

## Special Section on Drug Delivery Technologies—Minireview

# Impact of CEA-targeting Nanoparticles for Drug Delivery in Colorectal Cancer

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### ABSTRACT

Colorectal cancer (CRC) is one of the most common causes of cancer-related death in the world, mainly owing to distant metastasis events. Developing targeted strategies to treat and follow individuals in more developed stages is needed. The carcinoembryonic antigen (CEA) is a cell surface–overexpressed glycoprotein in most CRC patients, and the evaluation of its serum levels is recommended in the clinic. These reasons motivated the production of CEA-targeted nanotechnologies for monitorization of CRC progression, but only a few centers have reported their use for drug delivery. The cellular internalization of CEA-linked nanosystems occurs by the natural recycling of the CEA itself, enabling longer retention and sustained release of the cargo. The

functionalization of nanoparticles with lower affinity ligands for CEA is possibly the best choice to avoid their binding to the soluble CEA. Here, we also highlight the use of nanoparticles made of poly(lactico-glycolic acid) (PLGA) polymer, a well known material, owing to its biocompatibility and low toxicity. This work offers support to the contribution of antibody fragment–functionalized nanoparticles as promising high affinity molecules to decorate nanosystems. The linkers and conjugation chemistries chosen for ligand–nanoparticle coupling will be addressed herein as an elements essential to the modulation of nanosystem features. This review, to our knowledge, is the first that focuses on CEA-targeted nanotechnologies to serve colorectal cancer therapy and monitorization.

### Introduction

Colorectal cancer (CRC) is the third most incident and fourth most common cause of cancer-related death, and has the third highest 5-year prevalence (post-treatment) in the world (<http://publications.iarc.fr/Databases/Iarc-Cancerbases/GLOBOCAN-2012-Estimated-Cancer-Incidence-Mortality-And-Prevalence-Worldwide-In-2012-V1.0-2012>). This type of malignant neoplasm

arises from the mucosa of the colon or the rectum and could follow one of the three mechanisms of tumorigenesis, or a combination of them: chromosomal instability, microsatellite instability, and CpG island methylator phenotype. The chromosomal instability represents the pathway that most of sporadic colorectal malignant neoplasms follow (Kotelevets et al., 2016; Tariq and Ghias, 2016).

The major reason for treatment failure in CRC is the development of distant metastasis, most commonly liver metastasis. The production of technologies that specifically target CRC cells in more developed stages of their tumorigenesis may prove to be effective in overcoming the collateral damage caused by such “blind therapies” as standard chemotherapeutics. The nanocarriers targeted for drug delivery (Dinarvand et al., 2011) that specifically recognize cell surface–overexpressed molecules are already a field of interest. In this review, we investigate several promising molecules and focus on the potential of carcinoembryonic

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**ABBREVIATIONS:** CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CEACAM, carcinoembryonic antigen cell adhesion molecules; CDR, complementary-determining regions; CRC, colorectal cancer; EDC, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride; EGFR, epithelial growth factor receptor; EPR, enhanced permeability and retention; Fc, fragment crystallizable; GPI, glycosylphosphatidylinositol; Ig, immunoglobulins; mAb, monoclonal antibody; SNHS, *N*-hydroxysuccinimide; PAMAM, polyamidoamine; PLGA, poly(lactic-co-glycolic acid); scFv, single-chain variable fragment; sulfo-NHS, *N*-hydroxysulfosuccinimide; SMCC, succinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate; SM[PEG]<sub>4</sub>, succinimidyl-[(*N*-maleimidopropionamido)-tetraethyleneglycol] ester; TfR, transferrin receptor protein.

antigen (CEA), considered the protein most expressed in CRC (Tiernan et al., 2015), as a targeting moiety to direct a nanosystem, either for simple disease monitorization or targeted drug-delivery purposes.

In the context of targeted nanotechnology, our preference is to approach the functionalization of nanoparticles with antibody fragments, as they conserve the high affinity characteristics of a monoclonal antibody with more potential for oriented functionalization (Cheng and Allen, 2010; Shargh et al., 2016). A summary of antibody features will be given to complete the logical progression of the work.

We also defend here the functionalization of nanoparticles made of poly(lactic-co-glycolic acid) (PLGA) polymers, some of which are FDA-approved materials with huge impact, owing to their biocompatibility and low toxicity (Murthy, 2007). The most common antibody-conjugation strategies will be addressed, as they are important in the modulation of the nanosystem properties and are some of the most suitable linkers currently used.

We believe that in the near future CEA-targeting nanotechnologies may offer novel and more efficient anticancer theragnostic strategies.

### Cell Surface Molecules Highly Expressed on CRC

Targeted technologies—to diagnose, evaluate the prognostic or the predictive response to a treatment, and even treat tumors—rely on identifying molecular entities characteristic to, or at least highly expressed in, neoplastic rather than normal tissues. The histologic features and genetic signature of certain tumors permit stratification into distinct subtypes, providing in some cases a reliable predictor of response to a targeted therapy (Tiernan et al., 2013; Freidlin and Korn, 2014).

One of the most widely accepted definitions of a tumor marker was given by the National Cancer Institute, National Institutes of Health: entities, most of them proteins, produced by cancerous or noncancerous cells in response to malign or benign events; in the context of malignancy, these entities exist at higher levels and can be found in tissues or body fluids of some cancer patients (<https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis/tumor-markers-fact-sheet#q1>).

One factor that cannot be discarded is that the presence of a certain tumor marker in a patient is not always correspondent to a predicted clinical state or response to a treatment, and sometimes the variation between measurements in a population could be high, which invalidates the marker's utility (Strimbu and Tavel, 2010).

A nanosystem made to deliver a specific diagnostic probe or therapeutic agent to the inside of a cancerous cell requires first that it be highly targeted to a cell surface molecule and, ideally, one expressed specifically in the malign phenotype of study. For this reason, it is necessary to understand which molecular options remain available for targeting colorectal cancer cells. The cell surface molecules most commonly overexpressed in colorectal cancer are the cell adhesion protein CEA, the tumor-associated glycoprotein-72, the folate receptor- $\alpha$ , and the epithelial growth factor receptor (EGFR), which are present in 98.8%, 79.0%, 37.1%, and 32.8% of cases,

respectively, compared with matched healthy tissues (Tiernan et al., 2013). Another study suggests that CD44v6 overexpression, a hyaluronic acid (HA) receptor, represents a poor prognostic factor for colorectal adenocarcinoma patients (Köbel et al., 2004). Other authors confirmed the existence of a higher level of serum carbohydrate antigen 19-9 (CA 19-9) and of  $\alpha$ -fetoprotein in colorectal cancer patients, rather than in patients with nonmalignant colorectal disease (Wang et al., 2014). In addition, the vascular endothelial growth factor receptor and the transferrin receptor protein 1 (TfR1) are also upregulated in CRC (Hasan et al., 2011; Miljuš et al., 2015). Another relevant cell surface molecule is tyrosine kinase receptor c-MET, which is highly expressed in colorectal cancer and in liver metastases of this malignant neoplasm (Bradley et al., 2016). Finally, the death receptor 5 is a cell-surface receptor with proapoptotic characteristics that is overexpressed in stage II and III colorectal cancer patients (Schmid et al., 2014). Table 1 presents some of the currently most promising nanoparticle-based systems targeting cell surface molecules for gastrointestinal cancer treatment and monitorization.

