Antiepidermal growth factor receptor radiosensitizers in rectal cancer

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The activation of the epidermal growth factor receptor (EGFR) pathway correlates with a worse prognosis in many solid tumours. Hence, EGFR inhibitors have been developed as a treatment for cancer. The EGFR inhibitor cetuximab has been successfully combined with radical radiotherapy in head and neck cancer. In metastatic colorectal cancer, cetuximab and panitumumab have activity as single agents, and increased response rates are achieved when added to standard chemotherapy schedules. This approach of using EGFR inhibitors has also been extrapolated to the preoperative treatment of locally advanced rectal cancer. Counterintuitively, the combination of chemotherapy, EGFR inhibitors and anti-VEGF antibodies seem to show lower response rates, suggesting antagonism. In rectal cancer, disappointingly low pathological complete response (pCR) rates have often been observed in chemoradiation regimens using EGFR inhibitors. In this study, we aimed to examine the rationale for the integration of EGFR inhibitors into chemoradiation schedules for rectal cancer. We have reviewed the clinical evidence and potential mechanisms for an interaction when EGFR inhibitors are added to fluoropyrimidine-based preoperative chemoradiation, the majority of which have used cetuximab. The primary outcome measure used was pCR. The overall pooled pCR for cetuximab-based chemoradiation was 10.71% (38/356). The rate of G3/G4 gastrointestinal toxicity, in terms of diarrhoea, varied from 5 to 30%, with an overall pooled rate of 13.8% (49/353). A better understanding of the mechanisms involved in combining chemotherapy and radiotherapy might allow more effective future scheduling of biological and chemical agents in combination with radiation. *Anti-Cancer Drugs* **22:330–340 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Colorectal cancer (CRC) is one of the most common solid tumours, of which rectal cancer comprises approximately 40%. At the time of diagnosis, between 20 and 25% of patients with rectal cancer have overt metastatic disease and a further 30–40% will subsequently develop distant spread. Historically, there has been a high local recurrence rate in rectal cancer, and 10–40% of patients require surgical procedures, which lead to a permanent stoma. Hence, there is an established role for radiotherapy and chemoradiation in rectal cancer to reduce local recurrence.

Since the early 1980s, the fluororopyrimidine 5-fluorouracil (5-FU) alone, and more recently in the 1990s combinations of cytotoxic chemotherapy using oxaliplatin or irinotecan, have represented the mainstay of chemotherapy treatment for patients with advanced and metastatic CRC (mCRC). These combinations have been extrapolated into chemoradiation regimens in randomized trials in rectal cancer, potentially to increase the response to radiation and also to mirror the success of 5-FU and oxaliplatin in dealing with distant micrometastases in the adjuvant setting in colon cancer [1–3]. These combinations have been associated with greater acute toxicity and provided only moderate success in improving outcomes in rectal cancer [4–9]. Randomized phase III trials of neoadjuvant preoperative chemoradiation in resectable rectal cancer [4,6,10] show that the addition of 5-FU to preoperative radiation increases the pathological complete response (pCR) rate over radiotherapy alone [10,11] and improves locoregional control [6,10], but has not improved disease-free survival (DFS) or overall survival (OS).

In mCRC, three molecular-targeted agents have been integrated into standard chemotherapy regimens to improve response rates (RRs) or extend progression-free survival (PFS) and OS again with varying success [12–15], and have now entered routine clinical practice (i.e. cetuximab, panitumumab and bevacizumab).

Recent efforts to improve the outcome from chemoradiotherapy have focused on the addition of biological agents to avoid overlapping toxicities with chemotherapy. A landmark randomized phase III study, in patients with locally advanced head and neck cancer, showed that cetuximab in combination with radical radiotherapy significantly improved the OS [16] compared with radiation alone. The major principle behind this advantage is thought to be the inhibition of repopulation during the latter phases of the treatment. The strategy to incorporate these newer

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biologically active targeted agents into chemoradiation schedules has emerged before complete comprehension of their mechanisms of action, or the ideal sequence of chemotherapy, radiotherapy and biological agents required to avoid the potential for antagonism [17].

In this study, we aimed to examine the rationale for the integration of epidermal growth factor receptor (EGFR) inhibitors into chemoradiation schedules. We have reviewed the results of studies of preoperative EGFR inhibitors and fluoropyrimidine-based chemoradiation in rectal adenocarcinoma to test whether there is an evidence for additive effects and increased efficacy from the combination. We have also looked for the most effective chemotherapeutic partner for a biological agent, and have attempted to define future strategies with EGFR inhibition.

