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Monoclonal antibodies for targeted therapy in colorectal cancer

Sreeparna Banerjee[1,*] and Hernan Flores-Rozas[2-4]

[1]Department of Biological Sciences; Middle East Technical University; Ankara, Turkey; [2]Institute of Molecular Medicine and Genetics; [3]Department of Medicine; and [4]MCG Cancer Center; Medical College of Georgia; Augusta, GA USA

Key words: monoclonal antibody, EGFR, VEGF, metastatic colorectal cancer

Abbreviations: EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; ADCC, antibody dependent cellular cytotoxicity; CDR, complementarity determining regions; mCRC, metastatic colorectal cancer

Traditional therapeutic regimens of solid tumors such as chemotherapy and radiotherapy often do not distinguish between malignant and normal tissues, resulting in considerable side-effects. Monoclonal antibodies (mAbs), targeted against antigens dysregulated in cancers, have therefore generated great interest in both clinical and research settings. The antibodies are either chimeric or human(ized) and can bind to and inhibit target proteins overexpressed in both solid tumors and hematological malignancies. Some of these mAbs have shown efficacy in patients who are refractory to traditional chemotherapy. Examples of FDA approved antibodies against metastatic colorectal cancer include cetuximab, panitumumab and bevacizumab. This review summarizes the current knowledge of mAbs targeting growth factors in colorectal cancer and the importance of carefully screening patients to select candidates who will benefit most from these therapies.

Introduction

The prevalent use of antibodies as therapy against infectious diseases was first extended to treat malignant diseases by Ehrlich in early 20th century.[1] The science progressed relatively slowly until Kohler and Milstein described the first hybridoma cell line[2] in which antibody producing B lymphocytes were isolated from animals immunized with the target antigen and immortalized by hybridizing the lymphocytes with a murine myeloma cell line.

Although clinical trials exploiting this method of passive immunotherapy with murine antibodies against tumor antigens proved to be successful in the early 1980s,[3] the process of generating tailored antibodies for specific patients proved to be expensive and difficult. In addition, the murine antibodies (postfix—omab, e.g., edrecolomab) were immunogenic, leading to the secretion of human anti-mouse antibodies (HAMA) which precluded the possibilities of repeated administration of the antibodies.

*Correspondence to: Sreeparna Banerjee; Email: banerjee@metu.edu.tr
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To circumvent these issues, chimeric antibodies (postfix—ximab, e.g., cetuximab) were created where the constant region of the murine antibody was replaced with the human counterparts from the IgG1 heavy chain and κ light chain (**Fig. 1**). The choice of IgG1 was based on the ability of this class of antibody to activate complement and effector T-cells. These chimeric antibodies were also less immunogenic and had longer circulating half lives.

In a further step, humanized antibodies (95% human) were generated (postfix—zumab, e.g., trastuzumab) whereby the complementarity determining regions (CDR) at the antigen binding site of the murine antibody is grafted onto the human antibody.[4] Although low in immunogenicity, reports of decreased specificity compared to the murine antibody led to the development of humanized antibodies that were mutated in the CDR resulting in increased antigen specificity.[5]

Fully human antibodies were subsequently developed by the use of transgenic mice such as the Xenomouse® (postfix—mumab, e.g., panitumumab) which involved the humanization of the murine humoral immune system by replacing the mouse antibody generating loci with the human heavy and light chain loci.[6] These mice were capable of producing a vast repertoire of high affinity human mAb when challenged with the appropriate antigen and the antibodies had considerably lower immunogenicity and a much longer circulating half life.[7]

Monoclonal Antibodies in Use Against Colorectal Cancer

Of the mAbs that have been approved by FDA for cancer therapy, (**see Table 1**) three are used as singular therapy or in combination with chemotherapy for (metastatic) colorectal cancer—cetuximab, pantitumumab and bevacizumab. Cetuximab and pantitumumab bind to epidermal growth factor receptor (EGFR) while bevacizumab binds to and sequesters the ligand VEGF-A (vascular endothelial growth factor-A).

The Epidermal Growth Factor Receptor Signaling Pathway

The human EGFR family (ErbB/HER) of receptor tyrosine kinases (RTK) constitutes four members, EGFR, ErbB-2, ErbB-3

