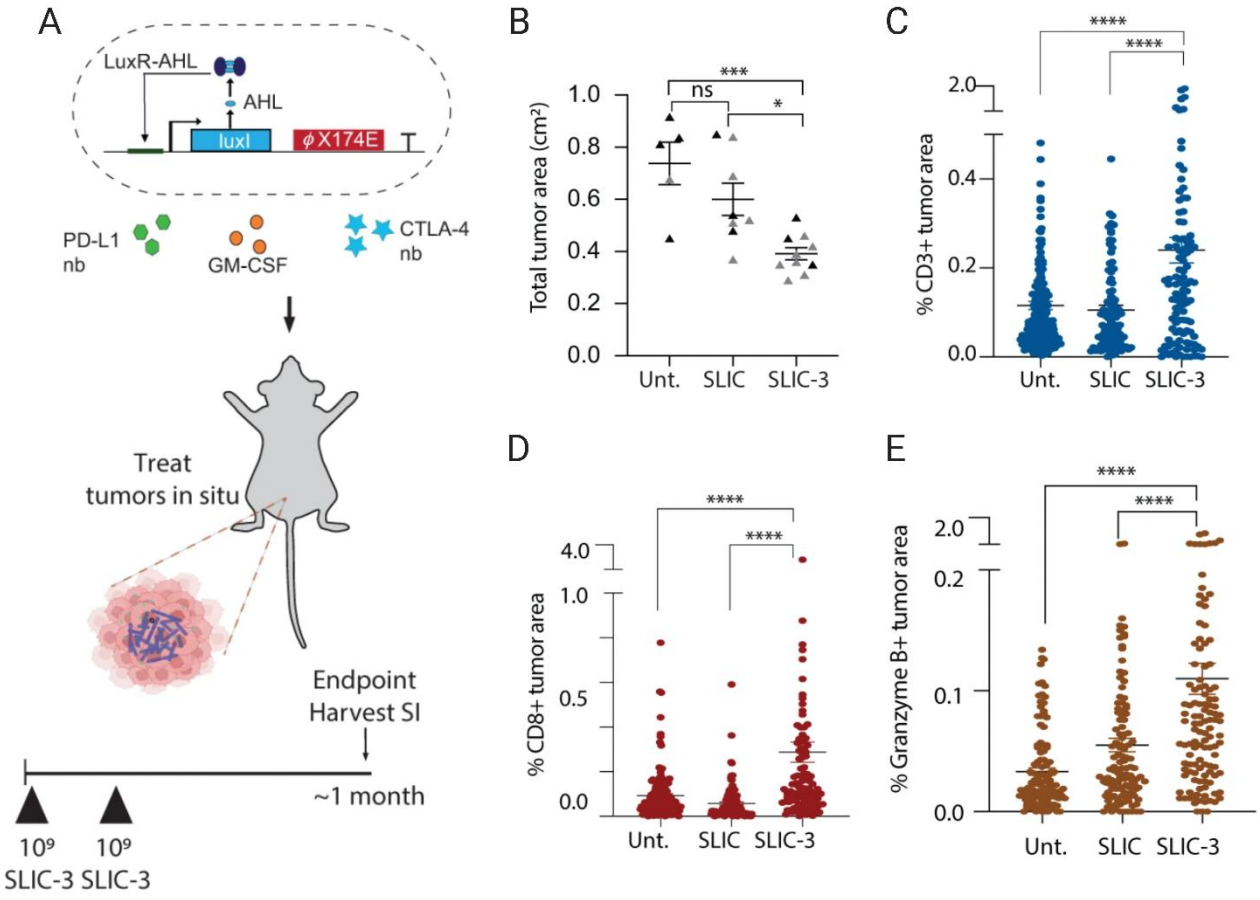


Figure 9. Oral administration of genetically engineered probiotics for CRC treatment. (A) Schematic of the EcN probiotic engineered to express immunotherapeutic proteins (top panel) and oral administration regimens for CRC treatment (bottom panel). (B) Quantification of the overall tumor area in different treatment groups (black: female mice; gray: male mice). (C-E). Quantification of the percentages of $CD3^+$ (C), $CD8^+$ (D) and granzyme B^+ (E) cells in the tumor area. Reproduced with permission from ref. [239].