The epidermal growth factor receptor pathway

The EGFR is a 170-kD a transmembrane glycoprotein whose gene is located on the short arm of chromosome 7p12. It is one of four members of the Erb-B family of proteins, and is also known as Erb-B1 or HER-1 receptor. Other members of the Erb-B family are Erb-B2 (HER-2), HER-3 and HER-4. These proteins are part of a complex and an inter-related signalling pathway, which when deregulated leads to malignant transformation. The receptors have an extracellular ligand-binding domain, a transmembrane region and an intracytoplasmic domain with tyrosine kinase activity. The identified ligands include EGF, amphiregulin, epiregulin, neuregulin, transforming growth factor-α and heparin-binding EGF-like growth factor [18].

On binding of the ligand to the extracellular domain, receptor heterodimerization or homodimerization leads to phosphorylation within the cytoplasmic domain and initiation of transduction signals regulating the cell growth, cell division, differentiation, proliferation and survival. The main downstream signalling pathways include the ras/raf mitogen-activated protein kinase, which controls the cell cycle progression and proliferation and the phosophoinositide 3 kinase/AKT pathway, which is antiapoptotic and promotes cell survival [19].

EGFR is overexpressed in a wide variety of tumour types and its overexpression has been associated with more aggressive tumour behaviour, adverse patient survival and poor tumour response to conventional therapy. EGFR activation also plays a role in acquired resistance to both chemotherapy and radiotherapy [20,21].

In CRC, EGFR seems to be overexpressed in 60–80% of tumours, either because of ligand overproduction by cancer cells, overproduction of the receptor or constitutive overactivation of the receptor. This overexpression is associated with a poor survival [22–24]. Perhaps surprisingly, more recent studies have shown that even with the addition of the monoclonal antibody panitumumab to chemotherapy, median PFS times were similar for patients with negative, low and high levels of EGFR expression [25]. Few reports have analysed the effect of EGFR expression in rectal cancer alone, although there is some evidence that it may be involved in tumour progression; it has been associated with a poor prognosis independent of lymph node status [26,27].

The rationale of integrating epidermal growth factor receptor into chemoradiation schedules

EGFR has a role in the repair of cellular radiation-induced damage. It is associated with the translocation of DNA-dependent protein kinase from the cytoplasm to the nucleus [28] and with the transcription and phosphorylation of DNA repair genes (*XRCC1* and *ATM*) [29]. Overexpression of EGFR has been linked to the failure of radiotherapy treatment [30,31]. Lammering *et al.* [32] have also shown that the exposure of tumour cells to ionizing radiation in the therapeutic dose range (1–5 Gy) results in the immediate activation of EGFR, and that repeated radiation exposures of 2 Gy lead to an increased EGFR expression. Radiation-induced EGFR activation contributes, at least in part, to the mechanism of accelerated proliferation. As fractionated radiotherapy may upregulate EGFR expression, even patients with initially EGFR-negative tumours may benefit from blocking this process.

Preclinical studies have shown that inhibiting EGFR signalling slows cell proliferation *in vitro* and *in vivo* and that additive effects are observed with radiotherapy [33]. There is an inverse relationship between the expressions of EGFR signalling and cellular radiosensitivity [19] and speculation that hypoxic cells express more EGFR and are more sensitive to EGFR inhibition [34].

There is also an inverse correlation between positive EGFR expression on immunocytochemistry in rectal cancer and pCR to chemoradiation [27]. A further analysis in rectal cancer with 77 patients [27] confirmed these initial results for pCR, and showed a correlation between EGFR-negative tumours and better DFS and metastasis-free survival. Other clinical studies of neoadjuvant radiation or chemoradiation in locally advanced rectal cancer have also shown lower rates of pCR and shorter DFS in patients who expressed high levels of EGFR [35–38]. The risk of locoregional recurrence may also be increased for high expression of EGFR [39]. However, our own results contradict this suggestion [40]. In a retrospective study of 59 patients treated with 5-FU-based chemoradiation, an increased expression of EGFR significantly correlated with an improved DFS on multivariate analysis ($P = 0.0038$).

High EGFR expression seems to be linked to a high Ki-67 and proliferating cell nuclear antigen [41]. Other studies suggest that both Ki-67 and proliferating cell nuclear antigen overexpression predict an improved response to

chemoradiation in rectal cancer. Debucquoy *et al.* [42] showed a reduction in tumour proliferation, as measured by Ki-67 expression, after a loading dose of cetuximab. Within this study, after cetuximab, EGFR expression was upregulated in 55%, downregulated in 30% (10/33) and remained unchanged in 15% of cases (5/33). In patients with an upregulated EGFR expression, an improved DFS was shown ($P = 0.02$). The investigators raise the hypothesis that upregulation could present a salvage response, which might make more EGFR receptors available as a target for cetuximab.

