Molecular Targeted Treatment and Radiation Therapy for Rectal Cancer

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Background: EGFR (epidermal growth factor receptor) and VEGF (vascular endothelial growth factor) inhibitors confer clinical benefit in metastatic colorectal cancer when combined with chemotherapy. An emerging strategy to improve outcomes in rectal cancer is to integrate biologically active, targeted agents as triple therapy into chemoradiation protocols.
Material and Methods: Cetuximab and bevacizumab have now been incorporated into phase I–II studies of preoperative chemoradiation therapy (CRT) for rectal cancer. The rationale of these combinations, early efficacy and toxicity data, and possible molecular predictors for tumor response are reviewed. Computerized bibliographic searches of Pubmed were supplemented with hand searches of reference lists and abstracts of ASCO and ASTRO meetings.
Results: The combination of cetuximab and CRT can be safely applied without dose compromises of the respective treatment components. Disappointingly low rates of pathologic complete remission have been noted in several phase II studies. The K-ras mutation status and the gene copy number of EGFR may predict tumor response. The toxicity pattern (radiation-induced enteritis, perforations) and surgical complications (wound healing, fistula, bleeding) observed in at least some of the clinical studies with bevacizumab and CRT warrant further investigations.
Conclusion: Longer follow-up (and, finally, randomized trials) is needed to draw any firm conclusions with respect to local and distant failure rates, and toxicity associated with these novel treatment approaches.

Key Words: Cetuximab · Bevacizumab · Chemoradiotherapy · Rectal cancer

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Molekular-zielgerichtete Therapie und Bestrahlung zur Behandlung des Rektumkarzinoms

Hintergrund: EGFR- (epidermaler Wachstumsfaktor-Rezeptor) und VEGF-Inhibitoren (vaskulärer endothelialer Wachstumsfaktor) zeigen beim metastasierten kolorektalen Karzinom in Kombination mit Chemotherapie einen klinischen Vorteil. Diese biologisch aktiven, zielgerichteten Substanzen werden als Dreifachtherapie zunehmend auch bei der Radiochemotherapie des Rektumkarzinoms eingesetzt.
Material und Methodik: Cetuximab und Bevacizumab sind in Phase-I–II-Studien zur präoperativen Radiochemotherapie des Rektumkarzinoms getestet worden. Die Rationale für diese Kombination, erste Wirksamkeits- und Toxizitätsdaten sowie mögliche molekulare Responsemarker werden dargestellt. Dazu diente eine Suchabfrage in Pubmed, in Referenzlisten publizierter Arbeiten sowie Abstracts von ASCO- und ASTRO-Konferenzen.
Ergebnisse: Cetuximab und Radiochemotherapie können ohne Dosiskompromisse sicher miteinander kombiniert werden. Zahlreiche Phase-II-Studien ergaben allerdings enttäuschende Raten an pathologisch bestätigten kompletten Remissionen. Der K-ras-Mutationsstatus und die Anzahl an Genkopien des EGFR scheinen die Tumorantwort zu prädizieren. Das bei Kombination von Bevacizumab mit einer Radiochemotherapie beobachtete Toxizitätsspektrum (Enteritis, Perforationen) sowie die postoperativen Komplikationen (Wundheilungsstörungen, Fistelbildung, Blutungen) erfordern weitere Untersuchungen.
Schlussfolgerung: Längere Nachbeobachtungszeiten (und schließlich randomisierte Studien) sind nötig, um Daten zu Lokalrezidiv- und Fernmetastasenraten sowie zur Toxizität dieser Kombinationstherapien zu erhalten.

Schlüsselwörter: Cetuximab · Bevacizumab · Radiochemotherapie · Rektumkarzinom

Introduction

Preoperative radiotherapy (RT) with or without concurrent chemotherapy or hyperthermia and total mesorectal excision surgery have optimized local control rates in rectal cancer patients [6, 15, 20, 31, 37, 40]. The development of distant metastases is now the predominant mode of failure. Thus, the challenge is to integrate more effective systemic therapy into combined-modality programs. Newer-generation cytotoxic

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chemotherapeutics, such as oral fluoropyrimidines, oxaliplatin and irinotecan, improved results for colon cancer patients when treated in the metastatic or adjuvant setting. These agents have now being incorporated into phase I–III studies for rectal cancer as well [39]. An emerging strategy to further improve outcomes is to incorporate newer, biologically active, targeted therapies.

Epidermal Growth Factor Receptor Inhibitors

Rationale to Combine Inhibitors of the Epidermal Growth Factor Pathway with Chemoradiotherapy

Epidermal growth factor receptor (EGFR) signaling is linked with increased proliferation, angiogenesis, and metastasis, and controls cell survival in response to exogenous stress via interaction with DNA damage repair and inhibition of apoptosis. This is mediated through two major EGFR-dependent pathways, the PI3K-AKT and the Ras-MAPK pathway. EGFR tyrosine kinase activity is increased in human cancer cells in response to irradiation, and addition of exogenous EGF can render cells radioresistant in vitro [46].

