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Overview

Combination of Novel Agents with Radiotherapy to Treat Rectal Cancer

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Abstract

Neoadjuvant chemoradiotherapy with fluoropyrimidines is an established treatment in the management of locally advanced rectal cancer. There has been a great deal of research into improving patient outcomes by modifying this regimen by the addition of further radiosensitising agents. One of the difficulties in advancing new combination therapies has been lack of consensus on which surrogate measures best reflect clinically important outcomes. Here we review combinations of the cytotoxic, biological and other agents currently under scrutiny to improve clinical outcomes for patients with colorectal cancer. We also discuss advances in biomarkers that may ultimately result in an ability to tailor neoadjuvant chemoradiotherapy regimens to the somatic gene profile of individual patients.

Key words: Cetuximab; fluorouracil; irinotecan; oxaliplatin; radiosensitisation

Statement of Search Strategies Used and Sources of Information

Data for this review article were identified through a search of Embase, Medline and Web of Science. The following terms were used together with any derivatives: colorectal cancer, radiosensitiser, radiotherapy, radiation, chemoradiotherapy, chemotherapy, drug therapy, novel agent, targeted agent, biological agent, bevacizumab, aflibercept, cetuximab and panitumumab. Only articles published in English were selected. The search also included the reference list for these articles and selected additional articles judged to be relevant.

Introduction

Although the original demonstration of significant radiosensitisation by a chemotherapeutic agent in combination with radiotherapy for rectal cancer was in the adjuvant setting [1], clinical practice has moved away from adjuvant radiotherapy to the current practice of neoadjuvant chemoradiotherapy (CRT) [2,3]. The combination of neoadjuvant radiotherapy with a fluoropyrimidine is now an accepted standard of care in the treatment of locally advanced rectal cancer [4].

Significant debate exists regarding the best primary end points for clinical trials testing the addition of a new radiosensitising agent to CRT. Pathological complete response (pCR), the absence of viable tumour cells within the resection specimen, is commonly used but evidence is indeterminate as to whether this translates into improved outcome in terms of overall survival and disease-free survival (DFS; the time from randomisation until local or distant disease recurrence, or death) [5]. Other potential surrogate outcomes include downstaging rate, R0 resection (complete resection with no residual disease at margin) or circumferential resection margin (CRM: the minimum distance between the nearest extent of the tumour and the resection margin). Failure to achieve a negative CRM is associated with a high risk of local recurrence, but it is unclear as to whether this reflects inadequate surgery or aggressive disease [5]. DFS has been found to correlate with overall

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survival in a meta-analysis of colon cancer adjuvant trials [6] and at present is considered the most meaningful primary end point in phase III randomised control trials (RCTs) of CRT, albeit with the need to control for adjuvant chemotherapy. DFS is being used as the primary end point in the ARISTOTLE phase III trial in the UK, which is assessing the addition of irinotecan to CRT for rectal cancer. On account of the controversy over the best end points to use in clinical studies, here we will detail the various clinical outcomes reported in neoadjuvant CRT trials.

Chemotherapy

Irinotecan

Pre-clinical data have suggested that the radiosensitising properties of camptothecin derivatives may relate to the inhibition of potentially lethal damage repair. Irinotecan stabilises topoisomerase-I, an intranuclear enzyme that relaxes supercoiled DNA, by introducing a single-strand break through which the intact strand passes prior to religation. Collision between the irinotecan–topoisomerase I complex and the replication fork results in the formation of double-strand breaks, leading to G2 phase cell cycle arrest and cell death [7,8].

Several early phase trials have assessed the addition of irinotecan to standard CRT with fluoropyrimidines for rectal cancer. Table 1 includes results from larger, published phase II trials, in which pCR rates varied from 13.7 to 37%. An abstract by Jung *et al.* [17] details one of two randomised trials of CRT ± irinotecan. With 142 participants, a pCR rate of 17.2% was achieved in the arm receiving 5-fluorouracil/leucovorin (5-FU/LV) CRT versus 24.2% with a combination of S1, irinotecan and radiotherapy ($P = 0.1$). A significantly higher response rate was found in the latter arm when combining those achieving complete and near complete response (57.6% versus 39.1%, $P = 0.035$).

In a RCT of 5-FU with hyperfractionated radiotherapy versus 5-FU, irinotecan and 45 Gy/25 fractions with boost, Mohiuddin and colleagues [11] showed a pCR rate of 26% in both arms with no difference in rates of tumour downstaging. However, there was a higher proportion of radiotherapy delays in the irinotecan arm, 45% versus 22%. Overall, grade 3–4 toxicity was 51% with irinotecan and 42% without the additional drug; gastrointestinal effects were most common in both arms (37% versus 28%) [11]. These rates were higher than those seen in single-arm trials (Table 1) where radiotherapy and chemotherapy dose intensity was largely maintained. Mohiuddin *et al.* [11] reported late toxicity rates of 6%, lower than expected and again gastrointestinal effects predominated.

Five year outcomes have been published by Yoon *et al.* [15] and Mohiuddin *et al.* [19]. The latter study revealed overall survival 61% (95% confidence interval 47–74%) versus 75% (95% confidence interval 61–85%), distant failure 16%/21% and locoregional failure (LRF) 16%/17% rates without and with irinotecan, respectively. By comparison, in a review of 115 patients who had undergone a regime of CRT with irinotecan/S1 in phase I/II trials, the overall pCR rate was similar at 24%, with 5 year overall survival higher at 87%, DFS 79%, distant failure 17% and LRF 2.6% [20]. The multicentre UK-based phase III ARISTOTLE trial, recruiting since 2011, aims to confirm the potential improvement in outcomes seen with the addition of irinotecan to CRT and is currently set to complete recruitment in autumn 2016 [21].

Oxaliplatin

Oxaliplatin acts as a radiosensitiser through a variety of mechanisms, including causing DNA damage through the formation of inter- and intra-strand cross-links, induction of G2/M cell cycle arrest and blockade of DNA repair [22,23].

The addition of oxaliplatin to neoadjuvant CRT regimens showed promise in early phase single-arm trials, with pCR rates from 13% [24] to over 20% in several trials [25–27]. Five large phase III RCTs have gone on to compare various fluoropyrimidine-based neoadjuvant CRT regimens with or without oxaliplatin (Table 2) with pCR rates between 13.3 and 19.5% in the experimental arm compared with 11.3–17.8% without oxaliplatin. CAO/ARO/AIO-04 was the only trial to show a significant difference between the two arms, with pCR rates of 13% in the control arm versus 17% with oxaliplatin ($P = 0.038$). The ACCORD trial found a difference of 13.9% versus 19.2% ($P = 0.09$) but was powered to detect an increase from 11% to 20% with CAPOX. Of note, a higher dose of radiotherapy was given in the arm receiving oxaliplatin, which makes the results difficult to interpret.

A meta-analysis carried out by An *et al.* in 2013 [37], including results from ACCORD, AIO-04, NSABP R-04 and STAR-01 trials, did favour CRT with oxaliplatin (odds ratio = 1.20; 95% confidence interval 1.01–1.42; $P = 0.04$) with an absolute pCR rate difference of 2.5%. However, PETACC-6 was not included in this analysis and with over 1000 participants showed no significant difference in pCR, 11.3% without versus 13.3% with oxaliplatin ($P = 0.31$). Downstaging rates were also similar at 43.5% versus 41.5%, higher than reported in NSAPB R-04 (23.5% versus 17.9%; $P = 0.2$).

In terms of survival outcomes, recently published results from CAO/ARO/AIO-04 [32] showed a significant increase in 3 year DFS in the investigational group of 75.9% versus 71.2% in the control group (hazard ratio 0.79; 95% confidence interval 0.64–0.98; $P = 0.03$). Of note, the former received oxaliplatin with both CRT and adjuvant therapy, with the control group receiving 5-FU alone. Although PETACC-6 also added oxaliplatin to neoadjuvant and adjuvant regimens in experimental arm B, interim 3 year results indicated no significant improvement in DFS, being 73.9% (95% confidence interval 69.5–77.8%) versus 74.5% (95% confidence interval 70.1–78.3%) in arm A, higher than anticipated. Follow-up is ongoing, but these results appear similar to the NSABP R-04 and ACCORD trials, which did not specify adjuvant chemotherapy regimens and reported no significant differences in DFS at 5 and 3 years, respectively.