Epidermal growth factor receptor inhibitors

The EGFR pathway can be targeted either through monoclonal antibodies, the small molecule tyrosine kinase inhibitors (TKIs), antisense nucleotides, ligand toxins and inhibitors of downstream effects of the EGFR signalling pathway. Two orally active TKs, gefitinib and erlotinib, act by inhibiting ATP binding and prevent phosphorylation in downstream signalling proteins. However, in CRC current established therapeutic options are limited to monoclonal antibodies. Surprisingly, monoclonals and TKIs have rarely been tested in combination, perhaps because they may confer unacceptable toxicity [43].

Cetuximab is a chimeric monoclonal antibody against the extracellular domain of EGFR, panitumumab is a fully humanized IgG2 monoclonal antibody against human EGFR and bevacizumab is an antiangiogenesis agent, which targets the vascular epidermal growth factor (VEGF). Cetuximab and panitumumab (but not bevacizumab) have some modest activity as single agents, but all the three are usually used in combination with chemotherapy. Clinical response has remained an important traditional end point in studies involving these agents. Well-recognized side effects include rash (less with panitumumab), diarrhoea, fatigue and hypomagnesiaemia, and their development seems to be associated with response.

The mode of action of these antibodies relies on binding to the extracellular domain, which leads to competitive inhibition of ligand binding and in turn prevents dimerization, activates the receptor and inhibits the downstream signalling pathway. Binding of the antibody also stimulates the cell to internalize and degrades the receptor. The consequences of this action include cell cycle arrest at G1, promotion of proapoptopic factors, decrease in levels of antiapoptopic factors and inhibition of angiogenesis. Cetuximab has also been suggested to induce antibody-mediated cellular cytotoxicity due to its human IgG1 backbone, which may contribute to its antitumour effects.

Cetuximab studies

Clinical studies in advanced CRC have confirmed the systemic efficacy of cetuximab in irinotecan refractory patients, both in terms of RR and PFS [44], and in RRs and PFS for the addition of cetuximab to folinic acid/5-FU/irinotecan (FOLFIRI) [45,46]. For patients with wild-type (WT) *KRAS* tumours, OS and PFS were significantly greater with the addition of cetuximab to FOLFIRI than with FOLFIRI alone [47].

These results have not been replicated in the COIN study, in which cetuximab was added to oxaliplatin and either 5-FU or capecitabine in the first-line setting [48]. This study shows no benefit for the addition of cetuximab in KRAS WT tumours apart from a small, nonsignificant, improvement in RRs. Recent results of the preliminary use of cetuximab in the adjuvant setting, combined with 5-FU and oxaliplatin in colon cancer have shown excess toxicity in high rates of more than 70%. No advantage in DFS has been shown and, indeed with excess toxicity in the over 70 s they may well have been disadvantaged by this approach [49].

Panitumumab studies

The efficacy of panitumumab monotherapy in patients with *KRAS* WT metastatic colorectal carcinoma refractory to standard chemotherapeutic agents has been shown in the pivotal open-label phase III study [47,50] in which panitumumab significantly prolonged PFS versus best supportive care (median 12.3 vs. 7.3 months, $P < 0.0001$). Disease control was also improved with 51 versus 12% benefiting from treatment (partial response, stable disease). OS was not significantly different between both groups, possibly because of the potential for crossover. An exploratory analysis excluding crossover supports this hypothesis.

The combination of panitumumab and folinic acid/5-FU/oxaliplatin (FOLFOX) for the first-line treatment has been investigated in a randomized study (PRIME) in which 1183 patients were randomized to FOLFOX4 with panitumumab every 2 weeks versus FOLFOX4 alone. The PRIME study showed that first-line panitumumab and FOLFOX4 significantly improved PFS in patients with KRAS WT tumours, with a median PFS of 9.6 months and a RR of 55% compared with a PFS of 8 months and a RR of 48%, respectively, in patients with unmutated KRAS treated with FOLFOX4 alone [51].

The phase II multicentre, Panitumumab Advanced Colorectal Cancer Evaluation study evaluated the efficacy and safety of adding panitumumab to combination chemotherapy with bevacizumab for the first-line treatment of mCRC [52]. A planned interim analysis showed that PFS and OS were worse in the panitumumab plus bevacizumab and chemotherapy arm compared with the standard bevacizumab and chemotherapy arm.

In the second-line setting, in the KRAS WT subpopulation, when panitumumab was added to FOLFIRI, the RR improved to 35% compared with 10%, and a significant improvement in PFS was observed (5.9 vs. 3.9 months; hazard ratio = 0.73; 95% confidence interval, 0.59–0.90; $P = 0.004$) [53]. No significant difference in PFS or OS was noted in patients with KRAS mutations.