In the clinical setting, EGFR overexpression has been associated with a more aggressive phenotype and poor prognosis in many human cancers, including rectal cancer [24]. Moreover, recent clinical studies have established EGFR expression as an independent predictor of poor tumor response and prognosis in rectal cancer patients treated with preoperative RT or chemoradiotherapy (CRT; Table 1).

Cetuximab is an IgG1 monoclonal antibody directed against the ligand-binding domain of EGFR. In preclinical models of several human cancers, cetuximab has been shown to be a potent enhancer of radiation-induced cell growth arrest [32]. A recent phase III trial in head-and-neck cancer has confirmed that cetuximab is a clinically active radiosensitizer [5]. Moreover, when combined with chemotherapy, the anti-EGFR antibody cetuximab has been shown to confer clinical benefit in metastatic colorectal cancer (mCRC) [11, 33].

Table 1. Predictive and prognostic impact of the EGFR status in series of preoperative (chemo)radiotherapy for rectal cancer. DFS: disease-free survival; EGFR: epidermal growth factor receptor; 5-FU: 5-fluorouracil; LV: leucovorin; pCR: pathologic complete response; UFT: uracil-tegafur.

Tabelle 1. Prädiktive und prognostische Bedeutung des EGFR-Status in Studien zur präoperativen Radio(chemo)therapie des Rektumkarzinoms. DFS: krankheitsfreies Überleben; EGFR: epidermaler Wachstumsfaktor-Rezeptor; 5-FU: 5-Fluorouracil; LV: Leukovorin; pCR: pathologisch bestätigte komplette Remission; UFT: Uracil-Tegafur.

Series	Patients (n)	Chemoradiotherapy	Outcome	Comments
Giralt et al., 2002 [17]	45	1.8 Gy to 45 Gy (concomitant 5-FU/LV in 21 patients)	pCR rate 3% of EGFR-positive and 38% of EGFR-negative tumors (p = 0.003)	Multivariate analysis: EGFR status only significant predictor for pCR (p = 0.013)
Azria et al., 2005 [1]	77	2.0 to 44–60 Gy (concomitant 5-FU ± oxaliplatin in 8 patients)	2-year local recurrence-free survival 94% in patients with EGFR < 25%, and 84% in patients with EGFR extent ≥ 25% (p = 0.06)	Multivariate analysis: EGFR expression independent factor for local failure (p = 0.037)
Giralt et al., 2005 [16]	87	1.8 Gy to 45–50.4 Gy (concomitant 5-FU/LV in 50 patients)	EGFR positivity significantly associated with a lack of pCR (p = 0.006). DFS significantly shorter among patients with EGFR-positive tumors (p = 0.003)	Multivariate analysis: EGFR expression significant predictor of DFS (p = 0.036)
Kim et al., 2006 [23]	183	1.8 Gy to 50.4 Gy (concomitant 5-FU/LV)	No correlation between EGFR status and pCR (p = 0.569). Low level vs. high level of EGFR significant for tumor downstaging (p = 0.012)	
Li et al., 2006 [26]	127	1.8 Gy to 50.4 Gy (concomitant 5-FU/LV)	Local recurrence-free survival: high level of EFGR associated with more local recurrences, but not significantly. High level of EGFR associated with significantly shorter DFS (p = 0.002).	Multivariate analysis: EGFR expression significant predictor of DFS (p = 0.041)
Spindler et al., 2006 [41]	77	2 Gy to 60 Gy + intracavitary boost 5 Gy (concomitant oral UFT/LV)	Major tumor regression in 34% of EGFR GG polymorphism homozygous patients compared with 65% in patients with replacement of G by T (p = 0.023)	G/T gene polymorphism
Bertolini et al., 2007 [3]	91	2 Gy to 50 Gy (concomitant 5-FU)	No statistical significance between pretreatment EGFR status and pCR. 4-year DFS rate 92% vs. 61.2% in cases of postoperative positive or negative EGFR expression (p = 0.019)	Multivariate analysis: positive expression of EGFR post treatment with a significantly higher risk of relapse than negative expression (p = 0.017)
Zlobec et al., 2008 [47]	104	High-dose-rate endorectal brachytherapy with 4 × 6.5 Gy (total 26 Gy) for 4 consecutive days	EGFR positivity significantly associated with pCR rate (p = 0.003)	Multivariate analysis: positive EGFR-expression independent predictive factor for pCR (p = 0.01)

Table 2a. Phase I studies of preoperative chemoradiotherapy for rectal cancer with EGFR inhibition. EGFR: epidermal growth factor receptor; RT: radiotherapy.

Tabelle 2a. Phase-I-Studien zur präoperativen Radiochemotherapie des Rektumkarzinoms mit EGFR-Inhibition. EGFR: epidermaler Wachstumsfaktor-Rezeptor; RT: Radiotherapie.

