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Is There a Best Radiosensitizing Agent in the Treatment of Locally Advanced Rectal Cancer?

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Abstract Over the past several decades, the management of localized rectal cancer has evolved from surgery alone as the definitive treatment to incorporating both radiation and chemotherapy to improve rates of local control and disease-free survival. Several chemoradiation regimens have been tested with different mechanisms of action, efficacy, and toxicity. There is little debate that concurrent radiation and a fluoropyrimidine (5-fluorouracil (5-FU) or capecitabine) is the current standard of care prior to total mesorectal excision (TME). Attempts to add additional chemotherapy, such as oxaliplatin or irinotecan, have not consistently improved results. Recent attention has been given to concurrent biologic therapies (vascular endothelial growth factor (VEGF)-, epidermal growth factor receptor (EGFR)-, Poly(ADP-ribose) polymerase (PARP)-inhibitors, etc.) which may improve the outcomes of multi-modality therapy; however, the evidence is limited to phase I/II trials. It is critical for oncologists to be aware of various radiosensitizing agents that have been investigated and which will provide the best chance of disease control. In this review, we describe the mechanisms of action and evidence supporting these regimens to determine if there is a best radiosensitizing agent. Furthermore, we describe the relevant studies investigating the recent use of biologic radiosensitizers and the future direction using those agents.

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Introduction

Forty thousand patients are diagnosed with rectal cancer in the USA annually [1]. Surgical resection remains the foundation of curative treatment for localized disease. To improve outcomes, radiation therapy (RT) and chemotherapy have been incorporated in the neoadjuvant and adjuvant setting [2]. RT inhibits cell proliferation through mitotic catastrophe and apoptotic cell death, thereby inhibiting tumor growth [3]. Radiosensitizing agents act in synergism with RT resulting in improved local control and distant recurrence rates. Secondary to its benefit in decreasing local recurrence, neoadjuvant chemoradiotherapy (CRT) preceding total mesorectal excision (TME) is the gold standard for the treatment of clinical stage II (T3–T4, node negative) and III (any T, node positive) rectal cancer according to the National Comprehensive Cancer Network (NCCN) guidelines version 2.2015 [4].

Concurrent CRT dates back to the 1950s when investigators began searching for chemical agents to enhance the effects of radiation [5]. The radiosensitivity of tumors and normal tissues is often similar, but in some cases, the tumor cells may be more resistant to treatment than surrounding normal tissues. External-beam photon radiation leads to the production of reactive oxygen species (ROS) and ionization of target molecules such as DNA, creating single-strand (SSB) or double-strand breaks (DSB), which can be lethal to cells if not repaired efficiently. When radiation is combined with traditional chemotherapy, these effects can be potentiated as a result of the disruption of normal cellular repair pathways.

In the USA, RT is typically delivered in 25 fractions (1.8 Gy/fraction), followed by a boost to the rectal tumor with a margin to 5.4 Gy in 3 fractions (cumulative rectal tumor dose 50.4 Gy). Short-course RT (25 Gy in 5 Gy fractions) is used at some institutions and more commonly in Europe [6]. The primary goal of RT is to decrease the risk of local recurrence, and in some cases, to downstage tumors to increase resectability and improve the chance of anal sphincter preservation. The introduction of total mesorectal excision (TME) alone reduced the local recurrence rate from approximately 30 to 10–20 % depending on the tumor stage [7•]. Prior to the implementation of TME, the addition of postoperative RT or CRT decreased local recurrence from 25–50 to 16–25 and 8–11 %, respectively [8–11]. The risk of distant metastases was 46 % with RT alone and 29 % with CRT in stage II/III patients [11].

Adjuvant CRT had been the standard of care until the German Rectal Cancer Trial showed superior local disease outcomes with neoadjuvant vs. adjuvant CRT [12, 13]. At 4 years of median follow-up, preoperative CRT was associated with a lower pelvic relapse rate (6 vs. 13 %, $p = 0.006$), which persisted with 10 years of follow-up (7 vs. 10 %, $p = 0.048$) [13], but the disease-free survival (DFS, 68 % each) and overall survival rates (OS, 76 vs. 74 % at 5 years, and 60 % each at 10 years) were similar for both groups. This study set the current standard of care in localized rectal cancer, upon which new approaches will be built in both contemporary and ongoing trials.

This review will discuss the mechanisms of action of chemotherapy, angiogenesis (vascular endothelial growth factor (VEGF)) inhibition, epidermal growth factor receptor (EGFR) inhibition, and novel radiosensitizers as we try to answer the question of whether there is a best radiosensitizing agent.

