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Efficacy and Immune Mechanisms of Cetuximab for the Treatment of Metastatic Colorectal Cancer

Hao ZHUANG[1]
Zhen-yi XUE[1]
Lu WANG[1]
Xiao-yan LI[1]
Ning ZHANG[1]
Rong-xin ZHANG[1,2]

[1] Research Center of Basic Medical Sciences, Tianjin Medical University, Tianjin 300070, China
[2] Department of Immunology, Tianjin Medical University, Tianjin 300070, China

Correspondence to: Rong-xin ZHANG
Tel: 86-22-2354 2082
E-mail: rxzhang@tijmu.edu.cn

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E-mail: editor@cocronline.org
Tel (Fax): 86-22-2352 2919

ABSTRACT Cetuximab is a chimeric immunoglobulin G1 monoclonal antibody that targets the ligand-binding domain of the epidermal growth factor receptor and inhibits downstream intracellular signals. Research has shown that cetuximab can stimulate the autoimmune system and produce antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity reactions, which can recruit cytotoxic lymphocytes to attack and kill cancer cells. Cetuximab is mainly indicated for patients with epidermal growth factor receptor-positive metastatic colorectal cancer who fail to respond to both irinotecan- and oxaliplatin-based regimens. The efficacy and safety of cetuximab as monotherapy or in combination with other treatment options were evaluated in a series of phase II and phase III trials. Identifying the clinical and molecular markers that can predict which patient groups may best benefit from cetuximab treatment is key to improving patient outcomes and avoiding unnecessary toxicities and costs. Herein, we discuss the mechanisms of action by which cetuximab exerts its antitumor effects, as well as the possible clinical and molecular markers that may help predict therapeutic benefits for patients with metastatic colorectal cancer.

KEY WORDS: colorectal cancer, cetuximab, epidermal growth factor receptor, immune mechanisms, prognostic marker.

Abbreviations: EGFR, epidermal growth factor receptor; CDC, complement-dependent cytotoxicity; mCRC, metastatic colorectal cancer; mAb, monoclonal antibody; ADCC, antibody-dependent cellular cytotoxicity; TA, tumor antigen; EGF, epidermal growth factor; NK, natural killer (cells); DC, dendritic cells; CTL, cytotoxic lymphocyte; BSC, best supportive care; OS, overall survival; PFS, progression-free survival; RR, response rate; APC, antigen-presenting cell; HLA, human leukocyte antigen.

Introduction

As metastasis is a major cause of death among cancer patients, any approach that can limit or prevent tumor metastasis would be of high value in disease management. Targeting cancer-specific molecules that are critical to the growth and metastasis of cancer cells is a promising approach for cancer therapy. The last few decades have witnessed considerable progress in the cellular, molecular, and genetic mechanisms of cancer development. The identification of epidermal growth

factor receptor (EGFR) signaling in cancer has considerably revolutionized anticancer treatment approaches.[1] EGFR signaling is now considered a highly promising molecular target in oncology.[2] Cetuximab is a monoclonal antibody (mAb) that binds to and blocks EGFR signaling, thereby inhibiting downstream intracellular signals. It may also induce antibody-dependent cellular cytotoxicity (ADCC), which can recruit cytotoxic T cells to attack and kill cancer cells, and complement-dependent cytotoxicity (CDC) reactions. As a tumor antigen (TA)-specific mAb, cetuximab was approved by the U.S. Food and Drug Administration in 2004 for the treatment of EGFR-expressing metastatic colorectal cancer (mCRC) as well as head and neck cancer.[3–9] However, not all patients respond satisfactorily to this agent.[10–12] Clearly, reliable clinical and molecular markers that can predict the best responders to cetuximab would be of great value. In this review, the efficacy of cetuximab in the treatment of mCRC and its underlying molecular mechanisms, with an emphasis on immune responses, are summarized. Potential clinical and molecular markers that can better predict who will be likely to respond to cetuximab are also discussed.

