New directions in neoadjuvant therapy for rectal cancer

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Neoadjuvant chemoradiation therapy in rectal cancer has yielded exciting data on response to treatment and is now widely accepted as essential for the optimum treatment of this disease. Although a variety of drugs (5-fluorouracil, irinotecan, oxaliplatin) and molecular targeted therapy (vascular endothelial growth factor inhibitors, epidermal growth factor receptor inhibitors, etc.) are being explored as single agents or in combination with radiotherapy, the results of treatment have plateaued with response rates of 65–70% and complete pathological responses of 10–20%. This review analysis makes it possible for the underlying factors in the continued lack of improvement and examines potential new directions for future approaches to management of this disease. ***Anti-Cancer***

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In 2010, colorectal cancer is the third most likely cancer diagnosis among men and women in the USA, with rectal cancer representing approximately 30% of the cases [1]. In patients younger than 40 years of age, the incidence of rectal cancer seems to be increasing out of proportion compared with colon cancer, according to the Surveillance Epidemiology and End Results database [2]. Naturally, finding long-term cures with functional preservation of the anal sphincter and with minimal side effects is the ultimate therapeutic goal for these patients. To understand a way forward in multimodality treatment, it may be of help to reassess our past approaches.

Forty years ago, surgery alone was the standard of care for rectal cancer, but high cure rates were seen only in the early stage (T1–T2 N0) of the disease. Locally advanced (T3–T4 N0, or N+) rectal cancers had an unacceptable local recurrence rate of 25–40% [3,4]. As surgical techniques improved including better anastomoses, pelvic lymph node evaluation, and en-bloc dissection, local control rates also improved but were still too high [5]. The addition of postoperative radiation improved the locoregional control (LRC) by 35% (in one meta-analysis), but still it did not impact disease-free survival (DFS), distant metastasis (DM), or overall survival (OS) [6]. During the period from the 1980s to the early 1990s, postoperative radiation for T3 or N1 disease became the US standard, and trials such as INT/NCCTG 86-47-51 showed that addition of chemotherapy to the radiation further improved local control [7,8]. Other studies such as NCCTG 79-47-51 and GITSG GI-7175 showed improvements in DM and DFS [9,10]. Importantly, the need for postoperative radiation despite a more extensive and meticulous surgery such as the total mesorectal excision procedure was highlighted by the fact that radiation still cuts the local failure rate by half [11].

Until the last decade or so, the field of rectal oncology has been relatively static from the point of view of chemotherapeutic agents. From the 1960s to the 1990s, the adjuvant treatment has largely centered on a single agent, 5-fluorouracil (5-FU), with recently reported studies such as INT-0114 and INT-0144 tweaking differences in delivery [comparing bolus, modified bolus, and continuous venous infusion (CVI)] or sequencing with or without biological modifiers (e.g. leucovorin, levamisole, semustine, etc.) [8,12,13]. The overall conclusion is that continuous infusion of 5-FU is more effective than bolus delivery by allowing a more sustained pharmokinetics that is useful when given along with the radiation. Capecitabine (Xeloda, La Roche AG, Basel, Switzerland) is a 5-FU prodrug oral agent that has been added to the arsenal because of its convenience in delivery over infusion. Unfortunately, as other agents such as cisplatin, taxanes, and gemcitabine have not been as effective in the adjuvant or metastatic setting for rectal cancer, they have not been tried in the definitive setting with radiation.

During this time, a number of single institutions had been pushing neoadjuvant chemoradiation therapy long before the phase III German study was finally reported in 2005 [14–16]. A review of all phase II and III neoadjuvant therapy studies from 1965 to 2004 including 3157 patients showed that the following three factors on multivariate analysis predict for an improvement in pathologic response rates: continuous infusion of 5FU, two-drug regimen (excluding folinic acid), and a higher radiation dose [17]. The overall pathologic complete

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response (pCR) rate reported was 13%, with a range of 0–67%. The German study finally overturned the National Cancer Institute postoperative standard when it showed improved local control (doubled), sphincter-preservation rates (doubled), and no detriment in the OS [18]. The treatment in this study used continuous infusion of 5-FU and conventional radiation doses of 45–50.4 Gy, thus representing the new standard of care. Although showing efficacy over the adjuvant technique, it is important to note that the pCR rate for neoadjuvant treatment in the German study was surprisingly only 10% for T3 tumors and 0% for T4 tumors.

Meanwhile, there has been an influx of new and promising chemotherapy agents for colorectal cancer that show a systemic efficacy for metastatic disease. Capecitabine offers an oral convenience with a similar pharmokinetics to continuous infusion of 5-FU. Other oral fluoropyrimidine agents such as uracil and tegafur and S1 (derivative that inhibits degradation) have been tried [19,20]. Irinotecan was added to 5-FU as FOLFIRI and has shown promise in DFS for colon cancer, and oxaliplatin was added as FOLFOX in the metastatic and adjuvant settings [21,22]. Biological agents such as epidermal growth factor receptor (EGFR) modifiers, such as cetuximab, and antiangiogenesis drugs, such as bevacizumab, are currently under investigation and have been detailed elsewhere in this issue of the journal.