Although in ACCORD, Dworak TRG score (hazard ratio 0.68; 95% confidence interval 0.59–0.79) was found to be significantly correlated with 3 year DFS on multivariate

Table 1
Phase II trials of neoadjuvant chemoradiotherapy (CRT) with fluoropyrimidine and irinotecan

Reference	Phase	Stage*	Systemic treatment (mg/m^2)	RT (Gy/fractions)†	*n*	pCR	Other end points	Grade 3–4 toxicity (non-surgical)	Surgical outcomes
[9]	I/II	Unresectable	5-FU 200/day Irino 60 days 1,8,15,22 ADJ: not defined	45/25	31	19%	cCR 29% Microfocal residual 15% Irino 97% had 4 cycles 5-FU 10% DR Median follow-up 24 months: 16% LRF, 26% DF, 10% mortality	Grade 3: diarrhoea 13%, constipation 6%, skin sores 3%, lethargy 3%, infection 3%, abdominal cramping 3%, chest pain 3% Grade 4: fever 3%	90% resected, 81% R0 1 death (AL)
[10]	II	uT3/4 or N+	5-FU 200/day Irino 50 days 1,8,15,22 ADJ: Up to 4 months 5−FU/LV	50.4/28	32	37%	DS 34% 5-FU DR 32% Irino DR 32% 6% metastases on restaging	Grade 3: diarrhoea 28%, mucositis 21%, skin sores 21%, abdominal cramps 9%, N&V 3%	100% resected, 50% SSS AL 3%
[11]	II	cT3/4, ≤9 cm from dentate line	A: 5-FU 225/day B: 5-FU 5 days/week + Irino 50/week ×4 ADJ: Recommended if pathologic residual disease	A: 45.6/38 BD (≥6 h apart) + boost 9.6 (T3) 14.4 (T4) B: 45/25 + boost 5.4 (T3) 9 (T4)	A: 50 B: 53	A: 26% B: 26%	T-DS 80%/80% RT delays ≥2 days 22%/45% pCR in T3 22/69 (32%) versus T4 5/27 (18%) 5 year: OS 61%/75%, LRF 16%/17%, DF 16%/21%	Grade 3–4: overall 42%/51%, GI 28%/37%, blood/marrow 9%/12%, skin 11%/4%, pain 4%/6% GU 0%/2%, CVS 2%/0% Late toxicity 6% overall, maximum severity grade 3 4%/6%, grade 4 0%/2% mainly GI	93% resected 1 death in each arm
[12]	II	T3–4 resectable, ≤15 cm from anal verge	5-FU 225 5 days/week Irino 50/week	45/25	74	13.7%	Partial response 61.6% Overall DS 49.3% T-DS 57.5% N-DS 66.7% RT DR 7% 5-FU DR 10% Irino DR 11%	Grade 3: diarrhoea 14%, asthenia 9%, abdominal pain 8%, rectal mucositis 8%, anorexia 5%, neutropenia 4%, anaemia 3%, constipation 3% Grade 4: neutropenia 3%, TCP 1%	98.6% resection 58% TME 59% SSS 84.9% R0 1 death (septic shock, not related to study treatment)
[13]	II	ct/uT3/4 or N+, M0/1, LR	Cape 500 BD daily Irino 50/week ADJ: discretion	50.4/28	36 (M1 $n = 3$, LR $n = 3$)	15%	Microfoci remaining 26% T-DS 55% N-DS 63% RT DR 14% Median received: Cape 100% Irino 95% Actuarial 2 year OS 83%	Grade 3: leukopenia 19%, diarrhoea 11%, N&V 6%, fatigue 3%, raised ALT/AST 3% Grade 4: leukopenia 6% 1 VF arrest (reversed)	94% resected, 82% SSS, 100% R0 DWH 26%, AL 12% 2 deaths (sepsis)
[14]	II	mrT3 (≤2 mm from mesorectal fascia or distal extent ≤5 cm from anal margin) or mrT4, ≤15 cm from anal verge	Cape 650 BD daily Irino 60/week weeks 1−4 ADJ: discretion	45/25 prone	107	22%	T-DS 67% N-DS 80% Mean dose delivered: RT 97.4%, Irino 94.3%, Cape 87.9% 3 years: LRF 4%, DFS 63.5%, OS 88.2%	Grade 3: diarrhoea 22%, fatigue 11%, radiation dermatitis 6%, anorexia 5%, neutropenia 5%, neutropenic fever/infection 2%	97% resected at median 62 days, 50% SSS, R1 8%

[15]	II	mr +/− uT3 or resectable T4	Cape 825 BD 5 days/ week Irino 40/week ADJ: Cape 1250 BD days 1−14/3 weeks ×6	50.4/28	48	25%	Dworak TRG 3 18.2% T-DS 68.2% Relative dose intensity: Cape 93.4%, Irino 94.4% RT 100% full dose, 1 interruption 5 years: DFS 75%, OS 93.6%, LRF 2% (also had DF), DF 25%	Grade 3: neutropenia, infection, infection, raised ALT, diarrhoea 2.1%	94% resected (6% cCR declined, 9.1% transanal excision), 86% SSS 2% AL, 2% DWH
[16]	II	cT3/4 N0-2	S1 40 BD 5 days/week, Irino 80 days 1,8,22,29 ADJ: S-1 + Irino (if internal iliac or obturator ypN+, 9%)	45/25 prone (only perirectal nodes included)	67	34.7%	86.6% completed CRT (CALGB criteria) G2 (good response) 32.8% T-DS 73.1%, N-DS 43.2%	Grade 3: diarrhoea, leukopenia, neutropenia 4.5%, anorexia & nausea 1.5%, TCP 1.5%	74.6% SSS (all had diverting ileostomy usually reversed at 6−12 months due to risk of AL)
[17]	II	cT3-4 or N+	A: 5-FU 400 + LV 20 bolus days 1−3/28 days ×2 B: S1 35 BD 5 days/ week + Irino 40/week ×5 ADJ: 5-FU	45−50.4/ 25−28	142	A: 17.2% B: 24.2% ($P = 0.1$)		Grade 3/4 A: 1.4% B: 7% ($P = 0.095$)	91.5% TME
[18]	II	Locally recurrent, unirradiated M0/1	Cape 625 BD 5 days/ week + Irino 50/week weeks 1−5	45/25 Boost 10-16/5-8	71	50% (7/14)	cCR 5.6% Complete symptom relief 56.6% of 53 symptomatic patients, partial in 32.1%	Grade 3: diarrhoea 22.5%, dermatitis 9.9%, leukopenia 5.6%, cystitis 4.2%, Grade 4: leukopenia 2.8%	R0 resection 20%

5-FU, 5-fluorouracil; LV, Leucovorin; Irino, irinotecan; Cape, capecitabine; OD, once a day; BD, twice a day; ADJ, adjuvant; DR, dose reduction; DS, downstaging (T, tumour; N, node); c, clinical; u, ultrasound; mr, magnetic resonance; GI, gastrointestinal; GU, genitourinary; CVS, cardiovascular; TCP, thrombocytopenia; N&V, nausea and vomiting; VF, ventricular fibrillation; ALT, Alanine transaminase; AST, Aspartate aminotransferase; TRG, tumour regression grade; TME, total mesorectal excision; SSS, sphincter sparing surgery; AL, anastomotic leak; DWH, delayed wound healing; RT, radiotherapy; pCR, pathological complete response; cCR, clinical complete response; LRF, locoregional failure; DF, distant failure; OS, overall survival; DFS, disease-free survival; LR, locally recurrent.

* M0 unless stated otherwise.

† RT starting at day 1.

Table 2
Phase III randomised controlled trials of neoadjuvant chemoradiotherapy (CRT) with fluoropyrimidine + oxaliplatin

Reference Trial	Disease stage*	Systemic treatment (mg/m^2)	RT (Gy/ fractions)†	*n*	pCR	Other end points	Grade 3–4 toxicity (non-surgical)	Surgical outcomes
[28] ACCORD 12/ 0405-Prodige 2	T2 (ant/lower) T3 T4 (resectable) Accessible to DRE	A: Cape 800 BD 5 days/week B: + Oxali 50/week ADJ: discretion	A:45/25 B:50/25	A: 293 B: 291	A: 13.9% B: 19.2% ($P = 0.09$)	Full dose RT: 100%/ 87% Dworak Score 3 or 2: 28.9%/39.4% ($P = 0.008$) 3 years: NSD in LRF 6.1%/4.4%, OS 87.6%/88.3% DFS 67.9%/72.7% [29]	Overall 10.9%/25.4% ($P < 0.001$), diarrhoea 3.2%/12.6% ($P < 0.001$). Grade 3: fatigue 0.8%/ 5.1% ($P = 0.004$)	97% TME CRM ≤ 2 mm 19.3%/9.9% ($P = 0.022$) NSD in rates of surgery (98/ 98.6%), conservative surgery rate (75% overall), Abdominopelvic distant metastases at surgery (4.2%/ 2.8%, $P = 0.4$) or R1 (12.7%/ 7.7%, $P = 0.17$).
[30] STAR-01	cT3-4 &/or N1-2 Resectable, within 12 cm anal verge	A: 5-FU 225/day B: + Oxali 60/week ADJ: 5-FU based	50.4/28	A: 379 B: 368	A: 16% B: 16% ($P = 0.9$)	Full dose 5-FU: 90%/ 80% ($P < 0.001$) Full dose RT: 92%/84% ($P < 0.001$) −discontinued due to toxicity 4%/17% Dworak TRG 3: 39%/ 45%	Overall 8%/24% ($P < 0.001$), diarrhoea 4%/ 15% ($P < 0.001$); nausea, abdominal pain 0%/2% ($P = 0.012$), radiation dermatitis 2%/5% ($P = 0.037$), SN 0%/1% ($P = 0.026$), asthenia 0%/ 3% ($P < 0.001$) 3 deaths (1/2)	Intra-abdominal distant metastases at surgery 2.9%/ 0.5% ($P = 0.014$) NSD in rates of surgery (96%/ 95%), APR or Hartmann's (21%/19%), CRM ≤1 mm (7%/ 4%), R0 94%/97%, postoperative complications (22%/24%), 60 day mortality (1%/1%).
[31] CAO/ ARO/ AIO-04	cT3-4 or cN+, within 12 cm anal verge	A: 5-FU 1000 days 1−5,29 −33 B: 5-FU 250 days 1−14,22 −35 + Oxali 50 days 1,8,22,29 ADJ: A: 5-FU B: 5-FU + Oxali	50.4/28	A: 623 B: 613	A: 13% B: 17% ($P = 0.038$)	Full dose: RT 96%/94%, NA chemo 79%/85% ADJ chemo completed 65%/44%. 3 years: DFS 71.2%/ 75.9% ($P = 0.03$), NSD in OS 88%/88.7% or LRF 4.6%/2.9% [32]	Overall 20%/23%, haematological 6%/5%, GI overall 15%/20%, diarrhoea 8%/12% 6 deaths (2/4)	R0 95%/94%, CRM ≤1 mm 6%/5%, APR 24%/25%, good quality TME 77%/76% 60 day mortality 4/3 deaths Complications grade 3–4 10%/13%, AL 5%/7%
[33] PETACC-6	T3/4 &/or N+ Resectable/potentially resectable, within 12 cm of anal verge	A: Cape 825 BD daily B: + Oxali 50/week A: Cape B: Cape + Oxali	45/25 + optional boost to 50.4	A: 547 B: 547	A: 11.3% B: 13.3% ($P = 0.31$)	≥45 Gy 98%/92% >90% chemo 91%/63% DS 43.5%/41.5% 3 year DFS 74.5%/ 73.9% LRF + DF 20%/18%	Toxicity (undefined) 7.7%/16.5% Deaths without progression 15 versus 26 [34]	R0 92%/86.3% SSS 70%/65% ($P = 0.09$)
[35] NSAPB R-04	cT3-4,N0 or cT1-4,N1-2 Within 12 cm anal verge, palpable on DRE or accessible by procto- or sigmoidoscope	A: 5-FU 225/day B: Cape 825 BD Post protocol amendment C: 5-FU 225 5 days/week D: C + Oxali 50 E: Cape 825 BD 5 days/week F: E + Oxali 50/week ADJ: not defined	45/25 Boost: T3 non-fixed, non-distal 5.4/3 T4 fixed +/or distal 10.8/3	A: 147 B: 146 C: 330 D: 329 E: 326 F: 330	No Oxali 17.8% With Oxali 19.5% ($P = 0.42$)	Dose delivered fluoropyrimidine C/E > D/F ($P < 0.05$) No Oxali versus with Oxali: DS 23.5%/17.9% ($P = 0.2$). 3 year LRF 12.1%/ 11.2% 5 year: DFS 64.2%/	No Oxali versus with Oxali: diarrhoea grade 3–5 6.9%/16.5% ($P < 0.001$), 2 in CAPOX arm died Arms C/D/E/F: Grade 3(%): nausea 0.3/0.6/1.3/2.2, fatigue 1.3/4/2.2/5.9,	No Oxali versus with Oxali: SSS 61% /57.8% ($P = 0.24$) NSD in postoperative complications arms A–F (%): Overall 30.7/37.4/37.3/37.5/ 36.2/40.5 AL 2.1/3.1/1.3/3.6/3.0/1.6 Death 0.7/0.0/0.3/0.7/0.7/0.0