Mechanisms Underlying the Antitumor Activity of Cetuximab

Cetuximab has been well identified and is now widely used for the clinical treatment of mCRC. It is a highly target-specific antibody, has better tolerance, and can induce tumor cell apoptosis.[13,14] This targeted drug shows intrinsic antitumor effects mediated by several mechanisms, which can be divided into two categories: mechanisms that require immune effector cells and mechanisms that do not.[15] TA-specific mAbs have been demonstrated to inhibit their specific receptors and induce apoptosis in the targeted tumor cells without influencing the immune cells in vitro. However, the TA-specific mAbs that induce ADCC and activate T lymphocyte-specific immune reactions also play an important role in the antitumor function. These mechanisms interact rather than function independently.

Inhibition of EGFR signaling transduction by cetuximab

EGFR is a transmembrane glycoprotein that forms part of the tyrosine kinase receptor protein. The EGFR family includes the transmembrane receptors EGFR/HER1/ErbB-1, HER2/ErbB-2/neu, HER3/ErbB-3, and HER4/ErbB-4.[16] Each EGFR family member is composed of an extracellular ligand-binding domain, a hydrophobic transmembrane domain, and an intracellular intrinsic tyrosine kinase domain (except the kinase-deficient ErbB3).[17] EGFR has seven genetically distinct ligands: epidermal growth factor (EGF), transforming growth factor-α, heparin-binding EGF, amphiregulin, epiregulin, betacellulin, and neuregulin G2b.[18–20] These ligands bind EGFR and trigger ErbB receptor aggregation, which in turn cause the formation and internalization of receptor homodimers and/or heterodimers.[17,21] The tyrosine kinase domain is then activated through protein phosphorylation at the C-terminal tail of EGFR within the cytoplasm.[22,23] This process initiates a cascade of intracellular signaling,[24] which can be terminated with the endocytosis of the phosphorylated receptor–ligand formation.[1,17] Activation of EGFR signaling may trigger a variety of downstream signaling events, including mitogenesis, apoptosis, protein secretion, altered cellular motility, cell differentiation, and cell dedifferentiation.[18]

Blockade of growth factors and their interactions with receptors influence downstream signaling and their physiological functions. EGFR-specific mAbs compete with ligands and bind to the extracellular domain of the receptor to prevent receptor tyrosine kinase activation, thereby weakening EGFR-mediated intracellular signaling.[25] All epithelial and mesenchymal cells express EGFR, although the abundance may vary.[18] Dysregulation or overexpression of EGFR has been widely reported in human malignancies.[26,27] Most cases of head and neck cancer and nearly 50% of CRC cases are positive for EGFR.[28,29] Cetuximab can specifically and competitively bind to EGFR to downregulate the EGFR signaling.[30] It inhibits the proliferation of a series of tumor cell lines in a dose-dependent manner[31,32] and induces cell cycle to arrest in the G_1 phase and apoptosis.[33,34]

TA-targeted cellular immunity and antitumor activity

The signaling transduction mechanism appears to be crucial to understand EGFR-specific mAb cetuximab therapy.[29,35,36] However, a growing body of evidence indicates that this mechanism of action may not be the only one regulating the clinical benefit of mAb-treated cancer patients. Some TA-specific mAbs may exert their effects through alternative Fc-based mechanisms, such as ADCC and CDC.[2,37–43]

ADCC and CDC depend on interactions between antibody Fc domains and such other receptors or proteins as cellular Fcγ receptors expressed on immune accessory cells and the complement-activating protein C1q. These two reactions can either activate mononuclear phagocytes, neutrophils, natural killer (NK) cells, and/or dendritic cells (DCs) or excite the secretion of interferon-γ, opsonins, tumor necrosis factor-α, and chemokines that recruit immune effector cells. Therefore, tumor cell proliferation and angiogenesis are constrained, antigen presentation is raised, and tumor cells are lysed.[42–44] Cetuximab has a human immunoglobulin G1 backbone, and chimeric immunoglobulin G1 antibodies have been reported to induce the ADCC activity of human effector cells, such as NK cells, macrophages, and monocytes, efficiently.[18] Kurai et al.[45] reported that low EGFR expression levels are sufficient for maximum ADCC activity mediated by cetuximab and show antitumor effects. Correale et al.[46] found that a cytotoxic lymphocyte (CTL) antitumor response was stimulated by DC-mediated cross-priming

