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Review

Recent Progress in Target Therapy in Colorectal Cancer

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Abstract. *Monoclonal antibodies are a new class of agents targeting at specific receptors on cancer cells. In addition to having direct cellular effects, antibodies can carry substances, such as radioactive isotopes, toxins and antineoplastic agents, to the targeted cells. Two of them, cetuximab (Erbitux®) and bevacizumab (Avastin®), seem to have acquired a significant role in the management of patients with radically resected and advanced colorectal carcinoma. Cetuximab plus irinotecan has been approved as second-line therapy in irinotecan-resistant colorectal cancer patients; bevacizumab plus 5FU/LV has resulted in higher response and longer survival than 5FU/LV alone in first line metastatic colorectal cancer; its combination with oxaliplatin has recently doubled results. The superior therapeutic efficacy of these molecular targeting agents over traditional chemotherapy has been shown by the survival benefit achieved by patients with advanced or recurrent cancers. Although the precise molecular mechanism by which these agents produce or enhance an antitumour effect, alone or in combination with anticancer drugs, is unknown, the specific inhibition of target genes critically involved in tumour progression and metastasis is clear. Further studies to determine which patient groups and anticancer drugs are more appropriate for combination therapy with these agents are needed. All the most important data obtained through recent studies are discussed, emphasizing their mechanisms of action, safety profiles and clinical applications.*

The use of chemotherapeutic agents against malignant tumours is successful in many patients but suffers from major drawbacks such as the lack of selectivity to tumour tissue which sometimes leads to severe side-effects and may limit efficacy and drug-resistance. To limit toxicity and to improve the efficacy of cancer therapy, some proteins generally overexpressed on the surface of tumour cells can be selectively targeted. Growth factor receptors are among the most often targeted proteins. Their implication in the pathogenesis and evolution of cancer has clearly been established and therefore, provides a rationale for therapeutic intervention. Monoclonal antibodies (mAbs) (whole molecule or fragments), bispecific antibodies, mAbs conjugated to drugs, toxins or radioisotopes, small peptidic and peptidomimetic molecules in free form or conjugated to drugs, anti-sense oligonucleotides, immunoliposomes-encapsulated drugs and small molecule inhibitors are some of those agents that selectively target and block their action. This review will focus on the current developments of target therapy, emphasizing mechanisms of action, safety profiles and clinical applications (1).

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Mechanism of Action

Epidermal growth factor receptor (EGFR). EGFR provides a rational target for cancer therapy, as it is commonly over-expressed in a variety of solid tumours and deregulation of its activity appears to be associated with resistance to chemotherapy and radiotherapy and a poorer prognosis. Despite preliminary data suggesting a relationship between intratumoural EGFR expression and drug efficacy, several other studies have failed to confirm this finding, because other factors, such as the activation of downstream signalling pathways, the presence of other activating growth factors and/or EGFR mutations influence cellular response. In this regard, the development and validation of immunohistochemical methods measuring the activation of EGFR-pathways with phosphorylation-specific antibodies could be relevant (2, 3). EGFR is a transmembrane glycoprotein that is a member of a subfamily of type I receptor tyrosine kinases including EGFR (HER1), HER2,

HER3 and HER4 (4). Moreover, it is constitutively expressed in many normal epithelial tissues, including the skin and hair follicle.

Cetuximab, formerly known as IMC-225 or C225, is one of the new monoclonal chimeric antibodies directed against EGFR. It has demonstrable activity in a number of tumour types both in combination with chemotherapy and radiotherapy and alone. It binds specifically to the EGFR, HER1 and c-ErbB-1 on both normal and tumour cells and competitively inhibits the binding of EGF and other ligands, such as transforming growth factor-alpha. Binding of cetuximab to the EGFR blocks phosphorylation and activation of receptor-associated kinases, resulting in inhibition of cell growth, induction of apoptosis and decreased matrix metalloproteinase and vascular endothelial growth factor (VEGF) production. *In vitro* assays and *in vivo* animal studies have confirmed that it inhibits the growth and survival of tumour cells that over-express the EGFR; no anti-tumour effect of cetuximab has been observed in human tumour xenograft lacking EGFR expression (5, 6).