Unfortunately, recent phase I or II neoadjuvant rectal studies have shown that these newer agents are merely being added to the current continuous infusion of 5-FU regimens while keeping all other factors the same, including the radiation dose. This may seem like the logical next step when extrapolating from metastatic disease, but it is not really marching in step with the ongoing improvements in surgery and radiation treatment delivery. Therefore, not surprisingly, there has been no real improvement as shown by the very recent failure of oxaliplatin.

In the phase III ACCORD 12/0405-Prodige2 trial reported in April 2010, 45 Gy with capecitabine (800 mg/m^2, twice daily) was compared with 50 Gy with capecitabine (800 mg/m^2, twice daily) and oxaliplatin (50 mg/m^2, every week) [23]. The pathological complete response rate was not statistically significantly different at 14% versus 19%, whereas the grade 3 toxicity was 1% versus 25%, respectively. In fact, the experimental arm represents a step backwards as the radiation dose was decreased to 45 Gy in the interest of adding the new drug because of the concern for the added toxicity. The investigators concluded that next time, capecitabine should be given with a radiation dose of at least 50 Gy. The multicenter phase III Italian STAR study of 747 patients treated with a radiation dose of 50.4 Gy also showed that the addition of oxaliplatin weekly to standard FU-based preoperative combined radiation therapy significantly increases toxicity without affecting the local tumor response [24]. The reduced pathologic metastatic rate suggested a potential effect on distant micrometastases. These two studies typify many other study designs in which the effective dose of the backbone, 5-FU, may be decreased to accommodate the new drug. If anything, by emphasizing only the new chemotherapy agent as the experimental variable, they suggest only small and incremental improvements in local control, sphincter preservation, and the decrease of systemic metastasis.

To further compound this locked-in paradigm in medical oncology (i.e. more is better), German researchers investigated the addition of cetuximab to the oxaliplatin and capecitabine regimen (triple therapy), in anticipation that the above two trials of doublet therapy would have been positive [25]. German investigators analyzing three prospective phase I–II trials using this schema showed that the pCR rate went down from 14 to 9% with the addition of cetuximab. They concluded that there was a 'subadditive interaction' with more agents, and that the impressive results of cetuximab seen in metastatic colorectal cancer may not be simply transferred to combined chemoradiation protocols.

Thus, in the last 5–6 years, the results of neoadjuvant therapy have shown little improvement despite the use of other multiple cytotoxic agents in a more intensified approach as part of the neoadjuvant strategy. There is usually more of toxicity and less efficacy. The new molecular targeted agents such as antivascular endothelial growth factor (VEGF) (bevacizumab) or anti-EGFR agents (cetuximab, panatumimab, etc.) seem to hold so much promise have also failed to have any meaningful impact on the rates of pCR or on the survival of patients [26–29]. The current pCR rates still range between 5 and 30%. The higher pCR rates reported in some studies are often a function of inclusion of patients in early stage with small volume disease rather than a true reflection of efficacy of neoadjuvant therapy.

Part of the problem is that the rationale for using a multidrug combination in the neoadjuvant setting is based on the success of such combinations in the treatment of metastatic colon cancer rather than rectal cancer *per se*. There is an assumption that there are no intrinsic differences in the biology of these tumors. These drugs in one setting may not provide the synergy with radiation in another. The approach of chemotherapy intensification as part of the neoadjuvant therapy, therefore, needs to be re-examined as to whether this is the right strategy when the role of chemotherapy is nominally as a radiation sensitizer to enhance local effects rather than as a systemic therapy, although the latter could be having a significant collateral benefit.

In short, the results of neoadjuvant therapy suggest that a third of rectal cancers will have pCR or a near pCR, a third will show good partial response (downstaging), and a third of the tumors will remain bulky and largely

unaffected. This differential response is clearly governed by factors such as tumor volume and by the genetic fingerprint of the tumor, which are directly related in that as tumors grow larger, they exhibit greater genetic mutations and growth independence leading to poorer outcomes. Clearly, therefore, a neoadjuvant strategy cannot be a 'one-size-fits-all' approach but requires optimization.

A new approach needs to change our current thinking by altering other variables than the addition of another chemotherapy agent. The first step would be radiation dose escalation (total dose and daily fraction size) as radiation is the most effective single cytotoxic agent that can also be delivered in a very targeted manner. The second step would be to re-examine the known effective radiosensitizers, which are not necessarily the most effective ones against colorectal cancer but which may enhance the effect of radiation though they have not been adequately tested in rectal cancer in the past pursuit of tweaking the 5-FU delivery. The third step would be to add chemotherapy agents in a sequential manner with concurrent radiation instead of suboptimal doses of drugs, all given together in the hope of maximal effects. The fourth step would be to tailor the treatment for individual patients based on the biology of their tumor.