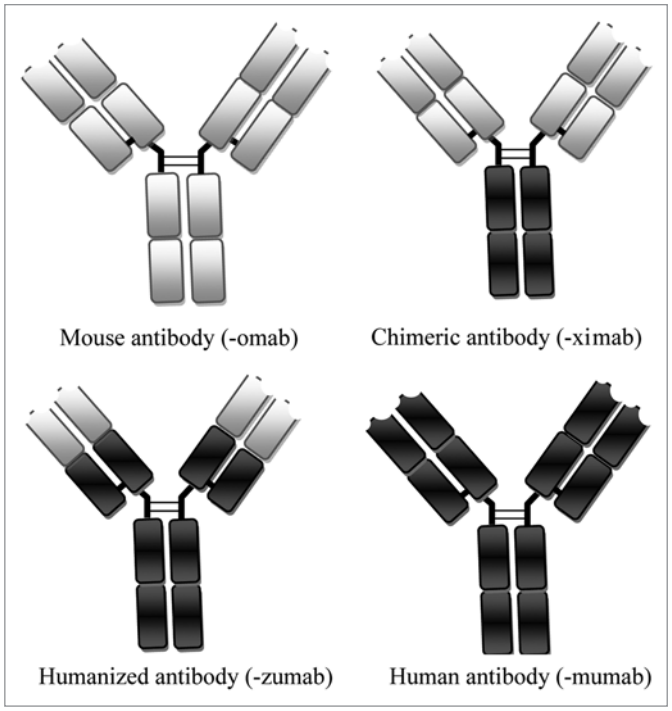


Figure 1. The structures of monoclonal antibodies used for therapy. The light grey portions represent mouse protein while the dark grey portions represent human proteins.

and ErbB-4.[8] These trans-membrane proteins have an extracellular ligand-binding domain and a cytoplasmic kinase domain bridged by a single hydrophobic transmembrane domain.[8]

In the absence of a ligand, the EGFR exists as a monomer. Receptor activation involves the sequential events of ligand binding, homo- or hetero-oligomerization, conformational changes in the receptor and autophosphorylation of the tyrosine kinase domains.[9] Downstream activation of pathways such as mitogen activated protein kinase (MAPK), Janus kinase/signal transducer and activator of transcription (JAK/STAT), as well as the phosphoinositol 3-kinase/v-akt murine thymoma viral oncogene homolog (PI3K/AKT) affect a multitude of cellular functions such as proliferation, motility, invasion, metastasis, adhesion, differentiation and inhibition of apoptosis (**Fig. 2**).[10] Receptor inactivation usually involves dephosphorylation by phosphotyrosine phosphatases or the internalization and ubiquitylation and degradation of the receptor in the lysosomes.[9]

The EGFR behaves as a proto-oncogene and is overexpressed in various epithelial tumors, particularly metastatic colorectal cancer (mCRC) where EGFR overexpression is seen in 65–70% of colon tumors and is associated with advanced stage of the disease and metastatic spread.[11] Dysregulated signaling in the EGFR pathway may involve aberrant receptor turnover and mutations in the receptor itself as well as alterations in ligand synthesis and processing.[12] EGFR amplification, rather than mutations, is most commonly associated with colorectal cancer. An increased copy number of the gene, as analyzed by fluorescence in situ hybridization (FISH), was found to be correlated to a positive response to EGFR mAb therapies.[13] However, the accuracy of FISH, owing to an absence of standardized EGFR scoring, has been called into question following tumor responses to mAb therapy in colorectal tumors without an increase of EGFR copy number.[14]

The Vascular Endothelial Growth Factor Signaling (VEGF) Pathway

Vasculogenesis, angiogenesis and lymphangiogenesis, catalyzed by the VEGF family via their tyrosine kinase receptors, involve a series of sequential events that are well coordinated and are necessary for normal development as well as for the survival of tumors.[15] Tumors that are more than 2 mm in size require neoangiogenesis to facilitate the delivery of oxygen and nutrients;[16] 40–60% of colorectal cancers express VEGF and the overexpression of this growth factor is associated with disease recurrence and reduced survival.[17]

In mammals, the VEGF family consists of five members: VEGF-A, placenta growth factor (P1GF), VEGF-B, VEGF-C and VEGF-D.[18] $VEGF_{165}$, one of four alternatively spliced isoforms of VEGF-A, is predominantly overexpressed in many different tumors. The VEGF ligands bind, with varying specificities, to three tyrosine kinase receptors: VEGFR-1, VEGFR-2 and VEGFR-3 [fms-like tyrosine kinase 4 (Flt4)]. All three receptors are characterized by the presence of three domains: an extracellular, immunoglobulin-like ligand binding domain, a transmembrane helix and an intracellular tyrosine kinase domain.[18] Once the ligand binds to the receptor, they dimerize, autophosphorylate each other via the tyrosine kinase domains and activate downstream signaling pathways such as the PLCγ (phospholipase-Cγ) and protein kinase C (PKC) pathway as well as the MAPK pathway (**Fig. 3**).[18]

The pathological role of VEGFs in tumor angiogenesis is mediated by enhanced endothelial cell proliferation and survival, endothelial cell motility and invasiveness, permeability of existing blood vessels and 'homing' of bone marrow derived endothelial cells and pericytes to sites of new blood vessel formation.[19]

Targeted Therapies in Colorectal Cancer

Traditional cytotoxic therapies such as chemotherapy and radiotherapy used in the treatment of solid tumors, often do not distinguish between malignant and normal tissues, resulting in considerable side-effects. Poor tolerance also leads to the use of lower therapeutic doses, which can lead to poor outcomes in terms of cure and survival. These factors highlight the need for developing targeted therapies that can spare the healthy tissues and exclusively target the tumor cells. In addition, tumor resistance may develop even with the most effective chemotherapy. We will describe here three monoclonal antibodies that target growth factors and have shown efficacy against mCRC.