5.2.4 Prebiotic-based therapies

Prebiotics are defined as substrates selectively utilized by host microorganisms to confer health benefits [241]. Most prebiotics are carbohydrate-based and are predominantly administered orally via dietary intake. Some prebiotics, including inulin, pectin, and dextran, have shown promising therapeutic effects on CRC [242]. Mechanistically, inulin and pectin stimulate the proliferation of beneficial microbial genera (e.g., the *Ruminococcaceae* family), individual bacteria such as Akkermansia muciniphila, and their metabolites, including SCFAs and cyclic diadenosine monophosphate (cyclic di-AMP) [243]. These metabolic products contribute to antitumor immunity. SCFAs increase cytotoxic $CD8^+$ T-cell activity [244, 245], whereas cyclic di-AMP activates STING-dependent type I interferon (IFN) production, stimulating the production of innate immune components such as monocytes, natural killer (NK) cells, and DCs, which in turn increase the responsiveness to immune checkpoint inhibitors [246]. Advances in drug delivery have enabled the development of prebiotics with increased colonic retention. Han et al. developed a colon-retentive inulin-based oral gel that effectively modulates the gut microbiota in situ, promoting the proliferation of commensal microorganisms involved in T-cell immunity. Such modulation facilitated the microbial metabolite-induced differentiation of $CD8^+$ T cells into stem-like memory $CD8^+$ T cells, synergizing with α-PD-1 therapy in a subcutaneous CRC mouse model [245].

Furthermore, prebiotics can encapsulate probiotics and protect them during GI tract transit. Zheng et al. engineered prebiotic-encapsulated *Clostridium butyricum* spores using dextran-based host–guest chemistry [118]. Dextran improved intestinal adhesion and increased fermentation through the production of *Clostridium butyricum* and SCFAs. The spore–dex system increased the abundance of SCFA-producing bacteria, such as *Eubacterium* and *Roseburia*, increasing the overall microbiota richness and shifting the phenotype from protumor to antitumor. In

subcutaneous and orthotopic CRC models, this system inhibited tumor growth by 89% and 65%, respectively. Interestingly, the reduced efficacy in the orthotopic model suggests a potential role of dysbiosis in promoting tumor growth [118]. Wang et al. developed a synbiotic hydrogel capsule by cross-linking dietary inulin with gelatin through hydrogen bonding to encapsulate *Limosilactobacillus reuteri*. This gelatin–inulin matrix markedly improved the gastric acid resistance, GI adhesion, and biological activity of the probiotic, resulting in synergistic antitumor effects. The capsule also restored intestinal barrier integrity, modulated the gut microbiota composition, and promoted SCFA production, thereby further increasing its antitumor efficacy. Notably, the capsule also improved the antitumor effect of 5-FU. This study provides novel insights and experimental evidence for the combined application of probiotics and conventional chemotherapeutic drugs [247].

5.2.5 Pathobiont killing therapies

CRC is associated with the presence of bacteria near the tumor. Scientists have reported the enrichment of protumor pathobionts (e.g., enterotoxigenic *Bacteroides fragilis* and oral anaerobe *Fusobacterium nucleatum*) [36, 37] in CRC patients. These findings highlight the significant potential of pathobiont-targeting strategies for CRC treatment via microbiota modulation.

Din et al. engineered a clinically relevant bacterium capable of synchronous lysis at a threshold population density, thereby releasing genetically encoded cytotoxic agents—a technique termed the synchronous lysis circuit (SLC) strategy [248]. Combining this approach (oral administration of an engineered bacterium) with chemotherapy (intraperitoneal injection of 5-FU) significantly reduced tumor growth and prolonged the survival of model mice with syngeneic hepatic colorectal metastasis transplantation [248]. Recently, Zhang et al. encapsulated engineered EcN within chitosan–sodium alginate microcapsules to develop an SLC nanovaccine for CRC treatment. These microcapsules protected the bacteria from extreme gastric acid conditions and facilitated targeted release in the colon. Subsequent SLC activation regulated the expression and release of recombinant antigens, enabling periodic antigen delivery. This system stimulated potent mucosal and systemic immune responses, achieving a tumor inhibition rate of 75% and a twofold increase in the T-cell response in a subcutaneous CRC mouse model [236]. These findings highlight the potential of SLC-based strategies for antitumor therapy and introduce the idea of limiting bacterial