Radiation dose

It is one of the mysteries of modern cancer therapy that the widespread strategy in the combined modality treatment of gastrointestinal (GI) cancers is to deliver a dose of 50.4 Gy of radiation at 1.8 Gy per fraction with chemotherapy, whether treating stage T1/2 cancers or bulky T4 cancers. This accepted practice (National Comprehensive Cancer Network, American Society of Clinical Oncology guidelines) is based on a notion of radiation tolerance limitations for the small bowel [30,31]. In a biologically dynamic system, using a physical agent that predicts a certain cell kill per unit dose means that it is illogical to expect that the response to larger tumors is likely to be the same as small tumors.

In true dose-escalation manner, the Lyon R96-02 study reported in 2004 on 88 patients with low rectum T2/T3 tumors with less than two thirds ofcircumferential involvement [32]. The patients received 39 Gy at 3 Gy/Fx with half of the patients receiving an additional endocavitary boost of 85 Gy in three fractions using contact X-rays. Twelve patients had a further brachytherapy boost of 20–30 Gy and seven patients never underwent surgery because of a good response. No concurrent chemotherapy was given. The study reported a clinical complete response of 2 versus 24% and a pathologic sterilization rate of 34 versus 57%, respectively, with a sphincter preservation rate of 44 versus 76% in favor of the boost. There was no difference in morbidity, local failure, or OS at 2 years. The 2-year local relapse-free survival (RFS) was 88% in the external beam radiation therapy group and 92% in the external beam radiation therapy and boost group. The study shows that dose escalation alone does have a significant effect on downstaging rectal disease.

The Princess Margaret Hospital (PMH) reviewed three phase II studies for T3–T4 or N + patients treated with continuous infusion of 5-FU in a dose-escalation manner of 40 Gy/20 Fx, 46 Gy/23 Fx, or 50 Gy/25 Fx. These cohorts showed a pCR rate of 15, 23, and 33%, respectively. In 2 years, the study showed an improvement in local RFS, DFS, and OS with a dose of 46 Gy or more [33]. A phase I dose-escalation trial of 45 Gy to the pelvis (1.8 Gy/Fx) with continuous infusion of 5-FU followed by concomitant boosts of 1.2 Gy twice daily to 54.6, 57, and 61.8 Gy was tolerable and resulted in 96% of patients with a negative margin at resection [34].

We have described earlier a clinical staging system based on volume and tumor fixity, which allows the radiation dose to be titrated to the extent of disease for optimized outcomes [35]. With modern surgical techniques, resections can be carried out safely even after high (> 50 Gy) or ultra high-dose reirradiation (> 85 Gy) of the pelvis [32,34,36]. Dose painting techniques combined with improved tumor imaging can deliver focally higher dose radiation to regions of primary tumor, and the fear of surgical complications should no longer be a limiting factor. The high-dose region of the rectum is likely to be sacrificed in the surgical specimen so that long-term toxicity is not a concern. In addition, the use of conformal, smaller portals of radiation to the gross disease only, will limit the toxicity to the bowel as seen in earlier studies with radiosensitizers. The benefit of increased pCR rates, disease control, and survival of the patient with T4 or bulky tumors, however, could be substantial.

Radiation dose per fraction

An improved dose response has been noted not just for total dose but dose per fraction. When treating at doses more than 2 Gy, the PMH reported that patients receiving 40 Gy at 2.5 Gy/Fx had a complete response in 49% of mobile tumors, 22% of partially fixed ones, and 9% of fixed cancers [33,37]. Derdel *et al.* [38] using a similar fractionation scheme as the PMH but without chemotherapy reported 62% downstaging (> 50% regression) with a pCR rate of 20% as compared with only 35% downstaging and 0% pCR rate at 45–50 Gy at 1.8 Gy/Fx. A prospective phase II study of preoperative short-course radiation for rectal cancer used 2.9 Gy twice daily (5.8 Gy/day) to a total dose of 29 Gy was well tolerated with 92% local control [39]. A dose-escalation phase I/II study to a total dose of 45–54 Gy with CVI of 5-FU but using 1.8 Gy to the pelvis with a twice-a-week concurrent boost of 2.7 Gy to the tumor reported an overall pCR rate of 24% and 50% for tumors less than 200 cc [40].

In-vitro tumor models of combined modality therapy shows that 1.8 Gy produces modest sensitization as it is largely at the shoulder region of the cell survival curve. Sensitization increases significantly within the exponential double-strand break region of the cell survival curve [41]. Our experimental data on 5-FU/radiation interaction suggest that 1.8–2 Gy induces thymidylate synthase induction and produces modest sensitization or additive effect. However, at higher dose/Fx, an exponentially greater sensitization is seen in wild-type cells and more particularly in mutant cell lines [42]. Therefore, in large rectal cancers in which mutations such as p53, K-ras, and other regulatory genes are common, treatment with higher dose/Fx (2.25–2.5 Gy) with chemotherapy may yield substantially higher pCR rates. Therefore, both the total dose and the dose per fraction need to be increased.