Chemotherapy Radiosensitizers

5-Fluorouracil

There are many potential mechanisms for the synergism between radiation and 5-fluorouracil (5-FU), but. the prevailing mechanism is thought to be the dysregulation of the G1/S checkpoint. This can cause the inappropriate progression of irradiated tumor cells through the S-phase of the cell cycle in the presence of 5-FU, leading to the inability to repair radiation-induced SSBs and DSBs, and mitotic catastrophe [14]. Several reports have demonstrated that 5-FU should be present for prolonged times before and after RT for best synergistic effects; hence, the use of infusional 5-FU with RT. Other potential mechanisms of sensitization include radiation-induced increase in intratumoral 5-FU levels and 5-FU dysregulation of the G2-M checkpoint, which cells are synchronized in after radiation leading to inability to repair damage prior to mitosis [15, 16].

Several randomized trials addressed the question of whether concurrent administration of chemotherapy with RT is needed for the treatment of rectal cancer. The EORTC 22921 trial randomized 1011 patients between 1993 to 2013, in a 2×2 fashion to preoperative RT ± bolus 5-FU and leucovorin (LV) vs. preoperative RT alone (45 Gy over 5 weeks) followed by surgery and either postoperative (adjuvant) 5-FU/LV or surveillance [38]. Though the adjuvant portion of the study was poorly adhered to, the neoadjuvant aspect of the trial was within expected tolerance and dosing. A higher pathologic complete response (ypCR) rate (14 vs. 5 %), less advanced pT and pN staging ($p < 0.001$), and less perineural or lymphatic invasion ($p = 0.008$) were noted with the preoperative CRT regimen vs. RT alone. The benefit of chemotherapy in regards to local control was seen in all chemotherapy groups, with an 11–15 % local relapse rate at 10 years, compared to 22 % with RT alone [39]. The 10-year DFS was similar in patients receiving preoperative CRT vs. RT alone (46 vs. 44 %), and no difference was noted in 10-year overall survival (51 vs. 49 %), irrespective of the use or not of adjuvant chemotherapy. Neoadjuvant CRT has become the standard of care given the improvement in local control, ypCR rates, and the ability for more patients to undergo sphincter-sparing surgery, despite the lack of improvement in DFS and OS in the EORTC 22921 and the German Rectal Cancer Trial [12, 13, 38, 39].

The schedule of concurrent chemotherapy varies between studies. Both bolus 5-FU alone and infusional 5-FU over 5 days with leucovorin on weeks 1 and 5 of RT have been used. In an adjuvant randomized study after curative surgery, compared to bolus 5-FU, continuous venous infusion (CVI) 5-FU during RT improved OS (4-year OS 70 vs. 60 %, $p = 0.005$) and DFS (4-year relapse-free 63 vs. 53 %, $p = 0.01$), but did not significantly decrease local recurrence ($p = 0.11$) [40]; these results established the use of continuous infusion 5-FU as the standard method to deliver 5-FU concurrently with RT.

Capecitabine

Capecitabine is an oral pro-drug of 5-FU [41], allowing for a more convenient continuous dosing during radiation, compared to CVI 5-FU. Several preclinical studies demonstrated radiation induces thymidine phosphorylase (TP), an enzyme that converts capecitabine to active 5-FU. Tumors often contain higher levels of TP then normal tissues at baseline and the addition of radiation can lead to further increased TP levels, thereby increasing the intratumoral concentration of 5-FU [41].

The efficacy and safety of substituting capecitabine for 5-FU was evaluated in a randomized, open-label, non-inferiority phase III trial [19]. Patients were randomized to receive neoadjuvant and adjuvant capecitabine or 5-FU, and both groups received CRT. During RT (50.4 Gy), capecitabine 1650 mg/m^2/day was administered continuously on days 1–38, and 5-

FU was dosed at 225 mg/m^2 daily as CVI. The 5-year OS in the capecitabine group was non-inferior to that in the 5-FU group (76 vs. 67 %, $p = 0.0004$), with a post hoc test for superiority p value of 0.05. The 3-year DFS was 75 vs. 67 % ($p = 0.07$), respectively. The local recurrence rate in each group was similar (6 vs. 7 %), but fewer patients developed distant metastases in the capecitabine group (19 vs. 28 %, $p = 0.04$). Adverse events of diarrhea (53 vs. 44 %, grade 3/4 9 vs. 2 %), hand-foot skin reaction (HFSR, 31 vs. 2 %), fatigue (28 vs. 15 %), and proctitis (16 vs. 5 %) were worse with capecitabine; however, leukopenia was worse in the 5-FU group (35 vs. 25 %). Use of capecitabine in place of CVI 5-FU is reasonable given its ease of use, acceptable toxicity profile, and non-inferiority.

Oxaliplatin

Oxaliplatin has a 1,2-diaminocyclohexane ring in its structure which slows the formation of platinum-DNA adducts, increases the local distortion of the DNA double helix, and inhibits DNA synthesis, cell growth, and repair of DNA damage. Studies in animal tumor models demonstrated it to be more effective than cisplatin in colorectal adenocarcinoma. Given the similarities between cisplatin, a known radiosensitizer, and oxaliplatin, the latter underwent testing in animal models [42]. Oxaliplatin causes cell cycle disruption, enhanced formation of platinum adducts with DNA in the presence of radiation-induced free radicals, and the inhibition of DNA repair of radiation-induced DNA breaks [43]. Oxaliplatin has also been shown to cause cell cycle arrest in G1 and G2, which are considered the most radiosensitive phases of the cell cycle [42].

