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The contribution of targeted therapy to the neoadjuvant chemoradiation of rectal cancer

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Abstract

Neoadjuvant chemoradiation therapy is a commonly used option aimed to make less aggressive surgery approaches and to improve quality of life allowing a high proportion of patients operated with sphincter-sparing surgical techniques in locally advanced rectal cancer (LARC). During the last 5 years a number of studies have tested the efficacy of more intensive chemotherapeutic approaches by combining irinotecan

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or oxaliplatin with fluoropyrimidines and standard radiation treatments as well as testing combined treatments with targeted agents directed against epidermal growth factor receptor (EGFR) or angiogenesis. Herein, we review the results and critiques of the published studies based on the introduction of novel targeted agents in neoadjuvant therapy of LARC.

1. Introduction

Colorectal cancer is the second leading cause of cancer worldwide and approximately one-third rises in the rectum. By SEER data, at the time of diagnosis 36% of patients affected by rectal cancer presents stage III disease [1]. Surgery remains the cornerstone in the treatment of rectal cancer and improvements in outcome are related to more radical surgical techniques, namely total mesorectal excision (TME). However, locally advanced rectal tumors (LARC) cannot be cured by using surgery alone. Randomized clinical trials demonstrated that infusional 5-fluorouracil (5FU) concomitant to preoperative radiation reduces local recurrence compared with preoperative radiation therapy (RT) [2,3] or adjuvant chemoradiotherapy (CRT) alone [4]. In comparison with preoperative radiation alone, fluorouracil-based preoperative CRT also results in downstaging/downsizing of the primary tumor mass in a large proportion of patients, obtaining in 10–25% of surgical specimens a pathologic complete response (pCR) [5]. In turn, this may facilitate radical resection of large advanced tumors and allows sphincter-preserving surgery in patients with distal tumors. Therefore, fluorouracil-based preoperative CRT is a standard option for LARC. However, despite these progresses, approximately 50% of patients with LARC eventually die of their disease.

Since newer cytotoxic agents such as capecitabine, oxaliplatin, irinotecan, as well as the targeted agents (TAs) bevacizumab, cetuximab and panitumumab demonstrated to improve clinical outcomes in patients with metastatic colorectal cancer (mCRC), several studies have been performed to incorporate these agents for therapy of LARC.

In this review we discuss the rationale of using TAs in combination with chemoradiation therapy and the evidence emerging from the published clinical studies where these agents have been used as part of neoadjuvant treatment of patients with LARC. Furthermore the challenges and some key issues for the future development of targeted drugs in rectal cancer are discussed.

2. Epidermal growth factor receptor (EGFR) inhibition

2.1. Rationale

EGFR signaling is linked to increased proliferation, angiogenesis and metastasis in response to exogenous stress via interaction with DNA damage repair and inhibition of apoptosis. EGFR tyrosine kinase activity is increased in human cancer cells in response to irradiation and the addition of exogenous EGF makes cells resistant to radiation treatment in vitro [6]. Bonner et al. demonstrated that cetuximab, an anti-EGFR monoclonal antibody, can be safely administered with conventional or hyperfractionated radiation therapy in patients affected by head and neck cancer with improved survival [7].

EGFR is overexpressed in 50–70% of primary rectal cancers [8] and it is related to decreased pCR, disease free survival (DFS) and overall survival (OS) [9,10].

2.2. Anti-EGFR monoclonal antibodies

Cetuximab and panitumumab are the two approved monoclonal antibodies that bind to EGFR (anti-EGFR moAb) with high specificity, blocking ligand-induced phosphorylation of the receptor: they have been shown to lead to longer progression free survival (PFS) and OS times for patients affected by mCRC who failed previous therapies [11–14]. It has been demonstrated, howewer, that in advanced colon cancer the benefit is limited to those patients with wild-type KRAS tumors. The discovery of KRAS mutations as a negative predictive marker for this class of agent in mCRC has rapidly been integrated into clinical practice [15,16].

2.2.1. Cetuximab

Cetuximab can be safely administered with conventional or hyperfractionated RT in patients with head and neck cancer with improved survival [7]. Based on the positive data in mCRC and synergy with RT in preclinical models there is a strong rationale to combine anti-EGFR moAb with neoadjuvant CRT in LARC (Table 1).

In an Italian Phase II study cetuximab and 5FU (225 mg/m²/day as continuous infusion) with RT (50.4 Gy in 25–28 fractions) were administered to 40 patients, with pCR in 3 patients (8%) [17]. In a Belgian Phase I/II study, Machiels et al. [18] tested the safety and efficacy of combining preoperative RT with capecitabine (650 and 825 mg/m² twice daily, continuously for the duration of RT (45 Gy in 25 fractions) and cetuximab. No unexpected toxicity was found (Table 2), but only 2 of 37 patients (5%) achieved a pCR and a total of 25/37 patients (68%) had only moderate or minimal tumor regression.

Velenik et al. [19] treated 37 patients with cetuximab added to capecitabine (825 mg/m² twice daily) continuously during RT (45 Gy in 25 fractions), with a pCR in 3 (8%) patients.

Table 1

Activity reported in phase I–II trials that evaluated the addition of targeted agents to preoperative chemoradiotherapy and/or to induction chemotherapy in LARC.

Authors	Phase	N. of pts	Treatment schedule	pCR %	
Cetuximab/panitumumab/EGFR-TKI					
Bertolini et al. 2009	II	40	Cetuximab followed by FU/RT 50.4 Gy	12	Not reported in details
Machiels et al. 2007	I/II	40	Cetuximab + capecitabine + RT 45 Gy	5	The complete disappearance of all tumour cells. The grading system of Wheeler et al. was also used
Velenik et al. 2010	II	37	Cetuximab + cape + RT 45 Gy	8	TMN staging system
Rodel et al. 2008	I/II	48	Cetuximab + cape + RT 50.4 Gy	8	The absence of viable tumor cells in the primary tumor and lymph nodes (ypT0N0)
Mc Collum et al. 2010	IIb	133	5 FU/RT 45 Gy (arm A) with cetuximab (arm B)	33	Not reported in details
Kim et al. 2011	II	40	Cape + iri + cetuximab + RT 50.4 Gy	20	TRG
Horisberger et al. 2009	II	50	Cetuximab + cape + iri + RT 50.4 Gy	8	TRG
Dewdney et al. 2012	IIb	164	Cape + oxa + RT 50.4 Gy without cetuximab (arm A)/with cetuximab (arm B)	7 11 ($p = .714$)	Absence of any residual tumor cells detected in the resected specimens
Pinto et al. 2011	II	60	Panitumumab + 5FU + oxa + RT 50.4 Gy	21	TRG
Valentini et al. 2008	I/II	41	Gefitinib + 5FU c.i. + RT 50.4 Gy	30	TRG
Bevacizumab as radiosensitizer					
Willett et al. 2009	I–II	32	Bevacizumab (5 or 10 mg/kg) on day −14 and then every 2 weeks (4 cycles); 5FU infusion (225 mg/m^2/24 h) during cycles 2–4; RT 50.4 Gy (28 fractions over 5.5 weeks)	16	ypT0 or Mandard TRG 1
Crane et al. 2010	II	25	Bevacizumab (5 mg/kg) every 2 weeks for 3 doses; capecitabine (900 mg/m^2 orally twice daily on days of radiation); RT 50.4 Gy (28 fractions, 5.5 weeks)	32	Absence of residual tumor in the rectum or regional lymph nodes in resection specimens
Velenik et al. 2011	II	61	Bevacizumab (5 mg/kg) on day −14 and then every 2 weeks (4 cycles); capecitabine (825 mg/m^2 twice daily on days 1–38); RT 50.4 Gy (1.8 Gy/day, 5 days/week for 5 weeks + three 1.8 Gy/day)	13	The complete disappearance of all tumour cells. Histological regression of the primary tumour was semi-quantitatively determined according to according to Dworak scale TRG scale
Spiegel et al. 2011	II	35	Bevacizumab (5 mg/kg) on days 1 and 15 (cohort A), or every 2 weeks (cohort B), 5FU (225 mg/m^2/day on days 1–42), RT 50.4 Gy (1.8 Gy/day or 28 fractions)	29	Not reported in details
Kennecke et al. 2011	II	42	Bevacizumab (5 mg/kg, days −14, 1, 15, 29) capecitabine (825 mg/m^2 twice daily days 1–14 and 22–35) + oxaliplatin (50 mg/m^2 on days 1, 8, 22, 29); RT 50.4 Gy (28 fractions including boost)	18	The complete absence of any viable tumour in the rectal specimen by central review
Resh et al. 2012	II	8[a]	Bevacizumab (5 mg/kg, days 1, 15, 29): capecitabine (825 mg/m^2 twice daily on RT-days weeks 1–4); RT 45 Gy (1.8 Gy/day in 5 weeks)	25	Histopathologic examination of the resected tumor followed the guidelines of the TNM system
Gasparini et al. 2012	II	43	Bevacizumab (5 mg/kg every 2 weeks for 4 cycles: days −14, 1, 15, 29); capecitabine (825 mg/m^2 twice a day for 5.5 weeks); RT 50.4 Gy (28 fractions over 5.5 weeks)	14	The absence of any residual tumor in the rectum and regional lymph nodes in resection specimens. Histological regression of the primary tumour was centrally evaluated, according to Mandard TRG scale
Martinez-Villacampa et al. 2012	IIb	90	Arm A: bevacizumab (5 mg/kg for 3 doses) capecitabine (825 mg/m^2 twice daily) Arm B: capecitabine (825 mg/m^2 twice daily) RT 45 Gy (25 fractions in 5 weeks)s	16 11 $p = 0.54$	Not reported in the abstract

Table 1 (*Continued*)

Authors	Phase	N. of pts	Treatment schedule	pCR %	
Bevacizumab in combination with induction chemotherapy					
Dipetrillo et al. 2010	II	25	Bevacizumab (5 mg/kg, days 1, 15, 29): + mFOLFOX6 (1 month) → bevacizumab (5 mg/kg, days 1, 15, 29) + 5FU (200 mg/m^2/day) and oxaliplatin (50–40 mg/m^2/week + RT 50.4 Gy (1.8 Gy/day, 5 days/week or 25 fractions)	20	No evidence of malignancy on the specimen
Noguè et al. 2011	II	47	Bevacizumab (7.5 mg/kg) + XELOX (capecitabine + oxaliplatin) for 4 cycles → bevacizumab (5 mg/kg every 2 weeks) + capecitabine (825 mg/m^2 twice daily on days 1–15) RT (50.4 Gy (28 fractions including boost)	36	yPT0,yN0, assessed using the Dworak scale
Bevacizumab in combination with chemotherapy only, with CRT and as consolidation therapy (before surgery)					
Schrag et al. 2011	II	31	Bevacizumab (dose not reported; cycles 1–4) FOLFOX 6 cycles No radiation therapy	27	Not reported in the abstract.
Liang et al. 2011	II	28	Bevacizumab (5 mg/Kg) biweekly + FOLFOX for 6 cycles during and after CRT RT 45 Gy (25 fractions/33 days)	25	Histopathologic examination of the resected tumor according to TRG

CRT, chemoradiotherapy; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; pCR, pathologic complete response; RT, radiotherapy.

