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Review

Monoclonal antibodies in the treatment of colorectal cancer

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Abstract

Monoclonal antibodies have been developed to target specific proteins involved in the development and progression of cancer. These reagents have the advantage of exquiste specificity, and as currently engineered, low toxicity. The impact monoclonal antibody therapy has recently been demonstrated in colorectal cancer, in which two pathways critical to carcinogenesis have been targeted. The targets are the epidermal growth factor receptor signaling pathway and angiogenesis. Antibodies directed to proteins in both pathways have shown significant activity especially in combination with chemotherapy, and studies in the adjuvant setting are in progress. We review the use of monoclonal antibodies in the treatment of colorectal cancer with particular attention to edrecolomab (Mab 17-1A), bevacizumab (Avastin), cetuximab (IMC-C225), ABX-EGF and EMD 72000. Additional compounds are in earlier stages of development, and the future of this approach in solid tumours is promising.

Keywords: Monoclonal antibodies; Colorectal cancer; Edrecolomab; Bevacizumab; Cetuximab; ABX-EGF; EMD 72000

1. Introduction

Colorectal cancer is the third most common cancer in the United States and the second cause of cancer death [1]. The incidence in the United States is approximately 130000 cases per year and over 50000 deaths per year result; both incidence and outcome in Europe are similar [2–4]. Approximately 25% of patients present with metastatic disease; the remaining 75% are treated surgically with cure as the objective [5], but even with complete resection the disease will eventually recur in 50% of these. Adjuvant therapy affords a risk reduction of at least one-third [with 5-fluorouracil (5FU) alone] in stages II and III disease [6]. Preliminary indications suggest an additional increment with oxaliplatin [7].

Advances in our understanding of the molecular mechanisms underlying the development and progression of cancer have resulted in the discovery of new therapeutic interventions that target specific molecular abnormalities. Among the numerous drug classes in development are the monoclonal antibodies (MAbs). Their specificity, and therefore their potential to bind preferentially and modify tumour-specific targets, sparing normal tissues and causing fewer side-effects than conventional cytotoxic agents, makes them an attractive therapeutic approach. For many years, research has been focused on the identification of cell-surface antigens with expression restricted to tumour cells. The success of rituximab in the treatment of lymphomas has not, however, been replicated in the case of solid tumours [8,9]. Indeed, relatively few antibodies capable of effectively targeting solid tumours have been identified. Among the factors adduced to explain these limitations are the molecular heterogeneity that characterises solid tumours as opposed to the clonality of lymphomas, the accessibility and the metabolism of tumour antigens, and the antibody affinity. The ideal antigen was long considered one that is preferentially expressed in high copies on the membrane of tumour cells, is genetically stable, is not shed or secreted, and plays a causal role in tumour development and/or progression. Now, the original paradigm that focused MAb research on antigens expressed on the membrane of tumour cells is being expanded. An example is the development of antibodies targeting the tumour vasculature. The formation of new blood vessels, angiogenesis, is a process that plays a key

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Table 1
Monoclonal antibodies as therapy for colorectal cancer

Name	Antibody type	Target	Phase of development
Edrecolomab (Centocor/GlaxoSmithKline)	Murine IgG2a	Ep-CAM	Phase III
Bevacizumab (Genentech)	Humanised IgG1	VEGF	Phase III
Cetuximab (Imclone/Bristol Myers Squibb)	Chimeric IgG1	EGFR	Phase III
ABX-EGF (Abgenix/Amgen)	Fully human IgG2	EGFR	Phase II
EMD 72000 (Merck)	Humanised IgG1	EGFR	Phase I

Ep-CAM, 17-1A antigen; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor.

role in the development of tumours and antigens expressed on tumour endothelial cells have the advantage of being genetically stable.

An important limitation of the first mouse-derived MAbs was the induction of a human antimouse immunoglobulin immune response in the majority of patients [10,11]. This resulted in rapid clearance of the antibody and reduced tumour targeting with subsequent dosing. To overcome this problem, strategies aimed to decrease the immunogenicity of the antibodies were developed. The chimeric MAbs were engineered by replacing the mouse constant domains with human constant domains [12]. A further improvement came with the creation of humanised antibodies that are derived from human cells or genetically engineered mice in which murine immunoglobulin genes have been replaced with human antibody genes [12,13].

This review will focus on the MAbs in development as therapy for colorectal cancer (Table 1). Five antibodies are currently in different stages of development: edrecolomab (Mab 17-1A), bevacizumab (Avastin), cetuximab (IMC-C225), ABX-EGF and EMD 72000.