In a phase II RTOG-0012 study combining total dose and daily fraction escalation, Mohiuddin *et al.* [43] reported on T3–T4 distal rectal tumors within 9 cm of the dentate line treated with CVI of 5-FU and radiation. One arm received a high-dose fractionation of 55.2–60 Gy at 1.2 Gy twice daily (2.4 Gy/day), whereas the other arm received irinotecan in addition to 5-FU with a standard radiation dose of 50.4–54 Gy at 1.8 Gy/Fx. The study reported a pCR rate of 28%, the highest in any multi-institutional study. For T3 tumors (72% of the total patients), the pCR rate was 35%. Even the T4 tumors (28%) had an 18% pCR rate. There were no increased postsurgical complications. These response rates are significant when put in the context of the RTOG-0247 study using standard 50.4 Gy along with capecitabine/oxaliplatin or capecitabine/irinotecan [44]. The pCR rate was 18 and 10%, respectively, which were both lower than the pCR of 28% in the high-dose radiation and CVI of 5-FU arm alone, despite the fact that RTOG-0247 had fewer (11%) T4 tumors. The German rectal cancer trial achieved a weak pCR rate of 25% for T2 tumors, 10% in T3 cancers, and 0% in T4 cancers using a dose of 50.4 Gy with infusion of 5-FU. The difference in response can be attributed to the higher dose of radiation in RTOG-0012.

Chemoradiotherapy

5-FU continues to remain the mainstay of chemoradiation combined therapy for GI cancers. It is still unclear as to whether this combination results in true sensitization or produces additive effects. It has been postulated that it is the radiation that sensitizes the 5-FU effect rather than the other way round [45]. 5-FU radiosensitization occurs at drug concentrations that are lower than those required for independent cytotoxic effect. This accounts for the widespread acceptance for 5-FU in designing chemoradiation approaches for GI cancers. The process of radiation sensitization of 5-FU is discussed in other papers of the journal. However, in large tumors drug delivery to cells may be insufficient to be fully exploited in the current chemoradiation schedules. Continuous infusion of 5-FU has, therefore, yielded better outcomes as compared with bolus infusion [8]. Capecitabine is an oral agent that mimics continuous infusion of 5-FU and may have a theoretical advantage in that the radiation induces intracellular thymidine phosphorylase, which is necessary for the conversion of capecitabine to the active metabolite [46]. However, it has never been tested as to whether 5-FU is necessary throughout the whole cycle of radiation or most effective at the beginning or at the end of the treatment.

In-vitro experiments with other agents have shown that enhanced tumor cell killing can be shown with any number of drugs especially the ones used for rectal cancer therapy (5-FU, irinotecan, oxaliplatin, etc.). However, the data from xenograft models and the failure of clinical benefit suggest that the conventional approach may produce a limited benefit and further intensification by expanding the number of related agents may be counterproductive, resulting in enhanced toxicity rather than improved tumor regression [19,47–49]. Tumor populations that respond to the addition of one drug with radiation may be the same population that responds to the use of a second or a third drug, so that in effect the same sensitive cells may be killed over and over by the multidrug regimen and radiation. The strategy therefore needs to focus not on the sensitive cells but on the cells that are not affected by the current chemoradiation combination.

Although 5-FU remains a key component in adjuvant therapy, other drugs such as mitomycin-C, which also sensitizes hypoxic cells, but might have a lower systemic profile for response in metastatic colorectal cancer, may in fact exhibit a higher synergy with radiation than 5-FU in the early phase of treatment and need to be systematically investigated [50].

Gemcitabine

Gemcitabine (2′2′-difluro-2-deoxycytidine) is a fluorine-substituted analog of cytarabine. It has shown antitumor activity in a number of murine tumor models and in human tumor xenografts, and may in fact be a better sensitizer to radiation than 5-FU [51]. Gemcitabine even at low concentrations, much lower than the therapeutic doses, decreases the intracellular deoxyribose nucleotide pools and enhances the radiosensitivity of cells *in vitro* [52]. In clinical practice, gemcitabine administration is targeted for systemic effects at 1–2 mg/m^2 but for radiation sensitization, a low dose, 24-h infusion is likely to produce optimum results. A dose of 400 mg/m^2 weekly over 7 weeks with 30 Gy in 10 fractions in 2 weeks has been the neoadjuvant protocol at MD Anderson Cancer Center for locally advanced pancreatic cancer [53,54]. When compared with 5-FU given with 30 Gy in 10 fractions and an intraoperative boost of 10 Gy, the gemcitabine regimen resulted in a pathologic partial response (> 50% nonviable tumor cells) of over 50 versus 40% and

a successful resection rate of 74% compared with 60% with 5-FU chemoradiation. More surprisingly, two patients had a complete pathologic response with the use of gemcitabine. The optimal dosing and combination of gemcitabine and radiation in pancreatic cancer has not been examined. A phase I study attempted dose escalation of weekly 350, 400, and 500 mg/m^2 with 30 Gy. This study suggested that a dose of 500 mg/m^2 was too toxic [55]. Yet, a Taiwanese study of 50.4–61.2 Gy with a dose of 600 mg/m^2 weekly, of gemcitabine versus 5-FU (500 mg/m^2 daily for 3 days every 14 days) showed an objective response rate of 50 versus 13% and a median survival of 14.5 versus 6.7 months, respectively [56]. The CALGB 89805 phase II study used 40 mg/m^2 twice weekly with 50.4 Gy with good LRC [57]. The University of Michigan protocol reported tolerable toxicities with a systemic dose of 1000 mg/m^2 (on days 1, 8, and 15 of the 28-day cycle) and a daily dose escalation of 0.2 Gy starting at 24 Gy at the rate of 1.6 Gy a day in 3 weeks [58]. They concluded that 36 Gy at 2.4 Gy/day was tolerable, only if the radiation encompassed only the gross tumor and not the regional lymph nodes. The phase II trial reported a disease control rate of 84.6% with 16 of the 17 resected specimens having no microscopic disease at the margin [59].