Series	Patients (n)	Concurrent chemoradiotherapy	Dose-limiting toxicity	Recommended dose
Hofheinz et al., 2006 [21]	14	Preoperative RT: 1.8 Gy to 50.4 Gy Capecitabine: 400–500 mg/m² bid d1–38 Irinotecan: 40–50 mg/m² d1, 8, 15, 22, 29 Cetuximab: 400 mg/m² loading dose (d1), followed by 250 mg/m² (d8, 15, 22, 29)	Grade 3 diarrhea	Irinotecan: 40 mg/m² d1, 8, 15, 22, 29 Capecitabine: 500 mg/m² bid d1–38
Machiels et al., 2007 [29]	10	Preoperative RT: 1.8 Gy to 45 Gy Capecitabine: 650–825 mg/m² during RT Cetuximab: 400 mg/m² loading dose (d–7), followed by 250 mg/m² (d1, 8, 15, 22, 29)	None	Capecitabine: 825 mg/m² during RT
Rödel et al., 2008 [38]	13	Preoperative RT: 1.8 Gy to 50.4 Gy Capecitabine: 500–650–825 mg/m² bid d1–14 and d22–35 Oxaliplatin: 50 mg/m² d1, 8, 22, 29 Cetuximab: 400 mg/m² loading dose (d–7), followed by 250 mg/m² (d1, 8, 15, 22, 29)	None	Capecitabine: 825 mg/m² bid d1–14 and d22–35

Clinical Phase I and II Trials with EGFR Inhibitors and Chemoradiotherapy for Rectal Cancer

Clinical studies of preoperative CRT have now been initiated to evaluate EGFR inhibitors as radiosensitizers in rectal cancer. Hofheinz et al. performed a phase I trial of preoperative RT with capecitabine, irinotecan and cetuximab (Table 2a) [21]. These authors demonstrated that such a combination can be safely applied without dose compromises of the respective treatment components. Machiels et al. have reported the safety and efficacy of combining preoperative RT with capecitabine and cetuximab in a phase I/II trial (Tables 2a and 2b) [29]. This combination was associated with no unexpected toxicity, and full doses of RT, chemotherapy, and cetuximab could be applied. However, only two of 37 patients (5%) achieved a pathologic complete response (pCR), and a total of 25/37 patients (68%) had only moderate or minimal tumor regression. The German Rectal Cancer Study Group conducted a multicenter phase I/II study to determine the tolerability and efficacy of adding cetuximab to preoperative RT with capecitabine and oxaliplatin. Again, only four of the 45 operated patients (9%) had pCR in the resected specimen, and 53% of patients had only moderate, minimal, or no tumor regression at all [38]. As shown in Table 2b, the disappointingly low rate of pCR rates achieved by the combination of CRT plus cetuximab has now been confirmed in several phase II studies. Intriguingly, the addition of gefitinib to CRT, a small molecule directed toward the intracellular tyrosine kinase domaine of EGFR, was feasible and associated with a 30% pCR rate in a recent study by Valentini et al. (Table 2b) [42].

Several mechanisms may contribute to the apparently subadditive interaction between CRT and cetuximab, including upregulation of cycline-dependent kinase p27 and G1 cell-cycle arrest, the redundancy of EGFR pathways, K-ras mutation status, as well as sequence dependencies. Intriguingly, recent in vitro data by Morelli et al. indicate a sequence dependency of the cetuximab-oxaliplatin combination with maximum synergy when oxaliplatin was followed by cetuximab, yet antagonistic effects when cetuximab preceded oxaliplatin [34].

Molecular Prediction of Response to Cetuximab Combined-Modality Treatment

The study of Machiels et al. included a translational part with biopsies taken at three time points, at baseline, after the loading dose of cetuximab but before start of CRT, and at surgery. Microarray gene expression analysis and proteomics revealed downregulation of invasion and proliferation pathways and an upregulation of inflammatory pathways and EGFR ligands after the first dose of cetuximab [28]. The immunhistochemically determined expression of Ki-67 and transforming growth factor-α correlated with T-level downcategorization. It has been established that the K-ras mutation status is a candidate marker for predicting survival in mCRC patients treated with cetuximab (the wild-type status is associated with a survival benefit) [13]. In the analysis of Machiels et al., a trend ($p = 0.06$) for better tumor regression was found for patients with wild-type K-ras [28]. Bengala et al. identified the gene copy number of EGFR as a significant predictor for better tumor regression in their study of cetuximab plus 5-fluorouracil-(FU-)based CRT; mutated K-ras was associated with reduced tumor regression, albeit not significantly ($p = 0.12$) [2].

Table 2b. Phase II studies of preoperative chemoradiotherapy for rectal cancer with EGFR inhibition. EGFR: epidermal growth factor receptor; 5-FU: 5-fluorouracil; (IO)RT: (intraoperative) radiotherapy.