2. Edrecolomab (Mab 17-1A)

Edrecolomab (Panorex, Mab 17-1A; Centocor and GlaxoSmithKline) is a murine IgG2a MAb tested in the adjuvant setting in patients with stages II and III colorectal cancer. Edrecolomab binds with low affinity to the tumour-associated antigen Ep-CAM (17-1A antigen) [14,15]. Ep-CAM is an epithelial cell-adhesion molecule implicated in the transport of calcium across the cell membrane that is expressed in normal epithelial tissues and in numerous tumours including carcinomas of the colon, rectum, pancreas, and stomach [16–18]. It is recognised that Ep-CAM plays an important part in tumour development. Its expression has been associated with increased epithelial proliferation and correlates negatively with differentiation [18]. The proposed mechanisms of antitumour activity of edrecolomab involve antibody-dependent cellular cytotoxicity (ADCC), complement-mediated cytolysis, and an anti-idiotype network [14].

Initial clinical studies evaluated the efficacy of edrecolomab both as monotherapy and as combination therapy with granulocyte-macrophage-colony-stimulating factor (GM-CSF) and interferon-γ (IFN-γ) in patients with gastrointestinal malignancies (Table 2). Khazaely and colleagues report a phase I study involving 25 patients with metastatic gastrointestinal carcinoma treated with one to four infusions of edrecolomab, 400 mg [19]. Another phase I trial explored repeated doses ranging between 200 and 500 mg in 24 patients with metastatic colorectal cancer [20]. The pharmacokinetic profiles of edrecolomab were similar after single and multiple doses, and were not affected by the human antimouse antibodies (HAMA), which were detected in all patients treated with the antibody. There was a wide interindividual variation in the mean maximum edrecolomab concentration (C_{max}), and the half-life ($t_{1/2}$) was inversely correlated with the C_{max}. Edrecolomab was not detectable in serum after 1 week from the last infusion in the majority of cases, but it could be detected in serum for 2 weeks in a small percentage of patients. In all studies edrecolomab was well tolerated, with the most predominant side-effects being diarrhoea, abdominal pain, nausea and vomiting, flushing/rash, fever and chills. A minority of patients developed allergic and anaphylactic reactions. Gastrointestinal toxicity was usually mild or moderate, reversible upon discontinuation of treatment and dose dependent.

Ragnhammar and colleagues treated 71 patients with advanced colorectal cancer with varying dosing schedules of edrecolomab (total dose ranging between 1 and 12 g over approximately 1 year) [21]. One patient achieved a partial response (PR) lasting more than 114 months and 10 patients had minor responses (MR) or disease stabilisation (SD) for more than 3 months. The overall survival was 11 months. There was a trend toward a decrease of response with increasing dose intensity. Based on *in vitro* data showing that the cytotoxic activity of MAbs is greatly enhanced by pretreatment of effector cells with GM-CSF [22], 20 patients with metastatic colorectal cancer were treated with the combination of edrecolomab (400 mg on day 3) and GM-CSF (250 μg/m^2 for 10 days) [23]. The overall response rate was 30%, with two patients achieving CR for 29 and 23 months, one patient MR and three patients SD for more than 3 months. Most patients experienced immediate-type allergic reactions against edrecolomab requiring reduction of dose and infusion rate. When edrecolomab

Table 2
Phase II and III clinical trials with edrecolomab in patients with colorectal cancer (CRC)

Type of trial	No. of patients	Regimen	Toxicity	Activity	Reference
Phase II (metastatic CRC)	20	Edrecolomab 400 mg on day 3 and GM-CSF 250 μg/m^2 4 times a day for 10 days	Diarrhoea, nausea, vomiting, allergic reactions	10% CR, 5% MR, 15% SD	[23]
Phase II (metastatic CRC)	15	Edrecolomab 400 mg on days 5,7,9,12 plus IFN-γ 0.1 mg/m^2 on days 1–15	Diarrhoea, nausea, vomiting	3/14 patients SD	[24]
Phase II (Dukes' C CRC)	189	Edrecolomab 500 mg followed by 4-monthly 100 mg doses vs. observation	Diarrhoea, nausea, vomiting	32% reduction of mortality, 23% reduction of recurrence	[25]
Phase III-157-001 (stage III colon cancer)	1839	Edrecolomab 500 mg followed by 4-monthly 100 mg doses vs. 5-FU 425 mg/m^2/LV 20 mg/m^2 daily for 5 days	Hypersensitivity reactions, diarrhoea, nausea, mucositis	3-years OS 81.6% vs. 78.9%	[26]
Phase III-157-002 (stage III colon cancer)	2761	Edrecolomab 500 mg followed by 4-monthly 100 mg doses plus 5-FU 425 mg/m^2/LV 20 mg/m^2 daily for 5 days vs. 5-FU/LV vs. edrecolomab alone	Diarrhoea, nausea, vomiting, hypersensitivity reactions	3-years OS 74.7% vs. 76.1% vs. 70.1%	[27]

