

# Immunotherapy for the treatment of colorectal tumors: focus on approved and in-clinical-trial monoclonal antibodies

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**Abstract:** Colorectal cancer is considered a disease of the elderly population. Since the number of geriatric patients continues to rise, monoclonal antibody therapy is the most promising therapy in the recent research. Presently, the monoclonal antibodies most frequently used in the treatment of colorectal tumors are bevacizumab, cetuximab, panitumumab, and ramucirumab. Bevacizumab is a monoclonal antibody that acts on VEGF. Cetuximab and panitumumab act on EGFR. Ramucirumab binds directly to the ligand-binding pocket of VEGFR-2 to block the binding of VEGF-A, VEGF-C, and VEGF-D. These monoclonal antibodies, alone or in association with radiotherapy or chemotherapy, are presenting good results and are increasing patient survival, despite the side effects. Due to the limited number of molecules available, several studies are trying to develop new monoclonal antibodies for the treatment of colorectal tumors. Among those being studied, some recent molecules are in phase I and/or II trials and are yielding advantageous results, such as anti-DR5, anti-Fn14, anti-IGF-1R, anti-EGFR, anti-NRP1, and anti-A33 antibodies. This has been successful in reducing side effects and in treating nonresponsive patients.

**Keywords:** monoclonal antibodies, colorectal tumor, bevacizumab, cetuximab, panitumumab, ramucirumab

## Colorectal tumor

Colorectal cancer is considered a heterogeneous disease, both in relation to the pathogenesis and the clinical signals.<sup>1</sup> The incidence of colorectal cancer is higher in people between 60 and 70 years of age. Twenty percent of cases occur before the age of 50. Colorectal cancer is more prevalent in the US, Canada, Australia, New Zealand, Denmark, Sweden, and other developed countries. The incidence is 30 times lower in India, South America, and Africa. In Japan, the incidence is increasing gradually, reaching similar rates in the UK, presumably as a result of changes in lifestyle and diet.<sup>2-7</sup>

The discovery of sequences of genes associated with adenoma–carcinoma has contributed to understanding the genetic and epigenetic alterations that drive the formation of colorectal cancer. Among the genetic alterations associated with colorectal cancer, abnormal activation of the Wnt/ $\beta$ -catenin signaling pathway has an important role, because *Wnt* pathway is crucial in cell proliferation and migration.<sup>8</sup>

Somatic mutations may play an important role as prognostic or predictive markers for the development of specific therapies in colorectal cancer. These mutations involve genes such as *KRAS*, *BRAF*, *PIK3CA*, *AKT1*, *PTEN*, *NRAS*, and *TGFBR2*.<sup>9-15</sup> Therefore, epigenetic and gene silencing events may increase the progression of

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colorectal cancer.<sup>16</sup> This finding opens new possibilities for high-precision laboratory tests such as the identification of molecular markers in screening tests for noninvasive colorectal adenocarcinoma.<sup>17</sup>

## Supporting treatment with monoclonal antibodies

### Approved monoclonal antibodies for therapy

Several monoclonal antibodies for different therapeutic targets have already been approved for use in patients with tumors. For example, cetuximab and panitumumab are monoclonal antibodies directed at the EGFR.<sup>4,18,19</sup> Bevacizumab is targeted to the VEGF.<sup>20,21</sup> Ramucirumab inhibits VEGF and VEGF receptor.<sup>22,23</sup> Moreover, for the CD20 target, rituximab, ofatumumab, tositumomab, and ibritumomab tiuxetan<sup>24–26</sup> are used. The antibodies trastuzumab, pertuzumab, trastuzumab, and emtansine are directed HER2/neu.<sup>27–33</sup> The monoclonal antibody that targets the CD33 molecule is gemtuzumab ozogamicin.<sup>31,34</sup> Alemtuzumab binds to CD52,<sup>35,36</sup> while brentuximab vedotin is directed at the CD30 molecule.<sup>37</sup> Ipilimumab binds to the CTLA4 molecule, among others.<sup>38,39</sup> Still under development are monoclonal antibodies directed at new or older potential targets, but are not yet approved for use in patients.<sup>18,25,40–43</sup>