Tabelle 2b. Phase-II-Studien zur präoperativen Radiochemotherapie des Rektumkarzinoms mit EGFR-Inhibition. EGFR: epidermaler Wachstumsfaktor-Rezeptor; 5-FU: 5-Fluorouracil; (IO)RT: (intraoperative) Radiotherapie.

Series	Patients (n)	Concurrent chemoradiotherapy	Toxicity	pCR (%)
Chung et al., 2006 [9]	20	Preoperative RT: 1.8 Gy to 50.4 Gy 5-FU: 225 mg/m^2 continuous infusion Cetuximab: 400 mg/m^2 loading dose (d1), followed by 250 mg/m^2 (d8, 15, 22, 29) and 4 additional weeks	Grade 3–4: diarrhea 10%, acneiform rash 15%, RT-field dermatitis 5%	12
Bertolini et al., 2007 [4]	40	Preoperative RT: 2.0 Gy to 50 Gy 5-FU: 225 mg/m^2 continuous infusion Cetuximab: 400 mg/m^2 loading dose, followed by 250 mg/m^2 weekly, three times, followed weekly concomitantly with chemoradiotherapy	Grade 3: acneiform rash 15% Grade 4: none	7.7
Machiels et al., 2007 [29]	30	Preoperative RT: 1.8 Gy to 45 Gy Capecitabine: 825 mg/m^2 during RT Cetuximab: 400 mg/m^2 loading dose (d–7), followed by 250 mg/m^2 (d1, 8, 15, 22, 29)	Grade 3: diarrhea 15% Grade 4: myocardial infarction (n=1), pulmonary embolism (n=1), sepsis (n=1)	5
Rödel et al., 2008 [38]	48	Preoperative RT: 1.8 Gy to 50.4 Gy Capecitabine: 825 mg/m^2 bid d1–14 and d22–35 Oxaliplatin: 50 mg/m^2 d1, 8, 22, 29 Cetuximab: 400 mg/m^2 loading dose (d–7), followed by 250 mg/m^2 (d1, 8, 15, 22, 29)	Grade 3–4: diarrhea 19%	9
Hofheinz et al., 2008 (personal communication)	50	Preoperative RT: 1.8 Gy to 50.4 Gy Capecitabine: 500 mg/m^2 bid d1–38 Irinotecan: 40 mg/m^2 d 1, 8, 15, 22, 29 Cetuximab: 400 mg/m^2 loading dose (d1), followed by 250 mg/m^2 (d8, 15, 22, 29)	Not given	8
Cabebe et al., 2008 [7]	23	Preoperative RT: 1.8 Gy to 50.4 Gy Capecitabine: 800 mg/m^2 bid Monday to Friday Oxaliplatin: 100 mg/m^2 d2 and d23 Cetuximab: 400 mg/m^2 loading dose, followed by 250 mg/m^2 weekly for 9 weeks	After 10 patients oxaliplatin was omitted due to "radiosensitizing properties"	17
Valentini et al., 2008 [42]	41	Preoperative RT: 1.8 Gy to 50.4 Gy ± IORT 10 Gy 5-FU: 225 mg/m^2 continuous infusion Gefitinib: 250–500 mg once daily	Grade 3: 41%; gastrointestinal: 20.5%, genitourinary: 10%	30

Vascular Endothelial Growth Factor Inhibitors

Rationale to Combine Inhibitors of the Vascular Growth Factor Pathway with Chemoradiotherapy

Angiogenesis is necessary for the survival and growth of tumors, however, tumor blood vessels are often characterized by a disorganized architecture that contributes to intratumoral regions of intermittent or chronic hypoxia. Preclinical data have suggested that proangiogenic factors, especially the vascular endothelial growth factor (VEGF), are upregulated in tumors in response to RT, and may increase resistance to RT [19]. These findings are now supported by clinical data in rectal cancer patients, such that VEGF expression has been linked to a worse prognosis (especially due to more distant metastases) in some, albeit not in all studies (Table 3).

VEGF-targeted therapy may lead to a "normalization" of the tumor vasculature, thereby leading to greater tumor oxygenation and drug penetration. When combined with RT, antibodies against VEGF induced additive to supraadditive tumor growth delay and cell death in colon cancer models [25]. Bevacizumab, a monoclonal antibody directed against VEGF, improves survival in patients with mCRC when combined with chemotherapy [22].

Clinical Phase I and II Trials with VEGF Inhibitors and Chemoradiotherapy for Rectal Cancer

Willett et al. have reported on a phase I study of preoperative bevacizumab, 5-FU and RT for clinical T3 or T4 rectal cancer [43]. Preliminary data indicate safety of this regimen and promising activity (six of seven evaluable patients demonstrated only microscopic disease in the surgical specimen 7 weeks after completion of neoadjuvant treatment). In a meticulous analysis of the first six patients performed 12 days

Table 3. Predictive and prognostic impact of the VEGF status in series of (chemo)radiotherapy for rectal cancer. DFS: disease-free survival; 5-FU: 5-fluorouracil; LV: leucovorin; OS: overall survival; pCR: pathologic complete response; VEGF: vascular endothelial growth factor.