69.2%, OS 79%/81.3% (NSD) [36]	dehydration 0.3/2.8/2.2/4 Grade 2–4(%): SN 0.6/5.6/2.2/6.5	

5-FU, 5-fluorouracil; Oxali, oxaliplatin; Cape, capecitabine; OD, once a day; BD, twice a day; ADJ, adjuvant; NA, neoadjuvant; DS, downstaging (T, tumour; N, node); DRE, digital rectal examination; c, clinical; GI, gastrointestinal; APR, abdominoperineal resection; SN, sensory neuropathy; CRM, circumferential resection margin; TME, total mesorectal excision; SSS, sphincter sparing surgery; AL, anastomotic leak; NSD, no significant difference; RT, radiotherapy; pCR, pathological complete response; OS, overall survival; DFS, disease-free survival; LRF, locoregional failure; DF, distant failure.

* M0 unless stated otherwise.

† RT starting at day 1.

analysis, the CAO/ARO/AIO-04 trial exploratory subset analysis of DFS indicated significant benefit in the investigational group for cN0 disease (hazard ratio 0.56; 95% confidence interval 0.36–0.86), but no difference was seen with pathological ypTNM subgroups (hazard ratio 1.20; 95% confidence interval 0.43–3.36). CAO/ARO/AIO-04 showed no significant difference in 3 year overall survival (88%, 95% confidence interval 85.3–90.7% versus 88.7%, 95% confidence interval 86–91.3%) or 3 year LRF (4.6%, 95% confidence interval 2.9–6.4% versus 2.9%, 95% confidence interval 1.5–4.3%) after R0/1 resection in control and investigational groups, respectively. Comparable results were seen in the ACCORD trial with 3 year overall survival 87.6% versus 88.3% (hazard ratio 0.94; 95% confidence interval 0.59–1.48). Three year LRF was 6.1% versus 4.4%, whereas Allegra *et al.* [36] in the NSABP R-04 trial found higher rates of 12.1% versus 11.2% without and with oxaliplatin, respectively.

Surgical outcomes were not significantly different with the addition of oxaliplatin. In ACCORD, exploratory analysis revealed a difference in CRM $\leq$2 mm of 19.3% versus 9.9% ($P = 0.022$) without and with oxaliplatin, but this difference was not seen at CRM $\leq$1 mm [28,30,31]. Whereas surgical toxicity was not altered with the addition of oxaliplatin, ACCORD, STAR-01 and NSABP R-04 all reported significantly increased CRT-related toxicity. Grade 3–4 diarrhoea was particularly problematic with rates up to 25.4% versus 10.9% without oxaliplatin in ACCORD and two deaths attributable in NSABP. Four trials showed a higher proportion of patients not receiving full dose chemotherapy or radiotherapy in the experimental arm [28,30,34,35]. The ACCORD trial assessed late toxicity at 3 years and found no difference between the arms in terms of bowel continence, erectile dysfunction or social life disturbance [38].

One randomised phase II trial [39] has compared the pCR rates of CRT with CAPIRI (capecitabine 600 mg/m^2 BD 5 days/week and irinotecan 50 mg/m^2 weekly four doses) or CAPOX (capecitabine 825 mg/m^2 BD 5 days/week and oxaliplatin 50 mg/m^2 weekly five doses). The pCR rate in the latter group was higher at 23.1% (95% confidence interval 12.5–36.8%) versus 11.8% (95% confidence interval 4.4–23.9%), with toxicity levels comparable and tolerable in both groups. Follow-up to 4 years revealed that both regimens showed efficacy with LRF 16% versus 18%, DFS 68% versus 62% and overall survival 85% (95% confidence interval 66–94%) versus 75% (95% confidence interval 60–85%), respectively [126]. The contradictory observation of higher overall survival in the irinotecan arm was noted, but this study was not designed to permit direct statistical comparisons between the two arms.

In conclusion, the addition of oxaliplatin to standard neoadjuvant CRT has shown improved pCR rates in one meta-analysis of four trials. Although this has not translated into improvement in survival measures to date, publication of long-term data is still awaited for two large phase III trials. The regimen does seem to be associated with increased acute toxicity and reduced treatment compliance. Nevertheless, several trials have added biological agents to oxaliplatin, fluoropyrimidine and radiotherapy. Pre-clinical

Table 3
Clinical trials of bevacizumab (BEV) with neoadjuvant chemoradiotherapy (CRT) for rectal cancer

Reference	Stage*	Systemic treatment	RT (Gy/fractions)†	Surgery	*n*	pCR	Other end points	Grade 3–4 toxicity (non-surgical)	Surgical outcomes
Bevacizumab with fluoropyrimidine-based neoadjuvant CRT (phase II)									
[45]	cT3/4	Cape 825 BD daily BEV 5, days −14,1,15,29	50/25	6–10 weeks	23	9.5% (2/21)	T-DS: 85.7% (18/21)	Grade 3: skin 17%, diarrhoea 9%, tenesmus 4% Grade 4: anal mucositis 4% 9% small bowel, 4% rectal wall perforation 1 death secondary to enteritis with uncontrolled bleeding	96% resected, 45% SSS, 91% R0 Rectovaginal fistula, peri-operative bleeding, pulmonary embolism, perineal dehiscence 5%
[46]	cT3/4	5-FU 225/day BEV 5, days −14,1,15,29 DL1:5 (PII dose) DL2:10	50.4/28	7–10 weeks	32 PI: 11 PII: 21	15.6% (5/32)	T-DS 50% N-DS 56.5% Actuarial 5 year OS 95%, DFS 68.9% [47]	Grade 3: overall GI 41%, diarrhoea 22%, hypertension 9%, Skin/RT dermatitis 6%; PPE, neurologic, wound separation 3%	100% resected, 94% R0 Wound infection 9%; haematoma, DWH 6%; AL, PE 3%
[48]	T3/4 or N1	Cape 900 BD 5 days/week BEV 5, days 1,15,29 ADJ: discretion	50.4/28	6–10 weeks TME	25	32.0% (8/25)	Microscopic residual 24% Overall DS 82% T-DS 64%, N-DS 79% Median follow-up 22.7 months: 4% LRF, 12% DF Actuarial 2 year DFS 77.3%	Grade 3: perianal desquamation 4%	100% resection, 100% R0, 72% SSS DWH, perineal wound dehiscence (required further surgery), AL 8%; anastomotic dehiscence (required further surgery), superficial wound abscess 4%
[49]	T3 or operable T4, N0/+ ≤12 cm anal margin	Induction: 4× 21 day cycles Cape 1000 BD days 1–15 OX 130 day 1 BEV 7.5 CRT: Cape 825 BD daily BEV 5, days 1,15,29 ADJ: CAPOX x4 recommended	50.4/28	6–8 weeks TME	47	35.6% (16/45)	Dworak TRG 37.8% 87% completed NA treatment	CRT phase: Grade 3: lymphopenia, hypertriglyceridemia, tenesmus 3%	96% resected, 97.8% R0, 67% SSS 58% postoperative complications: wound infection 22%, intra-abdominal infection 16%, AL 11%, stoma complications 4%, other 22% 24% further surgery
[50]	II/III	Non-randomised Cohort A: NACRT 5-FU 225/day, BEV 5, days 1,15 Cohort B:	50.4/28	A: 2–8 weeks	66 A: 35 B: 31	A: 29% (10/35)	A: Microscopic residual 11% Discontinued treatment due to toxicity: A 23%, B 16% Started ADJ	A (neoadjuvant CRT phase) Grade 3: diarrhoea 6%; neutropenia, fatigue, dehydration 3%, Grade 4: TCP 9% B (ADJ CRT phase) Grade 3: diarrhoea 26%, rash/	A: 89% resected, 32% TME, 39% SSS Complications: A: grade 4 rectal bleed, perforation, pelvic infection 3%. B: perforation, perianal infection