Other anti-EGFR antibodies, such as ABX-EGF, EMD 72000 and h-R3, and biospecific antibodies, such as M 26.1, MDX-447 and H 22-EGF, have shown a good safety profile and are now under early clinical investigation (2, 7).

ZD 1839 (Gefitinib; Iressa®) is a low-molecular-weight quinazolin derivate that selectively inhibits the activation of EGFR tyrosine kinase through competitive binding of the ATP-binding domain of the receptor (3). Several phase II studies have explored doses of ZD 1839 that were thought to be effective as a single agent or combined with standard chemotherapy. In general, the regimens were well tolerated, and no increases in toxicities were observed as compared with chemotherapy alone (3, 8-11). Based on the data of IDEAL-1 and -2 trials, the U.S. Food and Drug Administration approved (May, 2003) ZD 1839 (250 mg/day) as monotherapy treatment for third-line therapy in patients with non small cell lung cancer (NSCLC), after failure of both platinum-based and docetaxel chemotherapies.

OSI-774 (Erlotinib; CP-358,774; Tarceva®) is an orally available quinazolin that is a selective inhibitor of EGFR. Clinical responses have been observed in phase II studies conducted in patients with NSCLC, head and neck tumours and ovarian cancer (3).

Dose-selection of all these antibodies for phases II and III studies has been based on toxicity, as well as pharmacokinetic parameters, such as plasma concentrations above a biologically relevant level or saturation of clearance, which suggests a complete occupancy of drug-binding sites. Preclinical studies showed that there is a linear relationship between target inhibition and antitumour activity and that only tumours in which inhibition of the receptor results in inhibition of downstream signalling pathways are growth arrested (2). Tumour biopsy sampling before and after treatment is the simplest method to determine the biologically relevant dose, but it is difficult to obtain sequential tumour tissues. To overcome this barrier, investigators have used normal skin to develop pharmacodynamic surrogate markers of EGFR inhibition, but there is not necessarily a direct relationship between EGFR inhibition in epidermitis and cancer tissues, and it cannot be excluded that the downstream effects of EGFR inhibition are molecularly different in the tumour (2).

It is important to define the activity of these cytostatic compounds in phases II and III trials. Several methods, including functional imaging techniques, such as MRI and PET, and/or surrogate biomarkers, such as EGFR inhibition in the skin, are under investigation (3).

Vascular Endothelial Growth Factor (VEGF). Angiogenesis is an essential step in the growth and spread of solid tumours which are the cause of more than 85% of cancer mortality. Inhibiting angiogenesis should be a reasonable approach for preventing or treating cancer. However, tumour angiogenesis differs from normal angiogenesis in that the resulting vessels are tortuous, irregularly shaped and hyperpermeable (12). These abnormalities result in irregular blood flow and high interstitial fluid pressure within the tumour, which can impair the delivery of oxygen (a known radiation sensitizer) and drugs to the tumour. Emerging evidence suggests that antiangiogenic therapy can damage some tumour vessels and normalize the structure and function of the rest, thereby improving drug delivery and normalizing the tumour microenvironment. This normalization effect may underlie the therapeutic benefit of combined antiangiogenic and cytotoxic therapies. Several lines of evidence argue for angiogenesis inhibition in the treatment of colorectal cancer (12-14): i) angiogenesis (as measured by microvessel count) and the expression of pro-angiogenesis factors, such as VEGF, the key regulator of normal and pathological angiogenesis, have been reported to correlate with advanced disease and a worse prognosis; ii) the expression of VEGF has been shown to correlate with RAS mutations, alterations in the APC-WNT signalling pathway and overexpression of cyclo-oxygenase-2, which are all frequent in colorectal cancer; iii) bevacizumab, a humanized anti-VEGF monoclonal antibody, is a potent inhibitor of tumour growth of various colorectal cancer cell lines in murine xenografts; and iv) the addition of bevacizumab to systemic chemotherapy has been shown to be significantly superior to chemotherapy alone in terms of objective tumour response rate (RR), progression-free survival (PFS) and overall survival (OS) in patients with metastatic colorectal cancer, in the frontline, and more recently in the second-line setting, without worsening of chemotherapy-related toxicity. However, several potential specific adverse effects, such as thrombosis (related to