of antigens derived from cetuximab-covered cancer cells. The antitumor function can also be enhanced by NK cell–DC cross-talk, which ensues after the recruitment of both NK cells and DCs to the inflamed areas caused by cancer,[47] potentially decreasing the activity and the number of immunosuppressive regulatory T cells.[48,49] The resulting effective activating bidirectional signaling can shape not only the innate immune response in inflamed peripheral tissues but also the adaptive immune response within secondary lymphoid organs.[47] In addition, under the effect of cytokines released by DCs, NK cells are activated, regulating both the intensity and the quality of innate immune responses. In turn, DCs increase the cross-presentation and priming of T cells in response to the cytokines released by the activated NK cells. In summary, through direct secretion and interactions of cytokines/chemokines,[50–52] NK cells may act as helper cells,[53] as well as enhance DCs and broaden T-cell priming against multiple TAs.[54]

Most mAbs that mediate ADCC also activate the complement system.[55] Hsu et al.[37] were the first to demonstrate an antitumor growth function of complement-mediated immune response induced by cetuximab in vivo. They also observed CDC only against target cell lines with high EGFR expression levels, which limited the risk of complement-mediated side effects because of normal tissue EGFR expression at much lower levels than malignant cells.[56]

Clinical Efficacy of Cetuximab in mCRC

Cetuximab is effective when either irinotecan- or oxaliplatin-based regimens have failed and for patients who do not respond to irinotecan-based regimens. A series of phase II and phase III trials have tested the safety and efficacy of cetuximab as monotherapy and in combination with chemotherapy to treat patients with mCRC.[4–9,57,58] Most patients with mCRC who take cetuximab experience dermatological side effects, with the most common one being papulopustular skin rash, which occurs early during the course of treatment and can disturb the patient's quality of life. The severity of the rash is dose dependent and associated with the efficacy of treatment.[59]

Cetuximab monotherapy

Cetuximab is the first mAb to have demonstrated efficacy in colon cancer.[12] In a retrospective analysis, 22 of 105 participants who received cetuximab-containing chemotherapy were assigned to cetuximab monotherapy due to their irinotecan intolerance.[60] The median administration of cetuximab was eight cycles. Of the 22 patients, 4 experienced a stable disease and 4 others had a progressive disease; all the other patients were not evaluable for radiological response. The overall response rate (RR) and disease control rate were 9.1% and 27.3%, respectively. Cetuximab monotherapy was found feasible for irinotecan-intolerant mCRC, with the median progression-free survival (PFS) being 1.6 months and overall survival (OS) being 3.5 months.

Another study reported that cetuximab significantly improved the survival rate of patients with EGFR-expressing mCRC as compared with best supportive care (BSC) alone.[7] In the phase III CO.17 trial, Jonker et al.[7] randomly selected 572 patients with EGFR-expressing mCRC refractory to oxaliplatin, irinotecan, and fluoropyrimidine, among whom 285 were assigned to treatment with BSC alone and 287 were allocated to BSC with standard-dose cetuximab plus BSC. In comparison with the BSC-only treatment, the regimen with cetuximab remarkably improved OS and PFS. Twenty-three patients who received cetuximab had partial responses, whereas those who received BSC alone had none; 31.4% of the patients given cetuximab had a stable disease, but the percentage was only 10.9% among those given BSC only.

In a phase II open-label clinical trial, 57 patients with EGFR-expressing mCRC who showed no response to irinotecan treatment were given initial weekly intravenous cetuximab infusions of 400 mg/m^2 and subsequent weekly infusions of 250 mg/m^2.[57] The median PFS was 1.4 months, and the median OS was 6.4 months. Six patients had partial responses; 20 had minor responses with a tumor reduction of 25%–49% or a stable disease with either growth or shrinkage of less than 25% lasting for at least 12 weeks from the beginning of treatment.

According to these clinical trials, cetuximab showed potential for clinical benefit and was well tolerated as a single agent by patients with refractory mCRC. However, the overall RR of 9.1% with a median survival of 3.5 months illustrated that the efficacy of cetuximab monotherapy is modest and far from satisfactory.[60] More combination therapies clearly need to be explored to promote the clinical efficacy of cetuximab.