		ADJ CRT 5-FU 225/day, BEV 5 2- weekly from day 1. ADJ: modified FOLFOX6 x4 + BEV for 1 year					chemo: A 57%, B 84%	desquamation 13%; dehydration, mucositis 6%, fatigue 3%	6%; anal wound dehiscence, rectovaginal fistula 3%
[51]	II/III likely resectable	Cape 825 BD daily BEV 5, days −14,1,15,29 ADJ: Cape x4 (R0) or x6 (R1)	50.4/28	6–8 weeks TME	61	13.3% (8/60)	Dworak TRG3 15.0% Overall DS 75% T-DS 46.7% N-DS 65% Dose intensity: RT 100%, BEV 95.1%, Cape (≥95%) 91% 3 year OS 80.9%, DFS 74.1%, LRF 5.6% [52]	Grade 3: dermatitis 9.8%, proteinuria 6.5%, leukopenia 4.9%; diarrhoea, vascular, hypertension 1.6% Grade 4: vascular 1.6%	95% radical resection, 70% SSS 62.3% peri-operative complications: DWH 30%, infection/abscess 20%, AL 11.7%, pneumothorax 1.7% 10% further surgery
[53]	T3Nx potentially resectable	Cape 825 BD 5 days/week weeks 1–4 BEV 5, days 1,15,29	45/25	6–8 weeks TME	8	25% (2/8)	T-DS 37.5% Full dose: RT 87.5%, Cape 87.5%, BEV 75%	Grade 3/4: perianal bleeding, perianal or abdominal pain, diarrhoea 25%, anaemia 12.5% Trial terminated as 50% had grade 3/4 toxicity	88% resected Ileus grade 3, perineal wound dehiscence 14%
[54]	T2N+, T3/4	Cape 825 BD daily, BEV 5, days −14,1,15,29 ADJ: 5-FU/LV x12 ± BEV	50.4/28	6–8 weeks TME	43	14.0% (6/43)	Microscopic residual 16.3% T-DS 34.9% N-DS 41.9% Discontinued CRT 9.5% Median follow-up 16.7 months: LRF 11.6%, DF 7% Actuarial 3 year DFS 75.4%	Grade 3: diarrhoea 7.1%, neutropenia 4.7%; asthenia, hypokalaemia 2.4%	93% resected, 95% R0, 78% SSS Bowel perforation (81 days after BEV, died), anastomotic failure, abscess 2.5%
[55]	II/III MRI <15 cm anal verge	Randomised 1:1 A: Cape 825 BD daily, BEV 5, days 1,15,29 B: Cape 825 BD daily	45/25	6–8 weeks TME or PME	90 A: 44 B: 46	A: 16% (7/44) B: 11% (5/46) $P = 0.54$	A/B Mandard TRG 1–2: 36%/44% T-DS: 59%/39% ($P = 0.04$) CRT complete 93%/93% Median follow-up 18 months: LRF 0/2%, DF 14%/13%, Death due to disease 5%/0%	Grade 3: overall 16%/13% ($P = 0.70$), asthenia/fatigue 2%/0%, anorectal discomfort 2%/0%, PPE 0%/2%	98%/100% resected, 77%/78% TME, 61%/67% SSS Overall postoperative complications 43%/39%, anastomotic dehiscence requiring surgery 15.9%/6.5% Intra-abdominal distant metastases at surgery 0%/9%

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Table 3 (*continued*)

Reference	Stage*	Systemic treatment	RT (Gy/fractions)†	Surgery	*n*	pCR	Other end points	Grade 3–4 toxicity (non-surgical)	Surgical outcomes
[56]	T3/4 N0/+ <15 cm from anal verge	Cape 900 BD 5 days/week BEV 10, days −14; 5 days 1,15,29 ADJ: CAPOXx4 or FOLFOXx6 (ypT4/N+), Cape single agent x6 (ypT0-3N0)	45/25	6–8 weeks TME or PME	41	7.5% (3/40)	Microscopic residual 20% DS 77.5% ADJ chemo started 80% Actuarial 4 year: DFS 85.4%, OS 92.7%	Grade 3: leukopenia 7%; diarrhoea, radiodermatitis 2%. Neutropenia grade 1–3 7.3% Grade 4: vasospastic angina 2%	97.4% radical resection, 84.6% R0, 76.9% SSS, 69.4% TME Overall complications 33.3%, wound infection 15%, intra-abdominal collection 8%; paralytic ileus, pelvic/presacral collection, suture dehiscence 5%
Bevacizumab with oxaliplatin and fluoropyrimidine-based neoadjuvant CRT (phase I and II)									
[57] Phase I	II–IV	All: BEV 15, days 1; 10 days 8,22 Cape BD 5 days/week Oxali weekly weeks 1–5 DL1: Cape 625, Oxali 50 DL2: Cape 825, Oxali 50 DL −1,3 and 4 not reached	50.4/28	6–8 weeks TME	11 DL1: 3 + 6 DL2: 2	18% (2/11)	Microscopic residual 27% DS 82%	DL1 grade 3/4: diarrhoea 22%; nausea, vomiting, anorexia, dehydration 11% DL2 grade 3/4: diarrhoea, dehydration 50%	100% resected, 82% SSS, 18% concurrent hepatic metastasis resection Pre-sacral abscess, partial small bowel obstruction 9%
[58] Phase II	II/III	Induction: 5-FU 400 bolus + 2400/46 h Oxali 85/week BEV 5, 2-weekly for 1 month CRT: 5-FU 200/day Oxali 50 → 40 (toxicity in initial 3 patients) weekly ×6 BEV 5, days 1,15,29 ADJ: as induction x6	50.4/28	4–6 weeks TME	25	20% (5/25)	Discontinued chemo due to toxicity 40% Started ADJ chemo 60% Median follow-up 36 months: LRF 4%. DF 16%	Overall grade 3/4 76.0% Grade 3: diarrhoea 40%; pain, neutropenia 16%; fatigue, nausea, radiation dermatitis, anaemia, hypokalaemia 8%; neuropathy, mucositis, infection, bleeding with menstruation 4% Grade 4: diarrhoea, nausea, neutropenia, anaemia, weight loss, infection 4%	100% resected, 76% SSS 36% wound complications including infection 16%, DWH 12%, leak/abscess 8%, fistula 4% DVT 12% 1 death secondary to MI 10 days postop
[59] Phase II	II–IV	Cape 825 BD days 1–14, 22 −35	50.4/28	7–9 weeks TME	42	18.4% (7/38)	Dworak TRG3 31.6%, TRG2 39.5%	Grade 3/4: diarrhoea 24%, pelvic pain 10%, fatigue 10%, rash/PPE	90% resected, 92% R0 63% complications Grade 3/4: pain, fatigue, infection

		Oxali 50 days 1,8,22,29 BEV 5, days −14,1,15,29					Mean dose intensity: BEV 95%, Oxali 97%, Cape 91%, RT 97%	7%, hypertension 5%; pelvic infection, constipation 2%	13%, delayed healing 8%; AL, weight loss, hypertension, pelvic infection 5%; bleeding, anaemia 3% 11% further surgery
[60] Phase II	cT3/4 or N+, M0/1 (single resectable liver metastasis), <16 cm from anal verge	Cape 825 BD days 1−14,22−35 Oxali 50 days 1,8,22,29 BEV 5, days 1,15,29 ADJ: Recommended Cape 1250 BD days 1−14/21 x4	50.4/28	4−6 weeks TME	69	17.4% (12/69)	DS 44.9% Mean dose intensity: BEV 98.6%, Oxali 97.8%, RT 98.4%. Full dose Cape 90%	Grade 3: diarrhoea 3%; constipation, anal abscess, anaphylactic reaction, leukopenia 1.4% Grade 4: diarrhoea, nausea 1.4%	95.7% resection all R0, 68.1% SSS Postoperative complications: overall 36.2%, wound healing problems 9.8%, bleeding 2.8%; GI perforation, grade 4 ileus 1.4%
[61] Phase II	T3/4, ≤12 cm from anal verge	Cape 825 BD 5 days/week Oxali 50 weekly BEV 5, days 1,15,29 ADJ (if macroscopic complete resection): 2 weekly 5-FU/LV/BEV 5 x12 + Oxali x9	50.4/28	6−8 weeks TME	55	16.6% (9/54)	T-DS 59.3% N-DS 66% ADJ chemo started 51%, completed 30% Median follow-up 41 months: 5 year OS 80%, DFS 81% [62]	Grade 3: overall worst grade 53%, neutropenia 16%; rectal pain, leukopenia, fatigue 13%, diarrhoea 11%, dehydration 9%, SN 7%; abdominal pain, vomiting, TCP, anaemia 5%; weight loss, rash, anorexia, nausea 4%, proctitis 2% Grade 4: DVT, rectal pain, anaemia 4%; leukopenia, neutropenia, fatigue, nausea, diarrhoea, CNS haemorrhage, abdominal pain 2% Grade 5: Aspiration 2%, death related to cancer 2%	91% resected, 88% R0 Overall complications 18.3%. Wound infection 16%, fascial dehiscence 10%; intra-abdominal abscess, fistula, bowel obstruction, thrombosis/embolism 2% 6% further surgery Late complications 47%. Wound infection 47%, wound/fascial dehiscence 24%, bowel obstruction/ileus 10%, intra-abdominal abscess 4%, AL 2%
Bevacizumab with fluoropyrimidine-based neoadjuvant CRT + other agents (phase I and II)									
[63] Phase I	T3/4 or N+, ≤12 cm anal verge	All DL: BEV 5, 2-weekly ×3 Cape BD 5 days/week DL1: 650 DL2-4: 825 Erlotinib (mg) OD DL1-2: 50 weeks 1−3 DL3: 50 weeks 1−6 DL4: 100 weeks 1−6 ADJ: Discretion	50.4/28	Minimum 9 weeks TME	18 DL1: 2 DL2: 4 DL3: 4 DL4: 8	44% (8/18)	T-DS 83% ADJ chemo 100% Median follow-up 34 months: DF 6%, LRF 0% Estimated 3 year DFS 94%	Grade 3: hypertension 6% (DL2) MTD not reached	100% resected, 61% SSS Grade 3 postoperative complications: ileus, small bowel obstruction with pelvic abscess, cellulitis 6%