growth under a threshold population density during repeated growth cycles for antitumor treatment. However, it also highlights challenges, such as nonselective bacterial lysis. This approach could inadvertently eliminate both protumor and antitumor bacteria, potentially impeding probiotic colonization in the colon. Among the identified pathobionts, *Fusobacterium nucleatum* is well known for its significant role in CRC progression. While *Fusobacterium nucleatum* is commonly present in the oral cavity, it is rarely detected in the lower GI tract of healthy individuals but is enriched in CRC tumors, where it contributes to tumorigenesis [249]. Phages and antibiotics have been applied to kill *Fusobacterium nucleatum and* increase sensitivity to chemotherapy and/or immunotherapy. Zheng et al. isolated *Fusobacterium nucleatum*-targeted phage strains from human saliva and covalently linked them to irinotecan-loaded dextran nanoparticles [250]. This hybrid nanosystem specifically accumulated in CRC tumors after oral administration, effectively eliminating intratumoral *Fusobacterium nucleatum,* promoting the proliferation of endogenous *Clostridium butyricum,* increasing colonic SCFA levels, and increasing chemotherapeutic efficacy in orthotopic CRC tumors or spontaneously formed colorectal tumors in $Apc^{Min/+}$ mice [250]. Owing to the diversity of pathogenic bacteria associated with CRC, in addition to *Fusobacterium nucleatum*, other bacterial species can also serve as targets for phage-based antitumor therapy. Studies have revealed that *Bacteroides fragilis* is enriched in nonresponders to chemotherapy. Ding et al. employed the *Bacteroides fragilis*-specific phage VA7, which effectively cleared bacteria and restored sensitivity to chemotherapeutic treatment [251]. Antibiotics, such as silver ions, have long been utilized for their antibacterial effects [252]. Silver nanoparticles (Ag NPs) have been developed for improved targeting and retention in antitumor treatment [253]. Many studies have investigated the efficacy of Ag NPs for CRC treatment in vitro, including Ag/PVP nanoparticles [254], Ag/*Lactobacillus rhamnosus* GG nanoparticles [255], Ag NP-loaded sodium alginate and β-cyclodextrin hydrogel beads [256], and white tea Ag NPs [257]. Future modifications of these nanoparticles for oral delivery and in vivo efficacy studies could advance silver-based therapies for CRC treatment. Notably, the lack of specificity of silver may also eliminate beneficial antitumor bacteria residing in the colon or within the TME. Dong et al. constructed a phage-based bioinorganic hybridization system for targeted clearance of *Fusobacterium nucleatum* to remodel the TME [258]. They screened a specific *Fusobacterium nucleatum*-binding phage strain using an M13 filamentous phage display library [258]. They then assembled Ag NPs on the surface of the selected phages for enhanced antibacterial efficacy [258]. After oral administration, Ag NPs

selectively killed *Fusobacterium nucleatum* with the targeting function of the phages [258]. In addition, highly immunogenic phages induced DC maturation and decreased MDSC amplification at tumor sites, reversing the immune-suppressive TME [258]. When combined with immune checkpoint inhibitors (e.g., anti-PD1 antibodies) or chemotherapeutics (e.g., FOLFIRI), this strategy significantly inhibited tumor progression and prolonged overall survival in model mice bearing orthotopic CRC tumors (Figure 10) [258]. These findings highlight the promising potential of phage-based targeting of pathobionts as a novel anticancer strategy. Nevertheless, this approach faces crucial challenges that must be addressed before clinical translation. These include the variable abundance of *Fusobacterium nucleatum* among patients, the complex manufacturing and stability issues inherent to living biologic products such as phages, and the difficulties in defining appropriate clinical endpoints. Future research must carefully consider and overcome these hurdles.