[a] Accrual terminated because of grade 3 or 4 toxicities in ≥50% of patients at a programmed interim analysis.

The association of cetuximab with capecitabine (825 mg/m^2 twice daily, days 1–14 and 22–35), oxaliplatin (50 mg/m^2, days 1, 8, 22 and 29), and RT (50.4 Gy in 28 fractions) was also evaluated in 48 patients, with a pCR reached in 4 (9%) patients [20].

Preliminary results of a randomized phase II study evaluating continuous infusion of 5FU and RT, with or without cetuximab, have been recently reported: pCR was obtained in 33% and 31% of patients, respectively [21].

Two phase II trials evaluated the addition of cetuximab to capecitabine–irinotecan and RT. In the first study cetuximab was added to capecitabine (825 mg/m^2 twice daily, 5 days a week), irinotecan (40 mg/m^2, days 1, 8, 15, 22 and 29), and RT (50.4 Gy in 28 fractions) in 10 patients, with pCR obtained in 2 (20%) patients [22]. In the MARGIT study, 50 patients received cetuximab in combination with capecitabine (500 mg/m^2 twice daily continuously), irinotecan (40 mg/m^2 weekly), and RT (50.4 Gy in 28 fractions), with a pCR in 4 (8%) patients [23].

A large multinational randomized phase II study (the EXPERT-C study) compared neoadjuvant therapy with oxaliplatin, capecitabine, and CRT with or without cetuximab in 164 patients [24]. Patients with magnetic resonance imaging (MRI)-defined high risk rectal cancer received four cycles of capecitabine–oxaliplatin (CAPOX) followed by capecitabine chemoradiotherapy, surgery and adjuvant CAPOX (four cycles) or the same regimen plus weekly cetuximab (CAPOX + C). Ninety (60%) of the 149 assessable tumors were K-RAS or BRAF wild-type and in these patients the addition of cetuximab did not improve pCR, the primary end point (9% versus 11%, respectively; $p = 1.0$, HR 1.22) or PFS (HR 0.65, $p = 0.363$). However, cetuximab significantly improved response rate (CAPOX versus CAPOX + C: after

chemotherapy, 51% versus 71%, respectively; $p = 0.038$) and OS (HR 0.27, $p = .034$) [24].

2.2.2. Panitumumab

The efficacy of panitumumab combined to 5FU, oxaliplatin and RT was investigated as neoadjuvant treatment in high-risk LARC patients by Pinto et al. [25] (Table 1). Panitumumab at the dose of 6 mg/kg i.v. was administered 2 weeks before the start of CRT and after, in combination with CRT every 2 weeks, for a total of 3 times. 5FU (225 mg/m^2/day in continuous infusion) and oxaliplatin (60 mg/m^2 i.v. weekly for 6 courses) were given concurrently with RT (50.4 Gy delivered in 28 daily fractions of 1.8 Gy, on 5 consecutive days per week). Adjuvant chemotherapy with FOLFOX-4 regimen in combination with panitumumab for 8 cycles was planned after surgical treatment. The primary end-point of the study was pCR rate. All the 60 patients enrolled in 11 Italian centers were evaluable for safety, 57 for response; 55 (91.7%) patients underwent surgery and were assessable for pCR. A pCR rate of 21.1 (95% confidence interval: 10.4–31.6%) was obtained. Regarding the safety profile, the panitumumab combination treatment was associated with high incidence of grade 3–4 diarrhea that reached 38.9% (Table 2).

In the starPan (STAR-02) study, the primary end point was not reached, with a pCR rate of 21.1%, however, the addition of panitumumab to 5FU-oxaliplatin CRT showed a higher pCR rate as compared to the results of the phase II studies based on cetuximab-fluoropyrimidine combination with or without oxaliplatin. A possible explanation for the different results in terms of higher pCR of StarPan study may be related to different chemotherapy schedules: in this study 5FU was administered as continuous infusion and oxaliplatin weekly for 6 times. Moreover, in the StarPan study, the presence of

Table 2
Toxicity reported in phase I–II trials that evaluated the addition of targeted agents to preoperative chemoradiotherapy and/or to induction chemotherapy in LARC.

Authors	Phase	N. of pts	Treatment schedule	Toxicity
Cetuximab/panitumumab/EGFR-TKI				
Bertolini et al. 2009	II	40	Cetuximab followed by FU/RT 50.4 Gy	Skin rash G1/2 70%, G3/4 7.5%; hypersensitivity reactions G1/2 13%, G3/4 7.5%; gastrointestinal toxicity G1/2 47%, G3/4 13%; diarrhea G1/2 32%, G3/4 5%; stomatitis G1/2 2.5%, G3/4 2.5%; nausea/vomiting G1/2 0%, G3/4 0%; haematologic toxicity G1/2 2.5%, G3/4 2.5%; anemia G1/2 2.5%, G3/4 0%; trombocytopenia G1/2 and G3/4 0%; neutropenia G1/2 and G3/4 0%; febrile neutropenia G1/2 0%, G3/4 2.5%; systemic symptoms G1/2 5%, G3/4 0%; fatigue G1/2 5%, G3/4 0%; urologic toxicity G1/2 2.5%, G3/4 0%; cystitis G1/2 2.5%; G3/4 0%
Machiels et al. 2007	I/II	40	Cetuximab + capecitabine + RT45 Gy	G1/2 acneiform rash: 87%, diarrhea: 65%, fatigue: 57%; 2 pts with G3 dhiarrea followed by ileitis and occlusive syndrome discontinuated RT; 1 pt had G3 allergic reaction to cetuximab; G4 were reported by 3 pts (one myocardial infarction during CT-RT; one pulmonary embuslim, one fatal pulmonary infection with sepsis)
Velenik et al. 2010	II	37	Cetuximab + cape + RT45 Gy	G1/2 acneiform rash: 86%; G3 radiodermatitis: 16%; G3 dhiarrea: 11%; G3 hypersensitivity: 5%
Rodel et al. 2008	I/II	48	Cetuximab + cape + RT 50.4 Gy	Phase I: G3 hypersensitivity: 14%; G3 diarrhea: 14% Phase II: Leukopenia G1/2 17%, G3 2%, G4/5 1%[a]; thrombocytopenia 10%, G3 1%, G4/5 1%[a]; anemia G1/2 18%; diarrhea G1/2 27%, G3 8%, G4/5 1%[a]; nausea/vomiting G1/2 16%, G 3 2%, fatigue G1/2 6%, stomatitis G1 3%, constipation/ileus G1/2 1%, G3 1%; proctitis G1/2 7%, G3 1%; sensory neuropathy G1/2 11%, G3 3%; hand-foot syndrome G1/2 8%; radiation dermatitis G1/2 20%, G3 4%; acneiform rash G1/2 45%,[a] G3 2%; cardiac toxicity G1/2 1%, G3 1%; hyperbilirubinemia G1/2 8%; transaminases (GPT, GOT) G1/2 25%, G3 3%; alkaline phosphatase G1/2 13%; hypocalcemia/hyperkalemia G4/5 1%[a]; allergic reaction/hypersensitivity G1/2 5%, G3 1%, infection/fever G1/2 10%, G3 3%, G4/5 1 (death from multiorgan failure due to dihydropyrimidine dehydrogenase deficiency);[a] acneiform rash, G1 in 32 pts (68%) and G2 in 13 pts (28%)
Mc Collum et al. 2010	IIb	133	5 FU/RT 45 Gy (arm A) with cetuximab (arm B)	G3/4 diarrhea: 16/24%; G3/4 rash: 0/10%, mucositis: 5/5%
Kim et al. 2011	II	40	Cape + iri + cetuximab + RT 50.4 Gy	Leukopenia G1 17.9%, G2 30.8%, G3 7.7%, G 4 2.6%; neutropenia G1 15.4%, G2 20.5%, G3 5.1%, anemia G1 10.3%, G2 5.1%, G3 2.6%. AST abnormality G1 23.1%, G2 2.6%, ALT abnormality G1 17.9%, G2 10.3%, fatigue G1 12.8%, G2 5.1%, G3 2.6%, skin rash G1 41.0%, G2 46.2%, G3 2.6%, allergic reaction G1 5.1%, G2 10.3%, alopecia G1 2.6%, anorexia G1 43.6%, G2 5.1%; diarrhea G1 33.3%, G2 12.8%, G3 5.1%; ileus G3 2.6%, abdominal pain G1 17.9%, G2 7.7%, anal pain G1 10.3%, G2 41.0%, fever G2 2.6%, infection G2 2.6%, vomiting G1 2.6%, G2 10.3%, G3/4 19.4%; hand-foot syndrome G1 7.7%, G2 5.1%
Horisberger et al. 2009	II	50	Cetuximab + cape + iri + RT 50.4 Gy	Anemia G1 52%; G3 6% G4 2% thrombocytopenia G1 8%; leukocytopenia G1 34% G2 6% G3 2% G4 2%; nausea/vomiting G1 40%, G2 4%, G3 2%; Diarrhea G1 20%, G2 34%, G3 30%, abdominal pain G1 22%, G2 8%, G3 4%,; proctitis G1 26%, G2 26%, G3 2%, bilirubin elevation[a] G4 2%; ASAT/ALAT elevation G1 32%, G2 8%, G3 10%, hand-foot skin reaction G1 10%, G2 4%, acnelike skin rash G1 36%, G2 46%, G3 6%, anorexia + G1 4%., G2–3–4 0%

