

Targeted nanoparticles for colorectal cancer

Colorectal cancer (CRC) is highly prevalent worldwide, and despite notable progress in treatment still leads to significant morbidity and mortality. The use of nanoparticles as a drug delivery system has become one of the most promising strategies for cancer therapy. Targeted nanoparticles could take advantage of differentially expressed molecules on the surface of tumor cells, providing effective release of cytotoxic drugs. Several efforts have recently reported the use of diverse molecules as ligands on the surface of nanoparticles to interact with the tumor cells, enabling the effective delivery of antitumor agents. Here, we present recent advances in targeted nanoparticles against CRC and discuss the promising use of ligands and cellular targets in potential strategies for the treatment of CRCs.

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Colorectal cancer (CRC) is the fourth most widely diagnosed cancer worldwide and manifests as a malignant neoplasm in the mucosa of the colon or the rectum [1,2]. Based on the progression of cancer cells, the American Joint Committee on Cancer (AJCC) has classified CRC into five stages (Figure 1). Stage 0 is considered 100% cured after a surgical resection. The standard treatment for stages I–IIc is also surgical resection, with 5-year survival in the range of 37–74%. Patients diagnosed in advanced stages (stages IIIA–IV) receive adjuvant chemotherapy following surgical resection, but their survival rate decreases to 6% due to the high risk of metastasis and the recurrence (Table 1) [3,4].

A high cellular heterogeneity characterizes CRC due to several genetic and biological alterations, which are responsible for the high variability between of tumors [5]. Despite the broad repertoire of biomarkers of CRC described later as targets for advanced

nanoparticles; recently, a tremendous effort by the Cancer Genome Atlas Network has been made for characterizing the molecular genetics of CRC in 224 samples [6]. By genome sequence analysis, the samples were classified into nonhypermutated and hypermutated types of cancer. Among the nonhypermutated tumors, the most frequently mutated genes were *APC* (81%), *TP53* (60%), *KRAS* (31%), *TTN* (31%), *PIK3CA* (18%), *FBXW7* (11%) and *SMAD4* (10%), etc.; whereas the most commonly hypermutated tumors were *CVR2A* (63%), *APC* (51%), *TGFBR2* (51%), *MSH3* (40%) and *MSH6* (40%) among others [6]. Nonhypermutated tumors (~84%) exhibited a high frequency of DNA somatic copy number alterations with a microsatellite stable, and hypermutated tumors (~16%) showed either microsatellite instability due to defective mismatch repair (~13%) or DNA polymerase epsilon proof-reading mutations (~3%) [7]. The integrative analysis of the molecular genetics of CRC

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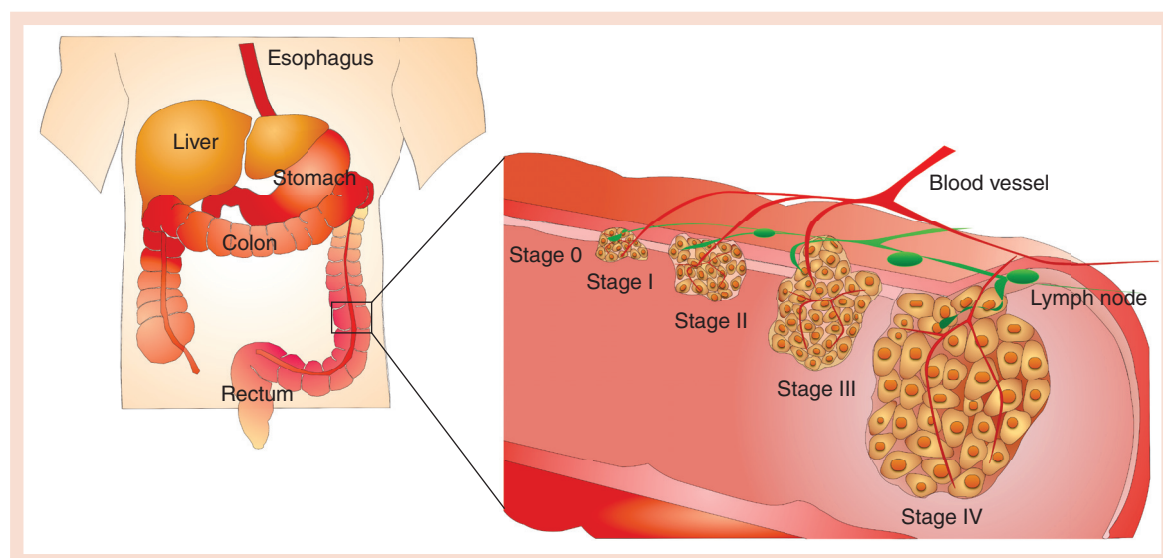