GM-CSF, granulocyte-macrophage-colony-stimulating factor; MR, minor response; SD, disease stabilisation; IFN-γ, interferon-γ: 5-FU, 5-fluouracil; LV, leucovorin; OS, overall survival.

(400 mg on days 5, 7, 9 and 12) was combined with IFN-γ (0.1 mg/m^2 on days 1–15), no CR or PR were observed. Three of 14 patients achieved SD and median survival was 56 weeks [24].

Edrecolomab was extensively evaluated for the adjuvant treatment of colorectal cancer (Table 2). Encouraging results were initially reported by Riethmüller and colleagues, who treated 189 patients with Dukes' stage C colorectal cancer with edrecolomab 500 mg loading dose, followed by four 100 mg doses given at monthly intervals [25]. After 7 years of follow up, edrecolomab resulted in a 32% reduction in the relative risk of overall mortality compared to no treatment [63% vs. 43%; hazards ratio, 0.57; 95% confidence interval (CI), 8–51%; $P < 0.01$] and a reduction in recurrence of 23% (52% vs. 68%; hazards ratio 0.66; 95% CI 1–43%; $P = 0.04$). When the site of recurrence was analysed, patients treated with edrecolomab showed significantly fewer distant recurrences compared to the group without treatment, suggesting that edrecolomab may preferentially act on isolated tumour cells preventing outgrowth of distant metastasis. HAMA were detected in 80% of treated patients, but there was no difference in antibody titre between patients who developed recurrences and those who remained tumour free. Additionally, no difference in anti-idiotype titre was detected in patients with and without relapse. These data prompted a more extensive evaluation of edrecolomab.

Three randomised phase III studies of edrecolomab for either stage II or III colon or rectal cancer were conducted in Europe and United States [26–28]. Two were designed to determine whether the combination of edrecolomab/5-FU/leucovorin (LV) improves overall survival relative to 5-FU/LV in patients with surgically resected stage III colon cancer [26,27]. In the 157-001 study conducted in North and South America, 1839 patients were randomised to receive 5-FU/LV (5-FU 425 mg/m^2 and LV 20 mg/m^2 daily for 5 days every 4–5 weeks), with or without edrecolomab administered in the same regimen as in the Riethmuller study. In the 157-002 study conducted in Europe, South Africa, Australia, New Zealand and Asia, 2761 patients were randomised to receive edrecolomab alone, edrecolomab and 5-FU/LV, or 5-FU/LV alone with the same schedule as study 157-001. Edrecolomab monotherapy was well tolerated, with the most common side-effect being diarrhoea. The addition of edrecolomab to 5-FU/LV did not, however, increase the incidence of diarrhoea or neutropenia. The two studies showed conflicting results. The North American study demonstrated a modest survival benefit by the addition of edrecolomab to 5-FU/LV for stage III colon cancer at 3-year follow up [3-years overall survival (OS): 81.6% vs. 78.9%, hazard ratio 0.785, 95% CI 0.638–0.967, $P = 0.023$]. In contrast, study 157-002 showed no additional benefit in either overall or disease-free survival by adding edrecolomab to 5FU/LV in the adjuvant treatment for stage III colon cancer. After 3 years, the OS was 74.7% and 76.1% for the combination and chemotherapy arms, respectively (hazard ratio 0.94, 95% CI 0.76–1.15, $P = 0.53$). The OS of the edrecolomab monotherapy arm was significantly lower than that of chemotherapy (OS 70.1%, hazard ratio 0.82, 95% CI 0.67–1.00, $P = 0.05$).