In the clinic, there are few tumor biomarkers that are also cell surface molecules currently used either for disease monitoring or diagnostic, prognostic, and predictive responses in colorectal cancer. The CEA is indicated for several situations: 1) stage II patients' prognosis, 2) preoperative evaluation of newly diagnosed cases, 3) postoperative surveillance, and 4) advanced disease monitorization. The CA 19-9 (a cell surface carbohydrate antigen) has emerged, although not yet FDA-recommended, as a postoperative surveillance marker in cases of metastatic disease when CEA is not upregulated (Duffy et al., 2014). The overexpression of MET and of human epidermal growth factor receptor 2 (HER2) confers *de novo* resistance to anti-EGFR immunotherapy (HER3 and EGFR mutations were not clearly associated). Despite this, the overexpression evaluation of EGFR, HER2, MET, or HER3 is not recommended for CRC patients (Van Cutsem et al., 2016). In the final analysis, CEA is an overexpressed protein in most CRC cases and the only cell surface molecule recommended for management of colorectal cancer patients. These reasons motivate the selection of CEA as a promising molecule for nanoparticle targeting systems in colorectal cancer.

### Carcinoembryonic Antigen as a Target for CRC-Directed Therapies

**CEA Features.** CEA is a glycoprotein that belongs to the 12-member family of carcinoembryonic antigen cell adhesion molecules (CEACAM), as represented in Fig. 1. In their turn, CEACAMs belong to the superfamily of immunoglobulins (Igs) and are generally characterized by harboring one variable (IgV-like) N-terminal domain, homologous to the Ig variable domain responsible for binding to homophilic and heterophilic cell adhesion molecules. This terminal N-domain is generally linked to none or a maximum of six constant domains (IgC2-like), also homologous to immunoglobulin nonvariable domains. In the specific case of the CEA protein, also known as CEACAM5 or CD66e, once produced it is covalently bound to glycosylphosphatidylinositol (GPI), and this post-translational modification leads to the anchorage of CEA at the external surface of the phospholipidic bilayer. This GPI-anchorage to the membrane does not allow CEA to perform by itself any

TABLE 1  
Targeting Nanoparticle-based systems to promising cell surface molecules for gastrointestinal cancer treatment and monitoring

Receptor	Cell Lines	Ligand	Formulation	Drug Delivered	Remarks	Reference
CEA	LS174T HCT-116	Monoclonal antibody (mAb)	Magnetic NPs	—	Maghemite NPs conjugated to anti-CEA (~550 nm) had greater uptake by CEA <sup>+</sup> CRC cells. The biocompatibility of the system was confirmed.	da Paz et al. (2012)
CEA and TAG-72	LS174T HT29	Anti-TAG-72 mAb and Anti-CEA mAb	Human serum albumin NPs		In vivo studies performed with LS174T and HT29 xenografts.	Cohen and Margel (2012)
EGFR	HCT-116	Cetuximab-Fab' fragment	Liposomes	Oxaliplatin	NPs with ~120 nm had specific binding for mice CRC tissues. Liposomes had ~120 nm, efficiency of encapsulation of ~32%, and a loading capacity of ~65 µg/mg. Fab'-liposomes induced cell-specific uptake, and cytotoxicity in EGFR <sup>+</sup> CRC cells.	Zalba et al. (2015)
VEGFR	SW-480 HT-29 SW-620 CT26	Polyclonal antibody	Dextran-coated iron oxide NPs	—	In vivo studies performed with CT26 xenograft.	Hsieh et al. (2012)
CD44	Colon-26	Hyaluronic acid (HA)	PLGA NPs	Camptothecin (CPT)/Curcumin (CUR)	Anti-VEGF-NPs had 65 nm, demonstrated in vivo tumor targeting, and efficient accumulation in tumor tissues. HA-functionalized PLGA NPs with ~300 nm. Codelivered CPT and curcumin (1:1) for CRC-targeted combination chemotherapy evidenced enhanced toxicity.	Xiao et al. (2015)
CD44v6	MKN74 (gastric cancer cell line)	Fab (fragment antigen binding)	PLGA-PEG NPs	—	NPs of ~300 nm and tagged with the Fab had specific cellular binding. NPs coated with Fab(CD44v6 <sup>+</sup> ) showed negligible binding to negative cells, as the Fab(CD44v6 <sup>+</sup> )-decorated NPs on the positive cells.	Kennedy et al. (2018b)
CD44v6	PANC-1 (pancreatic cancer cells)	Single-chain variable fragment (scFv)	Amphiphilic deblock copolymer of poly (ethylene glycol) and poly (D, L-lactide) [mal-PEG-PDLLA]	Arsenic trioxide (As <sub>2</sub> O <sub>3</sub> )	In vivo studies performed with PANC-1 xenografts. mal-PEG-PDLLA vesicles had ~200 nm and encapsulation efficiency of 65.8%. scFv-loaded-NPs (drug concentration of 8 mM) induced more apoptosis than the free drug or nonfunctionalized-loaded NPs.	Qian et al. (2013)
Folate receptor	HT-29	Folic acid (FA)	PLGA NPs	5-FU	Nanoparticles had ~200 nm, encapsulation efficiency of ~30% and drug loading of ~6%. FA conjugation of ~47% was obtained using 1, 3-diaminopropane as linker. 5-FU loaded FA-PLGA NPs showed cell toxicity at 50 µg/ml.	Wang et al. (2015)
CA 19-9	Pancreatic cell lines: AsPC-1, BxPC-3-Luc, KP4, PK-59	L-fucose	Liposomes	Cisplatin	In vivo studies performed with AsPC-1 and BxPC-3-Luc xenografts. L-Fucose-liposome cisplatin-loaded had ~200 nm. The greatest cytotoxicity was observed when using 50 µg/ml Fuc-liposomes, which were more cytotoxic than the free drug.	Yoshida et al. (2012)

(continued)

TABLE 1—Continued

Receptor	Cell Lines	Ligand	Formulation	Drug Delivered	Remarks	Reference
TTR	Caco-2	mAb	Liposomes	—	Anti-TTR-NPs with ~100 nm had 4.5-fold greater binding than the ones nonfunctionalized or coated with negative mAb.	Harel et al. (2011)
DR-5	HCT-116	mAb	PLGA-PEG NPs	Camptothecin	In vivo studies performed with HCT-116 xenografts. Nanoparticles had ~200 nm and association efficiency of ~18%. In mice treated with DR5-NPs, the malignant mass reduced ~35% over both PBS and control-IgG conjugated NPs.	Schmid et al. (2014)

mAb, monoclonal antibody; mal-PEG-PDLLA, poly(D,L-lactide)-poly(ethylene glycol)-maleimide; NP, nanoparticle; PBS, phosphate-buffered saline; PEG-PDLLA, poly(ethylene glycol) methyl ether-block-poly(D,L lactide); TAG-72, tumor-associated glycoprotein 72; VEGFR, vascular endothelial growth factor receptor.