Gefitinib and erlotinib

Few results from clinical trials are available for the treatment with TKIs of EGFR in patients with mCRC. The TKIs, gefitinib and erlotinib, have shown significant treatment-related toxic effects without a clear message of additional benefit. Gefitinib, which received Food and Drug Administration approval for the treatment in metastatic or locally advanced nonsmall-cell lung cancer, was given in combination with FOLFOX4 as a first-line treatment in patients with mCRC with a PFS of 7.8 months and an OS of 13.9 months. This is no better than any other first-line therapy. In contrast to cetuximab and panitumumab, neither gefitinib nor erlotinib show single agent activity.

Preclinical studies with gefitinib have suggested that there are additive effects when combined with both radiotherapy and chemotherapy [54]. In a small study of 41 patients with ultrasound-defined T3/T4 or N+ rectal cancer, Valentini *et al.* [55] have reported a pCR of 30%. Patients were treated with a combination of infusional 5-FU and gefitinib with pelvic radiotherapy [55]. Significant grade 3 toxicity was seen, 21% gastrointestinal and 26% hepatic, such that 61% of patients required dose reduction. We found one study in the form of an abstract that integrated erlotinib into radiotherapy in the neoadjuvant setting [56].

Predictive markers

EGFR testing is probably irrelevant, and cannot assist in predicting response [57,58] or clinical outcome in trials using cetuximab [59,60].

K-ras

Current interest has focused on the downstream signal transduction protein, K-ras. Mutations within the *K-ras* gene lead to a 'switching on' of the protein with the activation of further signalling pathways and stimulation of cell proliferation [61]. Numerous clinical studies have confirmed a lack of response to EGFR inhibition in K-ras-mutant patients [45,62,63]. Dose escalation of cetuximab does not add any additional benefit in K-ras-mutant tumours [64].

Wild-type K-ras is an imperfect biomarker, because only 30–50% of tumours expressing the nonmutated gene respond to cetuximab or have improved PFS or OS. Other studies have confirmed the validity of K-ras WT expression [65] but there seems to be no correlation between the WT K-ras status and tumour pCR [42,66,67].

BRAF

BRAF mutations are mutually exclusive to K-ras mutations, and are found in approximately 10% of colorectal carcinomas although they may be less in rectal cancer [68]. Several agents designed to inhibit the kinase activity of BRAF are either already approved or are in phase I and II studies. The treatment of patients with BRAF-mutated tumours using cetuximab/panitumumab in combination with a BRAF-inhibitor is both possible and logical.

Ligands

In addition, further upstream in EGFR signalling pathways, overexpression or very high expression of the EGFR ligands, amphiregulin and epiregulin, seem to be associated with a response to cetuximab [69,70].

Other potential biomarkers

Recent studies have evaluated functional germline polymorphisms of EGF and thymidylate synthase (TS) [71] and biomarkers such as K-ras status in combination with TS, VEGFR1 and VEGFR2d expression [72], which seem to predict histopathological response. Other potential markers of response include the TP53 mutation [73].

In an Italian study, the *EGFR* gene copy number was found to correlate significantly with tumour regression [66] and also identified gene copy number as a significant predictive factor for enhanced tumour regression ($P = 0.0016$).

Rectal and colon cancers have different gene expression profiles, different cytokeratin profiles, different levels of high microsatellite instability and different levels of mutations in K-ras and BRAF [74–77]. Thus, extrapolating results from colon cancer trials to the treatment in rectal cancer is not entirely logical and should, if possible, be avoided.

Results of clinical trials with cetuximab, panitumumab and oral tyrosine kinase inhibitors in combination with chemoradiation in locally advanced rectal cancer

In rectal cancer, information on local recurrence, DFS and OS can take many years to mature; therefore, phase I/II studies use the early primary end point of pCR as a surrogate for a long-term outcome. The overall pCR rate of 3157 patients included in a review of 77 phase II and III trials was 13.5% [78]. Recent reviews [79] and pooled analyses [80] showed that both on univariate and multivariate analyses, there is a significantly lower tumour regression grade and a nonsignificant trend towards a lower pCR rate (9 vs. 16%) when cetuximab was added to a combination of 5-FU/capecitabine and oxaliplatin.

A total of only 15 reports were identified from 12 phase I/II trials of preoperative chemoradiation in combination with cetuximab in rectal adenocarcinoma. Four studies reported the phase I [81] and I/II components of the same patient group [82–84]. In addition, we found two studies integrating gefitinib [55,85], one study integrating erlotinib and bevacizumab [56] and one study integrating panitumumab [86] (Table 1). There were no published phase III trials or meta-analyses.