Table 2 (*Continued*)

Authors	Phase	N. of pts	Treatment schedule	Toxicity
Dewdney et al. 2012	IIb	164	Cape + oxa + RT 50.4 Gy without cetuximab (arm A)/with cetuximab (arm B)	Neoadjuvant CT Arm A/Arm B: febrile neutropenia 1%/1%; diarrhea 9%/8%; lethargy 10%/10%; nausea and vomiting 2%/2%; hand-foot syndrome 1%/4%; stomatitis 0%/1%; neuropathy 0%/2%; rash 0%/10%; CRT Arm A/Arm B: diarrhea 1%/10%; rash 0%/9%; hand-foot syndrome 1%/4%;
Pinto et al. 2011	II	60	Panitumumab + 5FU + oxa + RT 50.4 Gy	Diarrhea G1/G2: 35.6%, G3/G4 38.9%, nausea G1/2: 28.8%, G3/4 5.1%, vomiting G1/2 18.6%, G3/4 1.7%, stomatitis G1/2 11.8%, acneiform rash: G1/2: 59.3%, G3/4: 18.6%, asthenia G1/2 30.5%, G3/4 3.4%, anorexia G1/2 22%, G3/4 3.4%, leucopenia G1/2 13.5%, G3/4 1.7%, neutropenia G1/2 8.4%, G3/4 1.7%, anemia G1/2 10.1%, G3/4 0; hand-foot sindrome G3/4 2%, periheral neuropathy G1/2 13.5%, G3/4 0%, one toxic death due to diarrhea
Czito et al. 2006	II 6 (10 PC; 6 LARC)		Gefitinib + cape + RT 45 Gy	Toxicity in patients affected by LARC: G4 diarrhea 16.6% (DLT); G1/2 diarrhea 33.2%; death: 1 pt (16.6%) due to arterial thrombosis complicated aspiration pneumonia
Valentini et al. 2008	I/II	41	Gefitinib + 5FU c.i. + RT 50.4 Gy	Diarrhea 12.8%, nausea/vomiting 7.6%; skin toxicity 15.3%; hepatic toxicity 25.6%; GU toxicity 10.2%; other G3 toxicities: (1 cardiovascular, 1 musculoskeletal, and 1 as constitutional symptoms). G1/2 proctitis/tenesmus 21.6%; hematologic 1%. G1/2 rectal blood spots 17%, metabolic/laboratory alterations 17%, neurologic disturbance 5%, ocular/visual 2.4%, pain 5%, pulmonary 1%
Bevacizumab as radiosensitizer				
Willett et al. 2009	I–II	32	Bevacizumab (5 or 10 mg/kg) on day -14 and then every 2 weeks (4 cycles); 5FU infusion (225 mg/m^2/24 h) during cycles 2 to 4; RT 50.4 Gy (28 fractions over 5.5 weeks)	G2/3 radiation dermatitis ($n = 7$); G2/3 diarrhea ($n = 12$); G2/3 hypertension ($n = 4$); G2/3 proctalgia/proctitis/colitis ($n = 13$); anastostomotic leak with presacral abscess requiring drainage ($n = 1$), abscess requiring drainage ($n = 1$), pelvic hematoma ($n = 1$), delayed healing of perineal incision ($n = 2$), ileus ($n = 2$), neurogenic bladder ($n = 1$), perforated ileostomy–stent related ($n = 1$), pulmonary embolus ($n = 1$), wound infection ($n = 3$)
Crane et al. 2010	II	25	Bevacizumab (5 mg/kg) every 2 weeks for 3 doses; capecitabine (900 mg/m^2 orally twice daily on days of radiation); RT 50.4 Gy (28 fractions, 5.5 weeks)	G2 gastrointestinal ($n = 3$; 12%); G3 perianal skin ($n = 1$; 4%); G2 hand-foot syndrome ($n = 6$; 24%). Major wound complications requiring surgical intervention ($n = 3$; 12%)
Velenik et al. 2011	II	61	Bevacizumab (5 mg/kg) on day −14 and then every 2 weeks (4 cycles); capecitabine (825 mg/m^2 twice daily on days 1–38); RT 50.4 Gy (1.8 Gy/day, 5 days/week for 5 weeks + three 1.8 Gy/day)	Dermatitis 10%, proteinuria 6.5%, leukopenia 5%. Delayed wound healing ($n = 18$, 30%), infection/abscess ($n = 12$, 20%); anastomotic leakage ($n = 7$, 11.7%). Surgical re-intervention for anastomotic leakage ($n = 3$), abdominal abscess ($n = 2$) and pneumothorax ($n = 1$)

Table 2 (*Continued*)

Authors	Phase	N. of pts	Treatment schedule	Toxicity
Spiegel et al. 2011	II	35	Bevacizumab (5 mg/kg) on days 1 and 15 (cohort A), or every 2 weeks (cohort B), 5FU (225 mg/m² /day on days 1–42), RT 50.4 Gy (1.8 Gy/day or 28 fractions)	G3/4 diarrhea (A cohort, 14%; B cohort, 29%), neutropenia (A cohort, 14%, B cohort, 23%), mucositis (A cohort, 23%, B cohort, 0%). Bowel perforation and pelvic infection (cohort A, $n = 1$ each), bowel perforation ($n = 2$), anal wound dehiscence ($n = 1$), perianal infection ($n = 2$), and rectovaginal fistula ($n = 1$) (cohort B)
Kennecke et al. 2011	II	42	Bevacizumab (5 mg/kg, days −14, 1, 15, 29) capecitabine (825 mg/m² twice daily days 1–14 and 22–35) + oxaliplatin (50 mg/m² on days 1, 8, 22, 29); RT 50.4 Gy (28 fractions including boost)	G3/4 diarrhea 24%; G3/4 hypertension 5%; Rash, hand-foot syndrome 7%; post-operative complications: pelvic infection ($n = 11$, 29%); delayed healing ($n = 7$, 18%; G3/4 in 3 patients, 8%); anastomotic leak ($n = 6$, 16%; G3/4 in 2 pts 5%); G1/2 fistulae ($n = 3$, 8%). 4 pts (11%) required a reintervention
Resh et al. 2012	II	8[a]	Bevacizumab (5 mg/kg, days 1, 15, 29): capecitabine (825 mg/m² twice daily on RT-days weeks 1–4); RT 45 Gy (1.8 Gy/day in 5 weeks).	G3 intestinal bleeding 25%; G3 diarrhea 25%; G3/4 perianal and abdominal pain 25%; G3 anemia 12.5%
Gasparini et al. 2012	II	43	Bevacizumab (5 mg/kg every 2 weeks for 4 cycles: days −14, 1, 15, 29); capecitabine (825 mg/m² twice a day for 5.5 weeks) RT 50.4 Gy (28 fractions over 5.5 weeks)	G3 diarrhea ($n = 3$ pts, 7.14%); neutropenia ($n = 2$ pts); G1/2 hypertension ($n = 3$ pts, 7.14%), G2 proteinuria ($n = 1$ patient, 2.38%. One patient died due bowel perforation; two patients presented failure to anastomosis and postoperative abscess, respectively
Martinez-Villacampa et al. 2012	IIb	90	Arm A: bevacizumab (5 mg/kg for 3 doses) capecitabine (825 mg/m² twice daily). Arm B: capecitabine (825 mg/m² twice daily) RT 45 Gy (25 fractions in 5 weeks)s	G3/4 toxicity rates were 18% and 13% (arm A versus B, $p = 0.50$); postoperative complications were reported by 19 (43%) patients in arm A compared with 17 (37%) in arm B
Bevacizumab in combination with induction chemotherapy				
Dipetrillo et al. 2010	II	25	Bevacizumab (5 mg/kg, days 1, 15, 29): + mFOLFOX6 (1 month) → bevacizumab (5 mg/kg, days 1, 15, 29) + 5FU (200 mg/m² /day) and oxaliplatin (50–40 mg/m² /week + RT 50.4 Gy (1.8 Gy/day, 5 days/week or 25 fractions)	Grade 3/4 diarrhea (40%), neutropenia (16%), and pain (16%). Postoperative complications: 9 pts (36%), infection ($n = 4$), delayed healing ($n = 3$), leak/abscess ($n = 2$), sterile fluid collection ($n = 2$), ischemic colonic reservoir ($n = 1$), and fistula ($n = 1$)

Table 2 (Continued)

Authors	Phase	N. of pts	Treatment schedule	Toxicity
Noguè et al. 2011	II	47	Bevacizumab (7.5 mg/kg) + XELOX (capecitabine + oxaliplatin) for 4 cycles → bevacizumab (5 mg/kg every 2 weeks) + capecitabine (825 mg/m² twice daily on days 1–15); RT (50.4 Gy (28 fractions including boost)	G3/4: diarrhea 11%, neutropenia 6%. Postoperative complication: 26 pts (58%), wound infection ($n = 10$), intra-abdominal infections ($n = 7$), anastomotic leak ($n = 5$), stoma complications ($n = 2$), other complications ($n = 10$). Re-surgery: 11 pts (24%)
Bevacizumab in combination with chemotherapy only, with CRT and as consolidation therapy (before surgery)				
Schrag et al. 2011	II	31	Bevacizumab (dose not reported; cycles 1–4) FOLFOX 6 cycles No radiation therapy	Two patients withdrawn the study due to cardiovascular side-effects (angina/arrhythmia) after 1–2 cycles. One patient had high output ileostomy post-operatively
Liang et al. 2011	II	28	Bevacizumab (5 mg/kg) biweekly + FOLFOX for 6 cycles during and after CRT; RT 45 Gy (25 fractions/33 days)	Postoperative complications: 21.4% (upper gastro-intestinal bleeding, deep vein thrombosis, pelvic abscess, wound infection, enterocutaneous fistula, and perineal fistula). The median blood loss was significantly greater than in the historic series without bevacizumab therapy. One operative mortality (3.6%) occurred

CRT, chemoradiotherapy; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; GI, gastrointestinal; GU, genitourinary; LARC, locally advanced rectal cancer; PC, pancreatic cancer; RT, radiotherapy.