VEGF regulation of vascular proliferation, permeability and endothelial cell apoptosis), haemorrhages, proteinuria (with the exception of those cases with significant baseline proteinuria which were excluded) (15), arterial hypertension (VEGF receptor [VEGFR] blockade results in decreased production of nitric oxide that could also lead to reduced renal sodium excretion, which is associated with persistent hypertension) (16) and bowel perforations have been described. Although 84% of hypertensive episodes were grade 3 or 4, these cases were easily managed. Antagonizing VEGF might decrease the renewal capacity of the endothelial cells in response to trauma, which in turn causes endothelial dysfunction and defects in the interior vascular lining exposing subendothelial collagen. VEGF antagonism may also cause decreased matrix deposition in the supporting layers of the vessels (17). Treatment with bevacizumab and chemotherapy results in elevated levels of factor VIII and von Willebrand factor (16). In the light of these data, while age-associated comorbidities are not an obstacle to the administration of chemotherapy regimens, safety evaluation of bevacizumab in a specific category of patients such as the elderly might instead limit access to this anti-angiogenic therapy.

Whether or not the antitumoural efficacy of bevacizumab could be increased when combined with low-dose (metronomic) chemotherapy or radiotherapy (in rectal cancer) is under investigation, as are other VEGF-targeted approaches (*e.g.,* dominant negative mutants, antisense oligonucleotides, antibodies directed against VEGFR, VEGFR tyrosine kinase inhibitors and soluble VEGFR) and other anti-angiogenesis agents (*e.g.,* thalidomide, celecoxib, angiozyme) (18).

SU11248 (semaxanib), a small molecule VEGFR TKI, is a pan-TKI that blocks the kinase activity of VEGF receptor-2 ($VEGFR_2$), platelet-derived growth factor receptor (PDGFR), tyrosine kinase receptor (KIT) and FMS-like tyrosine kinase 3 (FLT_3) (19-26). Clinical objective responses have been preliminarily observed in two phase I studies, in particular in renal cell carcinoma, neuroendocrine tumours and thyroid cancer and nearly 50% of patients had stable disease (SD) after treatment (19-21). In a phase I study in patients with gastrointestinal stromal tumour (GIST) resistant to imatinib, 5 out of the 32 treated cases had objective partial responses (PR) and about 60% of the patients had SD >4 months (22, 23); these good, even if preliminary, data appeared encouraging. A randomised phase III trial is ongoing to compare interferon *versus* SU11248 in metastatic renal cell carcinoma as first-line treatment (24).

Clinical phase I studies of PTK787/ZK222584, a multitargeted TKI, alone or in combination with cytotoxic agents have been conducted in patients with several metastatic solid tumours and acute myelogenous leukaemia or myelodysplastic syndrome (27-29). The once per day oral dose tolerated in most combination regimens was 1200–1250 mg. This compound, in combination with conventional chemotherapy, was investigated in more than 1000 metastatic colorectal cancer patients as first-line (CONFIRM-1) or second-line therapy (CONFIRM-2). The data from the CONFIRM-1 study suggested an advantage for the patients treated with the antiangiogenic compound (30) in terms of PFS, with a statistically significant difference as compared to 5FU/leucovorin (LV) and oxaliplatin (FOLFOX4) regimen in the subgroup with high lactate dehydrogenase (LDH) values ($p=0.002$). PTK 787 causes a higher incidence than FOLFOX4 regimen alone of hypertension, diarrhoea and thromboembolic events.

A phase I study has also demonstrated a safe clinical profile but no responses with ZD6474, a TKI anti-$VEGFR_{-2}$ and EGFR, at 100–300 mg/day/os (31).

Clinical experience of target therapy in the treatment of colorectal cancer. Two of the most promising monoclonal antibodies anti EGFR and VEGF in the treatment of colorectal cancer are cetuximab and bevacizumab. Clinical activity of both as single agents and in combination with chemotherapy has been demonstrated in phase II and phase III clinical trials (32).