Table 1 Studies with panitumumab and erlotinib/gefitinib chemoradiation

	No of patients	Fluoropyrimidine	Biological agent	Radiotherapy dose	G3/G4 diarrhoea	pCR	Good TRG
Czito *et al.* [85]	6	Capecitabine (650 mg/m^2, twice daily)	Gefitinib	50.4 Gy/28/38	16%	0/6	No data
Valentini *et al.* [55]	33	5-FU (225 mg/m^2) PVI +	Gefitinib	50.4 Gy/28/38 + 10 Gy IORT	12.8%	10/33 (30%)	7/33 (21%)
Star-02 Di Fabio *et al.* [86]	51	5-FU (225 mg/m^2) PVI + Oxaliplatin	Panitumumab	50.4 Gy/28/38	32%	No data	No data
Blaszkowsky *et al.* [56]	15	5-FU (225 mg/m^2) PVI +	Bevacizumab and erlotinib	50.4 Gy/28/38	24%	7/15 (47%)	No data
Total	105				22/90	17/54 (31%)	7/33

5-FU, 5-fluorouracil; IORT, intraoperative radiotherapy; pCR, pathological complete response; R0 resection, curative resection; TRG, tumour regression grade.

Table 2 Published papers and abstracts documenting pathological complete response in preoperative chemoradiation studies using cetuximab

	No of patients[a]	Cetuximab	Capecitabine	5-FU	Oxaliplatin	Irinotecan	Radiotherapy dose	pCR[b] (%)	R0 (%)	Good TRG (%)
Chung *et al.* [87]	20	Yes		Yes	No	No	50.4 Gy/28/38	2/17 (12)	17/17 (100)	NS
Machiels *et al.* [88]	40	Yes	Yes		No	No	45 Gy/25/33	2/40 (5)	>2 mm 27/40 (73)	Few cells only 10/40 (25)
Rodel *et al.* [89]	48	Yes	Yes		Yes	No	50.4 Gy/28/38	4/48 (8)	42/45 (93)	Good (>50%) 10/45 (21)
Hoffheinz *et al.* [81]	20	Yes	Yes		No	Yes	50.4 Gy/28/38	5/20 (25)	18/20 (90)	6/20 (30)
Horisberger *et al.* [84]	50	Yes	Yes		No	Yes	50.4 Gy/28/38	4/50 (8)	50/50 (100)	30/50 (60)
Bertolini *et al.* [90]	40	Yes		Yes	No	No	50 Gy/25/ 33–50.4 Gy/ 28/38	3/40 (7.5)	36/38 (95)	Dworak 8/38 (21)
Hong *et al.* [91]	10	Yes	Yes		No	Yes	50.4 Gy/28/38	2/10 (20)	10/10 (100)	2/10 (20)
Cabebe *et al.* [92]	23	Yes	Yes	No	First 10 patients only	No	50.4 Gy/28/38	4/23 (17)	NS	NS
Eisterer *et al.* [93]	28	Yes	Yes		No	No	45 Gy/25/33	0/28 (0)	NS	NS
Velenik *et al.* [94]	37	Yes	Yes	No	No	No	45 Gy/25/33	3/37 (8.1)	NS	TRG3 7/37 (18.9)
Kim *et al.* [67]	40	Yes	Yes	No	No	Yes	50.4 Gy/28/38	9/39 (23)		TRG3 3/39 (7.7)
Total	356	Yes (all)					45–50.4 Gy	38/356 (10.6)		

The terms Tmic and TRGs remain unvalidated surrogate end points, and the lack of consistency in their reporting, hinders their interpretation as a measure of response within rectal cancer trials.
5-FU, 5-fluorouracil; NS, not specified; pCR, pathological complete response; R0 resection, curative resection; RG, tumour regression grade.
[a]Number entering study.
[b]Number having had surgery.

We, therefore, retained only 11 studies with cetuximab (Tables 2 and 3). The least promising results in the chemoradiation studies have been seen with the combination of capecitabine, oxaliplatin, radiation and cetuximab. In total, 58 patients received additional oxaliplatin [89,92]. A total of 120 patients in four studies received additional irinotecan [81,84,91]. The pCR rate in the retained studies with a total of 356 patients ranged from 0 to 23%. Thirty-eight of 356 patients achieved a pCR, giving an overall pCR rate of 10.71%. In the irinotecan-containing studies, the pCR was 20/120 (16.7%). The most recent study [67] compares a pCR of 23% with the addition of cetuximab to 25% in a previous study of 48 patients from the same unit with capecitabine and irinotecan alone [96].