NSABP R-04 was a four-arm randomized phase III trial of 1608 patients comparing neoadjuvant CRT with 5-FU vs. capecitabine with or without oxaliplatin [22•]. 5-FU was administered as CVI 225 mg/m^2/day 5 days per week, and capecitabine 1650 mg/m^2/day was administered daily 5 days per week. Oxaliplatin was administered weekly at 50 mg/m^2. Capecitabine vs. 5-FU demonstrated comparable downstaging (21.1 vs. 21.3 %, $p = 0.95$), sphincter-sparing surgery (59.3 vs. 59.4 %, $p = 0.98$), and ypCR (20.7 vs. 17.8 %, $p = 0.14$). The addition of oxaliplatin did not improve ypCR (19.5 vs. 17.8 %, $p = 0.42$), sphincter-sparing surgery (57.8 vs. 61.0 %, $p = 0.24$) or surgical downstaging (17.9 vs. 23.5 %, $p = 0.20$) compared to no oxaliplatin. In an updated analysis, the 3-year local recurrence (11.2 vs. 11.8 %), 5-year DFS (66.4 vs. 67.7 %), and 5-year OS (79.9 vs. 80.8 %) were similar for 5-FU vs. capecitabine, and the addition or not of oxaliplatin did not significantly affect these outcomes (11.2 vs. 12.1 %, 69.2 vs. 64.2 %, and 81.3 vs. 79.0 %, respectively) [23]. Oxaliplatin did increase toxicity with a significantly higher rate of grade 3/4 diarrhea (16.5 vs. 6.9 %, $p < 0.001$).

STAR-01 was another randomized phase III trial which compared CRT with CVI 5-FU with or without oxaliplatin at 60 mg/m^2 weekly with a primary endpoint of OS. Preliminary results reported grade 3/4 toxicity of 24 vs. 9 % ($p < 0.001$), with no improvement in ypCR (16 % in both arms, $p = 0.904$) [18]. Similar results with no benefit in local recurrence, DFS or OS with the addition of neoadjuvant oxaliplatin to capecitabine-based CRT were seen in the ACCORD 12 [17] and the PETACC-6 trials [44, 45]. In contrast to the previously described negative trials, the German CAO/ARO/AIO-04 trial compared preoperative 5-FU-based CRT with 5-FU administered at 1000 mg/m^2 per day on days 1–5 and 29–33 during RT, and adjuvant bolus 5-FU for four cycles, to 5-FU-based CRT plus oxaliplatin, followed by 4 months of modified folinic acid, fluorouracil, and oxaliplatin (FOLFOX)6 [46]. The addition of oxaliplatin increased the ypCR rate (17 vs. 13 %, $p = 0.038$), and the 3-year DFS (76 vs. 71 %, $p = 0.03$) [24]. It is possible the benefit seen with the addition of oxaliplatin was due to the suboptimal 5-FU dosing schedule in the neoadjuvant setting and the fact that adjuvant FOLFOX is known to confer superiority to adjuvant 5-FU, especially for higher risk patients. In a recently reported phase II NRG 0822 trial, capecitabine and oxaliplatin (CAPOX) was studied with intensity modulated radiotherapy (IMRT) in an attempt to reduce toxicity. IMRT did not improve gastrointestinal toxicity as grade 2 or higher gastrointestinal adverse events were still high at 52 %, with grade 3/4 diarrhea of 17.6 %. The ypCR rate was 15 %, and locoregional failure was low at 7.4 %. The 4-year rates of DFS and OS were 61 and 83 %, respectively [47]. It has retrospectively been shown that adjuvant 5-FU mostly benefits patients who experience downstaging during neoadjuvant CRT [48], while adding oxaliplatin to adjuvant 5-FU may particularly benefit patients who do not experience downstaging with 5-FU-based CRT [49]. Although adjuvant 5-FU and oxaliplatin seems warranted, especially for stage III rectal cancer patients, neoadjuvant oxaliplatin should not routinely be used concurrently with RT.

Irinotecan

Irinotecan (CPT-11) is an analog of camptothecin, an alkaloid that inhibits the enzyme topoisomerase I (TOPO I), which normally produces single-strand breaks (SSB) during DNA synthesis. Irinotecan inhibits re-ligation through forming CPT-11-TOPO1-DNA complexes. Irinotecan has been shown in preclinical studies to synergize with ionizing radiation, which causes SSBs through the indirect effects of free radical formation [50]. As the advancing replication fork interacts with the aforementioned complex, the radiation-induced SSB is converted into a double-strand break, resulting in mitotic catastrophe. Furthermore, irinotecan is metabolized to SN-38, a more active metabolite. In vitro studies found synergy was dependent on drug concentration and timing, with increased radiosensitivity present only when the drug was present at the time of, or shortly after (24–48 h.) radiation [51, 52].