To explain the conflicting results obtained in these large clinical trials, several hypotheses can be offered. It is possible if not likely that edrecolomab has clinical efficacy but at a low level that could have been missed in the 157-002 study. Alternatively, the different outcomes

in these studies may reflect a different patient population. The studies were conducted in different geographic regions, and population differences in regulation of immune responses may exist. It is recognised that the level of expression of the target antigen on individual tumour cells and the antibody affinity for its target are important factors in MAb therapy [29]. Indeed, it was demonstrated that the antibody affinity and the presence of a minimal threshold number of antibodies bound to the target cells, which was dependent on antibody affinity, were of major importance for effective cell lysis through ADCC [29]. Additionally, the activity of MAb 17-1A is higher against cells expressing higher amounts of Ep-CAM [29]. Therefore, the low affinity of edrecolomab together with the tumour heterogeneity may partially account for the conflicting results reported in clinical studies, since a more heterogeneous tumour will probably require an antibody with higher affinity.

One continuing phase III study in North America and Europe is comparing the overall survival rates for patients treated with edrecolomab vs. surgery alone after curative surgery for stage II colon cancer (157-003). The results are expected in the near future.

3. Bevacizumab (Avastin)

Angiogenesis, the process leading to the formation of new blood vessels, has a key role in the survival of cancer cells, in local tumour growth and in the development of distant metastasis [30]. The development of blood vessels within the tumour mass is regulated by the production of several growth factors secreted by cancer cells to stimulate normal endothelial cell growth through paracrine mechanisms. Among these factors are vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and transforming growth factor-α (TGF-α) [31–34]. Enhanced expression of VEGF has been observed in human cancer cell lines and in cancer patients with different malignancies including colorectal, breast, non-small cell lung, and ovarian cancers, and is directly correlated with increased neovascularisation, as measured by microvessel density (MVD) within the tumour [34]. Studies in colorectal cancer have associated MVD with progression from adenoma to cancer, and from localised to metastatic disease [35–37]. High MVD confers a poorer prognosis, and might also determine the nature of the metastatic profile [36,37]. Therefore, agents that target the tumour vasculature represent an attractive approach to colon cancer treatment.

Bevacizumab (Avastin; Genentech, South San Francisco, CA) is a recombinant humanised MAb with selectivity against VEGF. Preclinical studies have shown that bevacizumab produced growth inhibition in several xenograft tumour models including colorectal cancer. When post-treatment tumours were analysed, a marked decrease in blood vessel density was observed compared to the animals treated with control agents, suggesting that bevacizumab is active though inhibition of neovascularisation [38].

Early phase I and II studies performed in patients with a variety of tumour types explored doses of bevacizumab ranging from 0.1 to 10 mg/kg over a 4- to 24-week period as a single agent or in combination with conventional chemotherapy [39–41]. Pharmacokinetic analysis showed a linear profile with doses $\geqslant 0.3$ mg/kg. The clearance (CL) and the central volume of distribution (V_c) were related to body weight and there was a 44% intersubject variability for the CL and 25% for V_c. In these initial clinical trials the predominant toxicities reported were bleeding episodes, headache, fever, asthenia, nausea/vomiting, arthralgia, dyspnoea and rash.

Following the phase I evaluation of the single agent, the drug was investigated in combination with cytotoxic chemotherapy (Table 3). Kabbinavar and colleagues report a randomised phase II trial comparing the combination 5-FU/LV/bevacizumab, with two administration schedules of bevacizumab (5 mg/kg or 10 mg/kg every 2 weeks) with 5-FU/LV alone (5-FU 500 mg/m^2/LV 500 mg/m^2 weekly for 6 of 8 weeks) in chemotherapy-naive metastatic patients with colorectal cancer [42]. The addition of low-dose bevacizumab to 5-FU/LV resulted in a modest improvement in response rate (RR) (17% vs. 40%), a longer median time to tumour progression (TTP) (5.2 months vs. 9 months), and longer median survival (13.8 months vs. 21.5 months). The results with the higher dose of bevacizumab were not quite as good, for reasons that are unclear. The most significant adverse event was thrombosis, which was fatal in 1 patient. Other side-effects were hypertension, proteinuria and epistaxis. The issue of the optimal dose of bevacizumab will have to be addressed in the future, and may need to be individualised.

By the time of the completion of the 5-FU/bevacizumab phase II study, the treatment of colorectal cancer included irinotecan. To ensure that the combination was tolerable and active, ECOG performed a phase II trial of bevacizumab (10 mg/kg every other week) in combination with irinotecan (100 mg/m^2) and 5-FU/LV (400–20 mg/m^2) weekly for 4 of 6 weeks in previously untreated patients (E2200) [43]. The overall response rate was 45% and the most common side-effects were diarrhoea, neutropenia, thrombotic events (pulmonary embolism in 4 patients), and bleeding episodes. This trial established safety of the combination, and supported more extensive testing.