Cetuximab. The administration of cetuximab in the treatment of irinotecan (CPT11)-refractory colorectal cancer is associated to a high risk of first infusion reaction (90% of cases) (33, 34). As with some conventional cancer therapy, the potential risk of developing interstitial lung disease is also present (<0.5% of cases). Other serious adverse reactions include fever (55%), sepsis (3%), kidney failure (2%), pulmonary embolism (1%), dehydration (5% cetuximab + irinotecan; 2% cetuximab only) and diarrhoea (6% cetuximab + irinotecan; 0% cetuximab only).

With respect to efficacy, cetuximab monotherapy in irinotecan resistant EGFR-positive metastatic colorectal carcinoma patients was evaluated in two subsequent, single arm, clinical studies (35, 36) (Table I). Five PR in 57 treated patients were observed (36), for a RR of 8.8%. Twenty-one additional patients had SD or minor responses (MR). The median times to progression (TTP) was 1.4 months and the median duration of response was 4.2 months. Median survival time (MST) was 6.4 months. The most commonly encountered grade 3 to 4 adverse events, regardless of relationship to the studied drug, were an acne-like skin rash, predominantly on the face and upper torso (86% with any grade; 18% with grade 3), and a composite of asthenia, fatigue, malaise, or lethargy (56% with any grade, 9% with grade 3). Two patients (3.5%) experienced grade 3 allergic reactions requiring discontinuation of study treatment. A third patient experienced a grade 3 allergic reaction that resolved and the patient continued on the study. Neither

Table I. *Cetuximab activity (at an initial dose of 400 mg/m^2 and then weekly 250 mg/m^2 i.v.).*

Reference	Drugs	No. of patients	RR (%)	TTP (mo)
II line				
(36)	cetuximab	57	8.8	1.4
(37)	cetuximab + CPT11	121	17	2.8
(38)	cetuximab + CPT11	60	20	5.1
(39)-BOND trial-	cetuximab + CPT11 *vs.* cetuximab	218 *vs.* 111	22.9 *vs.* 10.8 ($p=0.007$)	4.1 *vs.* 1.5 ($p<0.001$)
(41)	cetuximab + FOLFOX4 *vs.* FOLFOX4	102 (recruitment is ongoing)	about 18 *vs.* 8	still not available
I line				
(42)	cetuximab + CPT11 LDG *vs.* cetuximab + CPT11 HDG	10 *vs.* 13	67	nr

No.=number; RR=response rate; TTP=time to progression; *vs.*=*versus*; nr=not reported; LDG=low dose group; HDG=high dose group.

diarrhoea nor neutropenia were dose-limiting in any of the 57 patients treated.

Due to cetuximab increased activity in combination with chemotherapeutic agents, Saltz *et al.* (37) performed a phase II trial of cetuximab plus irinotecan in 121 patients, whose tumours tested positive for EGFR by immunohistochemistry (Table I). Patients were treated with cetuximab, 400 mg/m^2 loading dose followed by 250 mg/m^2 weekly, plus CPT11 at the same dose and schedule that the patient had previously progressed on. Toxicities attributable to cetuximab were allergic reaction (2% of grade 3 and 1% of grade 4) and acne-like skin rash/folliculitis (8% of grade 3). Other toxicities were those typically associated with CPT11 and did not appear to be exacerbated by cetuximab. The combination cetuximab-CPT11 was associated to a RR and TTP duplicated if compared with those observed with cetuximab administration alone (35, 36); an increased SD (31% of cases) was also reported. Similar results have also been recently observed in another trial (38) on 60 colorectal CPT11-refractory cancer patients and confirmed in a randomised phase II trial in Europe, comparing cetuximab plus irinotecan (218 patients) with cetuximab monotherapy (111 patients) (BOND [Bowel Oncology With Cetuximab Antibody] study) (39). In this study, results demonstrated a RR of 22.9% and 10.8% after cetuximab/irinotecan or cetuximab alone administration, respectively ($p=0.007$). Approximately two-thirds (63%) of patients had previously failed oxaliplatin treatment. Analyses were conducted in two pre-specified subpopulations: irinotecan refractory and irinotecan and oxaliplatin failures. The median duration of response in the overall population was about double in the combination arm than in the monotherapy arm ($p<0.001$). The MST was 8.6 and 6.9 months, respectively ($p=0.48$). Toxic effects were more frequent in the combination-therapy group, but their severity and incidence were similar to those that would be expected with irinotecan alone.