Non-randomized trials demonstrated encouraging results with the addition of irinotecan to fluoropyrimidine-based RT, but this effect has not been conclusively demonstrated in a randomized phase II trial, where both the control and the experimental arm had superior outcomes [20]. One hundred and six patients with T3/T4 distal rectal cancers were randomized in a phase II study to CVI 5-FU with or without irinotecan 50 mg/m^2 once weekly for 4 weeks. The ypCR rates were 26 vs. 30 % and locoregional recurrence rates were similar (17 and 16 %, respectively). The 5-year DFS rates were 85 vs. 78 %, and 5-year OS rates were 75 vs. 61 % with or without irinotecan, respectively. Generally, the treatment was well tolerated.

A retrospective analysis compared capecitabine (825 mg/m^2 BID continuously) vs. capecitabine plus irinotecan-based CRT (capecitabine 825 mg/m^2 BID Monday through Friday, irinotecan 40 mg/m^2 weekly) among 231 patients with T3/T4 tumors [53]. The 5-year local control rate (92 vs. 93 %, $p = 0.875$), relapse-free survival (81 vs. 76 %, $p = 0.685$), and OS (89 vs. 92 %, $p = 0.723$) were not different between the two groups.

Irinotecan has also been combined with S-1 (tegafur/gimeracil/oteracil) in a neoadjuvant phase 1/2 trial in 115 patients with T3/T4 tumors [54]. The ypCR rate was 35 % and secondary endpoints of 5-year local recurrence-free survival, OS, and DFS were 93, 87, and 79 %, respectively. In the Japanese population, this regimen was well tolerated, with only 6 % of patients experiencing grade 3 toxicities. Based on the current data, irinotecan should not be combined with fluoropyrimidine-based CRT in rectal cancer patients. Tables 1 summarizes landmark neoadjuvant chemoradiotherapy trials for rectal cancer.

Table 1 Landmark neoadjuvant chemoradiotherapy (CRT) trials for localized rectal cancer

Study/design	Eligibility	Radiosensitizing chemotherapy	Results
Gerard et al. (2010) [17]	T3–4	Cape	ypCR 13.9 %
Phase III		Cape-Ox	ypCR 19.2 %
ACCORD			
Aschele et al. (2011) [18]	T3–4 and/or N1–2	CVI 5-FU	ypCR 16 % both arms
Phase III		CVI 5-FU + Ox	
STAR-01			
Hofheinz et al. (2012) [19]	T3–4 and/or N1–2	5-FU	5-year OS 67 vs. 76 %
Phase III		Cape	*(non-inferior)
Mohiuddin et al. (2013) [20] Phase II	T3–4	CVI 5-FU	5-year DFS 78 vs. 85 %
		CVI5 FU + Irino	5-year OS 61 vs. 75 %
			no p values reported
Schmoll et al. (2014) [21]	T3–4 and/or N+	Cape	3-year DFS 74.5 %
Phase III		Cape + Ox	3-year DFS 73.9 %
PETACC-6			
O'Connell et al. (2014) [22•]	T3–4 and/or N1–2	CVI 5-FU	5-FU vs. Cape
Allegra et al. (2015) [23]		CVI 5-FU + Ox	3-year LR 11.2 vs. 11.8 %
Phase III, 2 × 2		Cape	5-year DFS 66.4 vs. 67.7 %
NSABP-R04		Cape + Ox	5-year OS 79.9 vs. 80.8 %
			Ox vs. No Ox
			3-year LR 11.2 vs. 12.1 %
			5-year DFS 69.2 vs. 64.2 %
			5-year OS 81.3 vs. 79.0 %
Rodel et al. (2015) [24]	T3–4 or N+	5-FU	3-year DFS 71.2 %
Phase III		5-FU + Ox	3-year DFS 75.9 %*
CAO/ARO/AIO-04			

Cape capecitabine, *CVI* continuous venous infusion, *CRT* chemoradiation, *DFS* disease-free survival, *Irino* irinotecan, *LR* local recurrence, *OS* overall survival, *Ox* oxaliplatin, *RT* radiotherapy, *ypCR* pathologic complete response after neoadjuvant therapy

*statistical significance $p < 0.05$

Anti-Angiogenesis Therapy

The mechanisms of interaction between angiogenesis-targeting agents and ionizing radiation are complex and involve the tumor stroma, vasculature, and the tumor cells themselves [55]. Anti-angiogenic agents destroy immature vessels and stabilize intact blood vessels thus delivering oxygen more efficiently. Given the need for oxygen as a substrate for radiation-induced free radical formation, improved oxygenation can improve the effects of ionizing radiation [56]. Preclinical and initial clinical reports demonstrated bevacizumab increased tumor blood flow, decreased vascular density and interstitial pressure, and reduced tumor regrowth after radiation [57, 58].