[a] Accrual terminated because of grade 3 or 4 toxicities in ≥50% of patients at a programmed interim analysis.

K-RAS and BRAF mutations in the pre-treatment biopsy was not correlated with pCR. In fact, the predictive significance of K-RAS and BRAF mutations in patients affected by rectal cancer submitted to preoperative CRT is not well established [25].

2.3. Small-molecule EGFR inhibitors

Gefitinib is an orally active anillinoquinazoline that reversibly inhibits EGFR tyrosine kinase autophosphorylation and inhibits downstream signaling. Preclinical studies in human colorectal cancer and other cancer cell lines have shown enhanced cytotoxicity when gefitinib is combined with CRT [26]. Gefitinib with 5FU-based chemotherapy appears to be feasible in patients with advanced colorectal cancer without a significant increase in severity of side effects [27,28]. A phase I trial combining gefitinib, capecitabine and RT in rectal cancer patients resulted in significant toxicity [29]. An Italian Phase II study evaluating infusional 5FU with gefitinib and RT showed a pCR rate of 30% in 41 patients with clinical T3/4 or lymph node-positive rectal cancer [30]. However, a dose reduction was required in 61% of patients due to the severe toxicities reported, including grade III–IV gastrointestinal (21%), hepatic (26%), skin (15%) and genitourinary (10%) toxicities (Table 2). Further studies are needed to establish the feasibility and safety of gefitinb plus chemoradiotherapy. Ongoing phase I/II studies are evaluating the tolerability and efficacy of these small-molecule EGFR inhibitors with conventional neoadjuvant CRT regimens in patients with LARC.

3. Vascular endothelial growth factor (VEGF) inhibition

3.1. Rationale

Angiogenesis is necessary for tumor growth and malignant progression, being the VEGF a key pro-angiogenic factor. High VEGF expression was associated to progressive disease and poorer survival in several malignancies, including colon and rectal cancers [31–33]. The efficacy of inhibition of VEGF pathway is demonstrated by improvement of clinical outcome in patients affected by several advanced cancers [34]. In particular bevacizumab, a humanized monoclonal antibody inhibiting VEGF-A, in combination with standard chemotherapy regimens was beneficial both in term of response rate and survival as first- and second-line treatment of patients affected by mCRC. However, bevacizumab did not improve outcome as adjuvant therapy in stage III colorectal cancer when associated to oxaliplatin-based chemotherapy [35].

In patients affected by LARC who underwent radical surgery and adjuvant chemoradiation, tumor VEGF overexpression is associated with a statistically higher risk of local recurrence and metastasis [36].

Preclinical data suggested that proangiogenic factors, especially VEGF, are upregulated in tumors in response to radiotherapy and may increase the resistance to radiotherapy. Tumor production of angiogenic factors generates neo-blood vessels with architectural abnormalities that are associated with increased interstitial pressure and contribute to intratumoral hypoxia that, in turn, negatively affect the efficacy of both radiotherapy and chemotherapy. Experimental studies in human tumor xenografts showed that VEGF blockade reduces tumor interstitial pressure, by structural and functional remodeling of abnormal tumor blood vessels. This vascular "normalization" may transiently reduce tumor hypoxia and facilitate drug penetration into tumor mass, thereby enhancing response to chemotherapy and RT [37–40]. These attractive findings stimulated the clinical evaluation of anti-VEGF therapy in combination with conventional primary CRT in LARC (Table 1).

3.2. Bevacizumab as radiosensitizer

In the seminal study by Willett et al. [41–43] bevacizumab was delivered as i.v. infusion once before and 3 times during RT with infusional 5FU administered during radiation treatment. The primary objective of this phase I–II study was to determine the maximum tolerated dose of bevacizumab when delivered concurrently with 5FU and RT in patients with cT3/T4 rectal cancer before surgery as well as to clarify in vivo, through correlative studies, the mechanisms by which bevacizumab inhibits angiogenesis. The results on 32 patients showed tumor regression from a mean tumor size of 5 cm (range, 3–12 cm) to an ulcer/scar with mean size of 2.4 cm (range, 0.7–6.0 cm) in all patients and a 16% of pCR rate. Postoperative complications included anastomotic leak with presacral abscess requiring drainage ($n = 1$), vaginal tear ($n = 1$), pelvic hematoma ($n = 1$), and delayed healing of perineal incisions. The actuarial 5-year local control and OS were both 100% and 5-year DFS was 75%, with 5 patients developing metastasis. The study also evaluated the biological effects of bevacizumab on rectal cancer before its concurrent administration with CRT. Before and 12 days after the first bevacizumab infusion patients underwent flexible sigmoidoscopy with tumor biopsy, tumor interstitial pressure measurement, perfusion computed tomography scan to measure blood flow, PET-FDG scan and analysis of blood and urine for a number of angiogenesis markers. Compared with baseline measurements evident antivascular effects were demonstrated, including lower tumor interstitial pressure, reduced tumor vascular density and increased pericyte coverage in tumor vessels.

The effect of bevacizumab on the activity of other radiosensitizer agents (infusional 5FU; capecitabine; capecitabine and oxaliplatin) has been evaluated in a number of phase II studies in LARC. In all these trials the primary end-point was pCR.

At the M.D. Anderson Cancer Center, Crane et al. [44] evaluated 25 patients affected by LARC (no T4 patients included) who received radiotherapy (50.4 Gy in 28 fractions over 5.5 weeks), capecitabine (900 mg/m² twice daily, 5 days/week concomitantly with radiation) and bevacizumab (5 mg/kg i.v. on days 1, 15, 29). Eight (32%) patients obtained pCR, and for 6 (24%) <10% viable tumor cells were detected in the surgical specimen. No patient experienced grade 3/4 hand-foot syndrome, gastrointestinal toxicity or hematologic toxicity. However, 3 patients required surgical intervention due to wound complications (Table 2). With a median follow-up of 22.7 months (range, 4.5–32.4 months) all patients were alive; the 2-year actuarial rate was 6.2% (one patient showed a recurrence in the pelvis and 3 had distant recurrences). The sphincter-sparing rate was not evaluated.

In the largest phase II trial evaluating the activity of bevacizumab on patients affected by LARC, Velenik et al. [45] evaluated 61 patients with MRI-confirmed stage II/III rectal cancer who received bevacizumab (5 mg/kg i.v. 2 weeks prior to CRT and on days 1, 15, 29), capecitabine (825 mg/m² twice daily on days 1–38) and concurrent radiotherapy (50.4 Gy in 28 fractions). The majority (67%) of patients had T3n+ tumors and another 8.2% had T4N2 tumors. Grade 3 adverse events included radiodermatitis (9.8%), proteinuria (6.5%) and leucocytopenia (4.9%). TME radical resection was achieved in 57 patients (95%), and 42 patients (70%) underwent sphincter-preserving surgery. Dworak-TRG 4 (pCR) was found in 8 (13.3%) patients and TRG 3 in further 9 (15%). Thirty-eight (62.3%) patients developed perioperative complications, including delayed wound healing (30%), infection/abscess (20%) and anastomotic leakage (11.7%). Six patients required surgical re-intervention for anastomotic leakage ($n = 3$), abdominal abscess ($n = 2$) and pneumothorax ($n = 1$) (Table 2).

Martinez Villacampa et al. evaluated in a randomized phase II trial the effect of adding bevacizumab (5 mg/kg for 3 doses) (arm A) to preoperative capecitabine-based CRT (825 mg/m² twice daily) (arm B) in 90 patients with stage II–III rectal cancer. In arm A, pCR was obtained by 7 (16%) patients compared with 5 (11%) patients ($p = 0.54$) in arm B. Overall grade 3/4 toxicity rates were 18% and 13% (arm A versus B; $p = 0.50$) and postoperative complications were 19 (43%) in arm A and 17 (37%) in arm B, respectively [46].

In a preliminary report of 23 patients with LARC from the Dutch Colorectal Cancer Group [47] bevacizumab (5 mg/kg on days −14, 1, 15, 29) with concurrent capecitabine (825 mg/m² twice daily) and radiation (50 Gy in 25 fractions) obtained pCR in 2 out 21 valuable patients. Grade 3 chemoradiation toxicities were experienced by 7 patients (skin $n = 4$, diarrhea = 2, tenesmus = 1). One patient had grade 4 anal mucositis. Another patient with enteritis and diffuse bleeding at CRT-end, died before surgery. Two small bowel perforations occurred. Another patient had an asymptomatic rectal wall perforation at the site of the primary tumor. Surgical complications consisted of one perineal dehiscence, one rectovaginal fistula, and 1 patient had high-volume bleeding.

The Austrian Breast and Colorectal Cancer Study Group (ABCSG) reported the preliminary results of a phase II trial

limited to cT3 rectal cancer patients [48]. The schedule of treatment included bevacizumab (5 mg/kg, days 1, 15, 29), capecitabine (825 mg/m^2 twice daily on radiotherapy-days weeks 1–4) concurrently to pelvic radiotherapy (45 Gy in 5 weeks). Surgery followed 6–8 weeks later. Two (25%) out of 8 patients obtained pCR and tumor downstaging was observed in 37.5% of patients. However, accrual had to be terminated according to protocol, since half of patients experienced grade 3 toxicity (intestinal bleeding, diarrhea, perianal/abdominal pain, anemia).