Hurwitz and colleagues have recently reported a phase III trial of bevacizumab in combination with IFL (irinotecan/5-FU/LV) as first-line therapy in patients with advanced colorectal cancer [44]. 800 patients were randomised to receive IFL/bevacizumab placebo or

Table 3
Phase II and III clinical trials with bevacizumab (BV) in patients with colorectal cancer (CRC)

Type of trial	No. of Patients	Regimen	Toxicity	Activity	Reference
Phase II	104	5-FU 500 mg/m^2/LV 500 mg/m^2 vs. 5-FU/LV plus BV 5 mg/kg q2 wks vs. 5-FU/LV plus BV 10 mg/kg q2 wks	Thrombosis, hypertension, bleeding	RR 17% vs. 40% vs. 24%; median survival 13.8 mo vs. 21.5 mo vs. 16.1 mo	[42]
Phase II	92	BV 10 mg/kg q2wks plus irinotecan 100 mg/m^2 and 5-FU/LV 400/20 mg/m^2 weekly	Diarrhea, neutropenia, thrombotic events, bleeding	RR 45%	[43]
Phase III	925	BV 5 mg/kg q2 wks plus IFL vs. BV-placebo plus IFL vs. 5FU/LV/BV	Hypertension, thromboembolism, bleeding	Median survival 20.3 vs. 15.6 mo, PFS 10.6 vs. 6.24 mo, ORR 45% vs. 35%	[44]
Phase III	223	BV 10 mg/kg q2 wks vs. BV 10 mg/kg q2 wks plus FOLFOX4 vs. FOLFOX4	Bleeding, thromboembolism, hypertension	Analysis ongoing	[46]

MR, minor response; SD, disease stabilisation; IFN-γ, interferon-γ: 5-FU, 5-fluorouracil; LV, leucovorin; OS, overall survival; FOLFOX4, oxaliplatin/5-FU/LV; RR, response rate; ORR, overall response rate; PFS, progression-free survival.

IFL/bevacizumab (5 mg/kg every 2 weeks). A third group of 100 patients received FU/LV plus bevacizumab, which was closed upon demonstration that the irinotecan-containing combination was safe in E2200. Patients who progressed on the bevacizumab arm could continue bevacizumab in combination with second-line chemotherapy. The study was strikingly positive: the median survival was 20.3 months in the IFL/bevacizumab group and 15.6 months in the IFL/Placebo ($P = 0.00003$), progression-free survival (PFS) 10.6 vs. 6.24 months ($P < 0.001$) and overall response rates (ORR) 45% vs. 35% ($P = 0.0029$). Significant increases in rates of hypertension (10.9% vs. 2.3%) and thromboembolism (19.3% vs. 16.1%) were seen, and gastrointestinal perforation was noted in a small number of patients. However, severe bleeding was not a serious toxicity, and the incidence and severity of the side-effects of chemotherapy were unaffected.

The results of large studies support a role for 5-FU/oxaliplatin in the initial therapy of colorectal cancer [7,45]. A key question is whether this benefit will be observed independent of the type of chemotherapy. A large randomised phase III study (E3200) comparing bevacizumab (10 mg/kg biweekly) alone, or in combination with oxaliplatin/5-FU/LV (FOLFOX4) and FOLFOX4 alone in patients with advanced colorectal cancer who had failed on 5-FU/LV and irinotecan has been completed [46]. An interim toxicity analysis shows that the addition of bevacizumab did not substantially alter the toxicity of FOLFOX4 and was overall well tolerated [46]. An increased incidence of bleeding, thromboembolism and hypertension was observed.

4. Cetuximab (IMC-C225)

Activation of epidermal growth factor-receptor (EGFR) signalling plays an important part in multiple cellular functions, including proliferation, differentiation, survival and angiogenesis, all of which are known to contribute to the neoplastic phenotype [47,48]. Alterations of EGFR signalling have been implicated in the development and progression of numerous tumours including colorectal cancer [47,48]. Expression of EGFR has been reported in 60–75% of colorectal cancers and has been correlated with poor prognosis [49,50]. As a result, EGFR represents a rational target for antitumour therapy. Two main strategies to block EGFR have consisted of MAbs that bind to the extracellular domain of the EGFR and inhibitors of the EGFR-tyrosine kinase (EGFR-TKI), which block its intracellular tyrosine-kinase domain.