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Table 3 (*continued*)

Reference	Stage*	Systemic treatment	RT (Gy/fractions)†	Surgery	*n*	pCR	Other end points	Grade 3–4 toxicity (non-surgical)	Surgical outcomes
[64] Phase I/II	T3/4, ≤15 cm anal verge	BEV 5, days −14,1,15,29 Erlotinib OD & 5-FU 225 (both omitted last day of RT) PI Erlotinib DL1: 50 mg DL2: 100 mg DL3: 150 mg	50.4/28	6–9 weeks TME	32 PI: 9 PII: 23	32.2% (9/27)	DL2 = MTD ($n = 27$) 33% DR RT break 22% Median follow-up 2.9 years: LRF 0%, 3 year DFS 75.5%	Grade 3/4: overall 46.9% Grade 3: lymphopenia 50%, diarrhoea 18.8%, acneiform rash 6.3%; mucositis, deranged LFTs 6.3%; colitis, cardiac ischaemia, dehydration, fatigue, hypertension, hypokalemia, hyponatraemia, proteinuria, radiation dermatitis, desquamation, rectal pain 3% Grade 4: lymphopenia 15.6%, febrile neutropenia, hyperuricemia 3%	97% resected all R0, 61.3% SSS Small bowel obstruction 17.9%, AL 14.3%, intra-abdominal infection 7.1%; PE, wound infection, fever 3.6%
[65] Phase II	T3 or N+	Amifostine (mg) day 1 500, day 2 750, day 3+ 1000 BEV 5, days 1,15 Cape 600 BD 5 days/week, weeks 1–4 ADJ: Cape single agent 4 months	34/10 (NTD 46 Gy)	6 weeks	19	36.8% (7/19)	Partial response 42.1% Amifostine 1000 mg received by 84.2% Median follow-up 21 months: OS 79%, DF 16%, LRF 5%	Grade 3: diarrhoea 10.5% Moderate-severe proctalgia 21.1% No significant late RT complications	100% resected No DWH/infection 1 death (PE)

5-FU, 5-fluorouracil (mg/m^2); LV, leucovorin; Oxali, oxaliplatin (mg/m^2); Cape, capecitabine (mg/m^2); BEV, bevacizumab (mg/kg); OD, once a day; BD, twice a day; P, phase; ADJ, adjuvant; NA, neoadjuvant; DL, dose level; MTD, maximum tolerated dose; DR, dose reduction; NTD, normalised total dose; DS, downstaging (T, tumour; N, node); MRI, magnetic resonance imaging; c, clinical; GI, gastrointestinal; CNS, central nervous system; SN, sensory neuropathy; DVT, deep vein thrombosis; TCP, thrombocytopenia; PPE, palmar plantar erythema; LFTs, liver function tests; TRG, tumour regression grade; TME/PME, total/partial mesorectal excision; SSS, sphincter sparing surgery; AL, anastomotic leak; DWH, delayed wound healing; PE, pulmonary embolism; MI, myocardial infarction; NSD, no significant difference; CI, confidence interval; LRF, locoregional failure; OS, overall survival; DFS, disease-free survival; DF, distant failure

* M0 unless stated otherwise.

† RT starting at day 1.

and clinical data have suggested that these combinations may have a synergistic effect; for example, bevacizumab enhances tumour blood flow and reduces tumour interstitial pressure and hypoxia [40,41], potentially improving the activity of both chemotherapy and radiation.

Biological Agents

Bevacizumab

Bevacizumab, a humanised monoclonal antibody against vascular endothelial growth factor (VEGF), has been evaluated as a potential radiosensitiser when added to current CRT protocols. Proangiogenic factors such as VEGF can lead to radio-resistance, which theoretically could be reversed by such agents as bevacizumab [42].

Several single-arm phase II trials have assessed the efficacy and safety of the addition of bevacizumab to standard CRT, many of which have been reviewed elsewhere [43,44] and are summarised in Table 3. Study size ranged from eight to 90 patients and of those completed, pCR ranged from 7.5 to 36.8%. When reported, tumour downstaging was seen in 34.9–100% of patients [45,46,54,55]. In a variation to standard treatment, Koukourakis *et al.* [65] added bevacizumab to hypofractionated radiotherapy. Using a historical cohort of patients who had undergone hypofractionated radiotherapy [66], the authors showed that the pCR for bevacizumab cohort was 36.8% compared with 21.4% for the previous study, although this was not statistically significant. The only randomised study to date recently published results [55]. Ninety patients received radiotherapy with concurrent capecitabine and bevacizumab 5 mg/kg once every 2 weeks, or the same schedule without bevacizumab. The patients receiving bevacizumab had a higher pCR rate, but this was not statistically significant (16% versus 11%, $P = 0.54$) and the predefined efficacy end point was not met. However, those receiving bevacizumab were more likely to achieve tumour downstaging (59% versus 39% B; $P = 0.04$).

One interesting study assessed bevacizumab as an adjuvant or neoadjuvant therapy in a non-randomised manner [50]. This trial was designed as a single-arm adjuvant trial. However, because many physicians preferred a preoperative option, a second arm was added. Thirty-five patients had neoadjuvant CRT and 31 had CRT after surgery. The 1 year DFS rates were 85% (95% confidence interval 67–93%) and 97% (95% confidence interval 79–100%), respectively, with a pCR of 29% achieved in the neoadjuvant cohort. Severe bevacizumab-related toxicity occurred in 12% of patients (both cohorts) but was manageable and reversible. Overall, most trials have concluded that toxicities from CRT with bevacizumab were expected and manageable. A direct comparison in the RCT by Salazar *et al.* [55] found no statistical difference in preoperative toxicities between therapy with and without bevacizumab. However, two single-arm studies were terminated early due to excessive toxicity [53,58].

Some trials have incorporated bevacizumab into a combination regimen (Table 3) of fluoropyrimidine, oxaliplatin and neoadjuvant radiotherapy. Tumour downstaging was seen in 44.9–81.9% and postoperative complications affected 18.3–63% of patients, where reported. However, the single-arm nature of these trials means that it is difficult to determine whether these are complications of oxaliplatin, bevacizumab or both. Within the phase II trials, pCR rates ranged from 16.6% to 18.4% in line with the previously described studies and similar to historic CRT trials without bevacizumab.

In conclusion, there is no definite evidence for which, if any, regimen is most appropriate to combine bevacizumab with standard neoadjuvant CRT and the addition of bevacizumab to CRT is therefore not currently recommended outside of clinical trials.

Anti-epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) is recognised as an important factor in colorectal cancer (CRC) initiation and progression. Elevated EGFR expression and activity frequently correlate with tumor resistance to radiotherapy, thought to be related to the activation of cell survival and proliferation pathways [67].

With the successful combination of cetuximab with radiotherapy in head and neck squamous cell carcinoma [68], there has been interest in its use as a radiosensitiser for other cancers. Cetuximab is already used routinely in the setting of metastatic CRC in patients with *KRAS* wild-type disease. Table 4 details clinical trials involving the addition of cetuximab to standard CRT with fluoropyrimidines. This regimen seems to be largely tolerable, with expected associated toxicities including rash, diarrhoea, mucositis and allergic reaction. Surgical toxicities where reported were not significantly increased [70,74,75]. However, an improvement in pCR, which ranged from 0 to 28.3%, has not been shown to date, even where *KRAS* status is taken into account. Two RCTs [74,76] retrospectively analysed *KRAS* status and in the wild-type group found no difference in pCR with and without cetuximab. In the EXPERT-C trial [74], pCR rates of 11% versus 7% ($P = 0.71$), respectively, were obtained, although of note, *KRAS* status was unavailable for eight patients who achieved pCR. However, there was evidence of increased response rates in the wild-type group who received cetuximab, 77.8% versus 53.8% ($P = 0.03$). In a pooled analysis of two phase II studies of CRT, one with irinotecan [91] and the other irinotecan and cetuximab [80], Kim *et al.* [92] found that pathological response, postoperative pathological stage and DFS did not differ significantly. This lack of difference in outcomes remained even among *KRAS* wild-type participants and was corroborated by Kripp *et al.* [93], who compared two trials of CAPIRI [13,94] with one adding cetuximab [79]. Sun *et al.* [75] in a single-arm trial also assessed *KRAS* status after treatment and noted no significant difference in pCR rates between wild-type and mutant, but did find higher levels of downstaging in the former, 86.4% versus 57.9% ($P = 0.02$). Numbers in this trial were small and the difference in downstaging was not confirmed by Eisterer *et al.* [77] or Bertolini *et al.* [72]. In terms of survival data, Sun *et al.* [75]

Table 4
Clinical trials of anti-epidermal growth factor receptor (EGFR) agents with neoadjuvant chemoradiotherapy (CRT) for rectal cancer