In the United States, a phase III trial (the EPIC study) (40) of cetuximab plus irinotecan (350 mg/m^2 every 3 weeks) *versus* irinotecan and another phase III trial (the EXPLORE study) (41) of cetuximab in combination with FOLFOX4 *versus* FOLFOX4 only, both as second-line treatment in patients with metastatic EGFR-positive colorectal cancer, are still ongoing. Actually, in the EXPLORE study, preliminary results have reported in arm A the double of response than in arm B (18% *vs.* 8%), confirming literature data of combination therapy with CPT11.

Encouraging results, with expected toxicities, have also been seen in a European phase II trial evaluating the safety and efficacy of cetuximab in combination with a FOLFIRI regimen as first-line treatment (42). Cetuximab was given at an initial dose of 400 mg/m^2 and then weekly 250 mg/m^2 *i.v.* FOLFIRI was administered every 2 weeks: CPT11 180 mg/m^2, FA 400 mg/m^2, 5FU 300 mg/m^2 bolus and 5FU 2,000 mg/m^2/46hours *c.i.* (low dose group, LDG) or 400 mg/m^2 bolus and 2,400 mg/m^2/46hours *c.i.* (high dose group, HDG). Dose limiting toxicity (DLT) was defined as neutropenia/leucopoenia, thrombocytopenia, phosphatase alkaline, bilirubin, ASAT, ALAT or skin toxicity > grade 3; neutropenia with fever or infection, anaemia, diarrhoea, mucositis, creatinine, or any organ toxicity related to treatment > grade 2 during the first 3 cycles. Similar RR were obtained in both arms; 22% SD were also observed. The combination of cetuximab with FOLFIRI appeared safe and feasible also in EGFR-positive metastatic untreated colorectal cancer, independently by the CPT11 dose. A phase III trial of cetuximab combined with irinotecan in first-line therapy for metastatic colorectal cancer (CRYSTAL) (43) has been recently conducted to confirm these data. Recruitment was completed with 1220 patients in December 2005. Results are pending.

Table II. *Bevacizumab activity (5 mg/kg every 2 weeks) in II line therapy.*

Reference	Drugs	No. of patients	RR (%)	MST (mo)	TTP (mo)
II line					
(48)-E3200 trial-	bevacizumab HD + FOLFOX *vs.* FOLFOX *vs.* bevacizumab	289 *vs.* 290 *vs.* 243	21.8 *vs.* 9.2 ($p<0.0001$) *vs.* 3	13.6 *vs.* 10.8 ($p=0.0018$) *vs.* 10.1	nr PFS: 7.4 *vs.* 4.8 ($p<0.0001$) *vs.* 3.6
(49)	bevacizumab + cetuximab + CPT11 vs. bevacizumab + cetuximab	76	35 *vs.* 23	nr	5.8 *vs.* 4

No.=number; RR=response rate; MST=median survival time; TTP=time to progression; *vs.*=*versus*; nr=not reported; na=not available; HD=high dose.

In conclusion, cetuximab in combination with irinotecan was approved in February 2004 by the US Food and Drug Administration and the Swiss Medicines Control Agency for the treatment of EGFR expressing metastatic colorectal carcinoma in patients who are refractory to irinotecan based chemotherapy. If administered as a single agent it is indicated for the treatment of EGFR expressing, metastatic colorectal carcinoma in patients who are intolerant to irinotecan-based chemotherapy. Data on first-line therapy are still pending. Cetuximab effectiveness is based on objective RR while, currently, no data that demonstrate an improvement in disease related symptoms or increased OS are available.