Cetuximab combination therapies

Several clinical trials have reported that cetuximab has significant improvements in combination with chemotherapies for mCRC. In the phase III CRYSTAL trial, 1198 patients with EGFR-positive mCRC were randomly assigned to be treated with FOLFIRI (folinic acid, fluorouracil, and irinotecan) plus cetuximab (n = 599) or FOLFIRI alone (n = 599).[8] Differences in the OS between the two regimens were not observed, which may be attributed to KRAS mutations, as subsequently discussed. In an updated survival analysis, Van Cutsem et al.[61] reported significant improvements in the median OS (23.5 *vs.* 20.0 months), median PFS (9.9 *vs.* 8.4 months), and RR (57.3% *vs.* 39.7%) of patients with wild-type KRAS who received FOLFIRI plus cetuximab in comparison with those given FOLFIRI alone. Despite the improvements in tumor control rates and OS, however, the combination therapy also resulted in a higher rate of resection of liver metastatic disease.

In another phase II trial, the efficacy and safety of cetuximab plus FOLFOX-6 (leucovorin, 5-fluorouracil, and oxaliplatin) as the first line of treatment were tested in patients with advanced CRC or mCRC.[58] Sixty-seven

of all 82 eligible patients showed positive results in EGFR expression, with an overall RR of 44.8%, and 30 patients had a stable disease. The median time to progression or death and median survival were 9.3 and 21.7 months, respectively. In another FOLFOX-6 trial, patients with wild-type KRAS tumors demonstrated improved PFS, OS, and overall RR compared with patients with mutated KRAS tumors.[62]

In the randomized phase II OPUS study, among 315 evaluable patients with mCRC, 159 received cetuximab plus FOLFOX-4 (oxaliplatin, 5-fluorouracil, and folinic acid) and 156 received FOLFOX-4 alone as their first-line treatment.[63] Patients with wild-type KRAS tumors who received cetuximab plus FOLFOX-4 had a 2.6-fold increased odds ratio of response and a 43% reduction in the risk of disease progression compared with those who received the FOLFOX-4 regimen alone. This trial provided strong evidence for the use of cetuximab combination chemotherapy in the treatment of mCRC. In a similar multicenter phase II study, among 67 eligible untreated patients with mCRC who received cetuximab plus FOLFOX-4, the objective RR was 64.2% and the tumor growth control rate was 94%.[64] Specially, 7 of 33 patients with initially unresectable liver disease were able to undergo resection. The median PFS and OS were 10.0 and 22.0 months, respectively. The treatment was well tolerated without mortality.

Conflicting results were discovered in a study of cetuximab in combination with the mAb bevacizumab as first-line treatment for mCRC.[65] Seven hundred fifty-five patients with previously untreated mCRC were randomly assigned in the CAIRO2 trial: 378 patients received oxaliplatin, capecitabine, and bevacizumab, whereas 377 others were treated with the same regimen combined with weekly cetuximab. Surprisingly, the combination therapy led to a remarkably shorter PFS (9.4 *vs.* 10.7 months), a lower-standard quality of life, and considerably higher costs.[66]

Major side effects

Cetuximab treatment has been generally well tolerated, and its safety has been confirmed. Most mCRC patients treated with cetuximab experience dermatological side effects, mainly papulopustular skin rash, xerosis, fissures, hyperpigmentation, and changes to the hair and nails. Other important but less common adverse effects include diarrhea, infusion reactions, and hypomagnesemia.[59]

Possible Mechanisms of Nonresponse to Cetuximab and Patient Selection

As previously mentioned, cetuximab-based immunotherapy has demonstrated increases in PFS and OS in some treated patients with mCRC. However, the mean effective rate of TA-specific mAb-based therapies is only 30% (range, 0%–60%).[15,67] Little is known about why such a limited percentage of the treated patients clinically respond to cetuximab treatment. The toxicities and costs associated with cetuximab therapy promote ways of effectively selecting patients who are most likely to benefit from it. The underlying mechanisms of nonresponse to cetuximab-based therapy have been widely investigated due to the clinical importance of the targeted drug.