Reference	Disease stage*	Systemic treatment (mg/m^2)	RT (Gy)†	*n*	pCR	Other end points	Grade 3–4 toxicity (non-surgical)
Cetuximab with neoadjuvant CRT phase I trial							
[69]	T3–4, locally recurrent	Cetux weeks 1–10 5-FU 225/day	50.4	20	12% (2/17)	100% R0	Diarrhoea 10%, acneiform rash 15%; stomatitis, radiation dermatitis, transaminitis 5%
Cetuximab with neoadjuvant CRT phase I/II trials							
[70]	T3–4 &/or N1–2	Cetux weeks −1–10 Cape BD daily DL1: 650 DL2: 825	45	40	5% (2/37)	Dworak TRG3 27% DS 38% 92.5% resected, 100% R0, 75.7% SSS	Grade 3: diarrhoea 15%, allergy Cetux 3% Grade 4: MI, PE, fatal sepsis 3%
[71]	T3/4 N0/1	Cetux weekly 5-FU days 1–5, 29–33 DL1: 750 DL2: 1000	50.4	20	5.5% (1/19)	MTD 5-FU 750 mg/m^2 90% resected, 100% R0, 67% SSS	Allergic reaction Cetux 5% DL2 DLTs: grade 3 diarrhoea, TCP Death 20% (3/4 due to metastatic disease progression)
Cetuximab with neoadjuvant CRT phase II trials							
[72]	T3/4 N0/1	Cetux weeks −3–5/6 5-FU 225	50 – 50.4	40	8% (3/38)	Dworak TRG 3 18% DS 60.5% 95% resected	Grade 3–4: skin rash, hypersensitivity reaction, diarrhoea 7.5%; stomatitis, liver enzyme, febrile neutropenia 2.5%
[73]	II/III	Cetux weeks −1–5 Cape 1250 BD week −3 and −2 then 825 BD weeks 1–5 ADJ: Cape x3 cycles	45	40	8% (3/37)	7% withdrew trial CRT due to cardiac ischaemia/chest pain Dworak TRG3 19% DS 73% R0 97%	Grade 3: radiodermatitis 16%, diarrhoea 11%, hypersensitivity 5% (Cetux discontinued after #1 in 11%); hepatotoxicity, infection, anorexia 3%
[74]	High risk operable: <1 mm CRM, Low T3 or T4, EMVI, Extramural extension >4mm	RCT A: NA CAPOX x4 Cape 825 BD daily + RT B: above + Cetux weekly	50.4	165 A:81 B:84	*KRAS/BRAF* WT A: 7% (3/44) B: 11% (5/46) $P = 0.71$	Median follow-up 32 versus 37 months. All: NSD PFS, OS, 91%/94% resected, 92%/96% R0, 73%/72% SSS. WT: radiologic response after CRT 75%/93% (P = 0.028), OS benefit with Cetux (HR 0.27, P = 0.034); NSD R0, SSS	Grade 3–5: diarrhoea 1%/10%, rash 0%/9%, PPE 1%/4% 2 peri-operative deaths (arm A)
[75]	T3/T4 (R0/1 achievable)	Cetux weeks 1–5 Cape 1250 BD week 1, 850 BD weeks 2–5	45	63	12.7% (8/63) *KRAS*: MT 10.5% (2/19) WT 13.6% (6/44) $P = 1.0$	All: DS 77.8% 3 year DFS 76.2%, OS 81% DS *KRAS* WT 86.4%/MT 57.9% (P = 0.02), 3 year DFS 76.7%/75%, 3 year OS 81.4%/80% (NSD)	Grade 3: radiodermatitis 16%; diarrhoea, acneiform rash 6%; dry skin, infection 3%
[76]	T3/4 or N+	RCT A: 5-FU 225 B: A + Cetux weeks −1–6	50.4	139 A: 69 B: 70	A: 28.3% (17/60) B: 26.6% (17/64) NSD	5 year PFS 61%/65%, OS 66/83%, LRF 3/4%	Diarrhoea 16%/22%, rash 0%/12%, mucositis 5%/6%, dehydration 5%/8%
[77]	cT3/4 potentially resectable	Cetux weeks 1–5 Cape 825 BD 5 days/week weeks 1–4	45	31	0% (0/28)	R0 96%, DS 46% *KRAS* status in 25 patients (56% WT, 44% MT) showed no correlation with DS	Grade 3: diarrhoea 10%, rash 6%, rectal itching/pain 3% Grade 4: diarrhoea 6%, weight loss 3%

Cetuximab and irinotecan with neoadjuvant CRT phase I or II trials							
[78] Phase I	uT3–4/N+	Cetux weeks 1–5 Irino weekly weeks 1–5 Cape BD 3 DLs: 1. Irino 40, Cape 400 2. Irino 40, Cape 500 3. Irino 50, Cape 500	50.4	20	26% (5/19)	DL2 accepted R0 95% T-DS 42% N-DS 67%	DL2 (10 patients) Grade 3: diarrhoea 20%, transaminitis 10%
[79] Phase II	cT3–4/N+	Cetux weeks 1–5 Irino 40/week weeks 1–5 Cape 500 BD	50.4	50	8% (4/50)	All R0 68% moderate to good tumour regression	Grade 3: diarrhoea 30%, transaminitis 10%, acne-like rash 6%, abdominal pain 4%; anaemia, leukopenia, N&V 2% Grade 4: 2% leukopenia
[80] Phase II	mrT3 or resectable T4 or N+	Cetux weeks −1–5 Irino 40/week weeks 1–5 Cape 825 BD 5 days/week ADJ: 5-FU/LV	50.4	39	23.1% (9/39)	T-DS 53.8% All resections R0, SSS 79.5% 3 year DFS 80%, OS 94.7% *KRAS* MT 13.2% (5/38). NSD in TRG, DFS versus WT	Grade 3: leukopenia 7.7%; neutropenia, diarrhoea 5.1%; anaemia, fatigue, rash, ileus 2.6% Grade 4: leukopenia 2.6%
[81,82] Phase II	cT2-4/N0-2	Cetux weeks −1–5 Irino 60/week weeks 1–4 Cape 650 BD 5 days/week	45	82	18% (14/76)	R0 89% T-DS 51% N-DS 78% 4 cCR did not have surgery	Grade 3: diarrhoea 25%, acne-like rash 9%, fatigue 8%; thrombotic event, febrile neutropenia 1% Grade 4: thrombotic event 6%, febrile neutropenia 1%
Cetuximab and oxaliplatin with neoadjuvant CRT phase I or II trials							
[83] Phase I	uT3-4/N1	Cetux weeks 1–9 Oxali 100 days 2,23 Cape 800 BD 5 days/week weeks 1–6	50.4	23	17% (4/23)	DS 57%	Oxali stopped after 10 patients (radiosensitising toxicities) 3 withdrew: 2 EGFR skin toxicity, 1 RT-induced ileus
[84] Phase I/II	cT2 N1-2/ T3–T4 N0–2 +/− M1	PI: Cape BD weeks 1–2,4–5 DLs 500/650/825, Cetux weeks −1–6, Oxali 50/week weeks 1–5 PII: Cape DL3 Cetux/Oxali as above	50.4	PI: 13 PII: 48	9% (4/45)	PII: 11% M1 RT given as prescribed 92% T-DS 47% N-DS 58% Resected 95%, R0 93%	PII: death multi-organ failure (DPD deficient) 2% Grade 3: diarrhoea 17%, radiation dermatitis 8%; transaminitis, infection/fever, SN 6%; leukopenia, N&V, acne-like rash 4%; TCP, ileus, cardiac toxicity, allergic reaction/ hypersensitivity, proctitis 2%
[85] Phase II	T3-4Nx/T2N+ M1	Cetux (regimen not stated) Oxali 50/week weeks 1–5 Cape 800 BD daily ADJ: FOLFOX/Cetux	50	19	Not given	63% CRT completed 68% resected	Grade 3: diarrhoea 26% Stopped at interim analysis (toxicity/ lack efficacy)
[86] Phase II	LARC *KRAS* WT	NA: 2 × 21 day cycles Cape 1000 BD days 1–14, Oxali 130 & BEV 7.5 mg/kg day 1 CRT: Cetux 400 2-weekly Cape 650 BD daily	50.4	10	50% (2/4)		Available for 8 patients Grade 3: proctitis 13%

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Table 4 (*continued*)

Reference	Disease stage*	Systemic treatment (mg/m^2)	RT (Gy)†	*n*	pCR	Other end points	Grade 3–4 toxicity (non-surgical)
Other anti-EGFR agents with neoadjuvant (C)RT phase I or II trials							
[87] Phase I	II–IV or recurrent	Gefitinib 250 mg OD Cape DL1 650 BD DL2 825 BD	50.4	6	0%	DLT at DL1, no recommended dose 67% resected, 75% R0	Grade 4 DL1: diarrhoea 16% (=1 patient, RT interrupted 2 weeks) Arterial thrombus 16% (=1 patient, died secondary to pneumonia. Had subclinical liver metastases).
[88] Phase I/II	cT2N1–2 or cT3N0–2	Gefitinib (mg) DL1: 250 DL2: 500 (dose for PII) 5-FU 225 ADJ: 5-FU/LV x6 (ypN+)	50.4	PI: 6 PII: 33	PII: 30.3% (10/33)	PII: Mandard TRG 2 21.2% RT and 5-FU break 12.8% Gefitinib DR 61.5%	Grade 3/4: overall 41% (no grade 4 in group A), skin toxicity 15.3%, diarrhoea 12.8%, GU toxicity 10.2%, N&V 7.6%; CVS, musculoskeletal, constitutional 3%.
[89] Phase II	cT3-4 or N+ *KRAS* WT	Panitumumab 6 mg/kg days 8,22,36,50 ADJ: recommended	45	19	0% (0/17)	DS 35% Dworak TRG 3 41% Median dose intensity: panitumumab 99.4%, RT 94.3% Median follow-up 53 months: DF 10%, LRF 5%, both 5%. 16% died due to disease progression.	Grade 3: leukopenia 26%; infection, hypophosphatemia 16%; anaemia, anorexia, acneiform rash, dermatitis, asthenia, cardiac arrhythmia, hypokalaemia 5%. Grade 4: infection, hypomagnesemia, hypophosphatemia, thromboembolic event 5%
[90] Phase II	cT3/4 or N+	Nimotuzumab 400mg days −6,1,8,15,22,29 Cape 825 BD daily ADJ: CAPOX for 4 months	50.4 prone	21	19% (4/21)	Dworak TRG 3 29% 3 year DF 33%, PFS 63.9%, OS 70%	Grade 3: diarrhoea 10%, leukopenia 5%, AST/ALT raised 5%