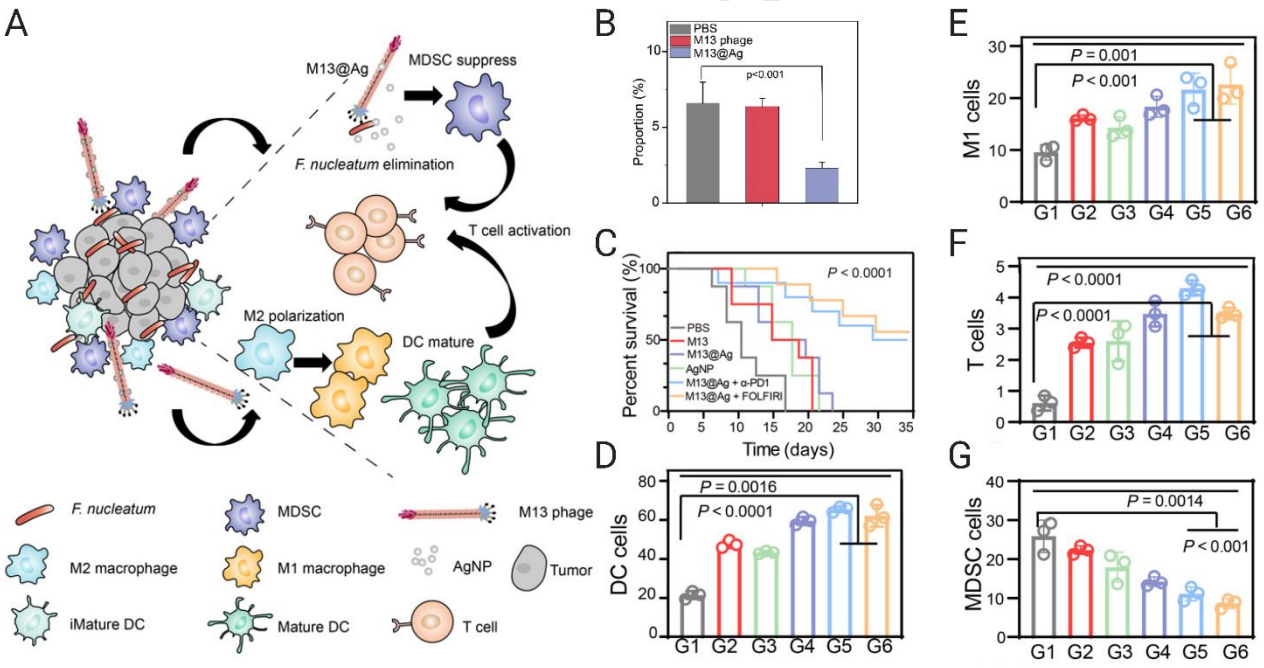


Figure 10. Oral administration of a bioinorganic hybrid bacteriophage for CRC treatment. (A) Schematic of the phage-based bio/abiotic hybrid system (M13@Ag) for the regulation of gut microbes in CRC-specific immune therapy. (B) qPCR analysis of *Fusobacterium nucleatum* levels in fecal samples from *Fusobacterium nucleatum*-colonized CRC model mice after various treatments. (C) Survival curves of orthotopic tumor-bearing mice after various treatments. (D-G). Orthotopic CT26-luc tumors were collected from mice at 14 days after treatment. Representative

flow cytometry analysis showing the relative quantification of immune cells infiltrating the tumor tissues, as indicated. (D) Maturation of double-positive $CD80^+$ $CD86^+$ DCs (gated on $CD11c^+$ cells). (E) TAMs of the classically activated M1 phenotype with high CD86 expression (gated on $CD11b^+$ cells). (F) The level of tumor-infiltrating $CD8^+$ cytotoxic T cells (gated on $CD3^+$ cells). (G) Percentage of $CD11b^+$ $Gr\text{-}1^+$ MDSCs among total $CD45^+$ lymphocytes. G1, PBS; G2, M13; G3, AgNP; G4, M13@Ag; G5, M13@Ag + α-PD1; G6, M13@Ag + FOLFIRI. Reproduced with permission from ref.[258].

Although microbiota-based intervention strategies hold significant therapeutic potential for CRC, their clinical translation remains constrained by several key limitations (Table 4). Currently, these approaches are largely confined to preclinical or early-phase clinical investigations, with major challenges including: (1) unstable efficacy and substantial interindividual variability, wherein the high heterogeneity of gut microbial composition often results in unpredictable treatment responses[198, 259, 260]; (2) safety concerns, particularly the potential risks of opportunistic infections, exacerbated inflammation responses, or horizontal gene transfer (especially with engineered bacterial strains) in immunocompromised patients [23]; (3) formulation and manufacturing difficulties, such as maintaining viability and achieving scalable, standardized production; and (4) inadequate mechanistic understanding and undefined regulatory pathways, as the modes of action for many microbial therapies are not fully understood, and standardized clinical evaluation criteria and regulatory frameworks are still lacking. These constraints significantly hinder their clinical translation and broad application, necessitating urgent resolution through interdisciplinary collaboration and technological innovation.

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Table 4. Summary of bacteria-mediated oral strategies for CRC.

Modulation strategies	In vivo performance	Advantages	Limitations
FMT	Enhanced antitumor immune response and improved sensitivity to PD-1 blockade.	-Demonstrated promising effects in clinical and preclinical studies.	-Safety concerns: potential transmission of pathogens or undesired microbes -Donor variability. -Inadequate mechanistic understanding and undefined regulatory pathways.
Probiotic-based therapies	Preventive and therapeutic effects; activation of antitumor immunity; augmentation of immune checkpoint inhibitor efficacy; alleviation of chemotherapy-induced dysbiosis	-Showed promising effects in many aspects for CRC, including prevention, treatment and management of side effects.	-Limited colonization and survival. -Limited robust and large-scale clinical trials.
Genetically engineered probiotic-based therapies	Probiotics expressing therapeutic agents enhanced responses to immune checkpoint blockade therapies.	-Synergizes probiotic benefits with therapeutic gene expression.	-Limited colonization and survival.