Potential immune escape mechanism of nonresponse to cetuximab

Immune escape mechanisms may explain why tumor cells evade mAb-induced antitumor immunity, as indicated by patients' differential clinical responses to TA-targeted mAb therapy.[68] Antigen-presenting cells (APCs), such as DCs, play an important role in initiating the tumor immune response. APCs process and present TA signaling to $CD4^+$ and/or $CD8^+$ T cells; they also mediate the tumor-specific CTLs. Therefore, multiple cells and multiple immune or immune escape mechanisms are involved in TA-specific mAb therapy. Whether the immune escape mechanism can cause the failure of cetuximab monotherapy is dependent on each patient's genetic or epigenetic background and/or the tumor microenvironment. FcγR genotypes are associated with clinical responses to TA-targeted mAb therapy.[69] Tumor cell expression of the NK cell inhibitory proteins human leukocyte antigen (HLA)-E and HLA-G also affects mAb-mediated tumor cell lysis.[70–72] T cells may affect the clinical efficacy of TA-specific mAb-based immunotherapy, and escape mechanisms may be relevant to T cell-based immunotherapies, which have been identified in several clinical studies.[15] Changes in the expression of TAs, HLA, and antigen-processing components, as well as regulatory T cells, regulate the interaction between tumor cells and T cells and the recognition of tumor cells by the host's CTLs.[53] Tumor cells may fail to express TAs (~10%–30%), causing variations in interlesional and intralesional heterogeneity in patients,[73] or express TA-derived peptide analogues, leading to abnormal T-cell activation.[74] Furthermore, there are approximately 10%–80% of tumor cells with downregulation or loss of HLA class I antigens.[74] All these render TA-specific T cells ineffective targets to malignant cells and may result in immune escape that ultimately leads to the failure of mAbs, such as cetuximab, to control antitumor immunity. Some of these abnormal cases show that cytokines can rebuild the expression of HLA class I antigens, which offers potential clinical benefit by combining TA-specific mAb-based immunotherapy with cytokine administration.

Potential role of KRAS mutation in nonresponse to cetuximab

According to the drug-target principle, the expression of EGFR can help identify both responders and nonresponders to anti-EGFR-based therapies. However, the current methods have failed to determine whether such expression is positive or negative among patients who have relapsing CRC or mCRC, although clinical benefit has already been observed in these patients.[75] Markers of patient selection are clearly needed to increase the probability of responding to cetuximab treatment.

KRAS is a signal transducer downstream of tyrosine kinase receptors, such as EGFR. Cetuximab blocks the EGFR signaling cascade, including those signals mediated by KRAS. With EGFR stimulation, wild-type KRAS is active for a short period; the signaling pathways downstream of RAF/mitogen-activated protein kinase/extracellular signal-related kinase are then activated, which are tightly controlled. Mutated KRAS protein becomes constitutively activated, making the cascade independent of EGFR upstream signaling. Thus, blocking of EGFR with cetuximab may not influence downstream events. Mutations within the KRAS gene leading to constitutive protein activity are discovered in approximately 30%–50% of all CRCs.[76–79] In line with this, KRAS forms a node in the downstream signaling pathway of EGFR, and it has been well understood that KRAS mutations are common in CRC. They are quickly recognized as a candidate molecular biomarker of the antitumor activity of EGFR-targeting agents.

KRAS mutations were identified in 35.6% of the 540 patients enrolled in the CRYSTAL trial.[77] For patients with wild-type KRAS, the addition of cetuximab to FOLFIRI yielded a longer PFS or a higher RR compared with the FOLFIRI-only treatment. In contrast, cetuximab did not markedly improve PFS and RR among the patients with KRAS mutations compared with FOLFIRI alone.

A meta-analysis including 2188 eligible mCRC patients demonstrated a more precise estimation of the relationship between KRAS mutations and therapy outcomes.[80] The patients with KRAS mutations demonstrated an overall RR lower than that in patients with wild-type KRAS (14% *vs.* 39%). Median PFS was markedly shorter in the mutant KRAS patients than that in the wild-type KRAS patients (3.0 *vs.* 5.8 months). Similarly, median OS was evidently shorter in the mutant KRAS patients compared with the wild-type KRAS patients (6.9 *vs.* 13.5 months). The meta-analysis evidently indicates that KRAS mutations are predictive biomarkers of unfavorable prognosis for tumor response and survival in mCRC patients treated with cetuximab.