Cetuximab (Erbitux; ImClone Systems and Bristol Myers Squibb) is a chimeric IgG1 MAb that binds competitively to the extracellular domain of EGFR, inhibiting EGF binding and receptor autophosphorylation, and inducing its internalisation and degradation [51–53]. Preclinical data indicate that cetuximab has antitumour activity in colon cancer xenografts [51–53]. Moreover, cetuximab blocks the production of pro-angiogenic factors such as VEGF, interleukin 8 and bFGF [54]. While immune mechanisms did not seem to play an important part *in vitro*, it is possible that they may contribute to its antitumour activity *in vivo* [55].

Phase I studies of cetuximab explored doses ranging from 5 to 400 mg/m^2. A loading dose of 400 mg/m^2 followed by a weekly maintenance dose of 250 mg/m^2 was the dosing regimen that resulted in continuous saturation of cetuximab clearance. Because cetuximab is cleared by binding to EGFR, this dose regimen was selected for phase II and III studies with the assumption that saturation of clearance indicated complete saturation of the EGFR-binding sites [51,56]. In support of this, and further indicating an effect on the target, inhibition of EGFR TKI activity was demonstrated in

sequential tumour biopsy samples obtained from patients in the phase I studies at this dose level [51]. Cetuximab was well tolerated, with the predominant side-effect being an acneiform rash. Other toxicities include asthenia, fever, nausea, elevation in aminotransferases and allergic reactions. Non-neutralising human antibodies against chimeric antibodies were detected in 4% of patients and had no effect on the pharmacokinetics of repeated doses of cetuximab.

Unlike the EGFR-TKIs, cetuximab has shown antitumour activity as a single agent in colorectal cancer (Table 4). In a multicentre phase II study, 57 patients with metastatic colorectal cancer who had failed on irinotecan and 5-FU were treated with cetuximab monotherapy (400 mg/m^2 loading dose then 250 mg/m^2 weekly) [57]. The response rate was 10.5%, and 36.8% of evaluable patients exhibited disease stabilisation. Based on preclinical data showing that the efficacy of cetuximab is augmented when combined with chemotherapy, the combination of cetuximab as 400 mg/m^2 loading dose followed by 250 mg/m^2 weekly and irinotecan at the same dose as the patients had progressed on was explored in a phase II study in 121 patients with metastatic colorectal cancer positive for EGFR and who had failed on 5-FU and irinotecan [58]. The response rate was 22.9%. The addition of cetuximab did not exacerbate the toxicities typically associated with irinotecan. Allergic reactions and an acne-like skin rash were the major side-effects attributed to cetuximab. Interestingly, the RR correlated positively with the development of skin rash, suggesting a pharmacodynamic or pharmacogenetic interaction with treatment efficacy [59]. Based on these initial encouraging results on a multiply treated patient population, a phase II trial of cetuximab in combination with weekly irinotecan/5-FU/LV was conducted in patients with previously untreated, EGFR-positive colorectal cancer [60]. 11 patients (44%) achieved PR and 5 patients had MR. A more recent European phase II study evaluated the combination of cetuximab (400 mg/m^2 loading dose followed by 250 mg/m^2 weekly) with a FOLFIRI regimen (5-FU 300 mg/m^2 bolus and infusional 5-FU 2000 mg/m^2/46 h or 400 mg/m^2 bolus and 2400 mg/m^2/46 h, LV 400 mg/m^2 and irinotecan 180 mg/m^2 every 2 weeks) [61]. Dose-limiting toxicities in the form of diarrhoea, allergy and neutropenia occurred in 3 patients in the high-dose group, and almost all patients developed skin rash. 8 of 18 evaluable patients had PR (44%) and four had SD (22%).

Finally, to determine if the addition of cetuximab to irinotecan can indeed revert chemotherapy resistance, and demonstrate activity greater than cetuximab alone, a randomised phase III study in patients with EGFR-positive, irinotecan-refractory colorectal cancer was conducted in Europe [62]. 329 patients (representing those entered on study from 470 EGFR-positive patients, from 576 screened) were randomised to receive cetuximab plus irinotecan, or cetuximab alone in a 2:1 randomisation. Patients were treated at the same dose and schedule of irinotecan on which they had progressed. Response rates of 17.9% (95% CI 13–27.7%) were observed with the combination cetuximab/irinotecan and 9.9% (95% CI 5.0–17.1%) in the arm receiving cetuximab monotherapy ($P = 0.074$). Time to progression favoured the combination (126 vs. 45 days). Taken together these results show consistent clinical activity of cetuximab in patients with colorectal cancer. They show that in addition to reproducible activity as a single agent, there is evidence favouring an interaction with chemotherapy that supports the combined use of the modalities.