Figure 1. Stages of colorectal cancer according to the American Joint Committee on Cancer. Stage 0; tumor confined to mucosa (carcinoma *in situ*). Stage I: tumor invades submucosa and muscularis propria. Stage II: tumor invades through the muscularis propria into pericolorectal tissues (IIA), then penetrates to the surface of the visceral peritoneum (IIB), then directly invades or is adherent to other organs or structures (IIC). Stage III: tumor invades muscularis propria with metastases in 1–3 regional lymph nodes or nearby tissue, or invades submucosa with metastases in 4–6 regional lymph nodes (IIIA). Then tumor penetrates to the surface of the visceral peritoneum with metastases in 1–3 regional lymph nodes or nearby tissue, or invades through the muscularis propria into pericolorectal tissues with metastases in 4–6 regional lymph nodes, or invades muscularis propria with metastases in 7 or more regional lymph nodes (IIIB). Then tumor penetrates to the surface of the visceral peritoneum with metastases in 4–6 regional lymph nodes, or invades through the muscularis propria into pericolorectal tissues with metastases in 7 or more regional lymph nodes, or directly invades or is adherent to other organs or structures with metastases in one or more regional lymph nodes (IIIC). Stage IV: metastasis confined to one organ or site (e.g., liver, lung, ovary, nonregional node).

provides many insights into the biology of CRC and identifies potential therapeutic targets.

Adjuvant chemotherapy, usually used in stages III–IV, includes a variety of chemotherapeutics intended to slow tumor growth and improve life expectancy. Despite the highly efficient chemotherapeutic agents used to treat CRC, their low specificity often produces a range of dose-limiting side effects including hair loss, nausea and vomiting [8]. In an effort to minimize side effects, current therapeutic protocols involve the coadministration of different chemotherapeutic agents in a series of cycles. The number of doses, frequency and duration of cycles depend on the needs and general state of each patient [9]. In addition, fluctuations in plasma levels of drugs over chemotherapeutic cycles can encourage the development of drug resistance in tumor cells [10]. When added to the high financial cost of treatment, such interventions can significantly decrease quality of life for CRC patients [11,12].

Over the last few years, several types of drug-loaded nanoparticles in the size range of 20–400 nm (i.e., liposomes, dendrimers, polymeric nanoparticles and micelles) have made a strong impact on drug delivery for chemotherapy [13–16]. In fact, such systems

are among the most promising developments in nanomedicine, which has grown exponentially: from simple nanoparticles loaded with drugs to multifunctional nanoparticles targeted to specific cancer cells through binding to unique cell-surface proteins [17–19]. Targeted nanoparticles exploit antigens differentially expressed on the surface of cancer cells, such as integrin [17,20] and folic acid receptors [21,22] and a number of such nanoparticles are currently undergoing clinical development [23]. There have been major advances in the use of nanoparticles as therapeutic platforms for the treatment of prostate [24,25], ovarian [26], breast [27,28] and lung cancers [29–31]. Nevertheless, despite the high morbidity and mortality associated with CRC, the clinical development of nanoparticles for treatment remains limited. In this review, we describe the state of the art in nanoparticles for CRC and discuss the tools available for future applications of such therapeutic strategies.

Current adjuvant chemotherapy against colorectal cancer

As stated above, much early-stage CRC is potentially curable by surgical resection [32]. Although adjuvant

Table 1. Stages, treatment options and survival rate for colorectal cancer.		
Stage (TNM criteria)	Standard treatment option	5-year observed survival rate
Stage 0	Surgical resection	Considered curative
Stage I	Surgical resection	74%
Stage IIA	Surgical resection	67%
Stage IIB	Surgical resection	59%
Stage IIC	Surgical resection	37%
Stage IIIA	Surgical resection Adjuvant chemotherapy	73% [†]
Stage IIIB	Surgical resection Adjuvant chemotherapy	46% [†]
Stage IIIC	Surgical resection Adjuvant chemotherapy	28%
Stage IV – liver metastasis	Surgical resection Local ablation Neoadjuvant chemotherapy Intra-arterial chemotherapy Adjuvant chemotherapy	6%
Stage IV and recurrent CRC cancer	Surgical resection Adjuvant chemotherapy	6%

[†]In this study, survival was better for some stage III cancers than for some stage II cancers. The reasons for this are not clear.
CRC: Colorectal cancer; TNM: Tumor, Node, Metastasis.
Data taken from [3,4].

chemotherapy clearly benefits patients with stages III and IV disease, its use in stage II is not usually indicated because of the curative effects of resection [33,34]. For many years, the only cytotoxic drug used in adjuvant chemotherapy for treatment of CRC was fluoropyrimidine 5-fluorouracil (5-FU), an analog of thymine that inhibits DNA replication [35]. Due to the variable gastrointestinal absorption of 5-FU, its preferred route of administration is intravenous (iv.) [36]. In addition, the discomfort associated with iv. administration of 5-FU prompted the development of more effective and less expensive oral formulations, which can be classified into three groups: 5-FU prodrugs such as Tegafur and Capecitabine, 5-FU prodrugs combined with dihydropyrimidine dehydrogenase inhibitor and 5-FU combined with a dihydropyrimidine dehydrogenase inhibitor [37].