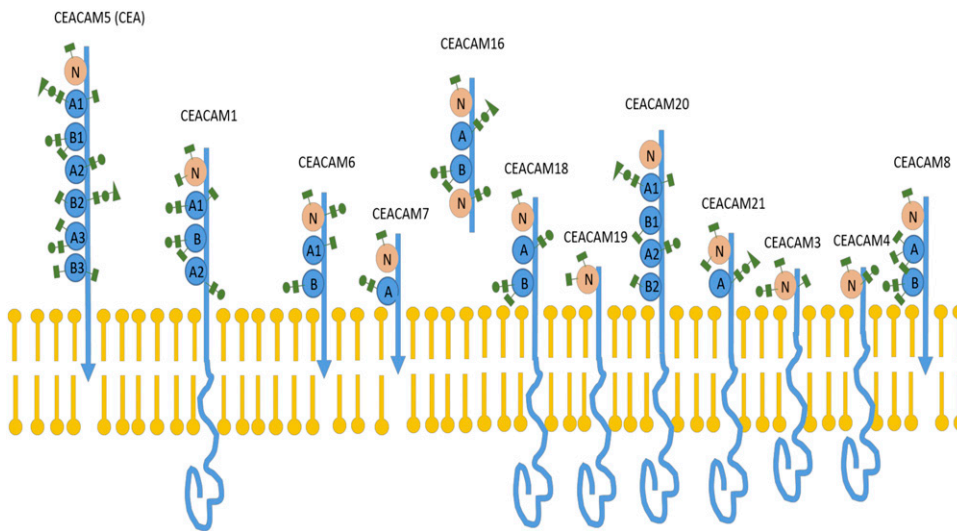
transduction of signal since it lacks intracellular domains and requires transactivation through other intracellular partners (Maeda and Kinoshita, 2011; Beauchemin and Arabzadeh, 2013).

CEA is produced in human gastrointestinal tract during early stages of embryonic and fetal development (from 9 to 14 weeks), and before birth its serum levels decrease and remain very low into adult life (Rodrigues et al., 2018). Nevertheless, there are some structures that still produce CEA. Its expression is mainly observed in goblet and columnar epithelial cells of the colon, principally in the free luminal surface and at the upper third of the crypt. It is also present in prostate, cervix, tongue, esophagus, stomach, and sweat glands (Hammarström, 1999). A healthy adult produces about 50–70 mg/day of CEA from the apical surface of mature enterocytes and releases it extracellularly into the gut lumen, from which it reaches the exterior environment by defecation (Hammarström, 1999; Rückert et al., 2010).

When referring to glycoproteins, the linkage between the polypeptide backbone and glycans typically occurs through two chemical strategies: 1) the binding of the nitrogen atom of an asparagine residue to a glycan chain (N-glycans), as in the case of CEACAM5, or 2) the binding of an oxygen atom of a serine or threonine residue to a glycan chain (O-glycans), like mucins. Glycoproteins such as CEA, either in normal or neoplastic forms, are highly N-linked to oligosaccharides (Reis et al., 2010). For instance, colorectal neoplasms produce high levels of CEA glycosylated forms that can reach the blood vessels and then be detected in the circulation. Indeed, in practice, the molecular mass of CEA is 180–200 kDa and about 60% of this value results from N-glycosylation. However, the theoretical molecular weight of the full-length protein, after deglycosylation treatment, decreases to approximately 80 kDa. Notably, the glycosylated patterns of CEA differ among tissues and cells. Isoforms have been described, the most abundant of which are the 60-kDa splice variant derived from isoform 5D and the estimated 40-kDa splice variant derived from isoform 3D (Hatakeyama et al., 2013).

Importantly, CEA protein expression is associated with melanoma, lung adenocarcinoma, and mucinous ovarian carcinoma. It is mostly seen in digestive tract cancers such as pancreatic, gastric, and colorectal carcinomas (Hammarström, 1999; Beauchemin and Arabzadeh, 2013). In contrast with a healthy context, in which colon cells express CEA only through the apical side, once the tumorigenic process occurs, there is no more defined basal lamina in the tissue, cells lose polarity, and CEA is expressed on the entire surface (Hammarström, 1999). The importance of CEA in oncology, primarily in colorectal cancer, has been highlighted by multiple clinical trials (Table 2).

**Recycling of the CEA Protein.** The oncofetal molecule is more often referred to as a noninternalizing antigen. Besides, Bryan et al. (2005) studied the internalization and biodistribution of CEA at several time points. To achieve this, they used two antibodies, an anti-CEA monoclonal antibody (mAb) and a known rapidly internalized monoclonal antibody, both labeled with a radionucleotide (copper-64). They tested labeled mAbs in mouse xenografts from LS174T colorectal cancer cells. The results revealed that CEA had a fast blood clearance, an increased liver uptake, and enhanced tumor vascular accumulation compared with the supposed fast internalized



**Fig. 1.** The carcinoembryonic antigen cell adhesion molecules family. Each molecule harbors one variable (IgV-like) N-terminal domain (pink oval), homologous to the Ig variable domain. The terminal N-domain is generally linked to constant domains (IgC2-like), represented here as the blue oval labeled A and B. CEACAMs 5–8 are covalently bound to the membrane by a GPI linkage (blue arrows), whereas CEACAMs 1, 3, 4, and 19–21 use transmembrane domains. CEACAM16 is the only fully secreted protein. CEACAMs are generally highly N-glycosylated (green shapes).

antibody. These events suggested that CEA is continuously secreted by the tumor to the bloodstream and right after is cleared by receptor-mediated endocytosis in the hepatic cells. The secreted CEA, as the authors suggested, is probably immediately coupled to the CEA-targeted mAb, establishing CEA-antibody complexes that could explain the fast appearance of radioactivity in the liver. Besides, the affinity of the antibody itself influences its cellular penetration, as the high affinity ones are more susceptible of binding to the soluble CEA first, leaving only a few to bind to membrane-linked CEA, decreasing in this way the antibody penetration of tumors (Bryan et al., 2005).

Once inside the body, an antibody is immediately exposed to the bloodstream and clearance, extravasation from capillary vessels, tumor diffusion, internalization, and finally, catabolic degradation in cancer cells (Jain, 2001). Another line of thinking was recently suggested by K. Dane Wittrup et al. They compared CEA detection using different antibodies, namely, the internalization rate constant ( $K_e$ ) of an mAb anti-CEA and two single-chain variable fragments (scFvs) anti-CEA, the Sm3E (Vigor et al., 2010), and shMFE (Schumacher et al., 2013). The authors' interest in evaluating different antibodies arose from the potential for transporting pharma, using only an antibody-associated drug or an antibody-tagged nanosystem to carry the drug. One factor that is certainly delaying the success of antibody technologies for drug delivery is precisely the lack of penetration of cancer cells (Shargh et al., 2016). The cellular internalization, followed by antibody-ligand binding, and consequent catabolism that occurred inside the cell decreased the penetration ability of the antibody and, in turn, the penetration of the associated drug (Schmidt et al., 2008). The monoclonal antibody tested, independently of its own affinity, exhibited a likewise slow uptake by CRC cells (10–16 hours), compatible with the metabolic turnover of the CEA protein (~15 hours). The uptake was enough to guarantee distribution and retention in the cells. Importantly, none of the antibodies tested triggered changes in CEA expression. The hypothesis that is given by K. Dane Wittrup's team infers that the uptake of the antibodies into CRC cells resulted from a nonspecific signaling mechanism and from the natural recycling of the CEA itself. This underscores once again the role of CEA as a GPI-linked

protein, with no known ability to trigger signaling transduction pathways. Antibodies with slower internalization rates, as surface molecules with slow turnovers, will probably better enhance the penetration and retention in the tumor cells (Schmidt et al., 2008). Once the internalization into a CRC cell occurs by nonspecific mechanisms, the use of ligands with lower affinity for CEA recognition would probably be the best choice when the main objective is the sustained intravenous release of drugs, avoiding thereby the binding to soluble CEA.

### Nanoparticles: An Opportunity for Safe Drug Delivery

Drug delivery systems have been developed to improve the transport of therapeutic entities through the biologic fluids of the body, enhancing their half-life time in circulation and decreasing their side effects, namely toxicity (Robert et al., 2017). The major role of drug delivery strategies not only comprises overcoming the poor solubility and stability of standard therapies and creating an opportunity to test known drugs that would be ignored otherwise but could even encompass novel therapeutic entities, giving them the opportunity to overcome biologic barriers and become more specific for tumor cells (Allen, 2002; Ferrari, 2005).