Bevacizumab

Bevacizumab is a monoclonal antibody blocking the action of vascular endothelial growth factor (VEGF). Several retrospective trials exploring bevacizumab with CRT demonstrated promising results [25]. A multicenter randomized phase II trial was conducted among 90 patients assigned to capecitabine 825 mg/m^2 BID daily with RT with or without bevacizumab 5 mg/kg on weeks 1, 3, and 5 [36]. Bevacizumab increased the incidence of fatigue (57 vs. 24 %) and HFSR (14 vs. 6 %). Surgery was performed after a median interval of 51 days after CRT completion, with no difference in postoperative complications between arms. The ypCR rates with and without bevacizumab were comparable (16 vs. 11 %, $p = 0.54$).

The ECOG 3204 phase II trial treated 54 patients with preoperative CRT with capecitabine, oxaliplatin, and bevacizumab followed by adjuvant FOLFOX plus bevacizumab, with the primary endpoint being a ypCR of at least 30 % [59]. The peri-operative toxicity of this regimen was significant. Grades 3 and 4 non-hematologic toxicities, mostly fatigue, dehydration, diarrhea, and rectal pain, occurred in 53 and 15 % of the patients, respectively. Acute postoperative complications among nine patients (18 %) included wound infection ($n = 8$), fascial dehiscence ($n = 5$), abscess, fistula, bowel obstruction, and thrombosis/embolism ($n = 1$ each), while late surgical complications occurred in 47 % of the patients. Only 54 % of patients received any adjuvant therapy. This regimen has not been further pursued due to a low ypCR rate (17 %, 90 % confidence interval (CI) 9–27 %) and the associated toxicity. An updated analysis recently reported the 5-year relapse-free survival of 81 % and 5-year OS for the ITT population of 80 %, similar with historical data with fluoropyrimidine-based CRT [35].

Aflibercept

Aflibercept (US Ziv-aflibercept) is an anti-angiogenic recombinant fusion protein that binds to VEGF-A, VEGF-B, and placental growth factor (PlGF). A phase II study combined neoadjuvant aflibercept with 5-FU-based CRT followed by 4 months of adjuvant mFOLFOX6 plus aflibercept [34]. Patients received standard CVI 5-FU and RT and aflibercept (4 mg/kg IV, days 1 and 15) for 6 weeks. Six weeks from the last dose of neoadjuvant aflibercept, patients underwent surgical resection. Among 39 patients treated, 95 % received all preoperative treatment, 82 % underwent resection, and 54 % received postoperative treatment. Postoperative complications included pelvic abscess ($n = 2$) and GI fistula ($n = 1$). The ypCR rate was 25 %, and with a peri-operative complication rate of 10 %, this regimen was not deemed to be of further interest.

Sorafenib

Sorafenib is a multikinase inhibitor that blocks the receptor tyrosine kinase vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and the RAF serine/threonine kinases along the RAF/MEK/ERK pathway. Given its multikinase activity, sorafenib 400 mg a day was evaluated in a phase I/II study among 54 KRAS mutated rectal cancer patients with capecitabine and RT [30]. The pCR rate is reported as 60 % comprised of 15 % complete response and 45 % near complete response. Another phase I study using CVI 5-FU and sorafenib with RT in 17 patients, found 200 mg daily sorafenib poorly tolerated secondary to skin toxicity and mucositis, but 400 mg BID dosed 5 days a week seemed well tolerated [37]. The study noted ypCR of 36 %, and downstaging in 86 % of all patients.

Anti-Epidermal Growth Factor Therapy

Epidermal growth factor receptor (EGFR) signaling promotes cellular proliferation, migration and invasion, transformation, differentiation, and angiogenesis. EGFR inhibition produces synergism with radiation mostly through blocking cellular proliferation and induction of apoptosis [60]. The most successful implementation of an EGFR inhibitor in combination with RT has been in locally advanced head and neck cancers [61]. Cetuximab and panitumumab are monoclonal antibodies targeting EGFR [62], and approved to treat metastatic KRAS wild-type colorectal cancer [63, 64]. Cetuximab with capecitabine-based CRT did not show activity and no patient achieved ypCR in an Austrian Breast and Colorectal Study Group phase II trial [29]. A phase I/II study treated 60 patients