Since the early 2000s, other drugs have also come into use. For example, irinotecan inhibits the enzyme topoisomerase I, hindering the uncoiling of DNA during replication [38]; and oxaliplatin forms cross-linking DNA, preventing transcription and replication [39]. However, in addition to cancer cells, cytotoxic drugs also kill healthy cells that grow and divide quickly such as white blood cells, red blood cells and platelets; for this reason some of these cytotoxic drugs are administered with leucovorin, a vitamin that strength-

ens the production of blood cells and improves treatment efficiency [40]. The drug combinations currently used in adjuvant chemotherapy for CRC are presented in Table 2. Another family of therapeutic agents for treatment of CRC are monoclonal antibodies, which can be directed against molecules on the surface or in the environment of tumor cells [41]. Two monoclonal antibodies are licensed for use in humans: bevacizumab binds to VEGF-A, which inhibits the formation of blood vessels, reducing tumor vascularization and inhibiting tumor growth [42,43]; and cetuximab binds to the extracellular domain of the EGFR to block ligand-induced receptor signaling [44].

Since its approval, the combination of monoclonal antibodies with cytotoxic drugs has become first-line treatment for CRC, extending both progression-free survival and overall survival [42,58–61]. However, despite the improvements in treatments involving adjuvant chemotherapy and biological agents, drug resistance remains a major challenge and general side effects (e.g., fatigue, hair loss, nausea and vomiting, diarrhea or constipation, anemia, immunosuppression and bleeding) have prompted researchers to explore advanced strategies based on nanotechnology, either to improve the pharmacological properties of classic chemotherapeutics or to specifically target tumor tissue and reduce side effects.

Table 2. Adjuvant chemotherapy for colorectal cancer.

A. Drug combinations in adjuvant chemotherapy for stage III colorectal cancer		
Regimen	Scheme	Ref.
FOLFOX4 regimen	Oxaliplatin (85 mg/m ²) administered iv. as a 2-h infusion on day 1; leucovorin (200 mg/m ²) administered iv. as a 2-h infusion on day 1 and day 2; followed by a loading dose of 5-FU (400 mg/m ²) bolus administered iv., then 5-FU (600 mg/m ²) administered iv. as a 22-h continuous infusion on day 1 and day 2, repeat every 2 weeks	[45,46]
FU/levamisole regimen	5-FU (450 mg/m ²) bolus administered iv. daily for 5 days, then weekly 28 days later plus levamisole (50 mg) administered orally three-times/days for 3 days, repeat every 2 weeks	[47]
Mayo Clinic or North Central Cancer Treatment Group (NCCTG) regimen	5-FU (450 mg/m ²)-leucovorin (20 mg/m ²) bolus administered iv. daily for 5 days, repeat every 4 weeks	[48]
Roswell Park Memorial Institute (RPMI) or National Surgical Adjuvant Breast and Bowel Project (NSABP) regimen	5-FU (500 mg/m ²)-leucovorin (500 mg/m ²) bolus administered iv. weekly for 6 weeks, repeat every 8 weeks	[49]
B. Drugs combinations in adjuvant chemotherapy for stage IV colorectal cancer		
Regimen	Scheme	Ref.
FOLFOX4 regimen	Oxaliplatin (85 mg/m ²) administered iv. as a 2-h infusion on day 1; leucovorin (200 mg/m ²) administered iv. as a 2-h infusion on days 1 and 2; followed by a loading dose of 5-FU (400 mg/m ²) bolus administered iv., then 5-FU (600 mg/m ²) administered iv. as a 22-h continuous infusion on days 1 and 2, repeat every 2 weeks	[45,46]
FOLFOX6 regimen	Oxaliplatin (85–100 mg/m ²) administered iv. as a 2-h infusion on day 1; leucovorin (400 mg/m ²) administered iv. as a 2-h infusion on day 1; followed by a loading dose of 5-FU (400 mg/m ²) bolus administered iv. on day 1, then 5-FU (2,400–3,000 mg/m ²) administered iv. as a 46-h continuous infusion, repeat every 2 weeks	[50]
FOLFIRI regimen	Irinotecan (180 mg/m ²) administered iv. as a 2-h infusion on day 1; leucovorin (400 mg/m ²) administered iv. as a 2-h infusion on day 1; followed by a loading dose of 5-FU (400 mg/m ²) bolus administered iv. on day 1, then 5-FU (2,400–3,000 mg/m ²) administered iv. as a 46-h continuous infusion, repeat every 2 weeks	[51]
FUFOX regimen	Oxaliplatin (50 mg/m ²) plus leucovorin (500 mg/m ²) plus 5-FU (2000 mg/m ²) administered iv. as a 22-h continuous infusion on days 1, 8, 22 and 29, repeat every 36 days	[52]
FUOX regimen	5-FU (2,250 mg/m ²) administered iv. as a 48-h continuous infusion on days 1, 8, 15, 22, 29 and 36 plus oxaliplatin (85 mg/m ²) on days 1, 15 and 29, repeat every 6 weeks	[53]
XELOX regimen	Oral capecitabine (1,000 mg/m ²) two-times/days for 14 days plus oxaliplatin (130 mg/m ²) on day 1, repeat every 3 weeks	[54]
IFL (or Saltz) regimen	Irinotecan (125 mg/m ²), 5-FU (500 mg/m ²) bolus administered iv. and leucovorin (20 mg/m ²) bolus administered iv. weekly for 4 weeks, repeat every 6 weeks	[55]
Douillard regimen	Irinotecan (180 mg/m ²) administered iv. as a 2-h infusion on day 1; leucovorin (200 mg/m ²) administered iv. as a 2-h infusion on days 1 and 2; followed by a loading dose of 5-FU (400 mg/m ²) bolus administered iv., then 5-FU (600 mg/m ²) administered iv. as a 22-h continuous infusion on days 1 and 2, repeat every 2 weeks	[56]
AIO or German AIO regimen	Irinotecan (100 mg/m ²) administered iv. as a 2-h infusion on day 1; leucovorin (500 mg/m ²) administered iv. as a 2-h infusion on day 1; followed by 5-FU (2000 mg/m ²) administered iv. as a 24-h continuous infusion weekly, repeat every 13 weeks	[57]