In our experience, 43 patients affected by LARC were enrolled in an Italian multicentre phase II trial to receive bevacizumab (5 mg/kg on days −14, 1, 15, 29), capecitabine (825 mg/m^2 twice a day for 5.5 weeks) concurrently with external-beam irradiation (50.4 Gy in 28 fractions over 5.5 weeks). Post-operative histologic examination was centrally performed and showed no residual cancer cells both in the primary site of tumor and lymphodes (ypT0, N0) in 6 of the 43 patients (14%; 95% confidence limits: 3.6–24.3%). In another 22 patients (51.2%) a v < 15% of cancer cells in residual areas of fibrosis/necrosis was found corresponding to Mandard TRG 2 or 3 classification. Tumor resection with negative circumferential margin of resection was achieved in 38 (95%) out of 40 operated patients. Sphincter-sparing surgery was performed in 31 (72.1%) patients. Primary tumor and lymph nodes downstaging was observed in 15 (34.9%) and 16 (37.2%) cases, respectively. G1-2 diarrhea, proctitis, rectal bleeding and hypertension were the most frequent side effects. Grade 3 toxicities were experienced by 5 patients, including diarrhea (n = 3, 7.14%), neutropenia (n = 2), asthenia and hypokalemia (n = 1), respectively. Four (9.52%) patients permanently discontinued CRT during the last week of treatment due to G3 adverse events. Of 7 patients who experienced serious adverse events one died of progressive disease and another died due to bowel perforation before receiving adjuvant treatment (1 month after surgery, 81 days after the last dose of bevacizumab). A patient presented failure of anastomosis (97 days after bevacizumab) and a patient experienced postoperative abscess (75 days after bevacizumab). Two patients were admitted to hospital due to G3 hypokaliemia and myocardial ischemia, respectively. In both cases, side-effects resolved with medical treatment. Only the latter patient was under adjuvant chemotherapy.

In this translational study aimed at identifying potential predictive indicators, we evaluated certain biomarkers related to microvessel density and expression of vascular endothelial growth factor receptor-2 (VEGFR-2), tumor associated macrophages (CD68 antibody) apoptosis (M30 antibody), cell kinetics (anti-Ki-67 labeling index), as well as anti-thymidine synthase and anti-thymidine phosphorylase being targets of fluoropyrimidines. No biomarker was significantly predictive of pCR nor of DFS. Pre-treatment vessel density by the panendothelial marker anti CD-34 antibody, post-treatment Ki-67 labeling index and VEGFR-2 cancer cells expression significantly correlated with residual tumor area [49].

In order to obtain better local control of disease from CRT, bevacizumab has been also evaluated in combinations with two cytotoxic chemosensitizers, such as 5FU/capecitabine and oxaliplatin in 3 studies.

In a pivotal phase I trial from Duke University the combination of capecitabine, oxaliplatin and bevacizumab was evaluated as primary treatment in patients with stage II to IV rectal cancer. Patients received escalating doses of capecitabine and oxaliplatin, with a fixed dose of bevacizumab. Two patients (18%) achieved pCR and 3 patients focal microscopic disease only. One patient experienced a postoperative abscess, one a syncopal episode during adjuvant chemotherapy, and one a subclinical myocardial infarction during adjuvant chemotherapy [50].

Kennecke et al. [51] evaluated the safety and efficacy of pre-operative chemoradiation, using capecitabine (825 mg/m^2 twice daily on days 1–14 and 22–35), oxaliplatin (50 mg/m^2 on days 1, 8, 22, 29) and bevacizumab (5 mg/kg on days −14, 1, 15, 29) with standard doses of radiation in 42 patients with high-risk LARC with 18 (43%) patients having cT4 and/or N2 tumors. TME was performed 7–9 weeks after CRT. Out of the 42 patients enrolled, 38 underwent radical surgery. Mean relative dose intensity was >90% for all systemic agents, and 97% for radiation. Grade 3/4 diarrhea occurred in 10 (24%) patients and pain in 4 (10%) patients pre-operatively, while grade 3/4 pain, fatigue and infection were each reported among 5 patients (13%) post-operatively. Four (11%) patients needed to be re-operated due to complications. The complete regression of primary tumor (ypT0) was seen in 9 patients (23.7%), but two had N1 disease, therefore the pCR rate (ypT0N0) was 18.4%. However, when the pathologic stage was centrally evaluated, the pCR was confirmed in six cases (16%).

In another phase II trial in 70 patients with LARC, the addition of bevacizumab (5 mg/kg on days 1, 15, 29) to capecitabine (825 mg/m^2 bid on days 1–14 and 22–35), oxaliplatin (50 mg/m^2 on days 1, 8, 22, 29) and standard RT (50.4 Gy in 1.8 Gy fractions) was well tolerated and did not lead to increased perioperative morbidity or mortality. Out of 69 patients who had surgery, 66 (96%) patients had a R0 resection, 12 (17.4%) obtained pCR. Pathological tumor downstaging as achieved in 32 (46.4%) patients. CRT was well tolerated (toxicities are not available in details). The reported surgical complications included multivisceral resections (14%), intraoperative (8%) and postoperative complications (43%), re-laparotomies (8%), re-bleeding (3%) and anastomotic leakages (5/40 anastomoses) requiring surgical interventions [52] (Table 2).

3.3. Combining bevacizumab with induction chemotherapy (before CRT)

The AVACROSS trial [53] recruited 47 patients affected by high-risk LARC defined by RMI criteria to assess the efficacy and toxicity of adding bevacizumab to induction chemotherapy followed by preoperative bevacizumab-based

CRT. Treatment consisted of four every 21-day cycles of bevacizumab (7.5 mg/kg) in combination with CT (XELOX regimen), followed by concomitant radiotherapy (50.4 Gy) plus bevacizumab (5 mg/kg every 2 weeks) and capecitabine (825 mg/m^2 twice daily on days 1–15). Surgery was scheduled for 6–8 weeks after CRT. The primary end-point of the study was pCR. Among the 45 patients who underwent surgery, pCR was achieved in 16 patients (36%; 95% confidence interval: 22.2–51.2%), and an additional 17 patients (38%) had Dworak TRG 3. R0 resection was performed in 44 patients (98%). Most grade 3/4 adverse events occurred during the induction phase and included diarrhea (11%), asthenia (4%), neutropenia (6%), and thrombocytopenia (4%). However, 11 patients (24%) required surgical reintervention.

Dipetrillo et al. [54] reported the results of a small phase II study evaluating induction bevacizumab plus modified infusional 5FU, leucovorin, and oxaliplatin (mFOLFOX6) regimen followed by concurrent bevacizumab (5 mg/kg on days 1, 15, 29), oxaliplatin (50 mg/m^2/week for 6 weeks), continuous infusion 5FU (200 mg/m^2/day), and radiation in patients with LARC. Patients received 1 month of induction bevacizumab and mFOLFOX6 followed by 50.4 Gy of radiation and concurrent bevacizumab with CRT. Because of gastrointestinal toxicity oxaliplatin dose was reduced to 40 mg/m^2/week. Resection was performed 4–8 weeks after the completion of chemoradiation. Unfortunately the trial was terminated early because of high grade toxicity after 26 eligible patients had been treated. Only 1 patient had significant toxicity (arrhythmia) during induction treatment and was removed from the study. During chemoradiation, grade 3/4 toxicity was experienced by 19 of 25 patients (76%). The most common grade 3/4 toxicities were diarrhea, neutropenia, and pain. Five (20%) of 25 patients had pCR. Postoperative complications were experienced by 9 (36%) out of 25, including infection ($n = 4$), delayed healing ($n = 3$), leak/abscess ($n = 2$), sterile fluid collection ($n = 2$), ischemic colonic reservoir ($n = 1$), and fistula ($n = 1$) (Table 2).

3.4. Bevacizumab as consolidation treatment (before surgery)

As consolidation chemotherapy following CRT may improve pCR [55], bevacizumab in combination with FOL-FOX [56] has been administered both concomitantly with, and following CRT, before surgery, in patients with LARC. However, these combinations were associated with an increased rate of post-surgical complications (21.4%).

The feasibility of neoadjuvant chemotherapy (including an anti-angiogenic agent) without radiotherapy has been evaluated in patients with clinical stage II–III rectal cancer (no T4 tumors), who were candidates for sphincter-sparing surgery [57]. The treatment regimen combined FOLFOX with bevacizumab without preoperative radiation and reported a pCR in 8/29 patients (27%). Glynne-Jones et al. are evaluating in a randomised phase II study the efficacy and toxicity of neoadjuvant FOLFOX/bevacizumab versus

FOLFOXIRI/bevacizumab in resectable rectal cancer where preoperative MRI suggests adverse features such as extramural vascular invasion, but the circumferential resection margin (CRM) is not threatened. The primary end point is pCR. RT is not planned unless patients are shown to progress [58].

4. Combining bevacizumab and EGFR inhibitors with CRT

Despite the negative results reported in the Panitumumab Advanced Colorectal Cancer Evaluation (PACCE) trial [59], bevacizumab and cetuximab have been sequentially evaluated in combination with preoperative CRT in LARC. Preliminary results of a phase II trial have been recently presented [60]. Ten patients with K-RAS wild type tumor received an induction treatment with capecitabine (2000 mg/m^2 day 1–14), oxaliplatin (130 mg/m^2) and bevacizumab (7.5 mg/kg) every 3 weeks for two cycles followed by capecitabine (1300 mg/m^2/day) continuously during radiotherapy and cetuximab 400 mg/m^2 every two weeks. The combination seems to be safe, with acceptable and manageable toxicities (Table 2).