### Approved monoclonal antibodies for colorectal tumors

At present, among biological molecules, a few antibodies have their use approved for the treatment of colorectal tumors (Table 1). The four approved monoclonal antibodies showing favorable results for use in the treatment of colorectal cancer are bevacizumab, cetuximab, panitumumab, and ramucirumab, which are detailed in the following section.<sup>42,44–46</sup>

#### Cetuximab

Cetuximab is a monoclonal antibody, IgG1 kappa human/murine chimeric, with constant regions and murine variable

regions. It recognizes the extracellular domain of the EGFR and therefore can block the tumor growth.<sup>47</sup>

After binding to the extracellular domain of EGFR, this antibody prevents the signaling and ligand-induced dimerization of the receptor. In this way, it blocks the signals of cell growth and tumor survival. Cetuximab may have the function of mediating antibody-dependent cellular cytotoxicity (ADCC). This monoclonal antibody only achieves dynamic equilibrium after the third week of infusion.<sup>48–56</sup> In addition, this biological molecule was approved by the US Food and Drug Administration (FDA) for patients with metastatic colorectal cancer (mCRC), especially for patients who do not tolerate irinotecan (a chemotherapeutic drug), and was also associated with irinotecan for patients who do not tolerate oxaliplatin, irinotecan, and 5-fluorouracil (5-FU).<sup>57,58</sup>

Some patients present an anaphylactic reaction. A study has shown that there is a higher incidence of anaphylaxis in people who have a history of allergies to red meat and tick bites.<sup>59</sup> Cutaneous reactions are considered severe with cetuximab. Cytokine release syndrome occurs usually 1 hour after the infusion. This reaction is usually more severe after the first infusion.<sup>60</sup> Electrolyte disturbances and neutropenia events may be related to the associated chemotherapy using platinum-based compounds. Eye infections such as ulcerative dermatitis can also occur.<sup>61</sup>

Cetuximab has demonstrated benefits only in a small subset of metastatic patients (KRAS wild-type patients and patients with limited metastatic disease). Alternative strategies that demonstrate promising results for the treatment of rectal cancer during stage II/III include short-course radiotherapy with adjuvant chemotherapy and neoadjuvant FOLFOX (folinic acid, 5-FU, and oxaliplatin)–bevacizumab without radiotherapy.<sup>62</sup>

#### Panitumumab

Panitumumab is a humanized IgG2 kappa antibody with complementary determining regions (CDRs) of murine origin. It is directed against the EGFR. When compared with cetuximab,

**Table 1** Approved antibodies for the treatment of colorectal tumor

Monoclonal antibody	Therapeutic target	Mechanism of action	Indication approved (FDA)
Bevacizumab (humanized IgG1)	VEGF	Inhibition of VEGF	Colorectal cancer – palliative treatment
Cetuximab (humanized/murine chimeric IgG1)	EGFR (ErbB1)	Inhibition of EGFR; ADCC	Colorectal cancer – palliative treatment
Panitumumab	EGFR	Inhibition of EGFR	Metastatic colorectal carcinoma – with EGFR expression
Ramucirumab	VEGF receptor-2	Blockage of VEGF-A, VEGF-C, and binding of VEGF-D	mCRC – in combination with FOLFIRI (irinotecan, folinic acid, and 5-fluorouracil)

**Abbreviations:** ADCC, antibody-dependent cellular cytotoxicity; FDA, US Food and Drug Administration; mCRC, metastatic colorectal cancer.

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it is a murine antibody with reduced portions. However, panitumumab is not able to mediate ADCC.<sup>56,63</sup> The mechanism of action of panitumumab is due to its binding to EGFR and blocking of this receptor. It inhibits the autophosphorylation of the receptor induced by the ligands of EGFR that internalizes their functions. In conclusion, the monoclonal ligation inhibits cell growth, induces cell apoptosis, and inhibits the production of interleukin-8 and VEGF.<sup>64</sup>

This monoclonal antibody is approved for use in adult patients. Patients with mCRC expressing EGFR in treatment with panitumumab have presented a better response.<sup>65</sup> The most frequently reported adverse reactions, in monotherapy or in conjunction with chemotherapy, were skin reactions, gastrointestinal disorders, nutrition and metabolic disorders, anorexia, chills, fever, and dyspnea.<sup>66</sup>