with neoadjuvant RT with capecitabine (825 mg/m^2 BID days 1–14 and 22–35); oxaliplatin (35–50 mg/^{2} on days 1, 8, 22, and 29); and cetuximab (400 mg/m^2 on day 7 followed by 250 mg/m^2 weekly) [28]. Among 45 patients who underwent surgery with curative intent, the ypCR rate was only 9 %, but no locoregional failures occurred, and the 5-year DFS and OS were 88, and 87 %, respectively. EXPERT-C was a randomized phase II trial of neoadjuvant capecitabine and oxaliplatin (CAPOX) with/without cetuximab, followed by capecitabine-based CRT with or without cetuximab, and adjuvant CAPOX with/without cetuximab in 165 high-risk rectal cancer patients [27]. The ypCR rates were similar with or without cetuximab (11 vs. 7 %, $p = 0.71$), but in the KRAS wild-type patients, the DFS rates were non-significantly higher (hazard ratio (HR) 0.65, $p = 0.363$) and the OS was improved in the cetuximab arm (median OS not reached, HR = 0.27; 95 % CI 0.07–0.99, $p = 0.034$). The addition of cetuximab mildly increased grade 3/4 toxicity during CRT: diarrhea 10 vs. 1 %, rash 9 vs. 0 %, and HFSR 4 vs. 1 %. Overall, cetuximab did not improve the primary outcome (ypCR), thus it was not felt to have contributed significantly to increased radiation-induced cytotoxicity. Given the role of *TP53* wild-type status and a functional p53 tumor suppressor gene in radiosensitization [65, 66], a retrospective analysis in EXPERT-C noted that *TP53* wild-type status was a predictive biomarker in favor of cetuximab-based therapy, albeit not for ypCR, with 5-year DFS and OS of 93 vs. 89 and 68 vs. 65 %, respectively ($p = 0.02$ each), in favor of the cetuximab arm [67]. Another phase II study tested RT with capecitabine (500 mg/m^2 twice daily), irinotecan (40 mg/m^2 weekly) and cetuximab (400 mg/m^2 on day 1 followed by 250 mg/m^2 weekly) [26]. Grade 3/4 toxicities were diarrhea (60 %), liver transaminase elevations (20 %), and acne-like skin rash (12 %). The ypCR rate was low at 8 %. Panitumumab (6 mg/kg biweekly) in combination with RT was evaluated in a small study of 19 KRAS wild-type rectal cancer patients, however, the ypCR rate was 0 [31].

Overall, there appears to be no role for the addition of EGFR-targeted therapy as radiosensitizers in the treatment of locally advanced rectal cancer [28]. Nevertheless, a pilot study of RT with personalized chemotherapy and biological therapy based on molecular markers (TOPO-1, ERCC1 expression, KRAS, BRAF, and PI3K) used capecitabine and either irinotecan (if TOPO-1 high) or oxaliplatin (if ERCC1 low), and either VEGF- or EGFR-targeted agents (if KRAS wild-type) among 16 patients with T3 or N1 rectal cancers [68]. The ypCR rate was promising at 50 %, which offers a potential basis for future molecularly driven larger studies. In addition, downstream from EGFR, the PI3K/mTOR pathway can be targeted. This pathway can not only modulate cellular apoptosis and senescence, but also affects angiogenesis and tissue perfusion, and prevents repair of DSBs induced by RT. PI3K/mTOR inhibitors have demonstrated preliminary radiosensitizing effects in preclinical models including KRAS wild=type and KRAS mutated rectal cancers [69, 70]. While clinical trials have not yet been reported in rectal cancer, several combinatorial approaches with RT have been tested or are ongoing in gliomas and head and neck cancers.

Novel Radiosensitizers

DNA Damage/Repair Pathways: Poly(ADP-Ribose) Polymerase Inhibitors

Poly(ADP-ribose) polymerase (PARP) plays a critical role in the recognition and repair of DNA SSBs and DSBs. Increased PARP activity has been documented in cancer cells with increased proliferative capacity, as well as chemotherapy and radiation resistance. This observation supports some of the observed selectivity of PARP inhibitors for sensitizing tumor cells compared with normal cells, towards chemo- and radiotherapy. PARP inhibition diminishes the ability of cancer cells exposed to ionizing radiation to repair radiation-induced SSBs and DSBs, leading to mitotic catastrophe. The extent of radiosensitizing effects from PARP inhibitors has been noted to depend on the homologous recombination status of tumor cells, such as the BRCA1 or BRCA2 deficiency or wild-type status [71], but other factors which affect DNA, such as the *TP53* status, play a role [72]. In colorectal cancer models, PARP inhibition was studied in cell culture analysis in both mismatch repair proficient (pMMR) and deficient (dMMR) cell lines. The mismatch repair (MMR) system maintains DNA integrity by correcting base substitution mismatches and small insertions or deletions generated during DNA replication. Inactivation of both alleles of one of the MMR genes (MLH1, MSH2, MSH6, PMS2) leads to defective MMR. While it has been hypothesized that MMR deficiency resulting in HR defects may lead to increased sensitivity to PARP blockade [73, 74], data suggests that at least when combined with topoisomerase inhibitors like irinotecan, sensitizing effects from PARP inhibition occur irrespective of MMR status. Veliparib (ABT888), a potent orally bioavailable PARP1/2 inhibitor, has been shown to enhance the antitumor activity of chemotherapy and RT in preclinical models [75]. When studied in vitro and in vivo in colorectal cancer, veliparib had independent radiosensitization effects and it was synergistic with chemotherapy, especially with irinotecan. Final results from a phase Ib dose-escalation study of veliparib plus capecitabine-based CRT were presented at ASCO 2015 [33]. Thirty-two stage II/III rectal cancer patients received capecitabine (825 mg/m^2 BID) with RT, and veliparib was administered in escalating doses from 20 to 400 mg BID (<400 mg, $n = 16$; 400 mg, $n = 16$) from day 2 until 2 days after CRT completion. Patients underwent surgery 5–10 weeks after completion of CRT. The most common treatment-related adverse events were fatigue (41 %), nausea