Cetux, cetuximab (given weekly as 400 mg/m^2 loading dose then 250 mg/m^2 unless otherwise stated); 5-FU, 5-fluorouracil; LV, leucovorin; Oxali, oxaliplatin; Irino, irinotecan; BEV, bevacizumab; Cape, capecitabine; OD, once a day; BD, twice a day; P, phase; ADJ, adjuvant; DL, dose level; DR, dose reduction; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; DS, downstaging (T, tumour; N, node); u, ultrasound; mr, magnetic resonance; c, clinical; EMVI, extramural venous invasion; WT, wild type; MT, mutant; GU, genitourinary; CVS, cardiovascular system; SN, sensory neuropathy; TCP, thrombocytopenia; PPE, palmar plantar erythema; N&V, nausea and vomiting; ALT, Alanine transaminase; AST, Aspartate aminotransferase; DPD, dihydropyrimidine dehydrogenase; TRG, tumour regression grade; CRM, circumferential resection margin; SSS, sphincter sparing surgery; AL, anastomotic leak; PE, pulmonary embolism; MI, myocardial infarction; NSD, no significant difference; LARC, locally advanced rectal cancer;RT, radiotherapy; p/cCR, pathological/clinical complete response; RCT, randomised controlled trial; LRF, locoregional failure; OS, overall survival; DFS, disease-free survival; DF, distant failure;PFS, progression-free survival.

* M0 unless stated otherwise.

† RT starting at day 1 given 1.8 Gy per fraction.

Table 5
Newer agents in combination with radiotherapy (RT) in early phase trials

Reference Phase	Stage*	Investigational medicinal product (IMP)	Neoadjuvant systemic treatment (mg/m^2)	RT (Gy/ fractions)†	*n*	pCR	Other end points	Non-surgical toxicity (grade 3–4)	Surgical outcomes
DNA repair									
[100] Phase I	Palliative CRC ($n = 16$), stomach ($n = 1$)	Vorinostat DL1: 100 mg DL2: 200 mg DL3: 300 mg DL4: 400 mg		30/10 pelvis	17 DL1: 1 DL2: 4 DL3: 6 DL4: 6	N/A	Volume reduction mean 26% (range −28%–54%) 100% RT completion MTD 300 mg	Grade 3 related to IMP: DL3: fatigue, anorexia 17% DL4: diarrhoea 33%; fatigue, anorexia, hyponatraemia, hypokalaemia 17%	
[98] Phase I	II/III	Veliparib 20–400 mg BD day 2 to 2 days post RT	Cape 825 BD 5 days/week	50.4/28	30	28%	DS 72% Recommended dose 400 mg BD	Grade 3/4: diarrhoea 7%; anaemia, lymphopenia 3% DLTs: 1 radiation dermatitis (70 mg; dose interruption), 1 N&V (400 mg; discontinued)	25 resected to date
PI3k/Akt									
[101] Phase 0/ I	M1	Nelfinavir 1250mg BD days −7 − 7		25/5	8	N/A	mrTRG 0–3 50%	Grade 3: rash, lymphopenia, diarrhoea 12.5%	
[102] Phase I	mrT3–4 N0–2	Nelfinavir DL1 750 mg BD DL2 1250 mg BD DL3 1000 mg BD (intermediate)	Cape 825 BD	50.4/28	11	27%	T–DS 45%, N–DS 73% DL1 = MTD	DL2 (3 patients): 1 raised transaminases, 1 cholangitis DL3 (4): 2 grade 3 diarrhoea, 1 raised transaminases	100% resected DL3: 1 grade 4 wound complication (unlikely related)
Tyrosine kinase inhibitors									
[103] Phase I	LARC	Cediranib OD from day −10- completion CRT DL1: 15 mg DL2: 20 mg DL3: 30 mg	Cape 825 BD	45/25	DL1: 3 DL2: 3 DL3: 3 (+ 3)	40% (2/5 to date)	2 pCR (DL2), 4 cCR (1 DL1, 1 DL2, 2 DL3), 2 partial response on mr (DL2) to date	Grade 3: DL3 Lethargy 1 patient	100% R0
[104] Phase II	T3-4 or N+ *KRAS* MT	Sorafenib 400 mg	Cape 825 BD	40/25	40	15%	DS 81.6% Dworak TRG 3 45% Median dose intensity RT 100%, Sorafenib 100%, Cape 98.6%	Grade 3: diarrhoea 15%, skin toxicity outside RT field 12.5%, pain 7.5%; radiation skin toxicity, proctitis, fatigue, cardiac ischaemia 5%; neutropenia, raised creatinine 3%. Grade 4: neutropenia 3%	94.7% R0, 89.5% SSS
Other									
[105] Phase I	cT3/4 or N+, 0-9 cm from dentate line or 3 −12 cm from anal verge	Celecoxib 400 mg BD	Cape BD 5 days/week DL0: 550 DL1: 625 DL2: 700 Irino days	50.4/28	14 DL0: 3 DL1: 3+3 DL2: 5	15%	DS 69% MTD = DL1 Median 30 month follow-up: DFS 72%, death 14%	Grade 3/4: DL1: diarrhoea, vomiting, dehydration 17%. DL2: diarrhoea, TCP 20%	93% resected, 61% SSS, 92% R0

(*continued on next page*)

Table 5 (*continued*)

Reference Phase	Stage*	Investigational medicinal product (IMP)	Neoadjuvant systemic treatment (mg/m^2)	RT (Gy/ fractions)†	*n*	pCR	Other end points	Non-surgical toxicity (grade 3–4)	Surgical outcomes
			1,8,22,29 DL0: 30 DL1: 35 DL2: 40						
[106] Phase I	uT3/4 or N+ M0/1	Bortezomib (mg/m^2) DL1: 0.7 DL2: 1.0 DL3: 1.3 DL4: 1.5 Biweekly weeks 1,2,4,5	5-FU 225/day from day 2	50.4/28	9 DL1: 3 DL2: 6	11%	Microscopic residual 11% MTD 0.7 mg/m^2	Grade 3/4: DL1 chest pain, diarrhoea, hyperglycaemia, lymphopenia 17%. DL2 diarrhoea 67%; hypoalbuminemia, hypokalaemia, ileus 33%	100% resected
[107] Phase I	T3/4 or N+ or local recurrence	TNFerade^{TM} (TNF-α) 4×10^{10} particle units direct tumour injection weekly × 5	Cape 937.5 BD 5 days/ week	45/25 + 5.4 −9 Gy boost	9	22%	Microscopic foci/no residual 78% Median follow-up 41.6 months: 1 death (metastases), 2 DF, 2 LRF (1 also DF)	Grade 3/4: lymphopenia 100%; hypophosphatemia, hyponatraemia 50%, neutropenia 38%; anaemia, diarrhoea, small bowel obstruction, LFT abnormalities 25%, enteritis 13% 1 patient stopped CRT	89% SSS
[108] Phase I	II/III	Nitroglycerin transdermal patches (mg/h) DL1 0.2 DL2 0.4 DL3 0.6	5-FU 225/day	45–50/ 25–28	13	17%	No DLTs 8% RT break	Grade 3: lymphopenia 31%; diarrhoea, headache, mucositis 8%.	92% resected, 67% SSS

5-FU, 5-fluorouracil; Irino, Irinotecan; Cape, Capecitabine; OD, once a day; BD, twice a day; DL, dose level; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; DS, down-staging (T, tumour; N, node); u, ultrasound; mr, magnetic resonance; c, clinical; MT, mutant; TCP, thrombocytopenia; TRG, tumour regression grade; SSS, sphincter sparing surgery; LARC, locally advanced rectal cancer; CRC, colorectal cancer; CRT, chemoradiotherapy; pCR, pathological complete response; N&V, nausea and vomiting; DFS, disease-free survival; DF, distant failure; LRF, locoregional failure;

* M0 unless stated otherwise.

† RT starting at day 1.

showed no difference in 3 year DFS or overall survival between *KRAS* wild-type and mutant groups. Updated analysis of the EXPERT-C data by Sclafani *et al.* [95] showed no significant difference in 5 year overall survival at 83.8% versus 70.0% ($P = 0.20$) with and without cetuximab in *KRAS* wild-type.

Table 4 lists trials in which cetuximab was added to combination chemotherapy regimens including oxaliplatin or irinotecan with fluoropyrimidine and radiotherapy. Combining three systemic agents seems to increase toxicity and two trials using oxaliplatin [83,85] were altered or closed early for this reason. pCR varied between 8 and 50% and downstaging rates did not seem to be different from those who received just two systemic agents.