The overall pooled pCR of 10.7% compares with an overall pCR rate of 13.5% seen with fluoropyrimidine-based chemoradiation schedules in a recent review [9], and 13.1% overall from the results of randomized trials using fluoropyrimidine-based chemoradiation (Table 4). In randomized phase III studies, the pCR rate with radiotherapy alone has consistently been in the region of 2.5–7% [5,10,97]. The rate with chemoradiation ranges between 8 and 17%. The pCR rate with the addition of cetuximab falls between that achieved with radiotherapy alone and that with fluoropyrimidine-based chemoradiation.

How should we interpret these results?

Caution must be taken in overinterpretation of these preliminary data and over-reliance on the importance of pCR. The heterogeneity of the data presented in this review of small disparate studies makes it difficult to draw firm conclusions. The potential for achieving a pCR depends on the case mix and clinical stage of patients on entry. Early surrogate end points such as pCR, have not been validated, and therefore may not in themselves be coupled to long-term end points such as DFS and OS.

Table 3 Published papers and abstracts documenting toxicity and surgical morbidity in preoperative chemoradiation studies using cetuximab

	No of patients[a]	Cetuximab	Oxaliplatin	Irinotecan	Radiotherapy dose	G3/G4 diarrhoea (%)	Sepsis (%)	Anastom leak (%)	Reoperation (%)
Chung *et al.* [87]	20	Yes	No	No	50.4 Gy/28/38	2/20 (10)	NS	NS	NS
Machiels *et al.* [88]	40	Yes	No	No	45 Gy/25/33	6/40 (15)	5/40 (12.5)	NS	5/40 (12.5)
Rodel *et al.* [89]	48	Yes	Yes	No	50.4 Gy/28/38	9/48 (19)	2/48 (4)	5/48 (11)	5/48 (11)
Hoffheinz *et al.* [81]	20	Yes	No	Yes	50.4 Gy/28/38	2/10 (20)	2/20 (10)	3/20 (15)	NS
Horisberger *et al.* [84]	50	Yes	No	Yes	50.4 Gy/28/38	15/50 (30)	NS	8/50 (16)	NS
Bertolini *et al.* [90]	40	Yes	No	No	50 Gy/25/33–50.4 Gy/28/38	3/40 (7.5)	1/40 (5)	1/40 (5)	1/40 (5)
Hong *et al.* [91]	10	Yes	No	Yes	50.4 Gy/28/38	1/40 (5)	NS	NS	NS
Cabebe *et al.* [92]	23	Yes	First 10 patients	No	50.4 Gy/28/38	4/23 (17)	NS	NS	NS
Milas *et al.* [95]	28	Yes	No	No	45 Gy/25/33	4/28 (15)	NS	NS	NS
Velenik *et al.* [94]	37	Yes	No	No	45 Gy/25/33	4/37 (11)	NS	NS	NS
Kim *et al.* [67]	40	Yes	No	Yes	50.4 Gy/28/38	2/39 (5)	NS	NS	NS
Total	316				45–50.4 Gy	49/352 (14)			

G3/G4, grade 3 and grade 4 toxicity; NS, not specified.
[a]Number entering study.

Table 4 Published papers of randomized single-agent fluoropyrimidine-based chemoradiation documenting pCR

Trial	Patient numbers	Chemoradiation	Radiotherapy dose	pCR Radiotherapy alone (%)	pCR Chemoradiation (%)
EORTC 1984 Boulis-Wassif *et al.* [97]	247	5-FU	34.5 Gy	2.5	5.0
NSABP RO3 Roh *et al.* [8]	267	FUFA	45 Gy/25/33 days	N/A	17.0
CA0/ARO/AIO-94 Sauer *et al.* [4]	394	120-h 5-FU infusion	50.4 Gy/28/38 days	N/A	8.0
Polish study Bujko *et al.* [98]	157	FUFA	50 Gy/25/33 days	N/A	16.0
FFCD 9203 Gerard *et al.* [10]	375	FUFA	45 Gy/25/33 days	3.0	11.4
EORTC 22921 Bosset *et al.* [11]	505	FUFA	45 Gy/25/33 days	5.0	13.4
ACCORD 12/0405 Prodige 2 Gérard *et al.* [9]	295	Capecitabine	45 Gy/25/33 days	N/A	13.8
STAR-01 Aschele *et al.* [7]	379	PVI (225 mg/m^2/day)	50.4 Gy/28/38 days	N/A	15.8
All	2372				312/2372 (13.1)

[ ], reference number; EORTC, European Organisation for Research and Treatment of Cancer; FFCD, Federation Francophone de Cancerologie Digestive; FUFA, 5-fluorouracil (5-FU) and folinic acid; N/A, not applicable; NSABP, National Surgical Adjuvant Breast and Bowel Project; OS, overall survival; pCR, pathological complete response; PVI, prolonged venous infusion.