The promising contributions of such technologies has attracted the attention of cancer researchers and physicians around the world. Chemotherapy, radiotherapy, and surgical resection remain the three “gold standard” anticancer therapies. Nevertheless, the majority of standard chemotherapies approved for clinical use have no ability to distinguish normal from cancer cells. This leads to severe side effects once the drugs act generally to impair mitosis, especially in fast-growing cells, including hair follicles and cells from bone marrow and gastrointestinal system, leading to hair loss, immune system failure, and infections, respectively (Banerjee and Sengupta, 2011; Labianca et al., 2013; Steichen et al., 2013).

Drug nanocarriers are solid and colloidal particles that emerge as safe drug vehicles, designed to generate many fewer toxic side effects and deliver high quantities of cargo to a

TABLE 2  
CEA-targeting systems for colorectal cancer monitorization and treatment in clinical trials

Ligand	Conjugate	Interventions	Clinical Indication	Route	Phase	State	Code	Sponsors
TF2 bispecific anti-CEA mAb	—	Pretargeted radioimmunotherapy	Metastatic colorectal cancer	IV	I	Terminated	NCT01273402 <sup>a</sup>	Immunomedics, Inc.
Anti-CEA diabody	<sup>125</sup> Iodine-labeled cT84.66 Diabody	Immunoscintigraphy	Colorectal cancer	IV	I	Completed	NCT00647153 <sup>a</sup>	City of Hope Medical Center
SGM-101	NIR fluorochrome-labeled anti-CEA mAb	Surgical resection histopathology	Colorectal and pancreatic cancer	IV	I/II	Recruiting	NCT02973672 <sup>a</sup>	Surgimab
SGM-101	NIR fluorochrome-labeled anti-CEA mAb	Surgical resection histopathology	Colorectal cancer and metastases of patients undergoing surgery	IV	III	Not yet recruiting	NCT03659448 <sup>a</sup>	Surgimab
M5A	Yttrium <sup>90</sup> (90Y) DOTA anti-CEA monoclonal antibody M5A	Treatment: Irinotecan hydrochloride Leucovorin calcium Fluorouracil Bevacizumab Yttrium <sup>90</sup> DOTA anti-CEA monoclonal antibody M5A	Metastatic colorectal cancer	IV	I	Completed	NCT01205022 <sup>a</sup>	City of Hope Medical Center
M5A	Cu <sup>64</sup> (copper -64) anti-CEA monoclonal antibody M5A	Positron emission tomography	CEA-expressing cancers, such as from gastrointestinal tract	IV	n.a.	Recruiting	NCT02293954 <sup>a</sup>	City of Hope Medical Center

IV, intravenous; n.a., not applicable.  
<sup>a</sup>www.clinicaltrials.gov.

very specific site of interest (Richards et al., 2017). Nanoparticles can be 1–1000 nm in diameter (Azevedo et al., 2018); however, those less than 200 nm are most suited for intravenous administrations, considering the width of the body's microcapillaries. Their advantages over microparticles (with a diameter >1  $\mu$ m) are notable; when the diameter of capillaries is 5–6  $\mu$ m, particles over 5  $\mu$ m could aggregate and drive an embolism (Singh and Lillard, 2009).

The novel therapies produced so far for use currently in colorectal cancer include targeted agents, such as monoclonal antibodies anti-VEGF like bevacizumab (de Gramont et al., 2012), or anti-EGFR agents such as cetuximab (Alberts et al., 2012) and panitumumab, the anti-VEGF recombinant fusion protein aflibercept, and the multikinase inhibitor regorafenib (Van Cutsem et al., 2014). For early colorectal cancer, no biologically targeted drugs are actually recommended (Labianca et al., 2013). Additionally, for metastatic CRC conditions, the majority of these therapies, primarily the monoclonal antibodies, only evidence clinical benefit when combined with standard chemotherapeutics (Van Cutsem et al., 2014, 2016). Most of the work that has been done on encapsulating such novel targeted molecules like monoclonal antibodies only intended the encapsulation of a single drug. Nevertheless, as most of them are only useful when combined with standard therapies, it is perhaps more interesting to encapsulate the whole combinatorial therapeutic scheme into the particles, instead of just one entity of it.

When developing a new formulation for therapeutic purposes, the main objectives to accomplish are: first, to guarantee that the system is biocompatible and stable in body fluids, which can be ensured by properly coating the particle surface with materials such as poly(ethylene glycol) that avoid the adhesion of opsonins, permitting an escape from immune system surveillance; second, to increase the concentration of drug into the tumor tissue by using materials that increase the tumor-enhanced permeability and retention effect, or simply by targeting the whole system to a molecule highly expressed in the tumor but not in healthy tissues; and finally, to reduce the toxic side effects of the drug, either by simple encapsulation or encapsulating the drug within a targeted system (Dawidczyk et al., 2014).

In the field of targeted drug delivery, strategies can be categorized as passive or active targeting. For both targeted systems, as with other nontargeted vehicles, delivery would be into the bloodstream. The difference is that the term “passive targeting” is used as synonymous with “blood circulation and extravasation,” meaning the passive accumulation of drugs in the vasculature surrounding the tumor, followed by an extravasation to tumor tissues, where it would be distributed (Park, 2013). The active targeting happens only after the “blood circulation and extravasation,” where a specific interaction occurs between a ligand from the drug/vehicle and a certain cancer cell molecule. The nanoparticle's surface can also be functionalized with molecules that have affinity for a specific cellular target of cancer cells, such as surface receptors and soluble proteins, to direct the whole system to a specific site (Zalba et al., 2015).

One characteristic that tumors have, although not exclusively, that may increase passive or active recruitment of nanoparticles is the enhanced permeability and retention effect, known as the EPR effect. The EPR effect is a phenomenon observed for macromolecules such as certain proteins and polymers whose molecular weight is higher than 40–50 kDa.



Such an effect favors the accumulation of molecules and nanoparticle delivery systems in neoplastic tissue rather than healthy tissue, increasing the local concentration of a given drug (Yin et al., 2014). The main reason for this behavior is the defective hyper-vascularization and lack of lymphatic drainage from the damaged tissues, so that molecules can invade the tumor tissue without being cleared for long time (Yin et al., 2014). The inherent properties associated with nanocarriers make them suitable for use in pharmaceutical formulations to enhance the accumulation of a drug into a solid neoplasm.

Nanocarriers can be sorted into organic (liposomes, polymeric micelles, polymeric nanoparticles, and dendrimers), inorganic (iron oxide nanoparticles, gold nanoparticles, mesoporous silica nanoparticles, carbon nanoparticles, and quantum dots), and hybrid organic-inorganic particles (Richards et al., 2017). One polymer that has become a success regarding polymeric nanoparticles is the poly(lactic-co-glycolic acid) (PLGA), mainly owing to its biodegradability and low cellular toxicity (Murthy, 2007). Some PLGA polymers are FDA-approved, and to date several formulations of PLGA nanoparticles have been clinically introduced; for example, Eligard, for advanced prostate cancer, delivers leuprolide, the luteinizing hormone-releasing hormone, to inhibit testosterone expression (Berges, 2005). Importantly, PLGA nanoparticles are versatile systems since, depending on the production method, they can deliver hydrophobic (Le Broc-Ryckewaert et al., 2013) or hydrophilic drugs (Gomes et al., 2017). The functionalization of this polymer with poly(ethylene glycol) turns the system less immunogenic, makes difficult its internalization and subsequent degradation by cancer cells, and enhances its stability in the body and its accumulation on solid tumors, all of them benefits from the EPR effect (Oliveira et al., 2012; Dawidczyk et al., 2014). For these reasons mentioned above, PLGA polymeric nanoparticles will receive more attention herein.