5-FU: 5-fluorouracil; AIO: Arbeitsgemeinschaft Internische Onkologie.

Nanoparticles in colorectal cancer therapy

The development of therapeutic strategies for cancer treatment based on nanoparticles has generated substantial advances in pharmacology, decreasing the side effects of cytotoxic drugs and improving their efficacy, solubility, pharmacokinetics and biodistribution. Over the last 50 years, several nanoparticles of diverse shapes, sizes and chemical natures have shown high efficacy in encapsulating different types of anticancer cargo, including siRNA [62], antibiotics [63] and chemotherapeutics [26]. These first-generation anticancer nanoparticles reach the tumor tissue passively, taking advantage of the enhanced permeation and retention effect offered by the vascular and lymphatic drainage of tumors; this allows the extravasation and accumulation of nanoparticles within cancer cells and improves therapeutic efficacy [64]. Liposome-based platforms are the most well established and were the first nanocarriers approved by US FDA for use in humans [65]. They are vesicles composed of a phospholipid bilayer with an internal and external aqueous phase that supports the encapsulation of both hydrophilic and hydrophobic drugs.

Liposome-based nanoproducts currently under clinical study for the treatment of CRC include CPX-1, LE-SN38 and Thermodox; CPX-1 (Irinotecan HCl: Floxuridine) has completed Phase II clinical trials [66]. One study focused on patients with advanced CRC who were already receiving chemotherapy including oxaliplatin or irinotecan [67]. Other researchers evaluated the liposome formulation LE-SN38 in HT-29 tumor-bearing mice; tumor growth was inhibited by 51, 79 and 90% after 10 days of treatment using doses of 10, 20 and 40 mg/kg (respectively) compared with the drug-free liposome group [68]. However, assessment of the effects of LE-SN38 in patients with metastatic CRC after progression on oxaliplatin (Phase II of clinical trial) showed that the drug did not slow cancer progression in patients treated with LE-SN38 35 mg/m² every 21 days for a minimum of 2 cycles [69]. Thermodox, another liposomal strategy in clinical trials, involves the use of thermally sensitive liposomal doxorubicin as an adjuvant therapy with radiofrequency thermal ablation in the treatment of recurrent or refractory colorectal liver metastases [70]. Although a study comparing Thermodox to radiofrequency thermal ablation monotherapy has been terminated, the results still are not available [71]. At present, several agents under preclinical development have shown promising *in vitro* results with potential applications for CRC, including oxaliplatin-loaded long-circulating liposomes (PEG-liposomal L-oHP) [72], liposomal curcumin [73] and doxorubicin-encapsulated liposome [74].

Since the 1980s, studies by Langer and coworkers have shown that biodegradable and noncytotoxic polymers such as poly(D,L-lactide-co-glycolide) and their derivatives [75], polycaprolactone [76,77] and chitosan [78] offer a versatile platform for the development of nanoparticles and drug delivery. Polymeric nanoparticles are spherical, with a hydrophobic core and a hydrophilic shell formed by the self-assembly of biocompatible amphiphilic block copolymers through aqueous or microencapsulation methods. They are considerably more stable than liposomes, permit the efficient encapsulation of drugs of different chemical natures and allow sustained release in response to changes in temperature or pH [23,79–80].