Other genetic markers of cetuximab response

As previously discussed, the percentage of patients who did not respond to cetuximab, due to KRAS mutations, was between 30% and 40% only. Recently, a retrospective consortium analysis showed that RRs to cetuximab were 24.4% in the unselected patients, 36.3% in the wild-type KRAS patients, and 41.2% in the wild-type KRAS, NRAS, BRAF, and PIK3CA (exon 20) patients.[10] Resistance to cetuximab in mCRC thus merits additional investigation. Further refinement of the biomarker selection criteria for patients who are most likely to benefit from cetuximab might be possible, by including, for instance, the presence of mutations in HER-3, c-MET, IGF1R, p.G13D, and TP53 as well as the levels of amphiregulin and epiregulin.[81–85] Other potential biomarkers include loss of PTEN expression, EGFR gene copy-number changes,[85] and EGFR promoter hypermethylation.[86]

According to the National Comprehensive Cancer Network, mCRC patients with KRAS mutations should not be treated with cetuximab. All the abovementioned markers demonstrated better predictive effects in combination with KRAS analysis. Most of these predictors are still in the preclinical testing phase, but they have promising applications in patient selection.

Skin toxicity

The function of EGFR in regulating epidermal basal keratinocytes is affected by the blockage of cetuximab, which leads to the characteristic papulopustular rash. In most clinical trials, the clinical benefit of cetuximab-based therapy is closely correlated with the incidence and severity of this type of rash.[8] Compared with patients without skin toxicity, those suffering from it were observed to have a remarkably longer average survival time.[12]

The ELSIE study recently analyzed the relationship between the early occurrence of acne-like rash and OS. Patients with acne-like rash showed a longer median OS than those without it (9.5 *vs.* 6.2 months). Median OS was shorter in patients with mild acne-like rash.[9]

Similarly, Saridaki et al.[87] demonstrated that, in comparison with patients who have a mild skin rash or none at all, mCRC patients with severe and moderate skin rash presented significantly higher PFS and OS values. Indeed, as an independent predictive factor in the multivariate analysis, the absence of severe (grade 3) or moderate (grade 2) skin rash formation does account for reduced PFS and OS. However, the biological mechanism between severe skin rash and tumor response remains unclear.

Summary

Cetuximab, an anti-EGFR therapy, has demonstrated clinical efficacy as monotherapy and in combination therapy among patients with mCRC largely limited to tumors with wild-type KRAS. The addition of cetuximab to chemotherapy contributes to better PFS and RR values in contrast to cetuximab-only treatment, and such combination therapy is the preferred regimen for patients who have irinotecan-resistant mCRC. As first-line therapy for mCRC, cetuximab, in combination with FOLFOX or FOLFIRI chemotherapy, has improved PFS and RR in patients with wild-type KRAS tumors.

Despite the efficacy of cetuximab in the treatment of mCRC, the RR values are still suboptimal. Selecting patients who are the ideal beneficiaries with the purpose of optimizing treatment with the targeted therapies is more than essential. Some clinical markers and several biological markers involved in EGFR intracellular signaling pathways have been identified as potential predictors of response to cetuximab. Among all biomarkers, KRAS is the most relevant one, and patients with KRAS mutations can obtain little benefit from cetuximab-based treatment. Studies of other promising biomarkers, such as BRAF, NRAS, and PIK3CA exon 20, are currently underway. The

epidermal reaction is also involved in the entire treatment process, but its value as a predictive marker is limited as the rash only appears after treatment.[88]

Research has shown that the immune escape mechanisms used by tumor cells to evade mAb-induced antitumor immunity may contribute to differential clinical responses to TA-targeted mAb-based immunotherapy. In some cases, the combination of TA-specific mAb-based immunotherapy and administration of cytokines or immune adjuvants may benefit patients.

EGFR transduction is complex, and tumor cells can use multiple ways to avoid the effects of cetuximab. Despite inhibiting EGFR and stimulating the ADCC reaction, cetuximab can also restrain the expression of VEGF and cancer angiogenesis. The existing predictive markers are not satisfactory, and progress in basic and clinical research into cetuximab-based therapies should address this issue.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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