Bevacizumab. Bevacizumab has been studied as an antiangiogenic therapeutic single agent and/or in combination with chemotherapy in patients with stage III and IV colon cancer (44, 45). A consistent dose-response relationship has not been observed in breast and colorectal cancer studies (16). It is possible that low-doses of bevacizumab result in lower intratumoral interstitial pressure and in improved delivery of chemotherapy, whilst higher doses may cause vascular collapse inside the tumour, thereby limiting the delivery of chemotherapy (16, 46). In six rectal carcinoma patients, a single infusion of bevacizumab decreased tumour perfusion, vascular volume, microvascular density, interstitial fluid pressure and the number of viable, circulating endothelial and progenitor cells, indicating that VEGF blockade had a direct and rapid antivascular effect in human tumours with "normalization" and improvement of tumour vasculature (47).

E3200 was the first phase III study of bevacizumab in combination with FOLFOX where an Author (48) (Table II) reported that patients with previously treated metastatic colorectal cancer lived longer when they received bevacizumab in combination with FOLFOX4, compared with FOLFOX4 alone or bevacizumab alone. Although E3200 was a second-line study, its survival benefit suggested that adding bevacizumab to first-line therapy with FOLFOX was likely to result in a survival benefit that was greater than could be achieved with FOLFOX alone. Safety data indicated that bevacizumab in combination with FOLFOX4 was generally well tolerated, with grades 3 and 4 hypertension, bleeding and vomiting reported. Bowel perforation occurred infrequently and only in patients treated with bevacizumab (alone or in combination with FOLFOX4). An observed increase in sensory neuropathy could have been related to the duration of treatment.

In another second line randomised phase II study in patients with irinotecan-refractory colorectal cancer cetuximab was administered with bevacizumab (49). EGFR expression was not required for study entry. Arm A received irinotecan at the same dose and schedule as last received prior to study, plus cetuximab 400 mg/m^2 loading dose then weekly at 250 mg/m^2, plus bevacizumab 5 mg/kg given every other week. Arm B received the same cetuximab and bevacizumab as arm A, but without irinotecan. No toxicities were encountered that would not have been expected from the single agents alone. The combination of cetuximab and bevacizumab, alone or with irinotecan, appeared tolerable. The RR and TTP seen with the addition of bevacizumab to either cetuximab or cetuximab/irinotecan appeared favourable compared to historical controls of cetuximab (PR in 9% to 11.6% of patients and SD in 21.6% to 36.8%) or cetuximab/irinotecan (PR in 17% to 22.9% of patients and SD in 31% to 32.6%) without bevacizumab. Studies of cetuximab/bevacizumab in conjunction with front-line combination chemotherapy regimens are warranted and have now been initiated.

Some of the most robust phase II data are from three randomised studies of chemotherapy (5FU plus LV) with or without bevacizumab in first-line metastatic colorectal cancer (50-52). In these studies, treatment with bevacizumab plus 5FU/LV has resulted in higher RR, longer median TTP and longer MST (Table III).

In the first randomised trial (50) 104 previously untreated patients with measurable metastatic colorectal cancer were randomly assigned to one of the following three treatment groups: 5FU (500 mg/m^2)/LV (500 mg/m^2 weekly for the first 6 weeks of each 8-week cycle) alone or 5FU/LV + low-dose

Table III. *Bevacizumab activity (5 mg/kg every 2 weeks) in I line therapy.*

Reference	Drugs	No. of patients	RR (%)	MST (mo)	TTP (mo)
I line					
(50)	5FU/LV *vs.* bevacizumab LD + 5FU/LV *vs.* bevacizumab HD + 5FU/LV	36 *vs.* 35 *vs.* 33	17 *vs.* 40 *vs.* 24	13.8 *vs.* 21.5 *vs.* 16.1	5.2 *vs.* 9 *vs.* 7.2
(51)	placebo + 5FU/LV *vs.* bevacizumab + 5FU/LV	105 *vs.* 104	15.2 *vs.* 26 (p=0.055)	12.9 *vs.* 16.6 (p=0.16)	nr PFS: 5.5 *vs.* 9.2 (p=0.0002)
(52)	5FU/LV or IFL *vs.* bevacizumab + 5FU/LV	241 *vs.* 249	24.5 *vs.* 34.1 (p=0.019)	14.6 *vs.* 17.9 (p=0.008)	nr PFS: 5.6 *vs.* 8.8 (p<0.0001)
(53)	placebo + IFL *vs.* bevacizumab + IFL *vs.* bevacizumab + 5FU/LV	100 *vs.* stopped *vs.* 110	37 *vs.* na *vs.* 40	15.1 *vs.* na *vs.* 18.3	nr PFS: 6.8 *vs.* na *vs.* 8.8
(54)-TREE1 *vs.* TREE2 trial-	FOLFOX/bFol/CapOx *vs.* bevacizumab + FOLFOX/ bevacizumab + bFol/bevacizumab + CapOx	147 *vs.* 213	41/52 *vs.* 42/59 *vs.* 27/46 (p=0.011)	nr	8.7/9.9 *vs.* 6.9/8.3 *vs.* 5.9/10.3