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			-Risk of horizontal gene transfer; safety concerns in immunocompromised patients. -Challenging to achieve precise delivery of therapeutic molecule to the tumor site. -Limited clinical trials.
Prebiotic-based therapies	Enhanced antitumor immunity; increased response to chemotherapy and immunotherapy in CRC models.	-Favorable safety profile compared to live bacteria. -Easier formulation and administration compared to live bacteria.	-Requires high dosage, which may be difficult to achieve in the colon. -Limited clinical evidence.
Pathobiont killing therapies	Enhanced mucosal and systemic immune response. Increased the efficacy of chemotherapy and immunotherapy.	-Selectively eliminates pro-tumorigenic bacteria. -Enhanced the efficacy of chemotherapy and immunotherapy.	-Nonspecific killing may disrupt beneficial antitumor microbiota. -Bacteria may evolve mechanisms to evade killing. -Limited clinical evidence.

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5.3 Synergistic strategies combining nanomedicine and bacterial modulation

Both nanomedicine and bacterial modulation have demonstrated promising therapeutic outcomes in the treatment of CRC. Nanomedicines, by virtue of their precise targeting, controlled release, and efficient delivery, significantly increase drug accumulation at tumor sites while reducing toxicity to normal tissues. Bacterial modulation, on the other hand, can impact gut microbiota dysbiosis, ameliorate the TME, regulate immune responses, and influence microbial metabolism. A synergistic combination of these two approaches is expected to yield enhanced therapeutic efficacy against CRC.

The oral administration of probiotics has demonstrated promising effects in CRC patients at multiple levels. However, the challenges associated with oral probiotics in vivo are important to note. Effective therapeutic outcomes require a high daily dosage of 10^6–10^9 viable cells, which are often compromised by the harsh GI environment. The acidic gastric fluids, digestive enzymes, and bile salts in the small intestine can degrade probiotics, limiting their survival, colonization of the colon, bioavailability, and antitumor efficacy. Advanced oral delivery technologies represent a potential solution, as they can increase the resistance of probiotics in the harsh GI environment and improve their mucoadhesive properties. Such modifications aim to increase oral bioavailability and facilitate intestinal colonization. Protective strategies include coating probiotics with acid- and enzyme-resistant or colon-targeting materials and modifying them with mucoadhesive groups. For instance, the encapsulation of engineered *Escherichia coli* with chitosan and alginate can protect the bacteria from the extremely acidic conditions in the stomach and facilitate their colonization of the intestine, thereby enabling targeted antitumor immunotherapeutic effects [236]. While research in this area for CRC treatment remains limited, insights can be drawn from analogous studies of treatments for IBD, considering the disease's location and associated dysbiosis. Examples include coating probiotics with acid- and enzyme-resistant polymers and colon-targeting polymers, such as multilayer coatings composed of alginate and chitosan [261]; conjugating probiotics with protective metal–organic frameworks [262]; and leveraging resistant biofilms, which are bioadhesive and protect against external threats [263]. Strategies such as coating probiotics with mucoadhesive polymers (e.g., HA) [191] or chemically modifying bacterial surfaces (e.g., converting primary amino groups to free thiols) [264] have also been explored to promote mucoadhesion. The application of these strategies specifically for oral CRC treatment remains

underexplored. Future work leveraging these advanced oral delivery technologies to facilitate the development of probiotic-based CRC therapies is promising and warrants further investigation.

Prebiotics can function as polymers to form nanoparticles, providing dual benefits in terms of microbiota modulation and efficient cargo delivery. Prebiotics such as chitosan, dextran, and pectin resist digestion by enteric enzymes [265], remaining intact through the upper GI tract and undergoing fermentation in the colon. This characteristic enables colon-targeted delivery [14]. Moreover, their improved safety profiles compared with those of other polymers make them ideal for drug delivery systems. For example, Ma et al. synthesized aspirin-conjugated dextran via click chemistry, which self-assembled into nanoparticles [266]. After oral administration, these nanoparticles increased drug accumulation in CRC tumor tissues, enabling the controlled release of aspirin and dextran. This system restored microbiota homeostasis, significantly increasing the abundance of anticancer microbes, such as *Lactobacillus* (6.66-fold) and *Akkermansia* (103-fold), while reducing the abundance of pathogen-associated microbes, including *Bacteroides*. In an orthotopic CRC mouse model, this nanosystem demonstrated a tumor suppression rate 2.1 times greater than that of free aspirin, achieving an overall suppression rate of 85% [266]. Physiochemical modification of prebiotics could further expand their utility in drug delivery. Lipid conjugation, for example, facilitates the oral delivery of lipophilic drugs. Xylan is a polysaccharide prebiotic with excellent water solubility, biocompatibility, and prebiotic activity [267]. It remains intact in the upper GI tract and is degraded exclusively by microbes in the lower GI tract—a property that makes it highly suitable for colon-targeted drug delivery. Based on this characteristic, Lang et al. synthesized a xylan–stearic acid conjugate (SCXN) for capecitabine delivery. The oral administration of SCXN delayed drug clearance, increased intratumoral drug concentrations and promoted probiotic proliferation and SCFA production in an orthotopic CRC mouse model. Compared with free capecitabine, SCXN significantly enhanced antitumor immunity, increasing the tumor inhibition rate from 5.29% to 71.78%, while exhibiting excellent biocompatibility and extending the median survival from 14 to 33.5 days (Figure 11) [267].