Phase I–II trials combined 1.25 Gy twice a day to a total dose of 50 Gy for locally advanced stage II–III rectal cancers [60]. The dose of gemcitabine in this study started at 10 mg/m^2 given twice a week and was dose escalated by 5 mg/m^2 to the final tolerated dose of 40 mg/m^2. Among the 36 patients who underwent surgery, 17 (47%) had a marked pathologic response, including six (17%) with a microscopically complete response and 11 (30%) with only microscopically residual carcinoma of less than 1 cm. All patients had clear surgical margins, and the 3-year actuarial OS rate was 85%, LRC was 94.5%, and DFS was 67%. Gemcitabine at a dose of 75 mg/m^2 weekly with a continuous infusion of 5-FU (225 mg/m^2) has been attempted with 45 Gy to the pelvis for locally advanced rectal cancer and is found to be tolerable [61]. It seems that gemcitabine is a potent radiosensitizer that is gaining momentum for use in GI tumors, but gemcitabine, either alone or in combination with 5-FU, as a radiosensitizer for primary rectal cancer has not been studied adequately and needs to be further explored.

Taxanes

There are no studies using taxanes in colorectal cancer but they are currently utilized clinically as effective radiosensitizers in the treatment of nonsmall cell lung cancer and head and neck cancer [62–64]. Taxanes disrupt chromatin structure and chromosome segregation in mitosis. Paclitaxel (Taxol, Bristol-Mayers Squibb, New York) and docetaxel (Taxotere, Sanofi-Aventis, Bridgewater, New Jersey, USA) bind to the N-terminal 31 amino-acid sequence of the B-tublin subunit of the tubulin polymers. Docetaxel has higher binding affinity for the tubulin-binding site and greater cytotoxicity *in vitro*, but clinically they have different toxicity profiles [65]. The taxanes cause G2/M cell arrest, which is a very radiosensitive phase. The timing of drug delivery is important, as the paclitaxel-induced G1 arrest can cause more resistance by preventing cycling to G2/M. Reexamining the role of taxanes to enhance radiosensitization either with 5-FU or other agents in rectal cancer may provide an enhancement of effect especially in mutant cell lines deficient in G2/M block.

The use of taxanes in abdominal malignancies is scant. Before gemcitabine, an MD Anderson pilot study of only 35 patients used paclitaxel (60 mg/m^2; 3 h given weekly) along with 30 Gy in 10 fractions as a preoperative radiosensitizer in resectable pancreatic cancer. This approach was deemed feasible, but did not show any advantages over 5-FU [66]. Another study using a dose of 50 mg/m^2/week with 50 Gy for locally advanced pancreatic cancer yielded a response rate of 26% and a median survival of 8 months [67]. RTOG 98-12 showed a 43% 1-year survival rate with paclitaxel/radiation for patients with locally advanced pancreatic cancer, representing a 40% improvement in survival rate compared with the earlier RTOG 92-09 study of 5-FU-based chemoradiation [68]. Perhaps, because of the successful use of taxanes in lung cancer, paclitaxel (50 mg/m^2, weekly) with cisplatin (25 mg/m^2, weekly) and concurrent radiation has been used in the most recent RTOG 0113 and 0436 studies of thoracic esophageal cancer. One of the advantages of taxane-based therapy over 5-FU was its less severe mucosal reactions, and the cisplatin/paclitaxel combination yielded a clinical complete response rate close to 35% [69]. Although encouraging, the method of drug delivery may explain the varying results of paclitaxel in these disease sites. Continuous infusion of paclitaxel (24 h for 5 or 7 days) has been used successfully with a higher dose irradiation for mesothelioma, nonsmall cell lung carcinoma, and locally advanced head and neck squamous cell cancers [63,64,70]. It may be that the dose–response relationship for radiosensitization depends more on exposure duration than the peak concentration [71]. Currently, the acceptable phase II levels for 5-day and 7-day infusions would be 21 and 10.5 mg/m^2/day, respectively [53]. Paclitaxel has not been used in rectal cancer so far and deserves evaluation either alone or in combination with other agents.