Preliminary results of a phase I/II trial of bevacizumab (5 mg/kg i.v. on days 1, 15, 29) and erlotinib (50/100/150 mg daily concomitantly with radiotherapy) in combination with infusional 5FU (225 mg/m^2/day) and standard pelvic radiotherapy for patients with cT3/4 rectal cancer have been reported [61]. No dose-limiting toxicities were registered. Erlotinib at a dose of 100 mg was chosen as the MTD. Seven (47%) out of 15 patients who completed the study treatment and underwent surgery obtained a pCR. At a median follow-up of 7 months, there were no local recurrences reported in patients who completed therapy. Grade 3/4 toxicities included lymphopenia (59%), diarrhea (24%), rash (12%), cardiac ischemia (6%), transaminitis (6%), and mucositis (6%). One patient had an anastomotic leak [62] (Table 2).

5. Current challenges and future areas of research

The advances in surgery, radiation and chemotherapy all integrated in a multidisciplinary approach have increased the rates of cure of patients with LARC. The use of TME has dramatically reduced local recurrence and more accurate radiation delivery techniques have lowered toxicity. Following the publication of the CAO/ARO/AIO-94 phase III trial, preoperative CRT with fluoropyrimidines followed by TME surgery is the standard therapy for LARC [63]. Local recurrence rate is now less than 7%, being distant metastases the predominant type of treatment failure. Recent randomized trials on combined modality treatments, using either preoperative short-course radiotherapy alone or preoperative radiotherapy combined with 5FU failed to demonstrate further survival benefit [64,65]. Unsatisfactory results both in term of OS and pCR have been reported during tha last 5 years

in phase III trials evaluating the addition of newer cytotoxic agents to standard radiation therapy.

Three TAs, cetuximab, panitumumab and bevacizumab, based on efficacy demonstrated in mCRC and on synergy with RT seen in preclinical models, have been evaluated in combination with CRT as neoadjuvant treatment of patients with LARC, being pCR the primary end-point in all the studies. These agents failed to improve pCR compared with standard regimens of fluoropyrimidine-based CRT.

However, in the absence up to now of direct comparative randomized phase III clinical trials is not possible to establish if the addition of TAs to standard CRT versus standard CRT alone improves or not clinical results in LARC. Concerning the side effects and the short time toxicity, bevacizumab enhances the risk of postoperative complications (bleeding, deep vein thrombosis, pelvic abscess, wound infection or dehiscence, perforation and enterocutaneous/perineal fistula). Similarly, the addition of EGFR inhibitors to standard CRT, besides the well know cutaneous toxicity, increases the incidence of gastrointestinal toxicities (abdominal pain, proctitis, diarrhea). The comparison of toxicity of the studies including TAs with the randomized clinical trials, such as CAO/ARO/AIO-94 and Dutch Colorectal Cancer Group trials, is not feasible because all the studies performed up to now on TAs in LARC are phase II studies, including very heterogeneous patient populations. An important question is if clinical evaluation of TAs in combination with CRT has been carried out in the optimal way. Several key issues should be reconsidered, including specific pharmacodynamic features of TAs and of their interactions with CRT, the parameters used to define efficacy of TAs when combined with CRT. Further aspects include the lack of reliable predictive factors of activity/efficacy and the analysis of possible biological and molecular peculiarities of rectal compared to colon cancer.

Bevacizumab and cetuximab exert their efficacy predominantly as cytostatic rather than cytotoxic agents, so it seems plausible that the benefits may not derive from an increased tumor shrinkage, but rather in slowering tumor progression. Therefore, it appears questionable that pCR is to be considered as the most suitable primary end-point [64,66].

While there has been much debate about whether pCR is associated with a favorable long-term outcome, a recently published pooled analysis of data from 3105 patients enrolled in 14 studies suggested that patients with pCR after standard chemoradiation had better long-term outcome than those without pCR [66].

The residual tumor cells in mesorectal lymph nodes have been proposed as the most relevant independent prognostic factor for survival, even after total regression of the primary tumor after preoperative therapy. Therefore, tumor grading systems should include not just the regression of the primary tumor but also the residual disease in mesorectal lymph nodes. In addition, the accuracy of clinical staging techniques is quite different in defining cT, cN and clinical "positivity" of the CRM, particularly in low-lying rectal cancer. Similarly, staging methods are non homogeneous in defining the CRM,

which is the most accurate baseline factor in predicting local and distant relapse of disease.

5.1. Clinicopathological issues

5.1.1. Predictive factors

Antiangiogenic agents lack up to now of reliable predictive factors [67–69]. The study performed by Sleijfer et al. [70] in advanced soft tissue sarcoma in the context of EORTC-STBSG suggests that in patients treated with pazopanib, the determination of VEGFR-2 (low levels) and placental-derived growth factor (PIGF) (high levels) is associated to elevated toxicity and poor response rate. Other studies [41–43,71] suggest that angiogenic cytokines and circulating endothelial cells and their progenitors are potentially useful predictive factor in rectal cancer. However, the potential clinical use of these biomarkers needs further evaluation in prospective clinical trials.

Conversely, K-RAS mutational status is a negative predictive factor for cetuximab and panitumumab therapy. Surprisingly, in the randomized phase II EXPERT-C trial evaluating capecitabine and oxaliplatin with or without cetuximab as induction regimen followed by CRT with capecitabine and concomitant radiotherapy (without cetuximab) in patients with high-risk wild-type-KRAS LARC, cetuximab significantly increased the response rate and overall survival, but not pCR (the primary end-point of the trial) [24]. These data are in line with results from other three independent trials where patients with LARC received cetuximab combined with chemoradiation [72,73]. The clinical significance of determination of K-RAS, BRAF, PI3K/PTEN/AKT mutations, having a predictive role in anti-EGFR moAb-treatment in patients with mCRC, remains to be specifically assessed in LARC patients.

An increasing number of studies performed with functional imaging techniques, including contrast-enhanced dynamic MRI/computed tomography and 18F-fluorodeoxyglucose (18FDG) positron emission tomography in patients treated with standard CRT, shows the capability to obtain an early prediction of response by comparing basal versus intermediate evaluations of response to therapy [74–81].

Changes in imaging parameters have been suggested as indicators of tumor vessel function after bevacizumab monotherapy [82,83]. In a small cohort of 32 patients affected by LARC treated with standard radiation therapy, infusional 5FU and bevacizumab, functional imaging parameters revealed significant vascular and tumor responses after completion of the neoadjuvant treatment [43]. Blood flow and permeability-surface area product measured by dynamic computed tomography significantly decreased at day 12 and presurgery compared to pretreatment. These data suggest that VEGF blockade alone and with CRT decreases vascular permeability and induces pruning of the rectal cancer vasculature. In contrast, the 18FDG uptake (a measure of tumor metabolic rate) was not changed by bevacizumab alone, but

significantly decreased by combination therapy [43]. However, these preliminary results need to be better evaluated in larger studies.

5.1.2. New biomarkers and pharmacogenomic studies

A plethora of potential histopathological, imaging and molecular predictive biomarkers have been evaluated by using single or multimarker assays and whole-genome analyses (Tables 3 and 4). Some of these biomarkers have great potential to stratify rectal cancer patients for personalized treatment regimens and to guide the implementation of targeted therapeutics as well. Gene and protein expression profiles appear to be the most promising approach since they better disclose the biological complexity of genetic and epigenetic aspects related to chemoradioresistance. However, none of these markers has been validated for selecting the optimal personalized treatment in individual patients. In addition, it should be considered that bioinformatics is an absolute requirement to analyse complex genomic data. Of particular interest appears the identification of a pharmacogenetic profile predictive of tumor response, following fluoropyrimidines-based CRT in rectal cancer patients. In a recent study [84], two polymorphisms were associated with response to CRT in a multivariate analysis: hOGG1-1245C>G, which can affect radiosensitivity and MTHFR-677C>T, which is involved in fluoropyrimidines activity. A differential tumor response has been associated to a specific genetic signature that allowed dividing patients into three groups with a different chance of tumor response. These data highlight that pharmacogenetic approaches could be useful to better identify patients who will benefit of neoadjuvant CRT. In addition, this approach may be used in defining the optimal personalized dose of radiosensitizer drugs, as shown for FOLFIRI in mCRC [85,86].

5.2. Pharmacodynamic considerations

5.2.1. Optimal sequence of targeted therapy and CRT

A relevant issue is the optimal schedule of sequence of TAs with CRT. Both cetuximab and bevacizumab have been proposed as radiosensitizers, since they antagonize tumor radioresistance induced by EGFR and VEGF, respectively. Irradiation may activate the EGFR-pathway stimulating proliferative and pro-angiogenic effects, evasion of apoptosis and tumor progression. EGFR inhibitors antagonize these effects, leading to reduction in DNA-repair activities, to cell cycle blocking and to reduced proliferation and angiogenic activity. Inhibition of the MAPK/AKT pathways specifically prevents recovery of the cells after irradiation, leading to cell death and to better tumor response [72,73]. However, it has been suggested that the cytostatic effect of cetuximab might impair activity of cytotoxic agents that mainly act on proliferating cells [72,73,87]. EGFR inhibitors given before or concurrently with chemotherapy may antagonize the effect of cell-cycle dependent chemotherapy by inducing G1 arrest. The G1 cell cycle arrest caused by cetuximab reduces the radiosensitizing activity of fluoropyrimidines, oxaliplatin or irinotecan, mainly exerted in the S/G2/M phases [20]. These negative interactions are overcome by delivering cytotoxics before EGFR inhibitors [88]. These suggestions have been confirmed by in vivo studies [89] and ongoing clinical trials are evaluating these new sequences [73].

Well conducted in vitro and in vivo studies, provided direct evidence that bevacizumab may induce a "normalization" of tumor vessels by inhibiting VEGF-A [41]. The architectural changes induce a number of biological events synergic with CRT, such as the reduction of interstitial blood pressure associated with increased permeability of vessels to drugs and decreased tumor hypoxia. Unfortunately, the "normalization window" is transient and, consequently, also the therapeutic benefits may be limited over time. In addition, antiangiogenic agents obtain a delay in tumor progression with a variable period of clinical benefit, rather than a tumor shrinkage [90–94]. Preclinical data suggests that the sequencing of chemotherapy, EGFR inhibition and radiation may be important. Other preclinical studies found that when antiangiogenc therapy is stopped, a rapid tumor revascularization is observed [93]. Similarly, when such agents are administered on discontinuous schedule tumors regrow [94]. Consistently, in clinical trials prolonged administration of bevacizumab has been associated to improved survival outcomes [95]. The suboptimal results obtained with bevacizumab-CRT clinical trials highlight that the optimal sequence of administering antiangiogenic agents in combination with cytotoxics and/or radiotherapy is yet to be optimized. There is also evidence that the sequence of oxaliplatin followed by cetuximab seems to be more effective than cetuximab before oxaliplatin [96].