Table 4
Phase II and III clinical trials with cetuximab (C225) in patients with colorectal cancer (CRC)

Type of trial	No. of patients	Regimen	Toxicity	Activity	Reference
Phase II (irinotecan refractory, EGFR + CRC)	57	C225 400 mg/m^2 followed by 250 mg/m^2 qwk	Acne-like skin rash, asthenia, allergic reactions	6/57 PR; 13/57 SD	[59]
Phase II (irinotecan/5-FU refractory, EGFR + CRC)	121	C225 400 mg/m^2 followed by 250 mg/m^2 qwk plus irinotecan	Acne-like skin rash, allergic reactions	17% PR; 31% SD	[57]
Phase II		C225 plus weekly irinotecan 125 mg/m^2 plus 5-FU 500 mg/m^2/LV 20 mg/m^2	Diarrhoea, neutropenia, acneiform skin rash	44% PR	[57]
Phase II	23	C225/FOLFIRI	Diarrhoea, neutropenia, skin rash	44% PR; 22% SD	[61]
Phase III	329	C225 400 mg/m^2 followed by 250 mg/m^2 qwk plus irinotecan vs. C225 alone		RR 17.9% vs. 9.9%; TTP 126 days vs. 45 days	[62]

EGFR, epidermal growth factor receptor; PR, partial response; SD, disease stabilisation; 5-FU, 5-fluouracil; LV, leucovorin; RR, response rate; FOLFIRI, see text; TTP, time to tumour progression.

5. ABX-EGF

ABX-EGF (Abgenix Inc/Amgen) is a human IgG2 MAb that binds with high affinity to the EGFR [63]. ABX-EGF blocks the binding of both EGF and TGF-α. Preclinical data have shown that ABX-EGF has antitumour activity in human pancreatic, renal, breast and prostate tumour xenografts and the expression of 17000 or more EGFRs per cell was associated with higher tumour inhibition [63]. Pharmacokinetic and pharmacodynamic analysis in tumour xenograft models indicated that a serum ABX-EGF concentration of 5 μg/ml produced 90% inhibition of A431 tumour growth (IC_{90}).

In a phase I study, 43 patients with prostatic, pancreatic, renal, colorectal, gastro-oesophageal and lung cancers expressing EGFR were treated with ABX-EGF at doses ranging from 0.01 to 2.5 mg/kg weekly [64,65]. Clearance was dose dependent due to progressive saturation of EGFR and a dose of 2.5 mg/kg/week resulted in trough levels exceeding the IC_{90} in the xenograft model. The incidence of skin rash increased with increasing dose of ABX-EGF, with a predicted 90% incidence at a dose of 1.9 mg/kg. All 15 patients receiving at least one dose of 2 or 2.5 mg/kg developed skin rash, and the phase 2 dose of 2.5 mg/kg weekly was the dose that resulted in 100% of patients developing skin rash. At doses producing more than 80% saturation, the interpatient coefficients of variation for areas under the curve (AUC) were <20%. Human antihuman antibodies (HAHA) were not detected. Two patients with colorectal and oesophageal cancer had stable disease.

Phase II studies were conducted in patients with advanced prostate, renal and colorectal cancers [66,67]. Meropol and colleagues recently reported an interim analysis of a continuing phase II study [66]. Twenty-three patients with metastatic colorectal cancer overexpressing EGFR who had failed previous therapy with fluoropyrimidine, irinotecan and/or oxaliplatin were treated with ABX-EGF, 2.5 mg/kg weekly. Three (13%) patients achieved PR and nine (39%) had SD. In clinical studies, ABX-EGF was well tolerated. As with other EGFR inhibitors, skin rash was the main adverse event observed. Other toxicities, all mild or moderate, included abdominal pain, asthenia, anorexia, conjunctivitis, and stomatitis.

6. EMD 72000

EMD 72000 (Merck KgA) is a humanised IgG1 MAb that binds with high specificity and affinity to the EGFR and has shown inhibitory activity in several human tumour xenograft models including gastric, pancreatic and lung cancers [68–70]. By blocking EGF binding to its receptor, EMD 72000 inhibits the EGFR downstream signalling pathway [68]. *In vitro* experiments have indicated that EMD 72000 may also act through ADCC by binding to FcR on immune cells [71]. Indeed, by enzymatically deglycosylating EMD 72000 and therefore blocking FcR binding and ADCC activity, a significant decrease was observed in the antitumour activity of EMD 72000 against a subcutaneous A431 tumour model in severe combined immunodeficiency (SCID) mice compared to the unmodified antibody. The deglycosylated antibody did not block growth or VEGF production by A431 epidermal carcinoma cells expressing large amounts of EGFR, suggesting that EGFR signalling was not inhibited by deglycosylation.