### Bevacizumab

Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to VEGF.<sup>21,67,68</sup> Bevacizumab has an important role in the treatment of colon tumors, but has been used in the treatment of other tumors with favorable results since its role in blocking angiogenesis is of fundamental importance in tumor biology.<sup>69</sup>

Bevacizumab, when used in association with chemotherapy, effectively reduces the progression of tumors in the lungs, colorectal tumors, and breast cancer. The proposed mechanism of action of bevacizumab is related to preventing the interaction of VEGF with VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). These receptors are proteins located in the cytoplasmic domain and are connected to the extracellular matrix. VEGF is on top of the cytoplasmic membrane. Bevacizumab binds to VEGF and prevents the proliferation of endothelial cells and formation of blood vessels, as well as decreases the blood supply and reduces tumor interstitial pressure. Thus, the ability of chemotherapy to reach the tumor is increased by the combinatorial treatment.<sup>20,70–73</sup>

In general, in patients with metastatic carcinoma of the colon or rectum, this antibody is used in combination with a fluoropyrimidine chemotherapeutic drug.<sup>74,75</sup> Bevacizumab is a monoclonal antibody with remarkable antiangiogenic capacity, but is associated with serious cases of gastrointestinal perforation and fistulas, bleeding, bleeding associated with the tumor, and hypertension. Injured or postsurgery patients should not make use of bevacizumab, since it can reduce healing, causing a wound dehiscence and even perforation or abscesses.<sup>76</sup>

### Ramucirumab

In 2015, ramucirumab was approved by the FDA for the treatment of patients with colorectal cancer.<sup>77</sup> It is a fully humanized

IgG1 monoclonal antibody that targets the extracellular domain of VEGF and VEGFR-2. The binding of the monoclonal antibody inhibits the activation and signaling of these molecules/receptors.<sup>78</sup> It is also believed that VEGF and VEGFR-2 mediated angiogenesis in colorectal cancer.<sup>22</sup> Ramucirumab, in combination with FOLFIRI (irinotecan, folinic acid, and 5-FU), is indicated for the treatment of patients with mCRC with the disease in progression or after therapy with bevacizumab, oxaliplatin, and fluoropyrimidine. The usual dose is 8 mg/kg ramucirumab plus FOLFIRI every 2 weeks.<sup>79</sup> Ramucirumab presents the risk of hemorrhage and gastrointestinal hemorrhage, including severe and sometimes fatal hemorrhagic events.<sup>23,77–81</sup>

## Monoclonal antibodies in clinical trials from 2012 to 2016

Bevacizumab is considered a milestone in the treatment of colorectal cancer and is helpful when used in combination with chemotherapy. On the other hand, the anti-EGFR antibodies, also relevant in practice, have limited application for cases of mutations of *K-ras* gene. Since the currently approved monoclonal antibodies act due to the inhibition of VEGF or EGFR, they did not present clinical efficacy in all patients with colorectal tumors. Due to this, the new antibodies under development are a therapeutic promise. These monoclonal antibodies present a different mechanism of action and the possibility of reduced side effects. However, detailed clinical trials are under way to verify if these new molecules are more effective than the already approved antibodies. Also, these trials are necessary to determine their drawbacks and adverse effects. The newest developments in monoclonal antibodies for immunotherapy are detailed in Table 2 and in the following section.

### Fibroblast growth factor-inducible 14 (Fn14) molecules

RG7212 is a monoclonal antibody in phase I immunotherapy study. It is a first-in-class, fully humanized IgG1 kappa monoclonal antibody. RG7212 blocks TNF (tumour necrosis factor)-like weak inducer of apoptosis (TWEAK) by binding to the Fn14 receptor. TWEAK and Fn14 are a ligand–receptor pair frequently overexpressed in solid tumors. TWEAK induces cell proliferation, survival, and NF- $\kappa$ B signaling. RG7212 had good tolerability and favorable pharmacokinetics in combination therapies in patients with Fn14-positive tumors.<sup>82</sup>

### Anti-DR5 antibodies

Tigatuzumab is a humanized monoclonal IgG1 agonist of human DR5, also known as member 10b of the TNF receptor