At present, several formulations for cancer therapy in clinical trials have already shown outstanding pharmacokinetic performance. For instance, NK105 (PEG-P[Asp]-paclitaxel) showed an area under the curve significantly higher than paclitaxel alone between 0 and 48 h after iv. administration (191,000 ± 32,100 vs 1500 ± 108 ng·h/ml, respectively) [81]. Other formulations in clinical trials such as NK911 (PEG-P[Asp]-doxorubicin) have shown high accumulation in solid tumors in mice [16], and SP1049C (Pluronic L61, F127–doxorubicin) exhibited notable single-agent activity in patients with adenocarcinoma of the esophagus and gastroesophageal junction with high efficacy and fewer side effects compared with drug alone [82]. Assays in mice model of metastasis have shown that an *in vivo* gene delivery formulation comprising a core of high-molecular-weight linear polyethylenimine complexed with DNA and surrounded by a shell of polyethylene-glycol-modified (PEGylated) low-molecular-weight linear polyethylenimine are selectively transfected in neoplastic cells. However, only a small fraction of those cells expressed the transgene [83].

Polymeric nanoparticles continue to be a popular subject of study in cancer therapy because they are a strong platform with which to encapsulate both hydrophilic and hydrophobic drugs [84–86]. However, most drugs are released into the extracellular matrix; their effectiveness depends on diffusion through the tissue, and low *in vivo* specificity also limits their application [87]. Thus, recent site-specific targeting of nanoparticles is a promising advancement in cancer treatment research. One successful approach is the BIND-014 technology, which consists of docetaxel-loaded polymeric nanoparticles capable of recognizing prostate cancer through targeting against PSMA, a tumor antigen on prostate cancer cells and the vasculature of most nonprostate solid tumors. BIND-014 is currently in Phase II clinical trials for non-small-cell lung cancer and metastatic castration-resistant prostate cancer, having already demonstrated significant

antitumor activity at a lower dose than conventional docetaxel in subjects with advanced or metastatic non-small-cell lung cancer [88].

Targeted nanoparticles for colorectal cancer therapy

The conjugation of ligands such as antibodies, fragments of antibodies, peptides, aptamers and other small molecules on the surface of nanoparticles for the purpose of cell recognition has yielded a new generation of nanoparticles for cancer therapy with enhanced *in vivo* specificity (Figure 2). The incorporation of these ligands is usually achieved by chemical modification during nanoparticle synthesis or through chemical bonding between ligands and polymers before synthesis [89,90].

Targeted nanoparticles are those that contain ligands on their surface and are capable of specifically recognizing cells. In applications against cancer, the promise of targeted nanoparticles is based on the fact that tumors express and/or overexpress some biomarkers, which can be used as targets for drug delivery. For example, Graf *et al.* described cisplatin prodrug-loaded poly(D,L-lactic-co-glycolic acid)-block-polyethylene glycol nanoparticles targeted with a cyclic pentapeptide c(RGDfK) that bind to the integrin receptor, which is highly upregulated in tumor-associated endothelial cells during angiogenesis [17].

One recent study used immunohistochemistry to analyze the expression of four biomarkers in mucosal and CRC tissues from 280 patients [91]. Carcinoembryonic antigen (CEA) was the marker most consistently overexpressed, that is, expressed in CRC 98.8% more than in normal tissue, followed by tumor-associated glycoprotein-72 at 79%, folate receptor- α at 37.1% and EGFR at 32.8%. This work supports the application of CEA as a potential cellular target for future development of targeted nanoparticles in the treatment of CRC [91] (see Figure 2). Markers have also been identified: IGF-1R [92], apolipoprotein A1 [93], the transmembrane receptor tyrosine kinase EphA4 [94], the receptor for hyaluronic acid-mediated motility [95] and $\alpha 2$ integrin [96].

Though the application of monoclonal antibodies (mAb) to CRC-targeting nanoparticles is still an emerging field, there are already many mAbs in preclinical and clinical development [59,97–100]. For example, the humanized A33 mAb (huA33 mAb) has shown great promise in clinical trials as an immunotherapeutic biological agent and also as a targeting ligand for CRC cells of polymer capsules formed by the layer-by-layer method [101,102]. As shown in Table 3, using targeted nanoparticles as a drug delivery system based on mAb is now one of the main approaches for

CRC therapy under preclinical development. However, the major limitation of mAb is their large size and complexity, posing a challenge to their conjugation on the surface of nanoparticles [103,104].

Peptides also represent a promising targeting alternative, given their small size and ease of attachment to nanoparticles. However, the use of peptides for CRC, for example, the tumor necrosis factor-related apoptosis-inducing ligand [119], and the peptide RPMrel (CPIEDRPMC) [120] as a targeting ligand, has not yet been well explored. One study has shown high cellular uptake of HEMA-copolymer-DOX conjugate with the oligopeptide GE11 in CRC cells that overexpress EGFR, achieving selective release of doxorubicin [114].