Phase I pharmacokinetic studies using doses of EMD 72000 ranging from 100 mg to 2000 mg weekly and different administration schedules have shown that increases of C_{max} and AUC were dose proportional [68,72,73]. The half-life increased in proportion to the dose and reached a plateau at higher dose levels. Additionally, these studies indicated that a loading dose is not necessary and an every 3-weeks administration schedule might be used. Finally, no HAHA were detected. Similar to results obtained with other EGFR inhibitors, pharmacodynamic studies have shown a significant decrease in the content of activated EGFR, mitogen-activated protein kinase (MAPK) and Ki-67 in skin biopsies after EMD 72000 treatment at doses between 800 and 1600 mg/week and with different schedules of administration [68,72]. A complete inhibition of EGFR phosphorylation was observed in basal keratinocytes of the epidermis after EMD 72000 therapy, while total EGFR expression was not altered. MAPK phosphorylation and Ki-67 expression were also significantly reduced.

Antitumour activity was observed in gastrointestinal malignancies in phase I clinical trials. Trarbach and colleagues treated 24 patients with EGFR-positive colorectal, gastric and cholangiocarcinomas with EMD 72000, 100–800 mg every week for 4 weeks [74]. One patient achieved CR (4%) and 9 (38%) patients had SD. In another phase I study, 22 patients with colon (15), gastric (3) and other (4) cancers received EMD 72000, 1200 mg weekly, at different administration schedules [72]. Two of the patients with colorectal cancer achieved PR and one had MR. A patient with colorectal cancer and one with renal cancer showed SD. In a European phase I trial, 22 patients with EGFR-positive oesophageal, colorectal, head-and-neck and cervical cancers were treated with doses of EMD 72000 ranging from 400 to 2000 mg weekly [68]. Two of 11 patients with colorectal cancer, 2 of 4 patients with head and neck cancer and 1 of 2 patients with oesophageal cancer achieved PR and 6 patients had disease stabilisation for 3–6 months. In all studies, EMD 72000 was well tolerated, with the main adverse events being acneiform skin rash, conjunctivitis, headache and fever. No haemato-

logical toxicities or anaphylactic reactions were reported.

7. Conclusions

Studies presented in the past year establish firmly the potential of MAbs in the treatment of a major solid tumour, colon cancer. Two targets of broad interest in cancer treatment have been shown to yield to MAb treatment, in both cases indirectly validating their importance as well. The positive results have been obtained in patients with advanced disease: studies are already under way in the cooperative groups to determine if this benefit will extend to the adjuvant setting. Issues to be addressed in future trials include dosing strategies, duration of therapy in the adjuvant arena, and integration with small-molecule inhibitors of the same pathways. But perhaps the most important question, in light of the economic pressure that the licensing of these molecules will put on health-care resources, is the selection of patients.

In the case of cetuximab, patients were preselected in the clinical trials: only those with at least 1 + immunohistochemical staining were eligible, and intensity of staining did not appear to be a determinant of response. This raises the issue of the sensitivity of the assay: is there a population with a lesser degree of EGFR expression that might also respond? These sensitivity assays for EGFR expression have dogged the development of the small-molecule inhibitors also, and no clear picture of target expression vs. response has emerged. Further, not all patients benefit: the selection of patients who should not get the drug is equally important, and all the continuing studies have associated translational analyses directed to this issue. A similar issue will be important for the ultimate use of bevacizumab: which patients respond to anti-angiogenic interventions, and why? Do measures of endothelial cell proliferation or maturity influence the probability of response? Can additional measures be taken to sensitise either endothelial or tumour cells, and are there differences in responsiveness according to expression of VEGF family proteins or receptors?

For both agents, unlike the receptor TKI of the EGFR, a positive interaction with chemotherapy is observed, and evidence suggests that both bevacizumab and cetuximab have greater efficacy when used in combination. Optimisation of this interaction is a challenge involving dosing and scheduling of the drugs, and an interaction with radiation therapy is being explored in phase I/II trials. Finally, the success of these molecules has permitted the development of novel monoclonals directed to alternative targets, and those structured to deliver various other classes of treatment directly to the cell. It is expected that these novel approaches will reinforce the importance of this class of agents in colorectal cancer.

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