Tabelle 3. Prädiktive und prognostische Bedeutung des VEGF-Status in Studien zur Radio(chemo)therapie des Rektumkarzinoms. DFS: krankheitsfreies Überleben; 5-FU: 5-Fluorouracil; LV: Leukovorin; OS: Gesamtüberleben; pCR: pathologisch bestätigte komplette Remission; VEGF: vaskulärer endothelialer Wachstumsfaktor.

Series	Patients (n)	Chemoradiotherapy	Outcome	Comments
Cascinu et al., 2002 [8]	79	Postoperative radiotherapy with 45 Gy and a boost to 54 Gy (adjuvant chemotherapy with six cycles of 5-FU/LV)	VEGF-positive tumors were significantly more often associated with distant metastases than negative tumors (p = 0.02)	Significantly higher proportion of relapsed patients with VEGF-positive than -negative expression (p = 0.003)
Giralt et al., 2006 [18]	81	1.8 Gy to 45–50.4 Gy (concomitant 5-FU in 45 patients)	No significant correlation between VEGF and pCR (p = 0.229), local relapse (p = 0.14). Higher levels of VEGF associated with metastasis-free survival (p = 0.016)	
Bertolini et al., 2007 [3]	91	2 Gy to 50 Gy (concomitant 5-FU)	No statistically significant association between baseline expression of VEGF and pCR, DFS, OS	VEGF expression significantly increased after treatment
Negri et al., 2008 [35]	57	2 Gy to 40–45 Gy (concomitant 5-FU and oxaliplatin in 19 patients)	No predictive value of VEGF for pCR (p = 0.31)	
Zlobec et al., 2008 [47]	104	High-dose-rate endorectal brachytherapy with 4 × 6.5 Gy (total 26 Gy) for 4 consecutive days	VEGF-negative expression significantly associated with pCR (p = 0.004)	Multivariate analysis: VEGF-negative expression independent predictive factor for pCR (p = 0.009)

after the first bevacizumab infusion, this group revealed a significant decrease in tumor blood perfusion and blood volume, and a significant decrease in tumor microvessel density. This was accompanied by an increase in pericyte coverage of tumor vessels and a decrease of the interstitial fluid pressure, indicating that a "normalization" of the tumor vasculature by anti-VEGF treatment may contribute to the high efficacy of bevacizumab in this and further trials with combined CRT and VEGF inhibition (Tables 4a and 4b).

Clinical studies investigating bevacizumab with chemotherapy established the toxicity profile with most common severe side effects occurring as hypertension, diarrhea, asthenia, pain, and leukopenia. Although infrequent (1–3%), arterial ischemic events, hemorrhage, wound healing delays, and bowel perforation have also been noted. Lordick et al. reported on three of 33 patients receiving bevacizumab (without concomitant RT) at their institution which developed severe bowel complications (acute ischemic colitis, n = 2, gastrointestinal perforation, n = 1) [27]. All three patients had previously undergone RT to the pelvis before treatment with bevacizumab, suggesting that there may be an increased risk of vascular bowel damage in previously irradiated tissues.

Willett et al. terminated the dose-escalating component of their study when two patients developed dose-limiting toxicities of diarrhea and colitis at 10 mg/m^2 bevacizumab [44]. Clearly the toxicity pattern (radiation-induced enteritis, perforations) and surgical complications (wound healing, fistula, bleeding) observed in at least some of the clinical studies (Table 4b) warrants further investigations of the interaction of RT with VEGF inhibition, both for tumor and normal tissues. Intriguingly, a protective effect of VEGF against the endothelial damage induced by radiation has been demonstrated [36].

Molecular Prediction of Response to Bevacizumab Combined-Modality Treatment

Correlative molecular investigations as part of the studies of Willett et al. showed that 12 days after the first bevacizumab administration tumor cell apoptosis significantly increased; however – unlike after cetuximab treatment – there was a clear trend (p = 0.06) for *increased* proliferation, possibly reflecting the improved tumor microenvironment subsequent to vascular normalization. This was also accompanied by a decrease in angiopoietin 2 expression, a molecule which promotes destabilization of blood vessels by inhibiting the recruitment of pericytes to blood vessels. Moreover, a decrease in blood concentrations of circulating endothelial cells, and an increase of the levels of plasma VEGF and plasma placental growth factor (PLGF) – a ligand of vascular endothelial growth factor receptor-(VEGFR-)1 – was noted [44]. The change in PLGF and the pretreatment VEGFR-1 in plasma correlated significantly with the extent of tumor regression [45].