## CEA-Targeting Nanotechnologies

Creating a targeted nanoparticle requires a tag at its surface, a molecule that will specifically bind to a cell surface receptor characteristic of a pathology, or at least overexpressed compared with normal tissues, or even any extracellular molecule of interest. The functionalization of nanoparticles with specific ligands is currently a field of development, and several types of molecules are being used, as appropriate to the desired application. The ligands explored until now include vitamins (Mallakpour and Soltanian, 2016), proteins (Wang et al., 2010), peptides (Ma et al., 2017), aptamers (Yang et al., 2015), monoclonal antibodies (Heister et al., 2009), and antibody fragments (Hu et al., 2010). The last one covers a variety of entities such as: 1) F(ab')<sub>2</sub>, Fab', Fab, and half-antibodies (hAb; ~67 kDa), native antibody fragments (Fig. 2B) that can be produced by introducing specific enzymes or chemicals to cleave strategic points of a total immunoglobulin (Kennedy et al., 2018a); and 2) single-chain variable fragments (scFv; ~27 kDa), single-domain antibody fragments (sdAb; ~13 kDa), and Heterodimeric bispecific antibody fragment (SS-Fc) bispecific fragments (~80 kDa), genetically-engineered antibody fragments (Fig. 2C) generally produced by recombinant technologies like phage display techniques (Kennedy et al., 2018a).

The high affinity properties found in antibodies has led to multiple applications in medicine, such as the emerging immunotherapy. Currently, antibody fragments are becoming more

prominent as a new and improved technology relying on full-antibody features to give conjugated nanoparticles greater advantages for tissue penetration (Richards et al., 2017).

Most of the applications of anti-CEA nanomaterials are used for detection of the secreted CEA protein itself, or even in the detection of CEA-overexpressing cells such as colorectal or pancreatic cancer cells (Vigor et al., 2010; Ramos-Gomes et al., 2018). Despite the huge potential of new tools to detect CEA for monitoring purposes, only a few researchers are working in CEA-targeting systems to enhance the efficiency of cancer therapy at more developed stages (Heister et al., 2009; Hu et al., 2010). Table 3 focuses on the CEA-targeted nanotechnologies that can be applied to colorectal cancer therapy and monitoring. In the following, we will concentrate on the contributions of antibodies, more specifically antibody fragments, as promising molecules to enhance nanoparticle-driven therapies.

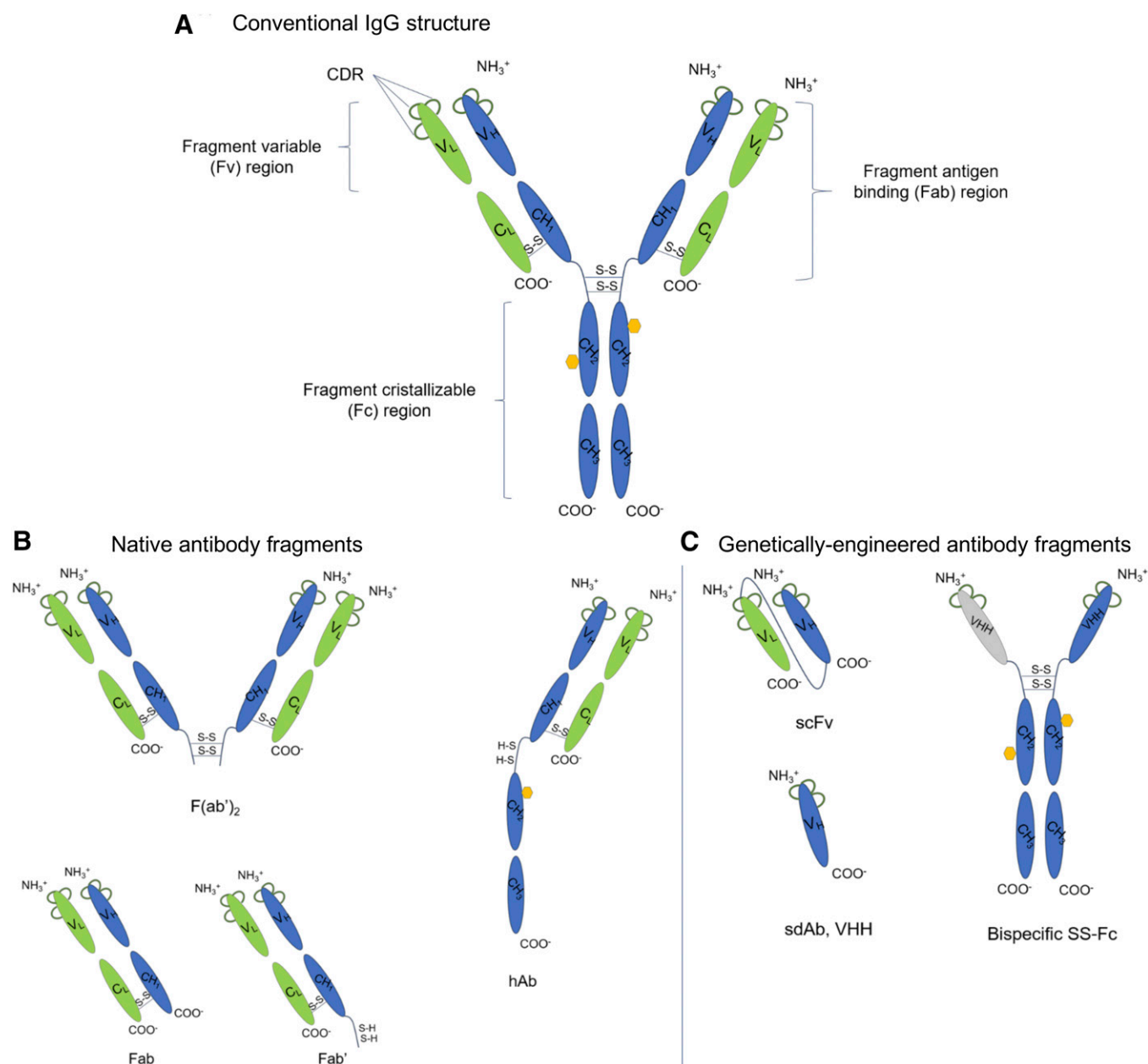
## Active Targeting Moieties

**Aptamers.** Aptamers are usually nonimmunogenic, single-stranded, synthetic oligonucleotides from RNA or DNA that can bind specifically to cell surface molecules. The small size of aptamers (from 20 to 50 nucleotides) allow them to work as delivery vehicles into the intracellular space. Although not able to passively permeate biologic membranes, these molecules overcome the phospholipidic bilayer by binding to specific cellular receptors that have turnover metabolisms compatible with the degradation time of the aptamer. Ultimately, they exhibit nano- to picomolar affinities for their targets (Orava et al., 2010; Yang et al., 2015; Li et al., 2016a).

## Monoclonal Antibodies

The soluble form of antibodies is produced by differentiated B lymphocytes (plasmocytes), and there exist several ways to fabricate antibodies against a desired protein epitope of an antigen. Each B lymphocyte clone produces antibodies that are specific for only a single epitope. A monoclonal antibody is in this way an antibody produced by a single clone of B cells. To produce monoclonal antibodies of interest, host animals are first immunized with a specific immunogenic sequence of a given antigen, the epitope. Once immature B cells, nonreactive to host-antigens, migrate to the host spleen, they follow the maturation step by which they are presented to the foreign antigen previously introduced. Still in the spleen, mature B lymphocytes, expressing at their surface the Ig receptors recognizing specifically the desired epitope, are selected and isolated. Those B cells are then fused with immortal B cancer cells, the myeloma cells, to constitute a highly proliferative hybridoma, immortal producers of that monoclonal antibody (Tomita and Tsumoto, 2011).