Platins

Cisplatin is a known radiosensitizer since the 1960s and has been used in a number of disease sites, including lung, head and neck, and gynecological malignancies. It seems to inhibit the DNA synthesis by intrastrand cross-links. With radiation, cisplatin seems to inhibit potentially lethal damage repair and cause radiosensitization of hypoxic tumor cells [72]. Unfortunately, cisplatin has not shown a systemic effect when used alone in rectal cancer

and so it has not been explored in combination with radiation for definitive treatment. In contrast, oxaliplatin seems to be effective in the treatment of systemic colorectal disease, and so medical oncologists extrapolate that it may be effective clinically in the definitive setting with radiation without many in-vitro studies. Oxaliplatin, such as carboplatin, has a different side effect profile and may not be as effective with radiation as cisplatin. This might account for the disappointing results when added to 5-FU and radiation in clinical studies to date [48]. Rather than extrapolate from one setting into the definitive one and assume similarity based on being in the same class, it would be more worthwhile to experiment using a true radiation sensitizer such as cisplatin itself, which is routinely used in esophageal cancer [69]. In anal cancer, the phase III RTOG 98-11 trial attempted to replace mitomycin C with cisplatin when given along with 5-FU and radiation [73]. Its failure in the treatment of anal cancer may be an example of overlapping effects in the same sensitive cell populations as compared with a true spatial cooperation.

Cetuximab (epidermal growth factor receptor)

EGFR overexpression is frequently detected in colorectal cancers and has been associated with poor prognosis and resistance to radiation therapy [74–76]. EGFR is a tyrosine kinase cell surface receptor encoded by the c-erbB-1 proto-oncogene that plays a role in signal transduction through phosphorylation of mitogen-activated protein kinase through the Ras/Raf pathway. EGFR can increase tumorigenicity by promoting cell cycle progression, angiogenesis, metastasis, and protection from apoptosis [77].

Cetuximab is an immunoglobulin G1 chimerized, monoclonal antibody that specifically binds to EGFR and competitively blocks other ligands, such as tumor growth factor-α [78]. The binding to EGFR blocks autophosphorylation and the activation of receptor-associated kinases, resulting in inhibition of cell growth, induction of apoptosis, and decreased matrix metalloproteinase and vascular endothelial growth factor production [79]. The drug has been used with great success as a single agent with radiation in head and neck cancers [80]. In 2004, cetuximab was approved for patients with metastatic colorectal cancer who are refractory or intolerant to irinotecan. However, the indications of use have narrowed with new studies and are limited to patients with wild-type KRAS only (approximately 60% of patients) [81].

Preclinical data regarding cetuximab show an enhanced tumor response of 1.59 with one dose of cetuximab and 3.62 with three doses when given with radiation, and are only associated with tumors expressing moderate-to-high levels of EGFR [82]. Unfortunately, phase I/II trials of cetuximab with 5-FU or capecitabine preoperatively show that the pCR rate ranges from 0 to 20%, with a pooled rate of 9.1% (29 of 316 patients) [83]. The gene copy number (GCN) of EGFR and KRAS mutation status were not prognostic for tumor regression in neoadjuvant chemoradiation for rectal cancer in an other retrospective analysis [84]. Outside the metastatic setting, it seems that cetuximab is not useful, even in patients selected for wild-type KRAS. The mature results of the MRC COIN study in 2010 showed that the addition of cetuximab to FOLFOX in stage III colon cancer did not improve the DFS, but increased the toxicity [85]. Newer studies are showing a lack of benefit with cetuximab in metastatic colon cancer even in patients selected for wild-type KRAS as per the CRYSTAL and OPUS trials [86–88]. These are largely disappointing results despite the success seen in head and neck cancers. Reasons may include a less critical role of repopulation in colorectal adenocarcinoma in the neoadjuvant setting, using a noncurative dose of radiation, or antagonism of chemotherapy agents in which the cells arrest in G_1 or G_2-M and fail to pass through the S phase. However, EGFR upregulation is a fact of life after radiation [82]. Therefore, although cetuximab has failed in the way it has been used, other strategies may find a more effective role for this agent than has been explored yet. The role of cetuximab as a radiosensitizer is also discussed in detail in a separate chapter of this issue of the Journal.

Bevacizumab

VEGF is a pivotal regulator of both normal and pathogenic angiogenesis and plays an important role in the survival of newly formed blood vessels and in endothelial cell migration and mitogenesis, induction of proteinases leading to remodeling of the extracellular matrix, and increased vascular permeability [89,90]. The biological effects of VEGF are mediated through its endothelial cell surface receptors such as Flt-1 (fms-like tyrosine kinase) and kinase domain region. During radiation, VEGF can induce overexpression of bcl-2, which inhibits apoptosis [91]. VEGF has been associated with increased microvessel tumor density and increased risk for lymph node metastases. A high expression of VEGF has been associated independently with disease progression and inferior survival in rectal cancer, with increased risk of local failure and metastases [92].

A promising antiangiogenesis inhibitor is bevacizumab, a recombinant humanized monoclonal anti-VEGF antibody. The use of an anti-VEGF monoclonal antibody to block VEGF has been shown to inhibit the growth of a variety of human tumor cell lines and has been approved as a chemotherapy agent for metastatic colorectal cancer in 2004 [93]. Bevacizumab is increasingly being used in combination with chemotherapy for the treatment of metastatic cancers and has also had initial testing in neoadjuvant regimens. Results of treatment, although initially promising, still show pCR rates ofless than 20%

[26]. Antiangiogenic agents have been shown to normalize tumor vasculature, reduce intratumoral hydrostatic pressure and hypoxia, potentially creating biological cooperation if not true sensitization [89]. Although bevacizumab is not a true radiosensitizer, it may nonetheless help promote the setting for improved radiosensitization. Alternatively, radiation can stimulate the upregulation of VEGF and can allow bevacizumab to be more effective [94]. Thus, bevacizumab may play a role sequential to radiation. A short treatment program that is initiated with antiangiogenic agents followed by chemoradiation may offer increased cell sensitivity and response.