(41 %), diarrhea (25 %), and vomiting (22 %); grade 3/4 events were rare diarrhea ($n=2$), anemia, lymphopenia, and pulmonary embolism ($n=1$ each). Dose-limiting toxicities (grade 2) were radiation-induced skin injury ($n=1$, 70 mg BID), and nausea and vomiting ($n=1$, 400 mg BID). The MTD was not reached and the recommended phase II dose for veliparib was 400 mg BID. The ypCR rate was 28 %, and sphincter-sparing surgery was performed in 70 % of 30 evaluable patients. Defining predictive biomarkers of benefit for combined PARP inhibition with RT or CRT will help define the patient population most likely to benefit.

Another class of agents which can affect DNA repair is cell cycle checkpoint (Chk1/2) inhibitors, which have demonstrated significant synergism with RT, as well as with fluoropyrimidine in preclinical models, including in rectal cancer [69]. Clinical trials in rectal cancer are anticipated.

Histone Deacetylase Inhibitors

Histone modification affects the expression of cancer genes by a tight balance between upregulation (induced by histone acetyltransferases, HATs) or transcriptional repression (via histone deacetylases, HDACs) [76–78]. HATs lead to uncoiling of DNA around histones by transferring an acetyl group to the histone and then promote genes transcription. HDACs remove the acetyl group and condense the chromatin, resulting in transcriptional repression. HDAC inhibitors block this interaction, maintaining DNA in an uncoiled configuration, and allowing gene transcription, but also affect signal transduction pathways, including activation of the cellular stress response, cell cycle and apoptosis regulation, and DNA repair. Given that the chromatin structure and gene expression are the main determinants of radiation response [76, 77], HDAC inhibitors have been shown to enhance radiosensitivity in multiple tumor models, including colorectal cancer [79]. In preclinical studies, radiation was delivered to colorectal cell lines and to tumor xenografts under normal or hypoxic conditions, with and without vorinostat, an HDAC inhibitor, and with or without capecitabine [78]. Exposure to hypoxic conditions during radiation increased radioresistance; but the addition of HDAC inhibition abrogated it, reversing the radioresistant hypoxic phenotype. Moreover, vorinostat enhanced tumor growth inhibition when added to capecitabine-based CRT in vivo. The Pelvic Radiation and Vorinostat (PRAVO) phase 1 study evaluated escalating doses of vorinostat (100 to 400 mg daily) with pelvic RT for advanced gastrointestinal malignancies, and determined good tolerability and the maximum tolerated dose (MTD) at 300 mg daily for vorinostat [80]. Further studies with HDAC inhibition and RT may be justified in locally advanced rectal cancer.

Heat Shock Protein 90

Heat shock protein 90 (HSP90) is a chaperone protein, which regulates the stability and trafficking of proteins involved in cellular proliferation and DNA repair. Ganetespib is a small molecule inhibitor of HSP90, which has been noted to induce G0/G1 arrest in colorectal cancer cell lines [81]. Similar to the in vitro effects, in colorectal tumor models, ganetespib significantly downregulated proliferative signaling pathways with decreased protein expression of EGFR, IGFR, pAKT, PI3K, pERK, RAF, and pJNK [81] and many of these client proteins can affect radiosensitivity [76]. Preclinically ganetespib downregulated the expression of thymidylate synthase (TS) leading to synergism with a fluoropyrimidine (5-FU, capecitabine) [81], and in addition, it independently increased radiosensitivity in colorectal cancer models [82].

Based on these data, a phase I study of ganetespib, and capecitabine-based CRT was performed in stage II/III rectal cancer patients. Capecitabine was dosed at 825 mg/m^2 BID and ganetespib was evaluated at dose levels 60, 80, 100, and 120 mg/m^2 on days 14, 11, 7, and 4 prior to CRT, and 1, 8, 15, 29, 36 concurrent with CRT [32]. Grade 3 or 4 toxicities included diarrhea (38 %), and the one DLT was grade 3 diarrhea for more than 4 days. The MTD was determined to be 100 mg/m^2. Preliminary results showed the ypCR rate was 25 % and an additional two patients had downstaging to pT1 tumors. It is clear HSP90 inhibitors have the potential to enhance the effects of CRT and should be studied further in rectal cancer patients.