The differential expression of FR α has been associated with several types of cancers including CRC [121]. Cell lines such as Caco-2 and HT29, which overexpress the folate receptor, selectively internalize nanoparticles conjugated with folate on their surface (i.e., functionalized) [113]. Sharma *et al.* described a multifunctional nanosystem based on methotrexate-loaded guar gum nanoparticles functionalized with folic acid (MTX-FA-GGNP), which released methotrexate at colonic pH (6.8) and displayed preferential *in vivo* uptake in colon tissue [122].

Imaging & detection in colorectal cancer

Nanoparticles may also be used to facilitate early diagnosis and monitor the efficacy of therapy. The design of nanoparticles could incorporate different contrast agents (e.g., radioactive, superparamagnetic or fluorescent), targeting groups and biocompatible coatings [123]. Since small molecular-weight gadolinium and metal chelate-based contrast agents have disadvantages such as low tissue specificity, rapid clearance and nonspecific extracellular distribution, nanotechnology may be used to modify such contrast agents to improve the sensitivity and specificity of CRC diagnostics [124]. Recently, He *et al.* described lectin core/shell nanoparticles formulated with iron oxide magnetite and gold (lectin-Fe₂O₃#Au NP), which allowed dual-modality imaging, that is, T₂-weighted MR and x-ray CT in nude mice bearing colorectal tumor (SW620) [125]. Another strategy that showed outstanding *in vivo* effectiveness by MRI, low cytotoxicity and extraordinary fluorescence stability was based on nanoparticles formulated with a core of superparamagnetic iron oxide nanocrystals, conjugated with quantum dots and targeted with a monoclonal antibody binding to CEA-related cell-adhesion molecules [126].

Near-infrared fluorescent (NIRF) endoscopic detection is a novel approach that may increase the sensitivity and specificity of surveillance colonoscopy of patients with CRC. Studies in a mouse model of coli-

tis-associated cancer monitored by NIRF endoscopy showed high efficiency in the detection of dysplastic foci within chronically inflamed colons [127]. Yang *et al.* reported the application of folic acid-conjugated chitosan nanoparticles loaded with 5-aminolaevulinic acid in NIRF endoscopy for CRC cells. The 5-aminolaevulinic acid is a precursor in heme group synthesis and is rapidly converted to the fluorophore protoporphyrin IX in normal cells. In cancer cells protoporphyrin IX accumulates intracellularly, because the degradation metabolism is slower than in normal cells, allowing its use in NIRF endoscopy and specifically on CRC cells, which overexpress the folate receptor [112].

Cancer stem cells in colorectal cancer

The biological basis of the recurrence of CRC after surgical treatment, chemotherapy and/or radiation still is not understood. Some authors suggest that factors like the stage of development of cancer, the age of the patient and the treatment received are a critical factor for the relapsed of patients; however, still the literature is controversial.

The cancer cells are recognized for a high rate of proliferation, and cellular division that promotes to boosting the number of mutations. The effect of cytotoxic drugs over the cancer cells generates a selective pressure that stresses the progeny and induces novel drug resistant mutants, which are responsible for relapses.

Interestingly, the relapse of those types of cancers that involve the drug resistant phenomenon is experienced shortly after the treatment; however, some tumors manifest relapse long time after the surgery or pharmacological treatment (months or years, and even when the treatment has been stopped). In those cases of cancer, the drug-resistant effect does not explain the relapse.

Several hypotheses are under investigation, and the common point of view is centered in the fact of the tumors are composed by clonal subpopulations of cancer cells, which differs in its growth rate, immunological characteristics, the ability to metastasize, the expression of proteins and sensitivity to treatments [128]. The authors also suppose a hierarchical interaction between the subpopulations of clones, which promotes the tumor progression. In this sense, it has been demonstrated the existence of cancer stem cells (CSCs) in various types of cancers including leukemia and CRC [129,130]. In this context, if the chemotherapeutic drugs affect the viability of cells under a highly rate of division, the CSCs, that are characterized by a slow proliferation rate, could promote the recurrence after a long time of the treatment.

Recent investigations have reported that CSCs in CRC could be characterized according to cellular markers such as CD44, CD133, CD166 and EpCAM [131–133]. At the signaling level, it has been proposed that both, WNT/ β -catenin and NOTCH/HES1 pathways,