Probably the optimal activity of TAs in LARC is related both to the direct on target activities and the off-target induced mechanisms of action with particular reference to antiangiogenic agents [97]. A deeper knowledge of the key molecular factors influencing tumor radioresistance would provide the opportunity to better modulate the activity of selective agents [98–102]. In several preclinical studies hypoxia modulating agents, inhibitors of the checkpoint kinases CHK1 and CHK2, of EGFR pathway kinases, of farnesyltransferase and of PI3K/ATM pathway and specific DNA repair inhibitors showed promising results [103].

5.2.2. Exploring the inhibition of new targets

Another strategy is to explore other key targets involved both in progression and in radioresistance of rectal cancer cells. It has been proposed that anti-VEGF therapy increases intratumoral hypoxia which, in turn, drives a genetic program resulting in elevation of c-Met expression and activity and EMT-mediated tumor cell invasion [104]. c-MET is also involved in signal transduction when EGFR and VEGFR are activated [105]. Multiple lines of evidence implicate hypoxia, HIF-1α, and c-Met activation in tumor aggressiveness. Blockade of both c-Met and VEGF signaling together, either by a combination of two selective agents or by a single multi-targeted agent, might reduce tumor invasion and metastasis.

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Table 3
A selection of the studies that evaluated the predictive potential of single- or multi-biomarkers in patients affected by LARC.

Authors	N. of pts	Preoperative treatment	Marker(s)	Method(s)	End-point(s)	Findings
Single or multiple biomarkers						
Giralt [112]	87	45 50.4 Gy	EGFR	IHC	pCR	EGFR-positive expression before radiotherapy is an indicator for poor response and low disease-free survival
Kim [113]	183	50.4 Gy/5FU + LV or cap + LV	EGFR	IHC	Tumor downstaging (defined as a reduction of at least one T-stage level)	On logistic regression analysis, a low level of EGFR expression was found as a significant predictive factor for increased tumor downstaging
Toyiama [114]	40	5FU-based CRT	EGFR, VEGF and HIF-1 expression	RT-PCR	TRG	The elevated expression level of each gene could predict low response to CRT
Carlomagno [115]	43	RT/cap + oxa	EGFR, VEGF, TS, Ki67, poly(adenosine diphosphate-ribose) polymerase-1, XRCC1	IHC	PR scored according to TRG (TRG1 = pCR versus TRG ≥ 2)	pCR was significantly associated with high TS, high Ki67 and low EGFR expression
Zlobec [116]	104	HDREB	EGFR, VEGF, Bcl-2, APAF-1, p53	IHC	pCR	In multivariable analysis, loss of VEGF and positive EGFR both demonstrated independent predictive value for pCR
Debucquoy [117]	99	RT (30/45 cGy) ± 5FU ± LV	EGFR, VEGF, CA IX, Ki67, COX2 and c-CK18	TMA-IHC	TRG according to Dworak	No predictive role for the biomarkers evaluated. Only pre-treatment VEGF was associated to poor response
Chang [118]	130	50.4 Gy/5FU + LV	Bax, Bcl2 p53, p21 WAF1/CIP1, Ki67, Ku-70, HDAC1, MBGR4	IHC	TRG	Bax higher expression in the CR group as compared with the PR group (54% versus 29%, p = .017)
Kikuchi [119]	60	45 Gy/S-1 + Iri	Ki67 LI, Bax, TS, DPD, MVD by CD34, and Grp78	IHC	TRG according to Dworak (responders: TRG 3/4; non-responders: TRG1/2)	On multiple logistic regression analysis, Ki67 LI, Bax, and TS scores were found to be independent predictive factors
Negri [120]	56	40–45 Gy ± 5FU + oxa	p53, p21,VEGF, TS, MSH	IHC	pCR versus partial responders versus non responders	No predictive value. High TS level was predictive of a higher pathological response in the CRT subset (p = 0.007)
Edden [121]	152	50.4 Gy/5FU or cap	APAF-1, Bax, BCL2, p53, p21, p27, Cox2, VEGF	IHC	TRG	On multivariate analysis, APAF-1 was found to be independently associated with good TRG
Bertolini [122]	91	50 Gy + 5FU	p53, p21, MLH1, MSH2, MIB-1, TS, EGFR, tissue VEGF	IHC	TRG (according to Dworak)	Patients with MLH1-positive tumors had a higher pCR rate (24.3% vs. 9.4%; p = 0.055)
Huerta [123]	117	50.4 Gy/Cap	MIB, Bcl-2, and Bax, Cyclin E, p21, p27, p53, survivin	TMA-IHC	pCR	MIB tumor expression was an independent predictor of response to CRT (p = 0.001)
Kim [124]	54	50.4 Gy/5FU or cap	Survivin, Cox2, EGFR, Ki67, p21, TS, VEGF	TMA-IHC	Tumor downstaging	Among molecular markers studied, only survivin expression was significantly related with tumor downstaging

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Table 3 (*Continued*)

Authors	N. of pts	Preoperative treatment	Marker(s)	Method(s)	End-point(s)	Findings
Single-nucleotide polymorphisms						
Ho-Pun-Cheung [125]	71	50 Gy/cap ± Oxa	128 SNPs over 76 genes	PCR and automated sequencing	TRG (Dworak)	The SNPs SOD2 rs4880 and L13 rs1800925 were significantly associated with TRG
Garcia-Aguilar [126]	132	50.4 Gy/5FU	23 genes	PCR and automated sequencing	pCR versus non-pCR according to ypTNM staging system	Patients with cyclin D1 G870A (AA) polymorphism, and MTHFR C677T (TT) polymorphism or *KRAS* mutation did not achieve a pCR
Conradi [127]	167	50.4 Gy/5FU/oxa	TS gene polymorphisms and expression	PCR and automated sequencing	TRG	No correlation was found between pretreatment TS expression or TS genotype and TRG
Stoehlmacher [128]	40	50.4 Gy/5FU	TYMS genotypes	PCR and automated sequencing	TRG	TS genotype and TRG were significantly correlated
Paez [129]	128	45 Gy/5FU or cap ± oxa	TS, EGFR, GSTP1, and DNA repair genes polymorphisms	PCR and automated sequencing	Pathologic response	The *3/*3 TS genotype was associated with a greater rate of pCR and microfoci residual tumor (59% in *3/*3 vs. 35% in *2/*2 and *2/*3; $p = .013$)
Spindler [130]	60	65 Gy/UFT	TS, EGFR Sp1-216 and EGF A61G gene polymorphisms	PCR and automated sequencing	TRG	The evaluated polymorphisms may be used in combination as predictive markers for pCR
Hur [131]	44	5FU-based CRT	TS gene polymorphisms and expression	IHC/PCR and automated sequencing	Tumor response (TRG; pTNM)	No significant difference in tumor response between patients homozygous for 3R/3R and patients heterozygous for 2R/3R
Chiorean [132]	28	Cap + Iri induction → 50.4 Gy + cap	CES1/2, TS, TP, DPD, TOPO I, UGT 1A1	PCR and automated sequencing	pCR	TP gene expression was higher in patients who obtained pCR
Balboa [133]	65	5FU or cap-based CRT	XRCC1, ERCC1, ERCC2, MTHFR, TYMS and EGFR polymorphisms	PCR and automated sequencing	TRG according to Mandard score	Only tumor XRCC1 appeared to be significantly associated with T-downstaging
Lamas [134]	93	50.4 Gy/5FU	XRCC1, ERCC1, MTHFR, EGFR, DPD, and TYMS	PCR and automated sequencing	TRG according to Mandard score (TRG 1/2: major response)	Only germline polymorphisms of XRCC1 G/G and of TS (2R/3G, 3C/3G, and 3G/3G) were independent predictors of a TRG1/2
Villafranca [135]	65	45–54 Gy/5FU + LV or tegafur + LV or 5FU + carbo or oxa	TYMS genotype from tumor DNA	PCR and automated sequencing	T-stage downstaging	TYMS2/2 and TYMS2/3 patients achieved higher T-downstaging than those with TYMS3/3 polymorphism
Tan [136]	135	45–50 Gy/5FU ± iri	Germline *TYMS* genotyping	PCR and automated sequencing	Pathologic T-downstaging (TDS)	High rates of TDS and ypT0 were achieved among the two risk groups (poor: TSER*3/*3 or TSER*3/*4; good: TSER*2/*2, *2/*3, or *2/*4) when treatment was based on *TYMS* genotype
Terrazzino [137]	125	45–50.4 Gy/5FU ± LV or 5FU + carbo/oxa	TYMS genotype from germline DNA (blood)	PCR and automated sequencing	TRG	No correlation was found between TYMS genotype and TRG

Table 3 (Continued)