Conclusion and Future Perspectives

Given the strong preclinical rationale to combine EGFR and VEGF inhibitors with CRT in rectal cancer patients, these combinations should clearly be investigated further. First results of phase II studies, however, have yielded disappointing results with respect to early tumor response rates, at least for the EGFR inhibitor cetuximab. As molecular targeted therapies exert their efficacy predominantly as cytostatic rather than cytotoxic agents, it is well conceivable that the benefit may not be manifested as an increase in tumor *regression* but rather as

Table 4a. Phase I studies of preoperative chemoradiotherapy for rectal cancer with VEGF inhibition. 5-FU: 5-fluorouracil; RT: radiotherapy; VEGF: vascular endothelial growth factor.

Tabelle 4a. Phase-I-Studien zur präoperativen Radiochemotherapie des Rektumkarzinoms mit VEGF-Inhibition. 5-FU: 5-Fluorouracil; RT: Radiotherapie; VEGF: vaskulärer endothelialer Wachstumsfaktor.

Series	Patients (n)	Concurrent chemoradiotherapy	Dose-limiting toxicity	Recommended dose
Willett et al., 2004 [43]	6	Preoperative RT: 1.8 Gy to 50.4 Gy 5-FU: 225 mg/m² continuous infusion Bevacizumab: 5–10 mg/m² d–14, 1, 15, 29 Surgery: 7–9 weeks after completion of RT	Diarrhea and colitis	Bevacizumab: 5 mg/m² d–14, 1, 15, 29
Czito et al., 2007 [12]	11	Preoperative RT: 1.8 Gy to 50.4 Gy Capecitabine: 500–625–825 mg/m² bid Monday to Friday Oxaliplalin: 50–60–75 mg/m² weekly Bevacizumab: 15 mg/m² (d1)/10 mg/m² d8 and d22 Surgery: 6–8 weeks after completion of RT	Grade 3–4 diarrhea	Preoperative RT: 1.8 Gy to 50.4 Gy Capecitabine: 625 mg/m² bid Monday to Friday Oxaliplatin: 50 mg/m² weekly Bevacizumab: 15 mg/m² (d1)/10 mg/m² d8 and d22

Table 4b. Phase II studies of preoperative chemoradiotherapy for rectal cancer with VEGF inhibition. 5-FU: 5-fluorouracil; RT: radiotherapy; VEGF: vascular endothelial growth factor.

Tabelle 4b. Phase-II-Studien zur präoperativen Radiochemotherapie des Rektumkarzinoms mit VEGF-Inhibition. 5-FU: 5-Fluorouracil; RT: Radiotherapie; VEGF: vaskulärer endothelialer Wachstumsfaktor.

Series	Patients (n)	Concurrent chemoradiotherapy	Toxicity	pCR (%)
Willett et al., 2008 [45]	25	Preoperative RT: 1.8 Gy to 50.4 Gy 5-FU: 225 mg/m² continuous infusion Bevacizumab: 5 mg/m² d–14, 1, 15, 29 Surgery: 7–9 weeks after completion of RT	No acute grade 4	20
Crane et al., 2008 [10]	25	Preoperative RT: 1.8 Gy to 50.4 Gy Capecitabine: 900 mg/m² bid Monday to Friday Bevacizumab: 5 mg/m² d1, 15, 29 Surgery: 6–11 weeks (median 7.3) after RT	No patients had grade 3 gastrointestinal toxicity Surgical: 3 wound complications that required surgical intervention	32
Marijnen, 2008 [30]	23	Preoperative RT: 2.0 Gy to 50 Gy Capecitabine: 825 mg/m² bid Bevacizumab: 5 mg/m² d–14, 1, 15, 29 Surgery: 6–10 weeks thereafter	Grade 3: skin (n=4), diarrhea (n=2) Grade 4: anal mucositis (n=1) Grade 5: enteritis with uncontrollable bleeding (n=1) Postoperative: 2/23 small bowel perforations, 1 rectal wall perforation Surgical: perineal dehiscence (n=1), rectovaginal fistula (n=2), bleeding 5,500 cm3 (n=1)	9
DiPetrillo et al., 2008 [14]	23	Two biweekly courses of bevacizumab 5 mg/m² and modified FOLFOX6, followed by bevacizumab 5 mg/m² biweekly, oxaliplatin 50 mg/m² weekly (subsequently reduced to 40 mg/m² due to grade 3 diarrhea), 5-FU 200 mg/m² continuous infusion with concurrent 50.4 Gy pelvic irradiation Surgery: 4–8 weeks after completion of RT	Grade 3 during chemoradiotherapy: 75% Grade 4: neutropenia (n=1), diarrhea (n=1)	25

an arrest in tumor *progression*. Thus, longer follow-up (and, finally, randomized trials) is needed to draw any firm conclusions with respect to local response rates, long-term local control, as well as toxicity. It also remains to be established whether the concurrent or sequential incorporation of targeted agents into the combined-modality treatment of rectal cancer patients will have an impact on distant tumor control. As a word of caution, the impressive results achieved with the addition of cetuximab to chemotherapy in mCRC, and to RT alone in head-and-neck cancer may not be simply transferred to combined chemoradiation protocols.