Cetuximab. EGFR, while essential in the normal maintenance of cellular growth and function, is dysregulated in 25–77% of colorectal cancers and 85% of mCRC.[20] Cetuximab (IMC-225, Erbitux®) is a chimeric IgG1 monoclonal antibody that competes with ligands for high affinity binding to the extracellular domain

Table 1. FDA approved monoclonal antibodies used for cancer therapy (US FDA, www.fda.gov/ accessed 2009)

FDA date	mAb	Trade name	Target	Effective against	Adverse reactions
1998	Trastuzumab	Herceptin®	HER2/neu	Breast cancer	Cardiomyopathy, infusion reactions, pulmonary toxicity
2000	Gemtuzumab	Mylotarg®	CD33	Acute myeloid leukemia	Hypersensitivity
2001	Alemtuzumab	Campath®	CD52	Chronic lymphatic leukemia	Cytopenia, infusion reaction, infections
2002	^{90}Y-ibritumomab	Zevalin®	CD20	B-cell lymphoma	Infusion reaction, cytopenia, severe cutaneous, mucocutaneous reactions
2003	^{131}I-tositumomab	Bexxar®	CD20	B-cell lymphoma	Hypersensitivity reactions, cytopenia
2004	Bevacizumab	Avastin®	VEGFR	Colorectal cancer	Gastrointestinal perforations, wound healing problems
2004	Cetuximab	Erbitux®	EGFR	Colorectal cancer	Infusion reactions, cardiopulmonary arrest
2006	Pantitumumab	Vectibix®	EGFR	Metastatic colorectal carcinoma	Dermatologic toxicity, infusion reactions

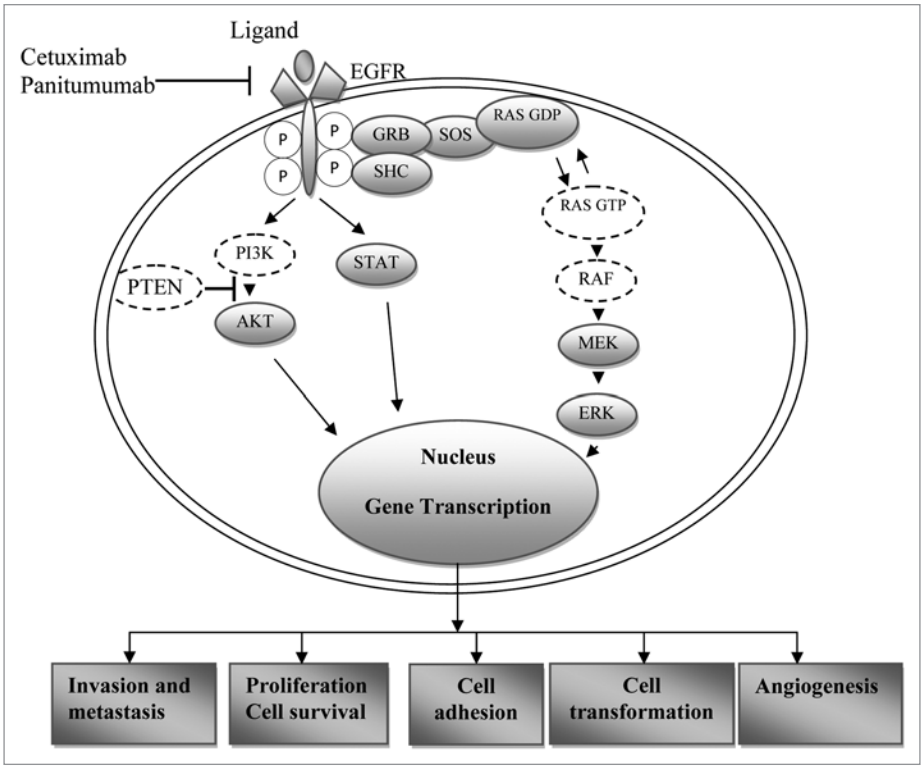


Figure 2. The EGFR signaling pathway. Ligand (EGF, TGFβ) binding to EGFR on the cell surface results in receptor dimerization, followed by phosphorylation of tyrosine residues and downstream activation of signaling cascades such as MAPK, PI3K/AKT and Jak/STAT pathways. These pathways then modulate several aspects of cell behavior such as survival, proliferation, adhesion and metastasis and angiogenesis. The molecules represented by dashed lines should have a WT phenotype for EGFR mAb therapy to work successfully.

of EGFR. Upon binding, the antibody causes internalization of the receptor and its degradation without concurrent autophosphorylation and signal transduction; resulting in a loss of the mitogenic signal (**Fig. 4**).[21] The recommended dosage of cetuximab is 400 mg/m^2 loading dose followed by weekly doses of 250 mg/m^2.[22]

The efficacy of cetuximab as an anticancer agent arises from several effector functions:

(1) Cell cycle progression is arrested at G_1 phase owing to hypophosphorylation of Rb resulting from higher expression of the CDK2 inhibitor p27^{KIP1}.[23]

(2) Apoptosis is induced as shown by the activation of caspase-3.[24]