**Table 2** New monoclonal antibodies under clinical trials for the treatment of colorectal tumor

Monoclonal antibody	Therapeutic target	Mechanism of action	Trials
RG7212 (fully humanized IgG1 kappa)	FnI4	Inhibits TWEAK; reduces proliferation, survival, and NF-κB signaling	Phase I trial for solid tumors
Tigatuzumab (CS-1008; humanized IgG1)	DR5 (TNFRSF10B or TRAIL-R2)	Agonist of DR5, apoptosis induction, and cytotoxic activity	Phase II trial for mCRC
Nimotuzumab (humanized IgG1)	Extracellular domain of EGFR	Inhibits EGFR	Phase II trial for local advanced colorectal tumor
Figitumumab (fully humanized IgG2)	IGF-1R	Reduces the activation of IGF-1R	Phase II clinical trial for refractory mCRC
Robatumumab (R-1507, I9D12, or SCH 717454; fully humanized IgG1 kappa isotype)	IGF-1R	Antagonist of IGF-1R, inhibits VEGF and angiogenesis	Phase II study for advanced colorectal cancer
KRN330 (fully humanized Ig)	A33 glycoprotein	Stimulates CTL response against A33-positive colorectal cancer	Phase I/II study, in combination with irinotecan for mCRC
I-huA33 (fully humanized IgG1)	A33 glycoprotein	Stimulates CTL response against A33-positive colorectal cancer	Phase I trial, in combination with capecitabine for mCRC
MNRPI685A (fully humanized IgG1 isotype)	NRPI	Targets the VEGF binding domain of NRPI; reduces angiogenesis, cell survival, migration, and invasion	Phase I trial, in combination with anti-VEGF therapies to advanced tumors
Dalotuzumab (MK-0646; fully humanized IgG1 isotype)	IGF-1R	Antagonist of IGF-1R; inhibits IGF-1- and IGF-2-mediated tumor cell proliferation, IGF-1R autophosphorylation and Akt phosphorylation	Phase I study, in combination with cetuximab and irinotecan for advanced colorectal cancer
Drozitumab (anti-TRAIL, PRO-95780, RG7425; fully humanized IgG1)	DR5	Agonist of DR5, apoptosis induction, cytotoxic activity	Phase I study, in combination with first-line mFOLFOX6 plus bevacizumab for mCRC

**Abbreviations:** 5-FU, 5-fluorouracil; CTL, cytotoxic T-lymphocyte; FnI4, fibroblast growth factor-inducible 14; mCRC, metastatic colorectal cancer; mFOLOFOX, oxaliplatin, folinic acid, 5-FU in combination with either panitumumab or cetuximab; TWEAK, tumour necrosis factor-like weak inducer of apoptosis.

superfamily (TNFRSF10B) or TRAIL-R2. DR5 is a protein overexpressed in many kinds of tumors. It is under clinical trial in patients with mCRC.<sup>83</sup>

Drozitumab is a fully humanized IgG1 that also binds with DR5. It is under phase I study combined with FOLFOX6 and bevacizumab. Patients with mCRC presented a partial response in this trial.<sup>84</sup>

**Anti-EGFR antibodies**

Nimotuzumab is a humanized monoclonal IgG1 antibody that binds to the EGFR with intermediate affinity.<sup>85,86</sup> It has presented good efficacy in phase II trials of radiotherapy and in combination with capecitabine in advanced rectal cancer.<sup>87</sup>

**Anti-IGF-1R antibodies**

Robatumumab is an IGF-1R monoclonal antibody under phase II study in patients with advanced colorectal cancer.<sup>88</sup> It acts by inhibiting tumor-derived VEGF and, indirectly, angiogenesis.<sup>89</sup> However, few patients appeared to benefit from treatment with robatumumab.<sup>88</sup>

Dalotuzumab (MK-0646) is a recombinant humanized IgG1 mAb, also against the IGF-1R. It is under a phase I study in combination with cetuximab and irinotecan and

is given to patients with advanced colorectal cancer.<sup>90</sup> Robatumumab acts by reducing IGF-1- and IGF-2-mediated tumor cell proliferation, IGF-1R autophosphorylation, and Akt phosphorylation.