Leveraging the ability of phages and antibiotics to eliminate pathogenic bacteria, their combination in nanomedicine offers a promising strategy for targeted drug delivery [198]. Zheng et al. covalently conjugated phages with irinotecan-loaded dextran nanoparticles via bioorthogonal chemistry to construct a phage-mediated nanodrug delivery system. Animal studies have

demonstrated that the phages specifically accumulated in tumors colonized by *Fusobacterium nucleatum*, thereby facilitating the targeted enrichment of nanodrugs within tumor tissues. By inhibiting the proliferation of *Fusobacterium nucleatum*, this approach effectively counteracted chemoresistance in colorectal tumors and significantly enhanced the efficacy of chemotherapy [250].

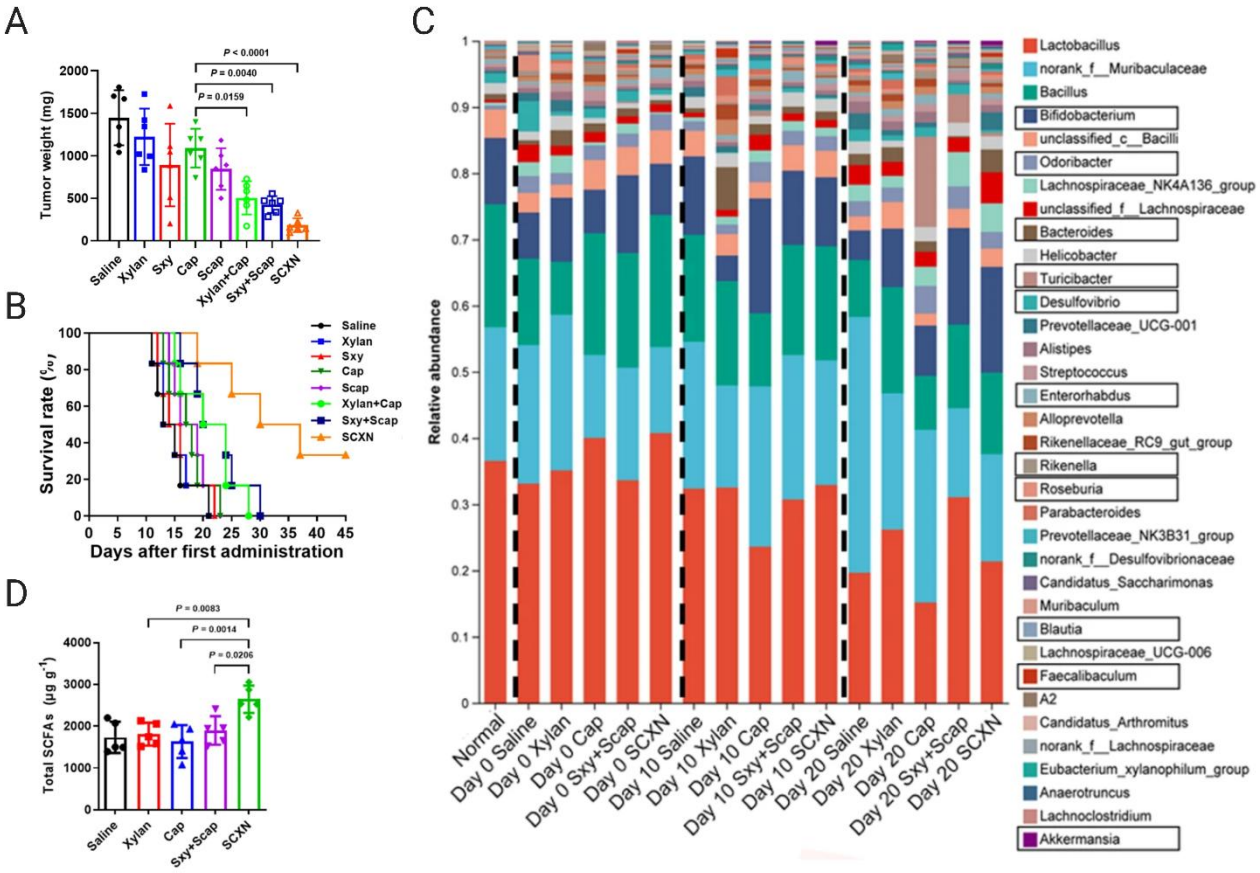


Figure 11. Oral prebiotic-based therapies for CRC treatment. (A) Average weights of tumors from MC38 tumor-bearing mice on day 21 after multidose treatment with different formulations. (B) Survival curves of CT26 tumor-bearing mice 45 days after the first administration. (C) Bar plot showing the relative abundance of various microbes in the gut at the genus level. (D) Summary of SCFA levels in feces from CT26 tumor-bearing mice after different treatments. Reproduced with permission from ref. [267].