As shown in Fig. 2A, each full-length immunoglobulin (~150 kDa) is composed by two heavy chains (H, in blue) and two light chains (L, in green). Within each chain there are two separated regions, the amino-terminal variable region (V), containing VH and VL domains, and the carboxyl-terminal constant region (C), containing CH1, CH2, and CH3 domains. Disulfide bridges are essential to link all chains and create the "Y" shape characteristic of an Ig. In addition, each heavy (VH) or light variable (VL) region contains a hypervariable domain, composed of three protein loops, the complementary-determining regions (CDRs). The CDRs have different amino acid sequences from antibody to antibody, which make them responsible for



**Fig. 2.** Structure of conventional whole immunoglobulin and antibody fragments. (A) Conventional IgG has one fragment crystallizable region and two fragment antigen-binding regions, each one containing one fragment variable region. The two heavy (H, on blue) and light (L, on green) chains contain the amino-ended variable region (VH or VL, respectively), and the carboxyl-ended constant region (CH1, CH2, CH3, or CL, respectively). The sites for antigen binding are given by three complementary-determining regions, CDRs (the green arches on the amine-ending). The Fc portion is also glycosylated (yellow hexagons). The disulfide bridges (S-S) stabilize the “Y” format of the Ig. (B) Native antibody fragments. F(ab')<sub>2</sub>, Fab', Fab, and half-antibodies (~67 kDa). (C) Genetically-engineered antibody fragments. Single-chain variable fragments (~27 kDa), single-domain antibody fragments (sdAb; ~13 kDa), and SS-Fc bispecific fragments (~80 kDa).

the variety of antigen epitopes that antibodies can specifically recognize (Kennedy et al., 2018a).

Moreover, the full antibody has two fragment antigen-binding (Fab) regions that integrate the sites for antigen binding (hyper-variable regions) and the constant regions from heavy (CH1) and light (CL) chains. The fragment crystallizable (Fc) region is the antibody portion that activates cells containing Fc receptors (FcR), namely, phagocytic cells. Phagocytes have in this way the ability to trigger an immunologic response through antibody-dependent cell-mediated cytotoxicity (ADCC). Fc fragments also initiate complement activation through the classic pathway, which ends with cell lysis (Kennedy et al., 2017). Interestingly,

immunoglobulins and albumin are the most abundant proteins present in human serum. To not waste much energy by producing these proteins *de novo*, the body has specific mechanisms to prolong their half-life in circulation. Particularly, neonatal Fc receptor (FcRn) appears as an intracellular Fc-receptor that recognizes antibody Fc domains and albumin, avoiding their degradation by lysosomes, which is an advantage of using whole Ig for targeting proposes (Martins et al., 2016).

### Antibody Fragments

Some drawbacks of whole antibodies are the immunogenicity and the clearance from bloodstream, both owing to binding



TABLE 3  
CEA-targeted nanosystems for monitoring and therapeutic applications

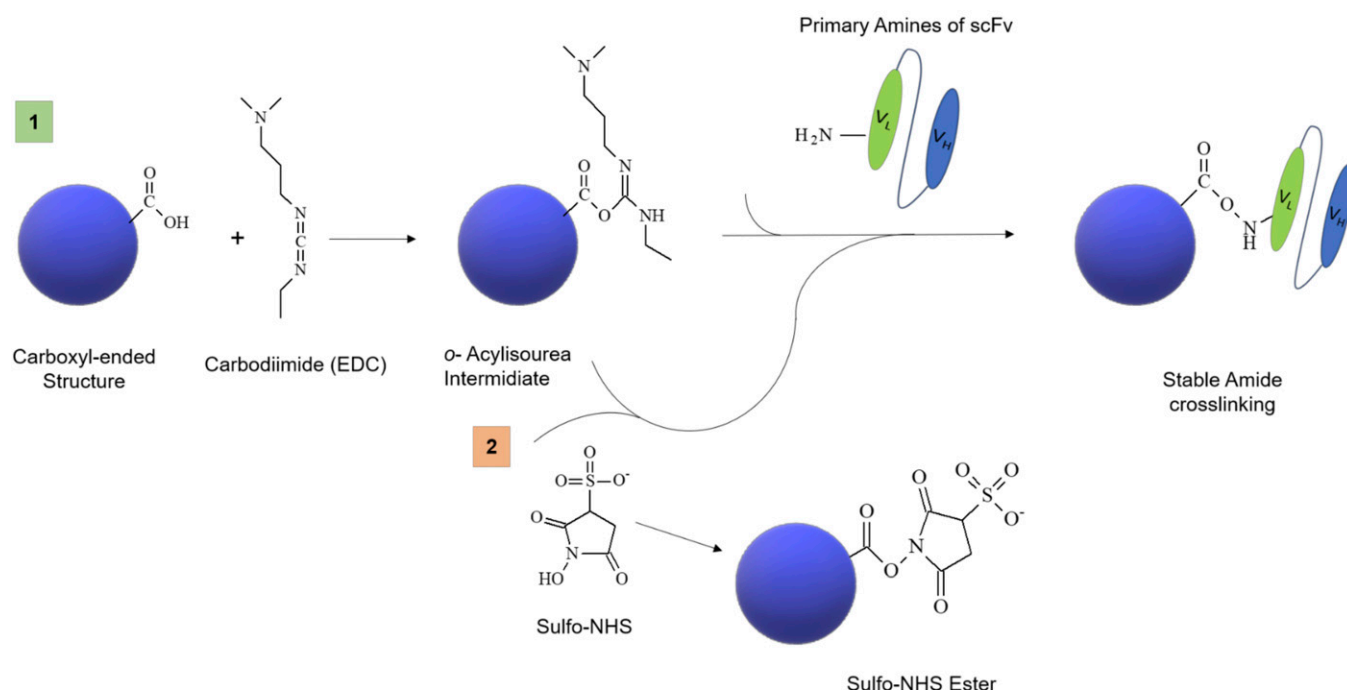
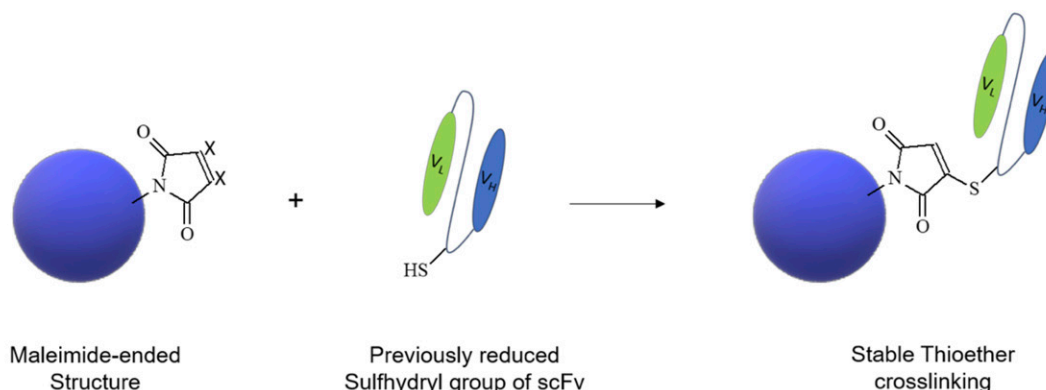
Ligand	Formulation	Linker	Drug	Features	Reference
CEA aptamer	Combination of silver nanoclusters (AgNCs) and gold nanoparticles (AuNPs)	Half-complementary DNA + CEA aptamer + half complementary DNA	-----	Detects CEA within a range of 0.01–1 ng/ml. The CEA detection limit was 3 pg/ml. DNA-Au NPs had 15.4 ± 0.7 nm and -37.3 ± 1.5 mV. This method was validated by testing CEA in healthy human blood samples.	Yang et al. (2015)
Amine-modified CEA aptamer	Upconverting nanoparticles (UCPs)	CEA amine modified aptamer + hexanedioic acid (HAD)	-----	The CEA detection occurred within a range of 4–100 pg/ml. The CEA detection limit was 1.7 pg/ml. The HSA-UCPs had 10–20 nm. The CEA aptamer was conjugated through carbodiimide chemistry.	Li et al. (2016a)
mAb anti-CEA	Silica nanoparticles	SMCC SM[PEG] <sub>4</sub> EDC/sulfo-NHS PAMAM dendrimers	-----	In vivo studies performed with LS174T xenografts. PAMAM dendrimer-conjugated particles had 71 nm. The CRC cell lines used for in vitro studies were LS174T, LoVo, and HCT116. CEA-targeted PAMAM dendrimer-conjugated NPs had the highest binding to CEA compared with the negative control.	Tieman et al. (2015)
mAb anti-CEA	Carbon nanotubes	BSA-fluorescein	Doxorubicin	A single SWCN had ~1 nm, AE of 87.5% (indirect method) and theoretical DL of 11.6%. The weight ratio of doxorubicin to oxidized SWCNs is 20:1. Carbodiimide chemistry was applied. CRC cell lines for in vitro studies: WiDr.	Heister et al. (2009)
mAb anti-CEA	PLGA nanoparticles	PEG-COOH	Paclitaxel	NPs had ~200 nm and -10.4 mV with a low PDI. They also had a practical DL of 16.6% and AE of 99.4%. Carbodiimide chemistry was applied and the NPs showed a sustained release up to 48 h and had no cytotoxicity in the CRC cells. CCR cell line CEA <sup>+</sup> was Caco-2 and CEA <sup>-</sup> was SW480.	Pereira et al. (2018)
Sm3E (scFv)	Superparamagnetic iron oxide nanoparticles (SPIONs)	Dextran-OH Dextran-PEG-COOH	-----	Sm3E was engineered with a C-terminal (6x His) tag and produced in yeast. The scFv K <sub>D</sub> was 30 pM. Carbodiimide conjugation strategy was applied. CRC cell line CEA <sup>+</sup> was LS174T and melanoma cell line CEA <sup>-</sup> was A375M.	Schmidt et al. (2008); Vigor et al. (2010)
shMFE (scFv)	PEG chain (5 kDa) Fluorescein Biotin Nitroxide spin label	Dibromomaleimide Dithiophenolmaleimide	-----	shMFE has tropism to the same CEA epitope as Sm3E and was also produced in yeast. The K <sub>D</sub> of shMFE to CEA was 8.5 nM and the K <sub>D</sub> of spin-labeled scFv in PBS was 1.91 ± 0.78 μM, whereas in plasma it was 4.35 ± 1.27 μM and in whole blood was 6.46 ± 1.7 μM. The CEA detection limit was 100 nM (spin-labeled scFv). Maleimide chemistry was applied. The PC cell line CEA <sup>+</sup> was CAPAN-1 and the melanoma cell line CEA <sup>-</sup> was A375.	Schmidt et al. (2008); Schumacher et al. (2013)