Genetics

A truly individualized approach to rectal cancer would take tumor genetics into account when choosing chemotherapy agents. KRAS genotyping was added to the National Comprehensive Cancer Network clinical practice guidelines for metastatic colon cancer in 2008. The new guidelines stipulate that only patients whose tumors have the wild-type (normal) KRAS genes should receive treatment with the EGFR inhibitors, cetuximab and panitumumab. In general, a low GCN of EGFR and mutated KRAS status predict for a worse prognosis on colon cancer, and also are significant predictors of response to preoperative chemoradiation [95]. The two markers are related as radioresistance to KRAS-mutated cells is mediated through EGFR-dependent pathways [96]. KRAS serves as a mediator between extracellular ligand binding and intracellular transduction of signals from the EGFR to the nucleus. In a study using cetuximab, 5-FU, and radiation therapy for locally advanced rectal cancer, a tumor regression grade of 3–4 was achieved in 52.4% of patients with a high GCN of EGFR as compared with 5.6% with low GCN ($P = 0.0016$) [97] KRAS-mutated tumors also had a lower rate of tumor regression grade 3–4 of 20% versus 38%. Therefore, for 40% of the patients who have mutated KRAS, there needs to be a more aggressive treatment strategy.

In the German prospective neoadjuvant radiochemotherapy protocol (CAO/AIO/ARO-94), the pretreatment biopsies of 44 patients were evaluated by immunohistochemistry for apoptotic index, Ki-67, p53, and bcl-2 [98]. Patients with a high apoptotic index were more likely to have a good response, and this correlated with Ki-67 but not with p53 or b-cl2. Apoptotic index and tumor regression predicted an improved RFS. A literature search of 1204 studies in locally advanced rectal cancer treated with neoadjuvant chemotherapy and radiation reviewed p53, EGFR, thymidylate synthase, Ki-67, p21, and bcl-2/BAX [99]. The researchers felt that EGFR, thymidylate synthase, and p21 were predictive, whereas p53, Ki-67, and bcl-2 were not. P21 and BAX were deemed to need further evaluation. A future strategy might be pretreatment and midtreatment biopsies to evaluate a change in genetic fingerprint that predicts a tumor response and the need to adjust chemotherapy agents [100].

Future strategies

Attempts to enhance the effects of radiation with the addition of systemic agents have been ongoing for many decades. Although it is well known that certain drugs potentiate the effect of radiation *in vitro*, there are significant challenges in obtaining predictable results *in vivo*. Variables such as drug metabolism, inconsistent distribution, effects of tumor microvasculature, hydrostatic pressure gradients, hypoxia, induced resistance, and a host of other factors make it harder to obtain predictable effects. For most drugs, we may understand the main biological mode of action, but these are rarely the only effects produced in physiological systems. In fact, cellular stress responses, development of alternate pathways to circumvent cellular injury, adaptive resistance are all too common. The addition of radiation adds an additional complexity in changing cell cycle dynamics, upregulating survival genes, interfering with drug effects, and/or changing the essential microenvironment. This is one of the reasons as to why radiation sensitization is poorly understood except at the most basic conceptual level.

The fundamental basis of combined chemotherapy and radiation is to use spatial cooperation in which toxicity is distributed in the system while a local effect can be enhanced. This clearly relies on the drug being effective against the tumor in the first place. Unfortunately, spatial cooperation has a limited potential because studies have shown that radiochemotherapy combinations also enhance radiation-related acute and delayed side effects thereby requiring a significant reduction of either the radiation or the dose of the radiosensitizing drug, often making the overall effort a zero-sum game, especially around critical normal tissue structures. Therefore, although the focus remains on biologically effective agents for metastatic disease, some true opportunities for radiation sensitization may be missed.