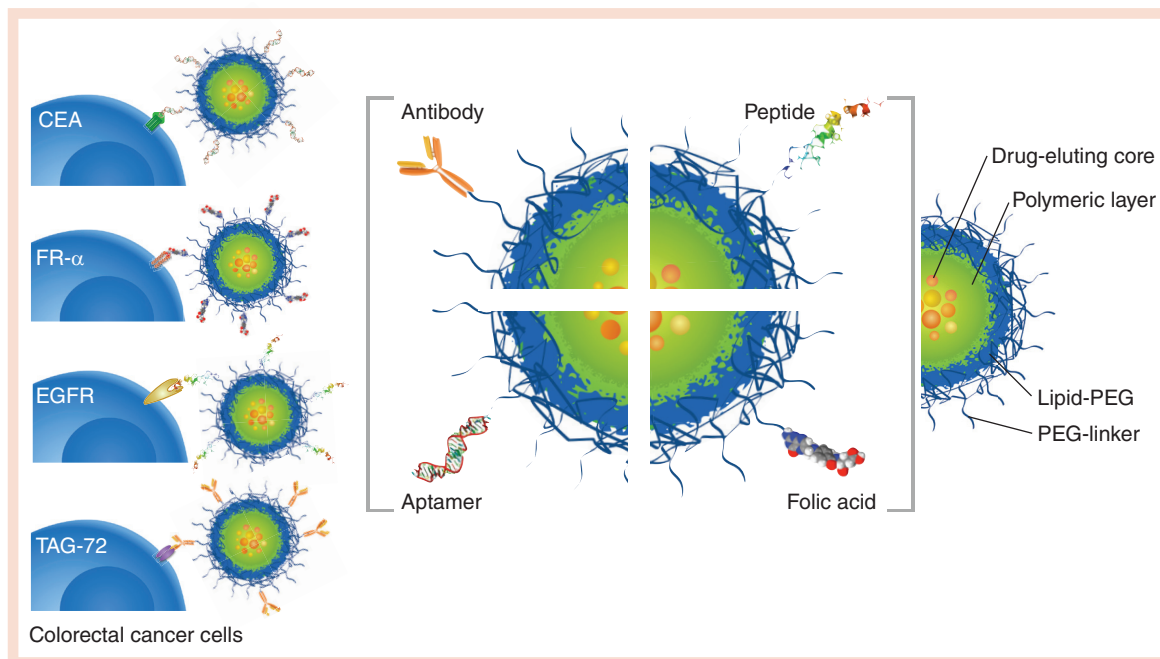


Figure 2. Targeted nanoparticle strategy for colorectal cancer. The most common CRC biomarkers overexpressed in the cellular membrane and the typical molecules/ligands used on the surface of nanoparticles in targeting strategies: FR- α , EGFR, TAG-72.

CEA: Carcinoembryonic antigen; CRC: Colorectal cancer; EGFR: EGF receptor; FR- α : Folate receptor- α ; TAG-72: Tumor-associated glycoprotein.

Table 3. Targeted nanoparticles for colorectal cancer under preclinical development.

Formulation	Ligand	Target	Cell population	Ref.
Nanosized maghemite particle	Antibody	CEA	High CEA-expressing cell line (LS174T) and a low CEA-expressing cell line (HCT116)	[105]
Dextran- and PEG-coated superparamagnetic iron oxide nanoparticles (abf-SPION)	scFv	CEA	LS174T, a CEA-expressing (CEA+ve) cancer cell line and A375M, a CEA-negative (CEA-ve) cancer cell line	[106]
Dye-doped silica nanoparticles conjugated with polyamidoamine dendrimers	Humanized anti-CEA monoclonal antibody A5B7	CEA	LS174T, LoVo and HCT116 cells and murine xenografts model	[107]
Conatumumab (AMG 655)-coated nanoparticles	Antibody	DR5	HCT116 cancer cells	[108]
Photosensitizer meso-Tetra(N-methyl-4-pyridyl) porphine tetra tosylate chitosan/alginate nanoparticles	Antibody	DR5	HCT116 cancer cells	[109]
Polymer capsules formed by the LbL technique	Humanized A33 monoclonal antibody (huA33 mAb)	A33 antigen	LIM1215 cells (antigen-expressing) SW480 (nonantigen-expressing)	[102]
Gold and iron oxide HNPs	scFv	A33 antigen	Colorectal cancer cell lines (SW1222 and HT 29 cells)	[110]
Poly(lactide- coglycolide) NP loaded with camptothecin	Antibody	Fas receptor (CD95/Apo-1)	HCT116 cells	[111]
Chitosan nanoparticles loaded with 5-ALA	Folic acid	FR	HT29 and Caco-2 colorectal cancer cell lines overexpressing folate receptor	[112]
FA-CS conjugates nanoparticles	Folic acid	FR	HT-29 cancer cells	[113]
HPMA-copolymer-doxorubicin conjugates	Peptide GE11	EGFR	HT29, SW480 and A431 cell lines	[114]
T22-empowered protein-only nanoparticles	18-mer peptide T22 (T22-GFP-H6)	CXCR4	HeLa cells	[115]
Chitosan nanoparticles encapsulating oxaliplatin (L-OHP)	HA	HA receptor	Colon cancer (HT-29) in C57BL mice	[116]
MSN	Coated with poly-(L-lysine) and HA	CD44 receptor	HCT-116 cancer cells	[117]
rHDL nanoparticles loaded with siRNA	Apo A-I	SR-B1	Model colorectal cancer metastasis in mice (HCT116 cells)	[118]