(3) Angiogenesis is inhibited by inhibiting the expression of VEGF, IL-8 and basic fibroblast growth factor.[25]

(4) Enhancement of antibody dependent cellular cytotoxicity (ADCC).[26]

The first line of treatment in advanced colorectal cancer normally involves the following chemotherapy drugs: Fluorouracil (5-FU) along with folinic acid/leucovorin (FA); Irinotecan with 5-FU and FA either as bolus (IFL) or infused (FOLFIRI); infusional 5-FU, FA and Oxaliplatin (FOLFOX); Oxaliplatin,

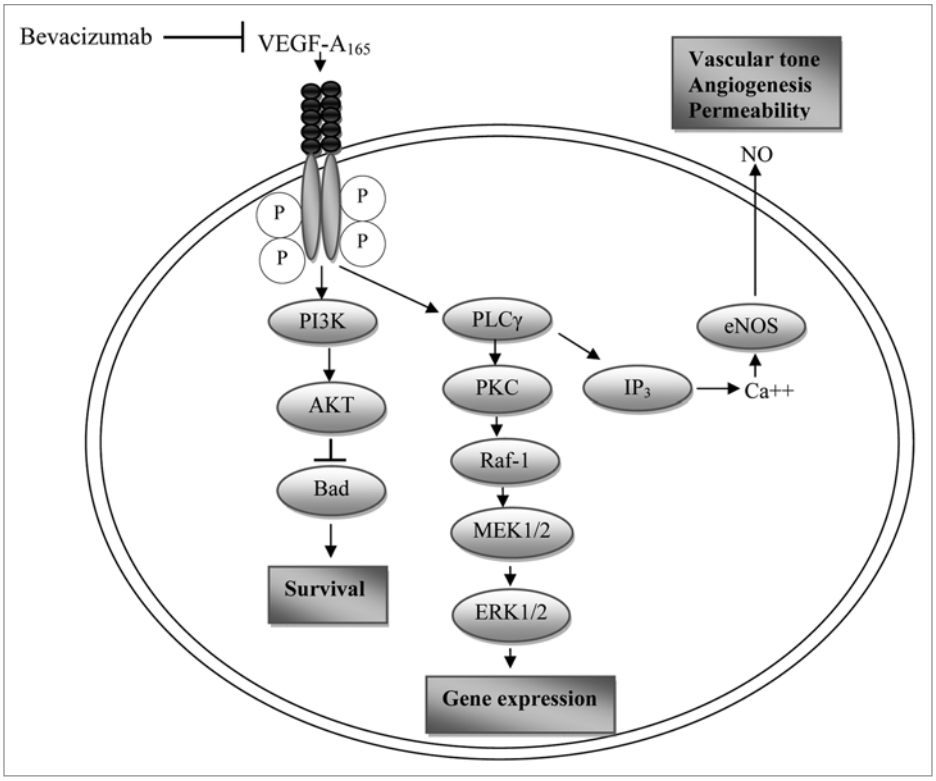


Figure 3. The VEGF signaling pathway. VEGF binding to KDR results in receptor dimerization and autophosporylation leading to the activation PI3K/Akt, MAPK and PLCγ pathways. Activation of these pathways result in cellular survival, angiogenesis, altered vascular tone and permeability.

infusional 5-FU and FA in a weekly schedule (FUFOX) and Capecitabine (tablet form of 5-FU) and Oxaliplatin (XELOX). Several Phase II and Phase III clinical trials combining cetuximab with these established first line chemotherapy regimens have shown statistically significant improvement in the response rate, median time to progression and median overall survival (**reviewed in ref. 10, see Table 2**). However, due to its chimeric nature, the use of cetuximab is associated with severe infusion reactions owing to the development of IgE antibodies against an oligosaccharide galactose-α-1,3-galactose on the Fab portion of the cetuximab heavy chain.[27] The main side effect observed is an acne-like rash. A positive correlation between the severity of the rash and a positive response to cetuximab has been suggested in several clinical trials (reviewed by Sipples).[28] Incidentally, the use of all EGFR inhibitors, whether they are immunoglobulins or small molecule tyrosine kinase inhibitors, tends to produce the rash. Normal EGFR activity is necessary for the highly regulated process of differentiation, migration and survival of keratinocytes in the skin. EGFR is also expressed in high amounts in the sebaceous epithelium. Sebaceous glands are localized in high density in the scalp, face and upper chest, where most of the rash observed with the use of EGFR inhibitors occurs. Inhibition of EGFR signaling in the basal keratinocytes is associated with growth, maturation and migratory abnormalities, which are manifested as the papulopustular rash.[29]