Authors	N. of pts	Preoperative treatment	Marker(s)	Method(s)	End-point(s)	Findings
DNA mutations in EGFR-KRAS pathway						
Luna-Perez [138]	37	50 Gy/5FU	*KRAS*	PCR-RFLP	ypUICC	Specific KRAS mutations (in codons 12-serine, 12 aspartate, 13-aspartate and 61-histidine) may be considered favourable tumor response markers to CRT
Zauber [139]	53	RT/5FU ± LV	*APC* and *DCC* LOH, KRAS mutations, MSH	PCR, DNA sequencing	TRG	*APC* and *DCC* LOH, MSH and KRAS mutation were not indicative of tumor regression after preoperative CRT
Kim [140]	82	50.4 Gy/iri + cap ± cetuximab	EGFR, KRAS, BRAF, and PIK3CA mutation status/EGFR and PTEN expression	Direct sequencing/IHC	Pathologic response rate (pRR), pathologic stage (ypTNM) and DFS	In Wt-KRAS cancer patients, pRR, ypTNM and DFS were not improved by the addition of cetuximab. EGFR or PTEN expression did not predict clinical outcome
Gaedcke [141]	94	50 Gy/5FU ± oxa	*KRAS, BRAF*	PCR, direct sequencing	Modified TRG as described by Gavioli et al., T-downstaging, ypUICC	KRAS mutation status was not correlated with response to preoperative CRT. Differential sensitivities were related to specific amino acid exchange in codons 12/13
Grimminger [142]	130	45–50 Gy/cap ± oxa ± cetuximab	EGFR, VEGF/R1-2, ERCC1, TS expression/KRAS and BRAF mutational status	RT-PCR/direct sequencing	TRG according to Dworak	High pretreatment tumor EGFR and VEGF mRNA expression levels and KRAS mutation status were predictive markers of pathologic non-response to cetuximab-based preoperative CRT
Bengala [143]	146	50 Gy/5FU or cap ± oxa	EGFR gene copy number/EGFR expression/KRAS mutational status	PCR/IHC/Direct sequencing	TRG according to Dworak	Neither EGFR gene copy number nor KRAS mutational status were statistically correlated to TRG

APAF-1, apoptosis protease-activating factor 1; Cap, capecitabine; CA IX, carbonic anhydrase IX; Carbo, carboplatin; CES1/2, carboxylesterase-converting enzymes 1/2; c-CK18, cleaved cytokeratin 18; COX-2, cyclo-oxigenase 2; CR, complete response; CRT, chemoradiotherapy; CT, chemotherapy; DPD, dehydropyrimidine dehydrogenase; DS, downstaging; GSTP1, glutathione S-transferase P 1; EGFR, epidermal growth factor receptor; 5FU, 5-fluorouracil; HDAC-1,histone deacetylase 1; HDREB, high-dose-rate endorectal brachytherapy; IHC, immunohistochemistry; IL13, interleukin-13; Iri, irinotecan; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; LI, labeling index; LV, leucovorin; MBGR-4, metabotropic glutamate receptor 4; MSH, microsatellite instability; MTHFR, methylenetetrahydrofolate reductase; MVD, microvessel density; Oxa, oxaliplatin; PCNA, proliferating cell nuclear antigen; PCR, polymerase chain reaction; PR, partial response; PTEN, phosphatase and tensin homolog; RT-PCR, real time protein chain reaction; SOD2, superoxide dismutase 2; SNPs, single-nucleotide polymorphisms; Tis, tumor in situ in the pathology specimen; TMA, tisse microarray, TOPO I, topoisomerase I; TP, thymidine phosphorylase; *TYMS*, thymidylate synthase gene; TS, thymidylate synthase; UGT, uridine-diphosphate glucuronosyl transferase 1A1; UFT, tegafur-uracil; VEGF, vascular endothelial growth factor; Wt, wild-type; XRCC1, X-ray cross-complementing group 1.

Table 4

A selection of the studies that evaluated the predictive potential of chromosomal aberrations or gene/protein expression profiling in patients affected by LARC.

Authors	N. of pts	Preoperative treatment	Marker(s)	Method	End-point(s)	Findings
Chromosomal aberrations						
Grade [144]	42	50.4 Gy/5FU	Chromosomal copy number alterations	CGH	T-downsizing	Chromosomal gains of 7q32–q36 and 7q11–q31 as well as amplifications of 20q11–q13 were associated with responsiveness to preoperative CRT
Chen [145]	95	50.4 Gy/5FU	Chromosomal copy number alterations	CGH	pCR	Chromosomal loss of 15q11.1–q26.3 was associated with non-pCR, while loss of 12p13.31 was associated with pCR
Gene profiling						
Nishioka [146]	17	40 Gy/S-1	Gene expression profile	DNA microarray/IHC	TRG (responders: 0–1 versus non-responders: 2–3)	17 genes were differentially expressed ($p < 0.05$) between responders and non-responders
Ghadimi [147]	30	50.4 Gy/5FU	Gene expression profile	DNA microarray	T-stage downstaging/TRG	A set of 54 genes was differentially expressed between responsive and resistant tumors. Expression profiles could predict tumor response (TRG) in 83% of patients
Rimkus [148]	43	45 Gy/5FU	Gene expression profile	DNA microarray	TRG according to Mandard	42 differentially expressed genes found, expression profiles could accurately predict 71% of responders and 86% of nonresponders
Kim [149]	46	50 Gy/5FU	Gene expression profile	DNA microarray	TRG according to Dworak	261 genes that were differentially expressed between 20 partial responders and 11 complete responders. Prediction accuracies were 84% (training set) and 87% (test set), respectively
Brettingham-Moore [150]	51	50 Gy/5FU	Gene expression profile	DNA microarray	TRG according to Mandard, metabolic response, and UICC downstaging	The sensitivity and specificity to predict outcome CRT was 82% and 89%, respectively
Watanabe [151]	52	50.4 Gy	Gene expression profile	DNA microarray	TRG (responders: grade 2/3; non responders: grade 0/1)	A set of 33 genes were differentially expressed between responders and nonresponders with a class prediction accuracy of 88.6%
Proteomic studies						
Allal [152]	17	50 Gy/5FU or Gem	Protein expression profile	MALDI-TOF	pCR versus partial response, versus no or minor response	Of the 56 landmark proteins, those of particular interest included tropomodulin, heat shock protein, b-tubulin, annexin, calsenilin, keratin type 1, Notch 2 protein homolog, and DNA repair protein RAD51L3
Smith [153]	20	50 Gy/5FU	Protein expression profile	SELDI-TOF-MS	TRG according to Mandard	A cohort of 14 protein peaks that differentiated good and poor responders to CRT with 87.5% sensitivity and 80% specificity

CGH, Comparative genomic hybridization; CRT, chemoradiotherapy; 5FU, 5-fluorouracil; Gem, gemcitabine; pCR, pathology complete response; S-1,; SELDI-TOF-MS, surface enhanced laser desorption/ionization time-of-flight mass spectrometry; TRG, tumor regression grade.

It has been recently reported that ionizing radiation induces overexpression and activity of the MET oncogene through the ATM/NF-κB signaling pathway. MET protects cells from apoptosis thus supporting radioresistance and promotes cell invasion. It has been demonstrated in xenograft models that the treatment with MET inhibitors enhanced tumor cell radiosensitivity [106].

Finally, data from gene expression profile along with existing evidence for distinct regional embryological origin support the concept that distal colon normal tissue is biologically different from the proximal counterpart. Consistently, right-sided colon cancers are distinct clinicopathologic entities compared with distal and rectal carcinomas and carcinogenesis pathways appear to be distinct in colon and rectal cancer [107–110]. These differences should be taken into account in the development of future therapeutic approaches.

6. Conclusion

The addition of TAs and/or newer cytotoxic drugs to preoperative fluoropyrimidine-based chemoradiation for patients with LARC is a feasible strategy, but used in the current modalities it does not enhance pathologic downstaging and the excess in toxicity is not counterbalanced by significant improvements in local or distant control of disease. According to data from the MERCURY study [111], rectal cancer is one of the best oncological settings to test the relevance of dynamic techniques aspotential predictive indicatorsof neoadjuvant treatment efficacy in LARC. Therefore, future translational studies should be also aimed to incorporate imaging studies in patients treated with new targeted anticancer agents.

The neoadjuvant CRT offers a unique opportunity to investigate new combinations and innovative treatment strategies coupled with translational studies to develop predictive factors, by evaluating the molecular and biological mechanisms underlying tumor sensitivity and resistance to treatment. Well designed translational studies in the setting of primary CRT of LARC will enable oncologists to provide the optimal patient-tailored treatment, by sparing unnecessary surgery to complete responders.

Conflict of interest statement

The authors declare no conflict of interest.

Reviewer

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Biographies

Francesco Torino received his MD *cum laude* in 1989 from the Catholic University of Rome, Italy. He specialized *cum laude* in Internal Medicine, Catholic University of Rome

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Roberta Sarmiento obtained her degree in Medicine and surgery at the University of Rome, La Sapienza, in 1997, *cum laude*, then she specialized in Oncology, at the University of Rome, La Sapienza, in 2001, *cum laude.* She spent her last year of fellowship in the United States, from January 2001 to March 2002, at the Brown University of Providence, RI, attending the laboratory of Clinical Pharmacology directed by Professor Paul Calabresi, working on tissue cultures. In 2004 she was appointed as assistant oncologist at the Division of Oncology of the Health District of Siena, then she moved to Rome in 2005, as oncologist at the Division of Oncology of the Azienda Ospedaliera "San Filippi Neri", where she works up to now. Roberta Sarmiento is the author of more than 30 scientific publications and 5 book chapters.

Giampietro Gasparini obtained his degree in medicine and surgery at the University of Padua in 1980. Subsequently he specialized in oncology, clinical pharmacology and radiotherapy. He was a fellow at the "Istituto Nazionale per la Ricerca e la Cura dei Tumori" of Milan, assistant at the "Centro di Riferimento Oncologico" of Aviano and then vice-director of the Division of Medical Oncology at the General Hospital of Vicenza. In 1997 Gasparini was appointed as the head and founder of the Oncology at the Azienda Ospedaliera "Bianchi-Melacrino-Morelli" in Reggio Calabria, and then as Director of the department of Oncology. Presently Gasparini is director of the Division of medical Oncology at the Azienda Ospedaliera "San Filippo Neri" and professor at the faculty of Medicine and Surgery-Specialty of Oncology University "Tor Vergata", Rome. Gasparini is the author of 300 publications in peer-reviewed journals and more than 350 abstracts at scientific conferences and meetings. The personal impact factor and the citation index up to June 2012 are 830 and 9800, respectively; *H*-index 44. Finally, he serves as a member of the editorial board of 25 international journals; recipient of national awards and reviewer for several journals and scientific organizations.