A large multinational randomized phase II study, EXPERT-C (NCT00383695), has compared neoadjuvant therapy comprising oxaliplatin, capecitabine and chemoradiotherapy with or without cetuximab in 164 patients [99]. The study was completed in July 2008, and results may throw more light on how to combine cetuximab with chemoradiation in the clinical setting in locally advanced rectal cancer. There are five other ongoing or recently closed phase III trials registered in the *http://clinicaltrials.gov* website:

Have we avoided the problem of overlapping toxicity with pelvic radiation? Diarrhoea has been observed as a common toxicity in randomized clinical trials of chemotherapy in patients with mCRC [100,101]. Yet, in rectal cancer the crude rate of G3/G4 gastrointestinal toxicity, in terms of diarrhoea, does not seem to be increased by the addition of cetuximab to chemoradiation in the above trials (Table 3).

What could be the explanation for these findings?

There may be differences between integrating cetuximab with radiotherapy alone in comparison with 5-FU-based chemoradiation. Rectal adenocarcinoma may be different to squamous cell carcinoma in the head and neck [16], because 45–50 Gy is not considered as a radical curative dose; thus, repopulation may be less crucial. Even when radical doses are used, neither overall treatment length nor treatment interruption seems to make an impact on the local control [102]. Alternatively, repopulation may be less inhibited by a continuous exposure to 5-FU or capecitabine chemoradiation.

In addition, the proportion of patients with rectal cancer (as opposed to colon cancer) with mutant K-ras varies between 12 [103] and 30% [104]. In a recent preoperative chemoradiation study using cetuximab, the K-ras mutant type was found in nine of 39 (23%) patients. Only one of these nine K-ras mutant patients (11%) showed a good pathological regression (TRG3 and 4) compared with 11 of 30 patients (37%; $P = 0.12$) with WT K-ras [66]. In contrast, neither did K-ras status significantly influence pCR in a Belgian study using cetuximab before and concurrently with capecitabine [42], nor a Korean study with capecitabine and irinotecan [91].

5-FU is S phase specific and acts by inhibiting TS and the synthesis of thymidine nucleotides required for DNA replication, thus preventing cell division. Additive effects with 5-FU and radiotherapy occur in cells, which are provoked into an inappropriate progression through S phase in the presence of 5-FU, arising from a disordered S phase checkpoint [105]. This is supported by evidence showing that if S phase entry is blocked resulting in G_1 arrest or the progression to S phase is inhibited, no additive effects are observed from the combination of 5-FU and radiation. Similarly, acquired resistance to 5-FU seems to work by cell cycle delay in the G_1 and G_1/S boundaries [106]. Cetuximab can lead to G_1 or G_2/M cell cycle arrest, and if only a small proportion of cells within the tumour are affected, this decrease in proliferation could make an impact on the chance of achieving a pCR. This hypothesis is supported by the evidence from one of the cited studies, which suggests that cetuximab upregulated several genes involved in proliferation (*PIK31*, *CGREF1* and *PLAGL1*), with a reduction in Ki67 [52]. This process might also affect oxaliplatin, which is mainly active in S phase, but would be less likely to be impacted by irinotecan.

Is sequence important?

Preclinical data suggest that the sequencing of chemotherapy, EGFR inhibition and radiation may be clinically significant, and that the sequence of oxaliplatin followed by cetuximab may be more effective than cetuximab before oxaliplatin [107]. Better efficacy might be achieved by integrating cetuximab in the latter portion of the radiotherapy or after chemoradiation. This strategy has already been proposed when integrating antimetabolites such as gemcitabine with EGFR inhibitors and radiation [108]. In the light of all these results above, in the United Kingdom, we have amended the protocol of an ongoing funded phase I/II study (XERXES ISRCTN11319909), to compare a schedule of capecitabine-based chemoradiation with a cetuximab sandwich approach (Fig. 1).

Where do we go from here?

These investigators recommend four potential future strategies with anti-EGFR radiosensitizers in rectal cancer. First, these investigators believe that trials in metastatic disease and locally advanced rectal cancer all suggest that the best cytotoxic partner if EGFR inhibition is to be investigated is irinotecan. In preclinical tumour models, there seems to be a synergistic effect with 5-FU and irinotecan and cetuximab [109]. Cetuximab seem to reverse irinotecan resistance *in vitro* [109]. In addition, inhibition of nuclear factor-κβ (NF-κβ) has been associated with an increased sensitivity to irinotecan (CPT-11). Altered expression of NF-κβ has been correlated with resistance to radiotherapy and chemotherapy because of a poor apoptopic response. Hence, the mechanisms, which promote additive effects with the combination of radiation, irinotecan and cetuximab, may rely on separate pathways.