Panitumumab. Panitumumab (ABX-EGF, Vectibix®) is a fully human monoclonal antibody of the IgG2 class that binds with very high affinity to EGFR. This mAb was FDA approved in 2006 (**Table 1**) for use as first-line treatment in patients with mCRC refractory to oxaliplatin, fluoropyrimidine and/or irinotecan-containing chemotherapeutic regimens.[30] The mAb competitively inhibits the binding of the ligands EGF and TGFα to EGFR and subsequent tyrosine phosphorylation and downstream signaling (**Fig. 4**).[30] After the mAb binds to EGFR, the receptor is internalized, but not degraded; however, cell growth is inhibited, with concurrent induction of apoptosis and a reduction in angiogenesis (through decreased production of VEGF) as well as synthesis of proinflammatory cytokines.[30] Preclinical studies indicated that panitumumab exclusively targets the EGFR overexpressing cells and the mAb was shown to be ineffective for tumor cells that do not express or express low levels of EGFR.[30] The recommended dose is 6 mg/kg given once every 2 w as a 1-h infusion applied intravenously.[31]

The FDA approved the use of panitumumab when a Phase III trial indicated superior progression free survival (PFS) compared to best supportive care (BSC)[32] (**Table 2**). The mAb was shown to be generally well tolerated, and being fully human, showed no incidence of human antihuman antibody (HAHA) response. The most significant side-effect was acneiform rash, the severity of which correlated positively with the clinical outcome.[33]

Why a second EGFR mAb? Both cetuximab and panitumumab target the extracellular portion of EGFR (domain III) and are approved for use in metastatic colorectal cancer.[10] Panitumumab, however, is a fully human mAb with no murine sequences whereas cetuximab is a chimeric molecule (**Fig. 1**). Owing to this, patients receiving cetuximab often develop

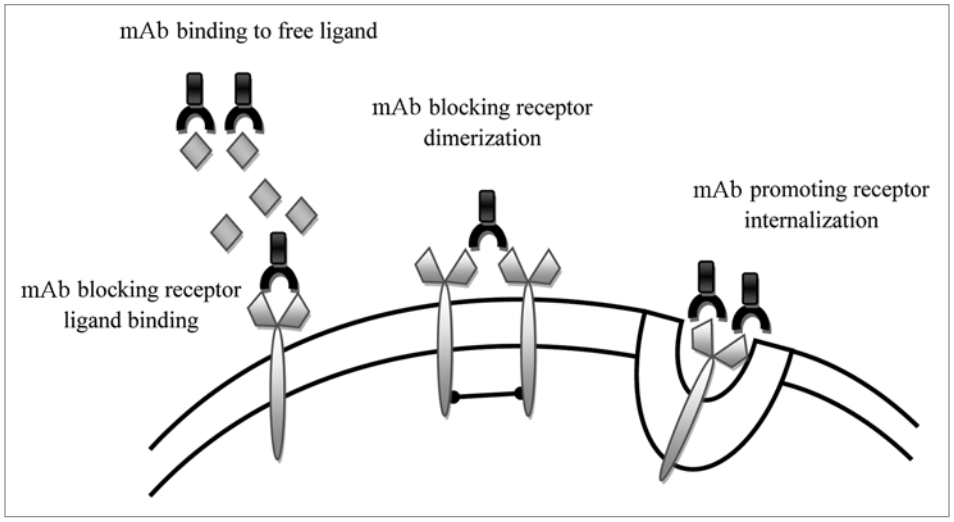


Figure 4. Mode of action of monoclonal antibodies. Antibodies can inhibit a signaling pathway by binding to the free ligand (e.g., bevacizumab binding to VEGF), by blocking the binding of the ligand to the receptor (e.g., panitumumab and cetuximab), by preventing receptor dimerization (e.g., cetuximab and panitumumab) and by promoting receptor internalization and degradation (e.g., cetuximab).

Table 2. Clinical trials of cetuximab, panitumumab and bevacizumab

Treatment	Other considerations	N	RR (%)	Study
Cetuximab + FUFOX	First line treatment	14 + 25	56	Arnold et al.[59] (Phase I/II)
Cetuximab + FOLFIRI	Presence of initially unresectable metastases	52	48	Raoul et al.[60] (Phase I/II)
Cetuximab + FOLFOX-4	First line treatment	43	72	Tabernero et al.[61] (Phase II)
Cetuximab ± Irinotecan	First-line fluoropyrimidine and oxaliplatin treatment failure	1298	16.4 vs. 4.2	Sobrero et al.[62] (Phase III)
Panitumumab	mCRC patients who failed fluoropyrimidine + irinotecan or oxaliplatin therapy	148	9	Hecht et al.[63] (Phase II)
Panituzumab + IFL	Progression after standard chemotherapy	19	46	Berlin et al.[64] (Phase II)
Panituzumab + FOLFIRI	Progression after standard chemotherapy	24	42	Berlin et al.[64] (Phase II)
Panitumumab ± BSC	Progression after standard chemotherapy	463	10 vs. 0	Van Cutsem et al.[32] (Phase III)
Bevacizumab + 5-FU/FA	Heavy pretreatment, refractory to Cetuximab	48	6.25	Vincenzi et al.[65] (Phase II)
Bevacizumab + XELOX/FOLFOX-4	First line treatment	1401	No improvement	Saltz et al.[66] (Phase III)

N, number of patients; RR, response rate.