6. Future perspectives

Although considerable progress has been made in the development of advanced oral therapies for CRC treatment, as described above, this field remains relatively underexplored. Current oral formulations continue to face major challenges in clinical translation, including unreliable therapeutic efficacy, poor stability, significant interpatient variability, complex manufacturing processes, difficult regulatory pathways, and potential safety risks [268-270]. This limited progress could be attributed to the significant barriers posed by the harsh GI environment and limited targets currently exploited. Overcoming these obstacles through future research would significantly advance the development and refinement of clinical treatment strategies for CRC. Valuable strategies can be drawn from the treatment of IBD, which shares the same anatomical location (the colon) and several pathophysiological features as CRC, to make progress in this area. For example, colon-targeted strategies have long been employed in IBD and have demonstrated safety and efficacy in clinical applications for decades [14]. Adapting these established systems for CRC treatment represents a compelling route to improve local drug delivery via oral administration. Moreover, several stimulus-responsive strategies, such as ROS-responsive systems, have shown promise as oral delivery strategies for IBD treatment [190-192]. Considering the elevated ROS levels present in the TME of CRC [137], the use of these strategies for oral advanced therapies in CRC treatment could yield promising results. Nanomedicine and bacterial modulation strategies utilize distinct delivery systems and therapeutic mechanisms, each possessing unique advantages and limitations (Tables 3 and 4). The synergistic integration of these two approaches has established a highly promising therapeutic modality. This combined strategy not only enables precise colon-targeted delivery of bacterial or pharmaceutical agents but also simultaneously regulates microbiota homeostasis and remodels the immune microenvironment, thereby significantly enhancing antitumor efficacy [236, 240, 266, 267].

Immunotherapy is a cutting-edge approach to cancer treatment and has revolutionized the treatment of immunogenic tumors such as those with melanoma and certain types of lymphoma [271]. Oral immune checkpoint blockade represents a paradigm-shifting advance in CRC treatment. Traditionally, immune checkpoint inhibitors such as anti-PDL1 antibodies have relied on intravenous injection, which can lead to systemic side effects and impose invasive administration,

high cost, and compliance challenges. Recent advances in oral delivery systems demonstrate the feasibility of oral anti-PD-L1 therapy, achieving effective mucosal absorption and antitumor efficacy comparable to those of intravenous injections in preclinical CRC models [104, 203]. This breakthrough broadens the accessibility of immunotherapy and highlights the unique potential of oral administration to modulate the tumor immune microenvironment through local treatment. Notably, approximately 85% of CRC cases are MSS subtypes characterized by low immunogenicity, which substantially limits the efficacy of immunotherapies in these patients [25, 272]. Future efforts could focus on activating both innate immunity, such as activation of the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway [273], and adaptive immunity, such as blocking immune checkpoints [274], to increase immune system activity. Moreover, combining immune activation with oral delivery strategies to overcome GI barriers may enhance both local and systemic antitumor responses.

Furthermore, the emergence of innovative oral delivery devices, such as autoinjector capsules [275], omnidirectional adhesive microneedles [276], and triggerable tough hydrogels [277], has led to significant progress in systemic delivery via the oral route. Although their application in colon-targeted delivery remains unexplored, they provide new possibilities for local antitumor treatment via the oral route. Future endeavors combining these innovative devices with advanced oral nanotechnology and/or microbiota modulation provide great promise for the oral treatment of CRC.

7. Conclusions

The increasing global burden of CRC underscores the urgent need for innovative treatment strategies that address the limitations of conventional therapies. Oral drug delivery represents a compelling solution, offering a noninvasive alternative that improves patient compliance while minimizing systemic side effects. The unique pathophysiological features of CRC further support the potential of oral therapy for local antitumor treatment. Recent advancements in drug delivery technologies, particularly nanomedicine-based systems, aimed at overcoming these barriers after oral administration have significantly improved the antitumor efficacy of a variety of drugs delivered orally. Additionally, the emerging field of microbiota-modulating therapies provides a novel approach to harness the microbiota to improve therapeutic outcomes and reduce the side effects of chemotherapies and immunotherapies. Continued interdisciplinary research, such as a

combination of nanomedicine- and microbiota-based therapies to facilitate the oral delivery of probiotics, will be key to advancing therapeutic efficacy. With these efforts, advanced oral therapies may transform the landscape of CRC treatment, offering therapeutic options that are more friendly, effective, and personalized for patients.