5-ALA: 5-aminolaevulinic acid; Apo A-1: Apolipoprotein A-I; CEA: Carcinoembryonic antigen; CXCR4: CXC chemokine receptor 4; DR5: Death receptor 5; FA-CS: Folate-chitosan; FR: Folate receptor; HA: Hyaluronic acid; HNP: Hybrid nanoparticle; LbL: layer-by-layer; MSN: Mesoporous silica nanoparticle; NP: Nanoparticle; rHDL: Reconstituted HDL; ScFv: Single-chain Fv antibody fragment; SR-B1: Scavenger receptor type B1.

are involved in the regulation of CSCs, and the self-renewal and maintenance of CSCs in CRC, respectively [134–136]. At present, several experimental drugs targeting to CSCs in combination with conventional chemotherapeutic drugs are in clinical trials for CRC and others type of cancers. The BBI608 targeted to STAT-3, and BBI503 targeted to Nanog and multiple kinases, have been developed by Boston Biomedical and currently are in Phase III on a clinical trial.

In summary, it seems to be that the elimination of all CSCs is critical to eradicating cancer and that fail-

ure to do so might be responsible for the occurrence of relapses and/or metastases frequently observed in the clinical management of CRC patients. Consequently, an adequate isolation and a profound identification of CSCs in CRC is essential for a better understanding of their role in the tumorigenesis process and the development of CSC-specific therapies.

Conclusion

Traditional cytotoxic drugs for CRC cause several side effects in part because of current therapeutic protocols,

which are based on a series of cycles of administration. Recent trends in nanomedicine include the use of combined therapy of cytotoxic drugs loaded in targeted nanocarriers, which allows sustained release and site-specific delivery, reducing or even eliminating cycles, depending on the efficiency of the strategy. The combined therapy can also include the use of DNA [137] or RNA [138] to assemble the nanoparticles that leading to the expression or knockdown of genes enhancing effectiveness of the cytotoxic drugs.

The deeper understanding gained in recent years of manipulating the physicochemical properties of nanoparticles for *in vivo* application bodes well for their use in treatment of CRC. Relevant parameters include optimal size to avoid the immune system response and clearance by glomerular filtration in the kidneys (range: 10–100 nm) [139,140] and the best shape to encourage longer circulation time and faster uptake by cancer cells (spherical) [141]. Moreover, other relevant parameters include the optimal surface charge to promote cellular binding and prevent complement activation (range: 0 to -10 mV) [142] and sufficient density of targeting ligands on the surface of nanoparticles to optimize tissue-specific targeting (range: 0.5–5%) [88].

On the other hand, optimizing the targeting technology for CRC therapy still faces several challenges, including the correlation of biomarkers with the early stages in the development of CRC and the identification of novel highly specific molecular targets. Thus far, peptides, aptides, aptamers and small molecules are the most attractive tools for targeting, given their small size, high affinity and ease of conjugation on the

surface of nanoparticles; they hold great promise for future drug development and medical translation.

Future perspective

The future perspective of targeted nanoparticles is brilliant because several of current promising formulations under preclinical and clinical developments for CRC soon and after overtaking the high standards of safety and efficacy for patients required by the FDA could be in the market. A future challenge is to implement systems or protocols to determine the molecular expression profile of tumors from patients with CRC and classify them according to the genetic profile, stage of development of tumor and putative targeting molecules. All above will support the rational administration of precise-targeted nanoformulations containing the most effective drug combination.

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Executive summary

Colorectal cancer & adjuvant chemotherapy

- Colorectal cancer (CRC) is the fourth most widely diagnosed cancer worldwide and despite notable progress in treatment still leads to significant morbidity and mortality.
- Based on the progression of cancer the standard treatment involves surgical resection, and adjuvant chemotherapy; however, since stages IIC–IV the survival rate over 5 years is lower than 50% due to the high risk of metastasis and the recurrence.
- Despite the highly efficient chemotherapeutic agents used to treat CRC, drug resistance remains a major challenge and general side effects.

Targeted nanoparticles for colorectal cancer

- The development of therapeutic strategies based on nanoparticles as a drug delivery system has become one of the most brilliant strategies for cancer therapy.
- The conjugation of ligands on the surface of nanoparticles for the purpose of cell recognition has yielded a new generation of nanoparticles (targeted nanoparticles).
- Targeted nanoparticles could take advantage of differentially expressed molecules on the surface of tumor cells, providing an adequate release of cytotoxic drugs.
- Since contrast agents have disadvantages such as low tissue specificity, rapid clearance and nonspecific extracellular distribution, targeted nanoparticles may be used to modify such contrast agents to improve the sensitivity and specificity of CRC diagnostics.

Future perspective

- The near future of precise-targeted nanoformulations containing the most effective drug combination for CRC is just around the corner due to the promising advances in this field of research.

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