human anti-chimeric antibodies, while neutralizing HAHA antibodies are rarely observed in patients receiving panitumumab.[22,31] Panitumumab, due to its IgG2 isotype, mediates its mechanism of action via EGFR blockade through high affinity interaction with the active site of EGFR (8-fold greater affinity than cetuximab), rather than ADCC, as is the case with cetuximab having an IgG1 isotype.[34] Additionally, owing to a longer half life for elimination of panitumumab compared to cetuximab, the former can be given once in two weeks whereas the latter is given every week.[22,31] Moreover, premedication with histamine antagonistsis recommended for use with cetuximab, whereas no such premedication is necessary for panitumumab.[22,30,31] Although the two mAbs have not been directly compared in prospective clinical trials, both seem to have similar objective response rates when used as monotherapy in metastatic colorectal cancer.[32,35] The time to disease progression, however, is slightly longer for panitumumab (2–3 mo vs. 1.5 mo)[32,35] affording a small advantage to panitumumab.

Bevacizumab. Bevacizumab (Avastin®) is a humanized monoclonal antibody of the IgG1 class that binds to VEGF and inhibits its binding to the VEGF receptors Flt-1 and KDR (**Fig. 3**). Preclinical studies indicated that administration of bevacizumab to xenotransplant models of colorectal cancer in athymic mice led to a decrease in neoangiogenesis.[36] Because the efficacy of cetuximab and panitumumab depends on the accurate determination of the EGFR status of the tumor and the presence of mutations in downstream signaling molecules, as well as the severely debilitating acneiform skin rashes associated with the use of EGFR inhibitors, FDA approval of bevacizumab holds promise for use in combination with chemotherapy for first and second line treatments of metastatic colorectal cancer. The drug is generally well tolerated although some adverse reactions such as gastrointestinal perforation, hypertension,

Table 3. Clinical trials of combination therapy using bevacizumab, cetuximab, panitumumab and chemotherapy

Treatment	N	RR (%)	OS (mo)	PFS (mo)	Study
Bevacizumab + Cetuximab ± Irinotecan	83	37 vs. 20	14.5 vs. 11.4	7.3 vs. 4.9	BOND-2[67] (Phase II)
Bevacizumab + XELOX ± Cetuximab	755	50 vs. 52.7	20.3 vs. 19.4	9.4 vs. 10.7	CAIRO2[39] (Phase III)
Bevacizumab + Oxaliplatin ± Panitumumab	823	46 vs. 48	19.4 vs. 24.5	10 vs. 11.4	PACCE[40] (Phase III)
Bevacizumab + Irinotecan ± Panitumumab	230	43 vs. 40	20.7 vs. 20.5	10.1 vs. 11.7	PACCE[40] (Phase III)

N, number of patients; RR, response rate; OS, overall survival; PFS, progression free survival.

proteinurea and thrombosis may occur (**Table 1**).[37] The current recommended dose is 5 mg/kg every 2 w.[36]

Based on the efficacy of initial trials on the safety and toxicity assessments of bevacizumab, several Phase I, II and III trials have been conducted to determine the effect of combination therapies with traditional 5-FU/LV, IFL and FOLFOX chemotherapy (**reviewed in ref. 38, Table 2**). Improvement in overall and PFS times and increases in the time to disease progression have been reported with the addition of bevacizumab to chemotherapy regimens.[37]

Combined Treatment with Bevacizumab, Panitumumab and Cetuximab

VEGF and EGFR share downstream signaling components; therefore a rationale was developed for the concurrent administration of both EGFR and VEGF targeting mAbs with potential for additive or synergistic effects. The results of these trials are summarized in **Table 3**.

The Phase II BOND-2 trial using a combination of bevacizumab and cetuximab, with or without irinotecan, showed promising overall response rate in patients refractory to irinotecan and toxicity comparable to the trials involving bevacizumab or EGFR mAbs alone (see **Table 3**). This prompted the design of larger scale Phase III trials. However, surprisingly, a negative risk benefit in terms of overall survival and progression free survival along with substantial toxicity (including skin toxicity, diarrhea, infection, nausea/vomiting) was seen to be associated with the combination of bevacizumab, an EGFR targeting mAb, and chemotherapy.[39,40] Although cetuximab and panitumumab have differing safety profiles, the lack of synergism with bevacizumab was related to a class based effect of EGFR inhibitors, as both the CAIRO2 (using cetuximab) and PACCE (using panitumumab) trials had very similar outcomes.

Potential explanations for the exacerbated toxicities include altered downstream signaling in either VEGF and/or EGFR signaling, affecting the crosstalk between the two pathways, pharmacokinetic interactions between the different agents as well as study design (open label) factors.[41]

Inactivating Mutations and the Efficacy of mAb Regimens

Optimal therapeutic response to EGFR targeted therapies requires two criteria: the growth of the cancer cells should be EGFR dependent and EGFR independent activation of the downstream signaling molecules should not occur.[42] For all EGFR mAb based therapies for metastatic colorectal carcinoma, a proportion of patients exist who either do not respond to the mAb or show disease stabilization. Although EGFR expression is detected by immunohistochemistry to assist patient selection, clinical experience indicates that the EGFR status is not sufficient to predict clinical efficiency.[43] The only marker that has been associated so far with the efficacy of cetuximab or panitumumab therapy is the acne-like rash on the skin. It has subsequently been reported that the mutational status and/or expression of *KRAS*, *BRAF* and *PI3K* could be critical in identifying those patients who are most likely to benefit from therapy with these agents.