No.= number; RR= response rate; MST= median survival time; TTP= time to progression; *vs.*= versus; HD= high dose; nr= not reported.

bevacizumab (5 mg/kg every 2 weeks) or 5FU/LV + high-dose bevacizumab (10 mg/kg every 2 weeks). Compared with the 5FU/LV control arm, treatment with bevacizumab (specially at the lower dose level) plus 5FU/LV resulted in higher RR, longer median TTP and longer MST. Thrombosis was the most significant adverse event and was fatal in one patient. Hypertension, proteinuria and epistaxis were other potential safety concerns. The encouraging results of this randomised trial supported further study of bevacizumab 5 mg/kg plus chemotherapy as first-line therapy for metastatic colorectal cancer.

In the second randomised trial (51), MST, median PFS and RR were also longer for the 5FU/LV/bevacizumab group than for the 5FU/LV/placebo group (p=0.16, p=0.0002 and p=0.055, respectively). Grade 3 hypertension was more common with bevacizumab treatment (16% *versus* 3%) but was controlled with oral medication and did not cause study drug discontinuation. Addition of bevacizumab to 5FU/LV as first-line therapy in colorectal cancer patients who were not considered optimal candidates for first-line irinotecan treatment provided clinically significant patient benefit, including statistically significant improvement in PFS.

A combined analysis of raw data from three randomised studies was performed to better assess the efficacy of bevacizumab with 5FU/LV (52). The analysis compared a combined control group receiving either 5FU/LV or IFL with a group receiving 5FU/LV/bevacizumab (5 mg/kg once every 2 weeks). Also in this case, the addition of bevacizumab to 5FU/LV provided a statistically significant and clinically relevant benefit to patients with previously untreated metastatic colorectal cancer.

Recently, a phase III, multicenter, double-blind, randomised, placebo-controlled trial has been designed to investigate the addition of bevacizumab (5 mg/kg every 2 weeks) to first-line irinotecan, 5FU and LV chemotherapy (IFL) (irinotecan 125 mg/m^2 *i.v.*, 5FU 500 mg/m^2 *i.v.* and LV 20 mg/m^2 *i.v.* given once weekly for 4 weeks every 6 weeks) (53). Nine hundred and twenty two patients were randomly assigned to receive IFL/placebo (control), IFL/bevacizumab, or 5FU/LV/bevacizumab. Before an interim analysis confirming acceptable safety for IFL/bevacizumab, 313 patients were concurrently randomly assigned to these three arms. After this analysis, the IFL/bevacizumab arm was discontinued. Adverse events consistent with those expected from 5FU/leucovorin- or IFL-based regimens were seen as modest increases in hypertension and bleeding in the bevacizumab arm, which were generally easily managed. Also in this case, median OS and median PFS were superior in the 5FU/LV/bevacizumab arm than in the control arm.

In a subsequent randomised study (54) the safety and tolerability of each of three oxaliplatin plus fluoropyrimidine regimens (bolus or bFOL, infusional or FOLFOX or oral fluoropyrimidine or CapOx) alone in TREE1, and with bevacizumab in TREE2 were also assessed. Addition of bevacizumab in TREE 2 caused increased grade 3/4 hypertension, impaired wound healing (5%) and bowel perforation (3%) in each arm. Grade 3-4 toxicity with first-line bevacizumab plus oxaliplatin-based chemotherapy was acceptable and less than reported for IFL. All regimens of fluoropyrimidine administration were active but FOLFOX had the best balance of response and toxicity (p=0.011). Probability of survival at 18 months (TREE1 *vs.* TREE2

respectively) were for FOLFOX 53% *vs.* 63%, bFOL 50% *vs.* 63%, CapeOx 49% *vs.* 68%. Addition of bevacizumab to oxaliplatin regimens improved RR and TTP with acceptable tolerability and no unexpected toxicity.