Total Neoadjuvant Therapy

Historically, local recurrence had been the main concern for patients with locally advanced rectal cancer. Secondary to improvement in surgical techniques, radiation, and chemotherapy, distant metastases are now the leading cause of recurrence. Adjuvant chemotherapy is often not administered secondary to a variety of reasons, including patients' desire not to delay ostomy reversal. Induction neoadjuvant chemotherapy (INCT) (neoadjuvant chemotherapy prior to CRT) [83] and consolidative neoadjuvant chemotherapy (CNCT) (neoadjuvant chemotherapy after CRT but before surgery) [84] are currently under investigation (NCT02008656) in patients undergoing TME or non-operative management (NOM) for rectal cancer. This trial administers 15–16 weeks of chemotherapy CAPOX or FOLFOX before or after capecitabine- or 5-FU-based CRT. Total neoadjuvant therapy (TNT) aims to increase the number of patients who receive systemic chemotherapy and eventually improve DFS and OS, meanwhile also identifying patients who may be able to avoid surgery secondary to clinical complete response. Table 2 summarizes most important trials using biological sensitizers.

Table 2 Clinical trials with biological radiosensitizers in localized rectal cancer

Study/design	Eligibility	Radiosensitizing therapy	Results
Crane et al. (2010) [25]	T3, N0–1	Bevacizumab	ypCR 32 %
Phase II ($n = 25$)		Capecitabine	DFS 77 % at 2 years
			OS not reached
Horisberger et al. (2009) [26]	T3, T4, N1–2	Cetuximab	ypCR 8 %
Phase II ($n = 50$)		Capecitabine	
		Irinotecan	
Dewdney et al. (2012) [27]	High-risk operable	Cetuximab	ypCR 11 vs. 7 %
Phase II ($n = 165$)		Capecitabine	WT PFS HR 0.65
		vs.	WT OS HR 0.27*
		Capecitabine	
Fokas et al. (2013) [28]	T3, T4, N1–2	Cetuximab	ypCR 9 %
Phase I/II ($n = 60$)		Cape-Ox,	
Eisterer et al. (2014) [29]	T3, T4	Cetuximab	ypCR 0 %
Phase II ($n = 31$)		Capecitabine	
Von Moos et al. (2014) [30]	KRAS MUT	Sorafenib	ypCR 15 %
Phase I/II ($n = 54$)	T3–4, N1–2	Capecitabine	nCR 45 %
Mardjuadi et al. (2014) [31]	T3, T4, N1–2	Panitumumab	ypCR 0 %
Phase II ($n = 19$)	KRAS WT		
El-Rayes et al. (2015) [32]	T3, T4, N1–2	Ganetespib	ypCR 25 %
Phase I		Capecitabine	
Michael et al. (2015) [33]	T3, T4, N1–2	Veliparib	ypCR 28 %
Phase Ib		Cape	
Acs et al. (2015, abs) [34]	T3, T4, N1–2	Aflibercept	ypCR 25 %
Phase II ($n = 39$)		5-FU	
Landry et al. (2015) [35]	T3, T4, N0-2	Bevacizumab	ypCR 17 %
Phase II (n = 53)		Cape-Ox	
Salazar et al. (2015) [36]	T3, T4, N0–2	Bevacizumab	ypCR 16 vs. 11 %
Phase II		Capecitabine	
		vs.	
		Capecitabine	
Kim et al. (2016) [37]	T3, T4, N1–2	Sorafenib	ypCR 36 %
Phase I ($n = 17$)		5-FU	

Cape-Ox capecitabine and oxaliplatin, *DFS* disease-free survival, *HR* hazard ratio, *nCR* near complete response, *OS* overall survival, *PFS* progression-free survival, *WT* KRAS wild-type, *ypCR* pathologic complete response after neoadjuvant therapy

*Statistical significance $p < 0.05$

Conclusion

The ideal radiosensitizing agent is one that will act selectively in the tumor, and increase efficacy with tolerable toxicity. The current standard of care for radiosensitization in rectal cancer remains single-agent fluoropyrimidine (5-FU or capecitabine) with low local relapse rates (<10 %) and pathological complete responses of 10–20 %. It is clear the combination of multi-agent chemotherapies with RT increased toxicity but did not enhance radiosensitization as local control remains unchanged. At this time, the role of novel radiosensitizers in rectal cancer is an area of high need, as cure rates remain suboptimal. Molecularly targeted therapies against VEGF/VEGFR or EGFR have not improved outcomes when added to CRT. Based on early reports, promising biological radiosensitizers include PARP inhibitors and HSP90 inhibitors, but only future randomized phase II or III trials with correlative biomarkers can help define the patient population most likely to benefit.

Compliance with Ethical Standards

Conflict of Interest Andrew L. Coveler, Patrick Richard, Smith Apisarnthanarax, and E. Gabriela Chiorean declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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