KRAS and BRAF mutations. The *RAS* family of proto-oncogenes (Harvey-Ras (*HRAS*), Kirsten-Ras (*KRAS*) and *NRAS*) are small G-protein molecular switches that can convert extracellular signals into specific cellular responses via growth factor receptors such as the EGFR and VEGFR signaling cascades (**Figs. 2 and 3**).[42] Activating mutations in *KRAS* (mutations in *NRAS* and *HRAS* are relatively rare in CRC) are found approximately in 30–40% of CRC.[42] Particularly, point and transversion mutations located in codons 12 and 13 of *KRAS* are associated with hyperproliferation of the colonic epithelium.[42] These mutations impair the intrinsic GTPase function of KRAS, resulting in the accumulation of GTP bound KRAS. Thus, the KRAS protein is constitutively activated, independent of upstream growth factor receptor signals.[42]

In the recent years, it has been reported that *KRAS* mutations may serve as an indicator for resistance to targeted therapy using mAb against EGFR (reviewed in ref. 42). This may be due to the fact that with a constitutively active KRAS, inhibition of the upstream EGFR would have minimal effect, causing resistance to cetuximab or panitumumab therapy.

For panitumumab, the authors reporting on a Phase III trial comparing the effect of mAb monotherapy to BSC[32] (**see Table 2**) have subsequently used PCR to determine the *KRAS* status of the tumors in 92% of the patients who participated in the trial.[44] These authors reported that 42% of the patients had mutations in codon 12 and 13 of *KRAS* and the response rates to panitumumab were 17% and 0%, for the wild-type (WT) and mutant groups, respectively.[44] Based on retrospective analysis of data from several randomized clinical trials, the American Society of Clinical Oncology released a Provisional Clinical Opinion (PCO) whereby it recommended that all patients with metastatic colorectal carcinoma and on a prospective cetuximab or panitumumab regimen should have their tumor tested for *KRAS* mutations in a CLIA-accredited laboratory.[45] It is worth mentioning here that in a randomized Phase III study of 230 patients who were treated with IFL in combination with either bevacizumab

or placebo, no significant association was observed between the clinical efficacy of bevacizumab and *KRAS* mutation status.[46]

The serine-threonine kinase BRAF is the primary effector molecule of KRAS and a *BRAF V600E* mutation is the most frequent oncogenic protein kinase mutation known. Since only 30–40% of patients who are unresponsive to cetuximab or panitumumab have the *KRAS* mutations, the status of *BRAFV600E* was also examined in patients who were treated with cetuximab or panitumumab for metastatic colorectal cancer.[47] The results indicated that the *BRAFV600E* mutations were present in 14% of patients who had WT *KRAS* and none of the patients with mutated *BRAF* responded to the treatment.[47] It was concluded from this study that a WT *BRAF* status may be required for the efficacy of EGFR targeting mAbs.

PIK3CA and PTEN mutations. Forty to 60% of patients with wild-type KRAS fail to respond to EGFR mAb treatment, leading to the notion that the mutation screening of other proteins in the EGFR signaling pathway may be necessary. Phosphatidylinositol 3-kinases (PI3Ks) are a family of membrane bound lipid kinases that phosphorylate phosphatidylinositol (PI) on the inositol ring.[48] The class IA PI3K proteins, consisting of the catalytic subunits p110α, p110β and p110δ and a regulatory p85 subunit, are involved in the regulation of proliferation and in tumorigenesis. In non-stimulated cells, p85 binds to and inactivates p110α (encoded by the gene *PIK3CA*). However, upon stimulation with a growth factor (such as EGF), the p85 binding and inhibition is relieved and the kinase function is activated in the plasma membrane.[49] PI3K may be further activated by an interaction between p110α and the GTP-bound form of the RAS oncoprotein.[49] Phosphatidylinositol 4,5-bisphosphate (PIP_2) is phosphorylated by p110α to form phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which can bind to and activate the serine-threonine kinase AKT via a kinase called PDK1 (3-phosphoinositide-dependent kinase 1) (**Figs. 2 and 3**). Activated AKT primarily mediates the growth and proliferation regulatory functions of PI3K. On the other hand, the tumor suppressor phosphatase, PTEN (phosphatase and tensin homologue deleted on chromosome 10), behaves as a negative regulator of the PI3K signaling pathway by dephosphorylating PIP_3 to generate PIP_2.[48] Cancer-specific gain of function mutations are located in the helical and kinase domains of the p110α subunit, particularly at the amino-acid residues E542, E545 and H1047.[48] Concomitant mutations in *KRAS* and *PIK3CA* have been observed in a significant subset of colorectal cancer patients.[50] *PTEN*, on the other hand, is mutated rarely in colorectal cancers and is usually associated with tumors with microsatellite instability.[14]