(continued)

TABLE 3—Continued

Ligand	Formulation	Linker	Drug	Features	Reference
MFE-23 (scFv)	Carbon nanotube	1-Pyrene-NHS ester Hexahistidine tag	-----	Ni-NPs had 20–60 nm and are linked to nanotubes through an electrochemical technique. scFvs have a hexahistidine tag in the C-terminal. The fragment was produced in bacteria.	Lo et al. (2009)
SS-Fc	Anti-Flag-FITC	Histag Flagtag	-----	In vivo studies performed with LS174T xenografts. SS-Fc was produced in bacteria. The Histag (6x His) and Flagtag (polypeptide chain) motifs were added to the C-terminal of anti-CEA-Fc and anti-CD16-Fc domains. The $K_D$ was 0.195 nM (for CEA) and of 5.75 nM (for CD16). The SS-Fc had potent toxicity against CEA <sup>+</sup> cells HT29 and LS174T. The ovarian cancer cell line CEA <sup>+</sup> was SKOV3.	Li et al. (2016b)
hAb anti-CEA	Lipid-polymer hybrid NPs	PEG-Maleimide	Paditaxel	hAb-NPs had 95 nm and –55 mV. The hAb-NPs had an $IC_{50}$ of 251 nM and nonfunctionalized particles had an $IC_{50}$ of 526 nM. The theoretical DL was 3.8%. The maleimide chemistry was applied, and NPs functionalized with hAb had more than 2-fold increase in toxicity comparing to naked NPs. The PC cell line CEA <sup>+</sup> was BxPC-3 and CEA <sup>–</sup> was XPA-3.	Hu et al. (2010)
sdAb-CEA	Quantum dots (QDs)	Sulfo-SMCC PMPI	-----	The $K_D$ was 8.3 nM and sdAb-QDs had $11.9 \pm 2.9$ nm. sdAb was engineered with a 6-Histidine tag chain in its C-terminal (sdAb-C17 his6Cys). Produced in bacteria. The CRC cell line CEA <sup>+</sup> was MC38CEA and CEA <sup>–</sup> was MC38.	Sukhanova et al. (2012); Ramos-Gomes et al. (2018)

AE, association efficiency; CRC, colorectal cancer; DL, drug loading; His, histidine;  $K_D$ , equilibrium dissociation constant; NPs, nanoparticles; PBS, phosphate-buffered saline; PC, pancreatic cancer; PdI, polydispersity index; PMPI, 4-(Maleimidophenyl isocyanate; scFv, single-chain variable fragment; sdAb, single-domain antibody fragment; SMCC, succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate; Sm3E, shMFE, type of single-chain variable fragments; SM[PEG]<sub>4</sub>, succinimidyl-[(N-maleimidopropionamido)-tetraethyleneglycol] ester; SS-Fc, heterodimeric bispecific antibody fragment; SWCN, single-walled carbon nanotube; WiDr, human colorectal cancer cell line.

**A** Crosslinking carboxyl-to-amine functional groups by using EDC (1) and sulfo-NHS ester (2) reaction scheme**B** Crosslinking maleimide-to-sulfhydryl functional groups by using maleimide reaction scheme

**Fig. 3.** Most common reaction chemistries to conjugate antibodies to other structures. (A) The linkage between a carboxylated structure and the primary amines of scFv (antibody fragment) could occur by adding two crosslinkers: EDC and NHS, (or its more water-soluble form, sulfo-NHS). Generally, when applying EDC (step 1) is also added sulfo-NHS (step 2) to increase the efficiency of the reaction. There are also circumstances where the carboxylated structure is already activated by sulfo-NHS, forming a sulfo-NHS ester structure, and in this situation (starting on step 2) there is no need to add any crosslinker. (B) In the linkage between a maleimide-ended structure and a thiolated scFv, maleimide works as the crosslinker and the X groups on it could be, most commonly, a simple hydrogen or preferably, any good-leaving group as a halogen. The thiol (-SH) and disulfide (S-S) groups on scFv should be previously reduced to guarantee that they are ready for conjugation.

of Fc receptor-containing entities to the antibody Fc region (Cheng and Allen, 2010). In addition, antibody size (~150 kDa) makes cell penetration difficult. However, the big advantage of using a full-length mAb for targeting systems is the presence of two antigen-binding regions (Fab), whereas some antibody fragments carry only one.