References

1. Azria D, Bibeau F, Barbier N, et al. Prognostic impact of epidermal growth factor receptor (EGFR) expression on loco-regional recurrence after preoperative radiotherapy in rectal cancer. BMC Cancer 2005;5:62.
2. Bengala C, Bettelli S, Bertolini F, et al. Predictive value of EGFR gene copy number and K-ras mutation for pathological response to preoperative cetuximab, 5FU, and radiation therapy in locally advanced rectal cancer (LARC). J Clin Oncol 2008;26:4125.abstract.
3. Bertolini F, Bengala C, Losi L, et al. Prognostic and predictive value of baseline and posttreatment molecular marker expression in locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy. Int J Radiat Oncol Biol Phys 2007;68:1455–61.
4. Bertolini F, Zironi S, Malavasi N, et al. Phase II study of pre-operative cetuximab, fluorouracil (5FU), and radiotherapy (RT) in patients with rectal cancer (RC). ASCO Gastrointestinal Cancers Symposium, Orlando, FL, USA, 19.1.2007.abstract 308.
5. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354:567–78.
6. Bosset JF, Collette L, Calais G, et al. Chemotherapy with preoperative radiotherapy in rectal cancer. N Engl J Med 2006;355:1114–23.
7. Cabebe EC, Kuo T, Koong A, et al. Phase I trial of preoperative cetuximab in combination with oxaliplatin, capecitabine, and radiation therapy for locally advanced rectal cancer. J Clin Oncol 2008;26:15019.abstract.
8. Cascinu S, Graziano F, Catalano V, et al. An analysis of p53, BAX and vascular endothelial growth factor expression in node-positive rectal cancer. Relationships with tumour recurrence and event-free survival of patients treated with adjuvant chemoradiation. Br J Cancer 2002;86:744–9.
9. Chung KY, Minsky B, Schrag D, et al. Phase I trial of preoperative cetuximab with concurrent continuous infusion 5-fluorouracil and pelvic radiation in patients with local-regionally advanced rectal cancer. J Clin Oncol 2006;24:3560.abstract.
10. Crane CH, Eng CB, Feig W, et al. Phase II trial of neoadjuvant bevacizumab (BEV), capecitabine (CAP), and radiotherapy (XRT) for locally advanced rectal cancer. J Clin Oncol 2008;26:4091.abstract.
11. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337–45.
12. Czito BG, Bendell JC, Willett CG, et al. Bevacizumab, oxaliplatin, and capecitabine with radiation therapy in rectal cancer: phase I trial results. Int J Radiat Oncol Biol Phys 2007;68:472–8.
13. De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol 2008;19:508–15.
14. DiPetrillo TA, Pricolo V, Sikov WM, et al. Neoadjuvant bevacizumab, oxaliplatin, 5-fluorouracil, and radiation in clinical stage II–III rectal cancer. J Clin Oncol 2008;26:15041.abstract.
15. Gerard JP, Conroy T, Bonnetain F, et al. Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3–4 rectal cancers: results of FFCD 9203. J Clin Oncol 2006;24:4620–5.
16. Giralt J, de las Heras M, Cerezo L, et al. The expression of epidermal growth factor receptor results in a worse prognosis for patients with rectal cancer treated with preoperative radiotherapy: a multicenter, retrospective analysis. Radiother Oncol 2005;74:101–8.
17. Giralt J, Eraso A, Armengol M, et al. Epidermal growth factor receptor is a predictor of tumor response in locally advanced rectal cancer patients treated with preoperative radiotherapy. Int J Radiat Oncol Biol Phys 2002;54:1460–5.
18. Giralt J, Navalpotro B, Hermosilla E, et al. Prognostic significance of vascular endothelial growth factor and cyclooxygenase-2 in patients with rectal cancer treated with preoperative radiotherapy. Oncology 2006;71:312–9.
19. Gorski DH, Beckett MA, Jaskowiak NT, et al. Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. Cancer Res 1999;59:3374–8.
20. Haustermans K, Roels S, Verstraete J, et al. Adaptive RT in rectal cancer: superior to 3D-CRT? A simple question, a complex answer. Strahlenther Onkol 2007;183:Special Issue 2:21–3.
21. Hofheinz RD, Horisberger K, Woernle C, et al. Phase I trial of cetuximab in combination with capecitabine, weekly irinotecan, and radiotherapy as neoadjuvant therapy for rectal cancer. Int J Radiat Oncol Biol Phys 2006;66:1384–90.
22. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–42.
23. Kim JS, Kim JM, Li S, et al. Epidermal growth factor receptor as a predictor of tumor downstaging in locally advanced rectal cancer patients treated with preoperative chemoradiotherapy. Int J Radiat Oncol Biol Phys 2006;66:195–200.
24. Kopp R, Rothbauer E, Mueller E, et al. Reduced survival of rectal cancer patients with increased tumor epidermal growth factor receptor levels. Dis Colon Rectum 2003;46:1391–9.
25. Lee CG, Heijn M, di Tomaso E, et al. Anti-vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. Cancer Res 2000;60:5565–70.
26. Li S, Kim JS, Kim JM, et al. Epidermal growth factor receptor as a prognostic factor in locally advanced rectal-cancer patients treated with preoperative chemoradiation. Int J Radiat Oncol Biol Phys 2006;65:705–12.
27. Lordick F, Geinitz H, Theisen J, et al. Increased risk of ischemic bowel complications during treatment with bevacizumab after pelvic irradiation: report of three cases. Int J Radiat Oncol Biol Phys 2006;64:1295–8.
28. Machiels JH, Debucquoy A, Gevaert O, et al. Prediction of pathological response to preoperative chemoradiotherapy with cetuximab in rectal cancer. J Clin Oncol 2008;26:4095.abstract.
29. Machiels JP, Sempoux C, Scalliet P, et al. Phase I/II study of preoperative cetuximab, capecitabine, and external beam radiotherapy in patients with rectal cancer. Ann Oncol 2007;18:738–44.
30. Marijnen CA. Preoperative chemoradiotherapy regimen with capecitabine and bevacizumab in locally advanced rectal cancer: a feasibility study of the Dutch Colorectal Cancer Group (DCCG). Proc Am Soc Clin Oncol 2008;27: abstract 15040.
31. Milani V, Pazos M, Issels RD, et al. Radiochemotherapy in combination with regional hyperthermia in preirradiated patients with recurrent rectal cancer. Strahlenther Onkol 2008;184:163–8.
32. Milas L, Mason K, Hunter N, et al. In vivo enhancement of tumor radioresponse by C225 antiepidermal growth factor receptor antibody. Clin Cancer Res 2000;6:701–8.
33. Moosmann N , Heinemann V. Cetuximab plus oxaliplatin-based chemotherapy in the treatment of colorectal cancer. Expert Rev Anticancer Ther 2008;8:319–29.
34. Morelli MP, Cascone T, Troiani T, et al. Sequence-dependent antiproliferative effects of cytotoxic drugs and epidermal growth factor receptor inhibitors. Ann Oncol 2005;16:Suppl 4:iv61–8.
35. Negri FV, Campanini N, Camisa R, et al. Biological predictive factors in rectal cancer treated with preoperative radiotherapy or radiochemotherapy. Br J Cancer 2008;98:143–7.
36. Paris F, Fuks Z, Kang A, et al. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. Science 2001;293:293–7.
37. Rhomberg W, Hammer J, Sedlmayer F, et al. Irradiation with and without razoxane in the treatment of incompletely resected or inoperable recurrent rectal cancer. Results of a small randomized multicenter study. Strahlenther Onkol 2007;183:380–4.
38. Rödel C, Arnold D, Hipp M, et al. Phase I–II trial of cetuximab, capecitabine, oxaliplatin, and radiotherapy as preoperative treatment in rectal cancer. Int J Radiat Oncol Biol Phys 2008;70:1081–6.