Mutations of *PIK3CA* and loss of *PTEN* (usually mutually exclusive events), which predict a resistance to cetuximab in mCRC patients overexpressing EGFR, have been reported in recent months.[51] The presence of WT *PTEN* would negatively regulate PI3K/AKT and restore the functionality of EGFR downstream signaling, while mutations in *PIK3CA* may lead to AKT activation in the absence of growth factors. Sartore-Bianchi et al.[51] reported that the presence of *PIK3CA* mutations and *PTEN* loss in colorectal tumors were statistically significantly associated with lack of response to panitumumab or cetuximab treatment in a study with 110 patients. Additionally, a negative correlation between *PIK3CA* mutations and/or loss of *PTEN* expression with progression-free interval was reported in the same study. These investigators suggested that a combined mutational analysis for *KRAS* and *PIK3CA* (loss of *PTEN* and/or *PIK3CA* mutation) would be beneficial in accurately screening out over 70% of patients with mCRC who are unlikely to respond to an EGFR-targeted monoclonal antibody.[51] However, the use of PIK3CA status as a marker for response to cetuximab therapy is controversial. A recent study carried out on 200 patients with metastatic colorectal cancer refractory to chemotherapy and treated with cetuximab alone or in combination with chemotherapy indicated that 12% of the patients had one of the *PIK3CA* mutations. However, the authors could not find any correlation between the presence of a *PIK3CA* mutation and impaired response to cetuximab and therefore discounted the use of these mutations as a predictive factor for cetuximab response.[52]

Aberrant EGFR signaling is thus clearly a key factor in the pathogenesis and progression of CRC. Inhibiting this receptor through the use of monoclonal antibodies, thereby altering downstream signaling, is of potential benefit for patients. However, careful patient selection with detailed monitoring of relevant mutations and informed choice of combinatorial treatment modes may help in the successful use of these therapies.

Future Perspectives and Conclusions

Monoclonal antibody therapy has many promises and an equal number of perils. New antibodies against EGFR have been designed that show improved tolerance over those currently available. Nimotuzumab, currently unlicensed in the USA, is a humanized IgG1 anti-EGFR mAb that prevents ligand binding and receptor dimerization, and having an IgG1 isotype, can induce ADCC.[53] A Phase II clinical trial of nimotuzumab, with or without irinotecan, in refractory CRC indicated overall disease control rates of 50% and overall survival of 9.3 mo, values that are similar to other EGFR mAbs used in similar settings.[54] Most importantly, the use of this mAb is not associated with the of incidence aceniform skin rash. Mathematical models indicate that the affinity of nimotuzumab to EGFR is 10 fold lower than cetuximab or panitumumab. As the EGFR expression in skin epithelial cells is low compared to tumors, nimotuzumab binding and inhibiting EGFR signaling does not occur, thereby avoiding the cutaneous toxicity.[53]

Further utilization of the concept of a therapeutic naked monoclonal antibody has been taken a step further by the use of combinatorial therapies with other drugs. Traditional chemotherapy drugs lack tumor cell specificity, which often results in life-threatening toxic side-effects. On the other hand, mAbs directly bind to specific markers on tumor cells and are therefore more 'targeted.' A combination of the two processes can be utilized in drug-antibody conjugates. Use of a conjugate of SN-38, the active form of the cancer pro-drug CPT-11, and labetuzumab (an anti-CEACAM5 antibody) in a colorectal cancer mouse xenograft model has shown therapeutic efficacy at non-toxic doses of the drug.[55] Additionally, the conjugation of mAb 2H9

(an anti EphB2 monoclonal antibody) to monomethylauristatin E resulted in the exclusive cytotoxicity of ephB2-expressing cancer cells, an effect that was not observed when the antibody was used alone.[56] A second way to utilize the specificity of mAb is by conjugating them to long-circulating, polyethylene glycol-coated nano sized liposomes that can also carry cytotoxic drugs. One such liposomal formulation Doxil®, which carries the drug doxorubicin, has been approved for clinical use. The in vivo targeting of Doxil® by the conjugation of the mAb 2C5, which targets a ubiquitous marker found on tumor cells but not normal cells, showed enhanced accumulation in tumors, superior therapeutic activity and smaller tumor volumes in preclinical studies in mouse xenograft models.[57]

Finally, the high cost for mAb treatments has dissuaded organizations such as the British National Health Service from providing cetuximab and bevacizumab under its auspices.[38] However, recent reports have demonstrated the use of a transgenic tobacco plant for the production of the mAb CO17-1A (IgG2a), which recognizes EpCAM (GA733), an antigen that is highly expressed on human colorectal carcinomas.[58] These transgenic plants could therefore be an inexpensive and large scale alternative for the production of mAbs, which benefit from the lack of animal pathogenic contaminants.

Based on these recent advances in the utilization of mAbs and expanding knowledge of who may benefit from mAb treatment, it is hoped that the future holds the promise of better use of this very powerful tool. Production of specific monoclonal antibodies and careful patient selection may enhance the efficacy of these therapies even further and bring us closer to designing a 'magic bullet' for cancer therapy.

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