Other EGFR inhibitors are now under evaluation with CRT in rectal cancer, including gefitinib, panitumumab and nimotuzumab. Erlotinib has also been studied in combination with bevacizumab [64] (Table 3). pCR rates range between 0 and 30% with downstaging rates of 21–35%. Jin *et al.* [90] had a high proportion of *KRAS* wild-type patients at 86%, which they postulate may have contributed to the pCR rate of 19% with nimotuzumab. However, in the study with panitumumab [89], which only included participants with *KRAS* wild-type, the pCR rate was 0%. The trials concluded that toxicity was generally manageable. The use of cetuximab or other EGFR inhibitors in combination with CRT therefore seems to be feasible, but there is little evidence of improvement in clinically important outcomes in early phase trials. Relevant and robust biomarkers of efficacy and toxicity will be required for further assessment of EGFR inhibitors in larger randomised trials.

Newer Agents

In recent years, advances in sequencing technology have seen a rapid expansion in our knowledge of the range of genetic mutations found in CRC and characterisation of the associated phenotypes. This knowledge has suggested potential new targets for therapy, some of which are relevant to radiosensitisation.

There has been increasing interest in novel radiosensitisers that prevent repair of DNA damage caused by ionising radiotherapy. Pre-clinical data have found that poly(ADP-ribose) polymerase (PARP) inhibitors prevent PARP from facilitating repair of single-strand breaks, leading to inability of DNA replication in S-phase and the formation of lethal double-strand breaks [96]. In CRC with microsatellite instability, there is evidence of a deficient double-strand break repair system related to mutations in coding microsatellites. This deficiency may be exploited using PARP inhibitors in a manner similar to their successful use in BRCA1-mutant breast cancers [97]. Czito *et al.* [98] recently published early results of a phase I trial of the oral PARP inhibitor, veliparib, in combination with CRT for rectal cancer. pCR was 28%, and 72% of 25 patients treated had tumour downstaging. Two dose-limiting toxicities (DLTs) were experienced and a recommended dose for phase II trials of 400 mg BD was advocated by the investigators. The histone deacetylase inhibitor, vorinostat, acts as a radiosensitiser by downregulating proteins involved in the signalling and repair of radiation-induced double-strand breaks [99]. Ree *et al.* [100] added vorinostat to palliative pelvic radiotherapy in 14 patients, most with CRC. The mean reduction in tumour volume was 26% (range −28%–54%) but toxicity was higher than with single-agent treatment (Table 5).

Genetic alterations in the RAS-MAPK and PI3-K/AKT pathways are common in CRC [109]. The latter is often overactivated in solid tumours and is an important tumour cell survival pathway with emerging evidence of involvement in hypoxia-related treatment resistance [110]. Nelfinavir, an AKT-inhibitor in the PI3-K pathway, is a radiosensitiser that has been shown in preclinical models to reduce tumour angiogenesis and hypoxia [111]. In an early phase study of patients undergoing short-course palliative pelvic radiotherapy, Hill *et al.* [101] found good mrTRG 0-3 in 50% of participants and the addition of nelfinavir was generally well tolerated. Buijsen *et al.* [102] undertook a dose escalation study of nelfinavir in patients receiving CRT with capecitabine and recorded a pCR rate of 27% in 12 patients. DLTs included grade 3 cholangitis (one), transaminase elevation (two) and grade 3 diarrhoea (two). By contrast, no DLT related to the study medication was found in studies evaluating nelfinavir for CRT in pancreatic and lung cancer [112,113]. The authors concluded that the combination is therefore feasible but that safety and efficacy, particularly in combination with pelvic radiotherapy, requires further assessment.

The addition of bevacizumab to CRT has had limited success in improving patient outcomes, as discussed previously. Other agents targeting this pathway are under investigation, including cediranib, a VEGF receptor Tyrosine kinase inhibitor (TKI), which has shown good tolerability and promising outcomes in terms of response (pCR = 25%, $n = 8$) [103]. The same study is also evaluating the MEK inhibitor, selumetinib, and final results are awaited. Van Moos *et al.* [104] combined sorafenib, a multikinase inhibitor of pathways including RAF/MEK/ERK and VEGF-R, with capecitabine and radiotherapy in a phase II study involving 40 patients with *KRAS* mutant disease. With pCR rates of 15%, downstaging of 81.6% and acceptable toxicity levels, they concluded that this regimen also warrants further investigation.

Bortezomib is a proteasome inhibitor that results in cell cycle redistribution and inhibition of transcription factors such as nuclear factor-κB (NF-κB), which promotes cell survival and proliferation and has been shown to be activated in CRC models in response to chemotherapy and radiation [114]. However, O'Neill *et al.* [106] saw mixed results on analysis of NF-κB gene expression in a clinical study of bortezomib with CRT. The trial was unable to recommend a biologically meaningful dose due to toxicity, similar to Edelman *et al.* [115] in lung cancer CRT, where delayed toxicity was a concern. Other phase I trials have analysed the effects of the cyclooxygenase-2 (COX-2) inhibitor celecoxib [105] and nitric oxide donor nitroglycerin [108] in combination with CRT, both of which showed potential as

radiosensitisers in preclinical models and, the authors suggested, warrant further investigation following phase I studies. Another early phase trial utilised intratumoral injections of tumour necrosis factor-alpha (TNF-α), a soluble cytokine that mediates cellular immune response, alongside CRT in rectal cancer and found that its use was feasible in this setting. Increased toxicity attributed to the TNF-α included fever, rigors and fatigue, all of which were low grade [115].

Increasing interest in the use of anti-cancer immunotherapy may indicate one future direction for novel radiosensitiser research. Radiotherapy has been noted to contribute to anti-tumour immunity through a variety of mechanisms, including release of tumour antigen through cell death, triggering of proinflammatory signals, which leads to activation of tumour-specific T-cells, and effects on the tumour microenvironment, which enhance T-cell infiltration [116]. In addition, a systemic anti-tumour immune response outside of the radiation field, termed the abscopal effect, has been described after radiotherapy. Pre-clinical models and early phase trials summarised in [117] have suggested a role for the promotion of T-cell priming with radiotherapy. The checkpoint inhibitors CTLA-4 and PD-1, which play a key role in the negative regulation of T-cell activity, are potential targets and blockade of the latter has been shown in animal models of CRC to enhance the therapeutic efficacy of radiotherapy [118]. Combining radiotherapy with other immunostimulatory agents may enhance their efficacy. The use of tumour neoantigens to stimulate immune response, for example through the creation of synthetic vaccines, has also been suggested as a novel treatment strategy with radiotherapy [116] and may be particularly relevant in cancers such as CRC, which have a high somatic mutation prevalence [119].

Discussion

Various explanations have been provided as to the limited improvement in outcomes seen with the addition of radiosensitising agents to standard CRT. It has been hypothesised that optimal radiotherapy with fluoropyrimidines already maximises the local response with little scope for increasing the radiosensitiser effect [30]. Systemic agents may target different phases of the cell cycle, and radiosensitisation may be partly dependent on cell cycle synchronisation of the tumour cell population. Given that fluoropyrimidines are S-phase specific and oxaliplatin is also mainly active in this phase, cell cycle delay or arrest may limit effectiveness and increase time for DNA repair. This may be of concern with agents such as cetuximab, which can lead to G1 or G2/M cell cycle arrest [120]. An understanding of the underlying biological mechanisms of novel radiosensitisers will be fundamental to future research in this area.

Toxicity is a significant consideration when combining radiosensitising agents with radiotherapy. There is evidence that delivery of radiotherapy and therefore treatment efficacy could be compromised due to the toxicity of additional therapies such as oxaliplatin [37]. Nonetheless, the importance of radiotherapy quality assurance should not be overlooked when investigating systemic agents in this context. For example, Bratland *et al.* [121] found that toxicity with vorinostat and pelvic radiotherapy was considerably higher than with the drug alone, but upon reanalysis of their data, radiation dose-volume effects were identified as a potential contributory factor. Toxicity has also limited the combination with CRT of higher than radiosensitising doses of chemotherapy, which had been postulated to provide a survival benefit through the treatment of systemic micrometastases [30].

In the case of biological agents, there is currently a paucity of direct comparison with placebo and limited ability to make accurate patient selection. Gollins *et al.* [82] in their study of CRT with cetuximab found retrospectively that in 22% of the participants where EGFR pathway mutation status was available, this differed between biopsy and surgical resection specimen, leading them to question the validity of using biopsy-derived mutation status to select treatment. Effective predictive biomarkers are required to select patients who would benefit most from targeted therapy. Cubillo *et al.* [122] have started to personalise CRT based on molecular markers with some success and this may indicate the direction of future research, in a similar vein to the molecular selection strategy seen in the FOCUS4 trial in metastatic CRC [123]. In addition, future work with biological therapies must also strive to further characterise the safety profile, given the mixed evidence of increased perioperative toxicity seen, particularly with anti-angiogenesis agents.

Finally, choosing appropriate end points will be essential for any future research. Although pCR has been found to reflect good outcomes for that patient group [124,125], Kennecke [59] reported that central review changed pathological stage in six cases (16%) in a trial of CRT with oxaliplatin and bevacizumab, highlighting a potential issue with this measure. Clinically relevant, robust outcomes will provide valuable evidence for any change to standard CRT regimens for locally advanced rectal cancer.

Conclusions

The addition of further cytotoxic agents to standard CRT has thus far provided largely disappointing results, with little improvement in outcomes, often at the expense of greater toxicity. With the advent of biological therapy, it was hoped that their use as radiosensitisers would provide significant synergistic effect without the side-effects of cytotoxic agents. However, in the case of bevacizumab and cetuximab, their addition to CRT with fluoropyrimidines has not proved successful so far. Debate exists as to whether the use of surrogate end points such as pCR adequately reflects long-term clinical outcomes, and which primary end point to use will remain an important consideration for future research. Nevertheless, the ability to tailor targeted therapy based upon a greater understanding of tumour genetic information, increasing

availability of predictive biomarkers and a growing range of novel agents still has the potential to improve patient outcomes in the future.

Conflict of Interest

Charles Dearman is a freelance managing editor for Elsevier.

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