Discussion

The future of cetuximab lies in its use in combination with antineoplastic agents and/or radiation therapy in the treatment of colorectal cancer, head and neck cancer, NSCLC and pancreatic cancer. As understanding of the mechanisms of action of EGFR molecular inhibitors increases, more rational clinical designs which examine EGFR inhibitors combined with radiation and chemotherapy will emerge. In parallel, the ability to select patients (tumours) whose molecular profile renders them likely to respond to specific EGFR inhibitory strategies will improve. The lack of a predictive marker that would allow clinicians to select patients who are most likely to benefit from cetuximab therapy, especially taking into consideration the high costs of this medication, remains a challenge.

Bevacizumab, used in combination with intravenous 5FU-based chemotherapy, is indicated for first-line treatment of patients with metastatic carcinoma of the colon or rectum (55) but the optimal sequence of treatment is an unresolved issue. The mechanisms for a synergistic effect of chemotherapy with targeted therapy may be related to increased access of the cytotoxic drug as a result of the enhanced permeability related to antiangiogenic effects on endothelial cells, increased blood flow, oxygen delivery and decreased interstitial pressure. Hypoxic tumour cells up-regulate hypoxic inducible factor-α and VEGF expression (56). In experimental models, the combination of radiotherapy and anti-VEGF therapy has potent antitumour effects, blocking the ability of cancer cells to escape by inhibiting VEGF-dependent angiogenesis (56). Metastatic disease is also often marked by the production of multiple angiogenesis factors that may represent a potential obstacle to the successful use of a therapy targeting VEGF. Treating unselected heavily pretreated patients with advanced and bulky disease the potential value of this agent can be underestimated (16, 57). In fact, most of preclinical studies have documented that this agent is most effective in experimental models of minimal tumour burden and when administered by frequent low doses capable of maintaining active and constant concentrations at the target level, rather than at high dosages with long periods of resting between subsequent bolus injections (56). Given the complexity of tumour angiogenesis, several interesting combinations of this drug with other molecular targeted agents, such as erlotinib, trastuzumab and rituximab, are also under clinical evaluation (16, 57). The combination of multiple agents targeting a number of cell pathways may yield potent pro-apoptotic or growth inhibitory effects. Ongoing trials in colorectal cancer are examining the combination of cetuximab and bevacizumab with or without irinotecan in irinotecan-refractory disease, irinotecan with or without cetuximab in oxaliplatin-refractory disease, and FOLFOX4 with or without cetuximab in patients receiving first-line irinotecan treatment. Extended access of these agents to more patients in a cost-effective way is a matter of debate.

Patients and the scientific and medical communities have pressurised the health-care system to increase the speed at which these highly active agents are approved for reimbursement. The high costs of these treatments are, in fact, a substantial obstacle to their widespread use, posing challenges to clinicians who care for patients with cancer. For instance, the cost-per-year in Italy of bevacizumab given every 2 weeks for metastatic colorectal cancer has reached Euro 29,288.00; the cost-per-year of cetuximab given every week for metastatic colorectal cancer has reached Euro 36,869.00. When FOLFOX (Euro 598.485 per FOLFOX4 administration) or FOLFIRI regimens (Euro 521.56 per FOLFIRI administration) are added to these drugs, costs increase more and more (58). To overcome this serious issue, identification of patients who are most likely to benefit from these treatments (with less empirical and more individualized prescriptions) should be the primary objective of academic researchers and the pharmaceutical industry. According to some authors (59), the high costs of these agents should be seriously reconsidered and renegotiated regularly. In fact, as the number of patients given these drugs increases, the cost of treatment per patient should decrease, ensuring better access to the new therapies for all patients in poor and rich countries, and economic safety for the pharmaceutical industry. Rigorous and realistic pharmacoeconomic studies must be part of new drug development.

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