Also, figitumumab is a monoclonal antibody under a phase II clinical trial for patients with refractory mCRC. It is a humanized IgG2 isotype that binds and reduces the activation of IGF-1R. However, no partial or complete responses occurred in this trial, and further study of figitumumab was not supported.<sup>91</sup>

**Anti-A33 antibodies**

KRN330 is a fully humanized monoclonal antibody directed against A33. A33 is a 43 kDa transmembrane glycoprotein of the immunoglobulin superfamily that is highly expressed in intestinal epithelium and expressed in 95% of colorectal cancers. This monoclonal antibody seems to stimulate cytotoxic T-lymphocyte (CTL) response against A33-positive colorectal cancers. In the phase II study, the prespecified objective response rate to the treatment with KRN330 and irinotecan was not met.<sup>92</sup>

I-huA33, another anti-33 monoclonal antibody, combined with capecitabine, is under a phase I trial for mCRC. In this trial, I-huA33 is achieving specific targeting to colorectal cancer metastases and is safely combined with chemotherapy.<sup>93</sup>

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### Anti-NRPI antibodies

MNRP1685A is a fully humanized IgG1 directed against NRPI. NRPI is overexpressed by certain tumor cells. This monoclonal antibody has potential antineoplastic activities by preventing coupling of NRPI to VEGFR-2 and potentially inhibiting VEGF-mediated signaling and angiogenesis. MNRP1685A, in combination with anti-VEGF therapies, may present an enhanced antiangiogenic effect and is under phase I trials in patients with advanced solid tumors.<sup>94</sup>

### Future perspectives

Presently, new strategic trials are being developed due to the elevated cost of new agents and reduced number of molecules available. Furthermore, molecular-targeted agents such as antiangiogenic agents (bevacizumab, aflibercept, and regorafenib) and anti-EGFR agents (cetuximab and panitumumab) have been associated with chemotherapeutic drugs (fluoropyrimidine, oxaliplatin, and irinotecan), as described in the following section.

The combination of monoclonal antibodies anti-VEGF or anti-EGFR with chemotherapy has shown good clinical activity in cases of metastatic colorectal tumors. However, the use of both molecules combined with chemotherapy presented several adverse outcomes. Clinical trials are under development to test the effects of bevacizumab plus erlotinib, as well as bevacizumab plus tegafur/uracil and leucovorin, as a first-line treatment for patients with mCRC.<sup>95</sup> Also, STRATEGIC-1, a phase III trial, is in progress with the purpose of creating global information on the treatment sequence for patients with unresectable RAS wild-type colorectal cancer. In this trial, FOLFIRI + cetuximab + oxaliplatin + bevacizumab is compared to OPTIMOX + bevacizumab + irinotecan.<sup>96</sup>

Panitumumab or bevacizumab with FOLFIRI (SPIRITT) is also under evaluation as a second-line treatment. This combination presented similar efficacy as oxaliplatin-based chemotherapy with bevacizumab, with expected toxicities.<sup>97</sup>

Necitumumab plus modified FOLFOX6 (oxaliplatin, folinic acid, 5-FU in combination with either panitumumab or cetuximab) was recommended as the first-line treatment for patients with RAS mutation and as first-line treatment for locally advanced cancers or mCRC and is also in evaluation.<sup>98</sup>

Conversely, dalotuzumab plus irinotecan and cetuximab did not improve survival outcome in KRAS wild-type mCRC.<sup>99</sup> Meta-analysis studies showed that anti-EGFR monoclonal antibodies alone or in combination with others (FOLFIRI ± cetuximab; FOLFOX4 ± cetuximab; oxaliplatin or

irinotecan-based chemotherapy, bevacizumab ± panitumumab; bevacizumab, capecitabine, oxaliplatin ± cetuximab, and irinotecan ± cetuximab) did not present efficacy in patients with mCRC containing KRAS mutations.<sup>100–102</sup>

### Conclusion

This review concludes that the repertoire of approved monoclonal antibodies used to treat patients with colorectal tumors, especially in cases of malignant and metastatic tumors, is very limited. Nowadays, these biological molecules belong to only two classes of monoclonal antibodies: specific binders of VEGF or of EGFR. Despite the occurrence of side effects and higher costs of the treatment, researchers are constantly looking for new monoclonal antibodies for the treatment of colorectal tumors. New classes of molecules are under clinical trials with promising results.

A further possibility for identifying tumors is the use of biomarkers to optimize therapeutic efficacy and reduce toxicity. One of the current trends is the individualization of systemic treatment, which has become possible due to molecular biology tools and statistical analysis based on biologically plausible principles.

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### Disclosure

The authors report no conflicts of interest in this work.

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