Second, there is a strong rationale for consolidation or maintenance treatment inhibiting EGFR after chemoradiation. Preclinical data show that cetuximab increases radiation-induced apoptosis [21,110]. Cells that survive radical chemoradiation may express a variety of factors, which may promote cell survival and aggressiveness by virtue of AKT activation, increased VEGF secretion and enhanced transcription of EGFR and transforming growth factor-α. In theory, these signals could be blocked by EGFR inhibition.

Cetuximab strongly enhances the curative effect of fractionated radiation, and its effect was greater if administration was extended beyond the end of radiotherapy [95]. In another xenograft model from the same group, the

Fig. 1

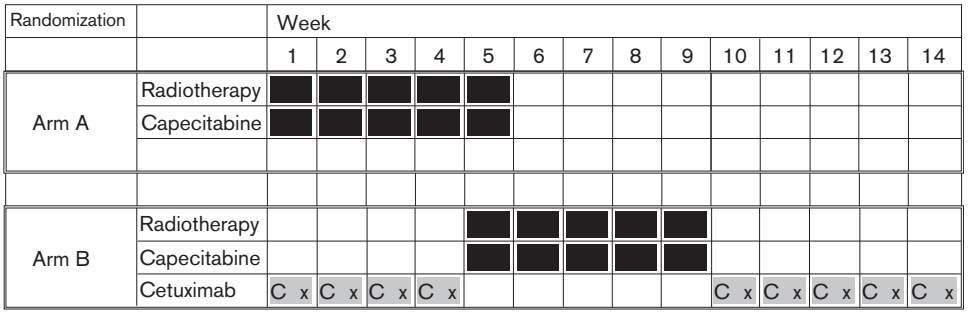

Diagram of trial schedule. C x, weekly cetuximab.

preirradiated tissue microenvironment made tumour cells more susceptible to the cytostatic and cytotoxic actions of cetuximab [93]. Both these findings support the extended use of maintenance of EGFR inhibition therapy after completion of radiotherapy. There is further preclinical [111] and clinical rationale for this approach [112]. Hence, a future clinical strategy could use consolidation or maintenance treatment inhibiting EGFR after chemoradiation.

Third, better selection for the potential efficacy of EGFR inhibition by molecular markers could be appropriate in the future [66]. A recent study in rectal cancer examining a combined analysis of VEGF and EGFR identified a subgroup of EGFR-negative and VEGF-positive patients who seemed resistant to radiotherapy, of whom only two of 34 (6%) achieved a pCR [38].

Finally, combination with EGFR inhibitors and other molecular targets, such as the proteasome inhibitor bortezomib, may be a promising avenue of research [113].

Conclusion

Biological agents targeted at growth factors and their receptors, when combined with conventional cytotoxic drugs, increase RRs and improve PFS in mCRC. However, we do not really know what underlies their clinical efficacy. Defining who does and does not benefit from EGFR inhibitors in chemotherapy regimens is partially within our grasp. We know that responsive CRC tumours usually carry WT K-ras/BRAF alleles and tend to have an increased copy number of the *EGFR* receptor gene. Some trials with K-ras-mutant patients suggest an antagonistic interaction with EGFR inhibition [114]. In addition, phosophoinositide 3 kinase and PTEN mutations may further clarify resistance, and the activated ligands, epiregulin and amphiregulin, may predict response. We also have a very strong biological correlate in that patients who do not develop a skin rash are unlikely to benefit from these agents, making it possible to maximize effectiveness and minimize toxicity.

Integration of these targeted drugs into preoperative chemoradiation schedules in rectal adenocarcinoma is therefore attractive in principle to enhance response, but involves the further complexity of radiation. In contrast to mCRC, trials in rectal cancer overall show a smaller proportion of patients with mutant K-ras [67]. There is no significant difference in pCR between mutant and WT tumours when EGFR inhibitors are added to preoperative fluoropyrimidine-based chemoradiation. However, the combination may yet reproduce long-term improvements in outcome (DFS and OS), achieved when cetuximab has been combined with radiation alone in studies of head and neck cancer [16] or with chemotherapy in squamous cell carcinoma of the oesophagus [115].

The use of irinotecan and consolidation with anti-EGFR radiosensitizers after chemoradiation are recommended. More rationally designed preclinical and translational studies (with recognized negative predictive factors such as K-ras mutations, BRAF mutations, EGFR and VEGF expression and *EGFR* gene copy numbers) might, therefore, help to select out inappropriate patients, and to determine the optimal sequence of such chemotherapy and biological triple combinations. Only then can we move on to conduct large randomized phase III trials. The era of pharmacogenomics, although long awaited, has not yet arrived for rectal cancer.

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