Antibody fragments, excluding SS-Fc ones (Li et al., 2016b), have multiple advantages in comparison with mAb with respect to their use in intracellular drug-delivery systems. First, they are less immunogenic than a whole Ig, owing to the lack of the Fc region, and they retain almost the affinity and

specificity found in whole immunoglobulins. Second, they are able to couple in a more oriented manner to a nanoparticulate system (Cheng and Allen, 2010; Shargh et al., 2016). For nanoparticle-decorating purposes, the size of the ligands is also important, making antibody fragments certainly very promising.

### Antibody Conjugation Strategies

To covalently link two compounds, it is first necessary to understand the reactive groups that are present in each of

them. Next, it is required to choose the most appropriate crosslinker to participate in the selected conjugation reaction. When referring to antibody conjugation systems, there are two main chemistries that might be applied: the carbodiimide and the maleimide. Importantly, the conjugation chemistry that is selected to bind an antibody to a nanoparticle can influence the specific binding to a desired epitope (Tiernan et al., 2015). As explored below, the linker chosen for ligand-nanoparticle coupling is essential to the modulation of nanosystem characteristics.

**Carboxyl-to-Amine Conjugation Reaction.** This strategy is often applied to covalently link the amine-containing residues (lysine, histidine, and arginine) to a carboxylated structure or carboxyl-containing residues, such as aspartic acid and glutamic acid to a primary amine structure.

The first of two-steps in the reaction of carbodiimide chemical conjugation, where 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), a carbodiimide linker, reacts with the carboxylated structure, is represented in Fig. 3. The production of a relatively more stable and water-soluble ester complex is achieved through the addition of *N*-hydroxysulfosuccinimide (sulfo-NHS), representing the second step of the reaction. Thereby, the carboxyl-activated groups of the structure react with the primary amine groups of the antibody fragment (scFv), producing ultimately a stable amide between both. More importantly, the carbodiimide is known as a “zero-length” linker, meaning that the unstable intermediate *o*-acylisourea will not participate in the final product of the reaction. The same happens when carbodiimide is used in combination with NHS or sulfo-NHS (NHS linked to a sulfonate group,  $\text{SO}_3^-$ ) (Carter et al., 2016). Interestingly, Tiernan et al. (2015) tested two different linkers to conjugate a monoclonal antibody to nanoparticles by the carbodiimide chemistry: the EDC/NHS and the polyamidoamine (PAMAM) dendrimers. These dendrimers have primary amine groups at their surface that could bind either to the carboxylated silica nanoparticles or to the antibody. This group studied the specificity of the conjugated systems by conjugating separately with a negative control monoclonal antibody. Overall, they demonstrated that the EDC/NHS linkers provided 1.7-fold more binding compared with the negative control, although not sufficient to guarantee specific binding. Moreover, the PAMAM dendrimers linked via carbodiimide chemistry showed a maximum binding of 12.3-fold compared with negative control. These results could be explained by the amplification of the conjugation when crosslinkers that bind to multiple molecules are used. In this case, each PAMAM dendrimer binds a single nanoparticle to several antibodies, amplifying the number of ligands that exist in the system, and thereby increasing the available ligand epitopes for CEA receptor targeting. This report also recognizes the importance of using negative control antibodies to confirm that the binding of an antibody-functionalized nanoparticle is only owing to the affinity of the antibody to its target epitope, and not the result of nonspecific interactions that may occur.

**Maleimide-to-Sulfhydryl Conjugation Reaction.** This chemical reaction is mostly used to conjugate antibodies containing a cysteine residue that is the only amino acid containing a terminal thiol group. Such ligands can have just one sulfhydryl group ( $-\text{SH}$ ) or multiple cysteines from which disulfide bridges ( $-\text{S}-\text{S}-$ ) are originated. A free cysteine amino acid is considered a relatively rare constituent of proteins, and this feature is used as

an advantage to artificially modify antibodies and other proteins of interest to produce the chemical conjugations desired through sulfhydryl binding reagents (Jones et al., 2012). A structure containing the maleimide group (crosslinker) could then react with the previously reduced thiol groups of the scFv antibody fragment, as shown in Fig. 3B. The final product would be a stable thioether linkage between both compounds. Regarding the maleimide molecule, the “X” groups on it (Fig. 3B) will not participate in the final product of the reaction. Such groups are generally any hydrogen atom ( $-\text{H}$ ) and preferably any the good-leaving groups, for instance the bromine atom ( $-\text{Br}$ ) and other halogens. Once the maleimide reaction is known as an irreversible one, it could change the conformation of the antibodies, which could affect the affinity for the target. Baker and colleagues used halogen-substituted maleimides as dibromomaleimides, which have the ability to create a rigid two-carbon bridge between two cysteines (not represented). This strategy confers a reversible linkage and maintains the stability of the antibody (Schumacher et al., 2011, 2013). Moreover, James P. Tiernan and coworkers tested two different crosslinkers: succinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and succinimidyl-[(*N*-maleimidopropionamido)-tetraethyleneglycol] ester (SM[PEG]<sub>4</sub>), with the main goal of linking the amine groups previously added to the silica nanoparticles to the thiol groups of a monoclonal antibody (Tiernan et al., 2015). First the linkers reacted with the amine-coated silica particles, and after that the antibodies were added. Both crosslinkers have an NHS and a maleimide terminal group, one at each side, that will trigger, respectively, the binding of the amine groups of silica particles to the carboxyl-activated linker and the binding of the antibody thiol groups to the maleimide molecule. Both linkers showed no specific binding to the neoplastic cells.

## Conclusions

Colorectal cancer is one of the deadliest diseases worldwide, primarily owing to metastatic events. Designing new targeted strategies to treat and monitor individuals in more developed stages is needed.

CEA glycoprotein appears as a cell surface molecule overexpressed in most CRC patients, and the evaluation of its serum levels are recommended in the clinic. This promising protein has a slow half-life ( $\sim 15$  hours), which enables longer retention of ligand-CEA complexes inside the cell. In its turn, this could enhance the sustained release of nanoencapsulated drugs, in the case of therapeutic applications, or specific dyes, in the case of colorectal cancer monitorization.

CEA-targeting technologies that have already been produced are mainly focused on monitorization of colorectal cancer evolution. Only a few address the specific guiding of drug-delivery systems. The affinity of the ligands used for the functionalization of nanoparticle-based systems could also modulate the tendency to bind to the membrane-linked CEA or to the serum-available soluble CEA secreted by tumor cells. Independently of the affinity of the ligand, the internalization into a cancer cell occurs by nonspecific mechanisms and the use of lower-affinity ligands for CEA recognition is probably the best choice when an intravenous administration is desired. Here, we envisage that the use of antibody fragment-decorated nanoparticles, with high affinity characteristics and the probability of performing an oriented

functionalization, might be a successful approach for CRC treatment and monitorization. Among the nanocarriers most suitable for this purpose, PLGA nanoparticles have a huge impact, owing to their biocompatibility and low toxicity features and are expected to achieve important advances in the near future.

Overall, we have highlighted the great potential for development of CEA-targeting nanocarriers for drug delivery into colorectal tumors, a field that remains poorly explored but worthy of development.

#### Authorship Contributions

Wrote or contributed to the writing of the manuscript: Sousa, Oliveira, Sarmento.

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