39. Rödel C, Sauer R. Integration of novel agents into combined-modality treatment for rectal cancer patients. Strahlenther Onkol 2007;183:227–35.
40. Sauer R, Becker H, Hohenberger W, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med 2004;351:1731–40.
41. Spindler KL, Nielsen JN, Lindebjerg J, et al. Prediction of response to chemoradiation in rectal cancer by a gene polymorphism in the epidermal growth factor receptor promoter region. Int J Radiat Oncol Biol Phys 2006;66:500–4.
42. Valentini V, De Paoli A, Gambacorta MA, et al. Infusional 5-fluorouracil and ZD1839 (fefitinib-IRESSA) in combination with preoperative radiotherapy in patients with locally advanced rectal cancer: a phase I and II trial (1839IL/0092). Int J Radiat Oncol Biol Phys 2008;72:644–9.
43. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004;10:145–7.
44. Willett CG, Boucher Y, Duda DG, et al. Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. J Clin Oncol 2005;23:8136–9.
45. Willett CG, Duda DG, Xu L, et al. Correlation of blood and physiologic markers with effect of bevacizumab (BV) with chemoradiation therapy in rectal cancer (RC). J Clin Oncol 2008;26:4096.abstract.
46. Wollman R, Yahalom J, Maxy R, et al. Effect of epidermal growth factor on the growth and radiation sensitivity of human breast cancer cells in vitro. Int J Radiat Oncol Biol Phys 1994;30:91–8.
47. Zlobec I, Vuong T, Compton CC, et al. Combined analysis of VEGF and EGFR predicts complete tumour response in rectal cancer treated with preoperative radiotherapy. Br J Cancer 2008;98:450–6.

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