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Declaration of Interest Statement

Yining Xu and Ana Beloqui are coinventors on a patent (application filed by the Université catholique de Louvain: PCT/EP2020/064766). Ana Beloqui has served within the past 12 months as a consultant to Johnson & Johnson.

Declaration of the use of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to improve the readability and language of the work. After using this tool/service, the authors reviewed the content and take full responsibility for the content of the publication.

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Declaration of Interest Statement

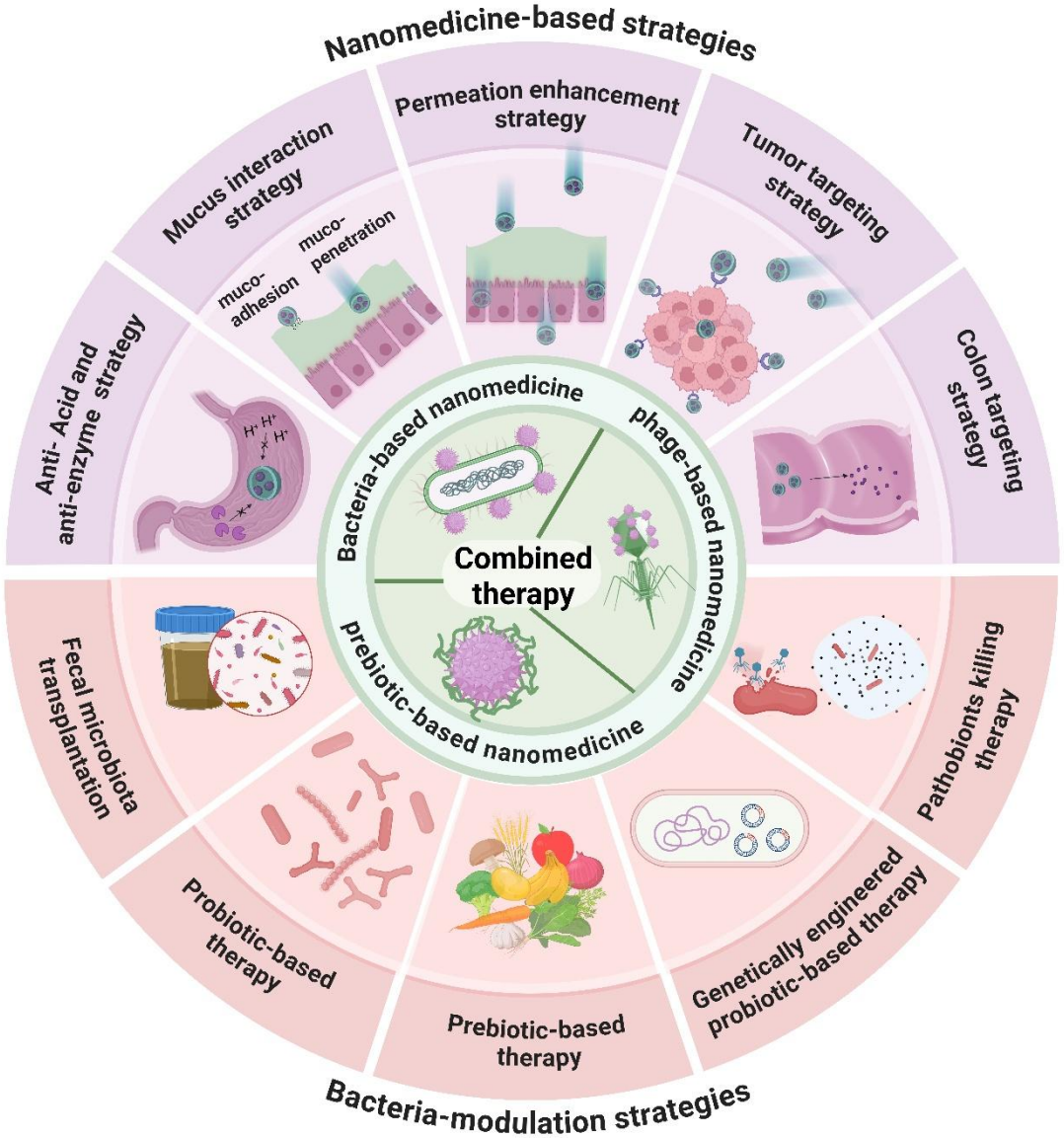
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☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Y.X. and A.B. are coinventors on a patent (application filed by the Université catholique de Louvain: PCT/EP2020/064766). A.B. has served within the past 12 months as a consultant to Johnson & Johnson.

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