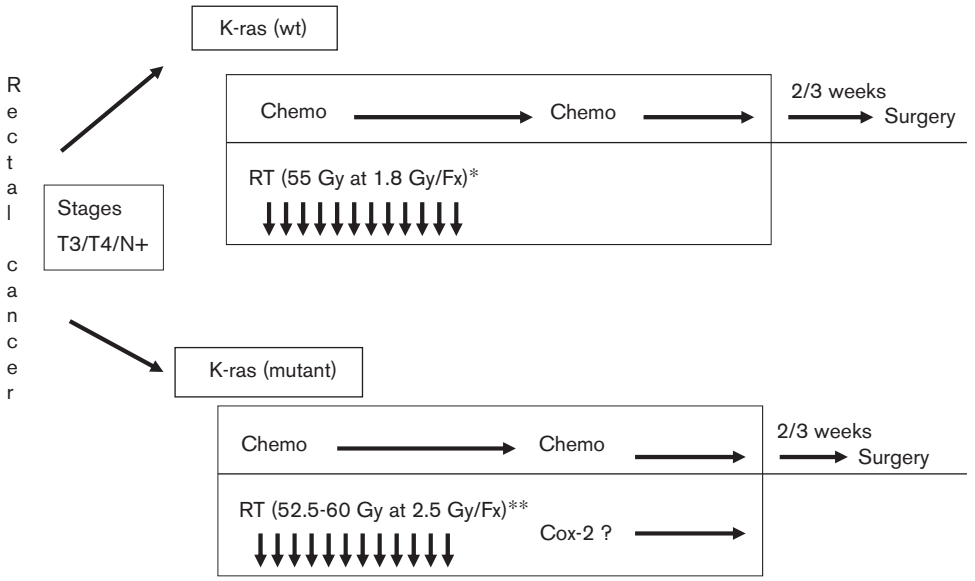
Cytotoxic enhancement usually requires the drug to be present at the time of radiation, but this may be variable because of the patient-to-patient pharmacokinetic and pharmacodynamic differences. Although these differences are unpredictable, an early recognition of biological effects (such as by functional imaging) may allow for better individualization and optimization either by changing the drug strategy, changing the radiation dose per fraction, or time interval between the first and the last dose of treatment. All of these factors need to be explored in expanding the optimum use of combined modality chemoradiation.

Chemosensitization of radiation is a complex process that we understand only superficially. It is unlikely that in-vitro data can be fully translated into clinical outcomes. Although in-vitro results may give us clues and some

biological bases for appropriate combinations, future improvements will still be determined by imaginative clinical trials in a physiologically dynamic system. A higher radiation dose titrated to the volume of disease combined with a selective approach to radiation dose/time fractionation based on the individual tumor gene profile has to be a cornerstone in extending the full benefits of combined chemoradiation. Overcoming re-accelerated tumor growth and preventing the development of adaptive resistance in partial responders is also critical in reducing rates of DM in these tumors.

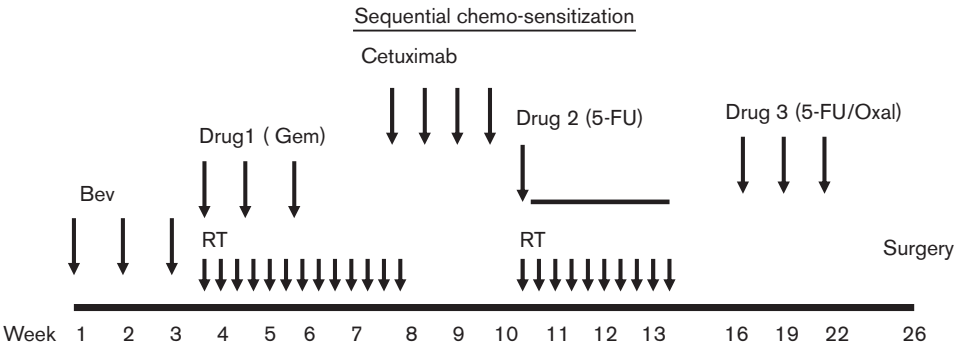
Future approaches to treatment requires a paradigm shift in the management of this disease, in which chemoradiation should be considered as the primary treatment approach, as in anal canal cancer, and surgery of the local lesion considered as the consolidation adjuvant phase of treatment. A suggested option for future trials is shown in Fig. 1. Radiation treatment volumes should be limited to the true pelvis for stage T1–T3 cancers and only for T3/4 or N+ cancers larger volumes should be considered. Rectal cancers can be divided into favorable or advanced based on their T stage and biology (K-ras and EGFR GCN). Favorable tumors can receive the standard daily fractionation or a twice-a-day boost per RTOG-0012. Unfavorable tumors will need more total dose compared with smaller volumes of the tumor using larger (2.5 Gy) fraction sizes. The schema of combined chemoradiation for locally advanced disease is shown in Fig. 2. Neoadjuvant chemotherapy can be given in the form of

Fig. 1



A suggested option for future trials. *45 Gy to pelvis and 10.8 Gy boost at 1.8 Gy/Fx or 45.6 Gy to pelvis and 14.4 Gy boost at 1.2 Gy/Fx (RTOG-0012). **45 Gy to pelvis at 1.8 Gy/Fx/52.5-60 Gy to tumor at 2.5 Gy/Fx.

Fig. 2



The schema of combined chemoradiation for locally advanced disease. 5-FU, 5-fluorouracil; Bev, bevacizumab; Gem, gemcitabine; Oxal, oxaliplatin.

bevacizumab to normalize the blood vessels feeding the tumor. This would allow more oxygenation needed for radiosensitization and an increased chemotherapy drug delivery to the tumor. The first drug, gemcitabine, can be given with concurrent radiation therapy. Midtreatment evaluation can be done with functional imaging or MRI to evaluate early response. If response is suboptimal, the drug can be switched to 5-FU in a sequential manner. This approach is similar to switching between neoadjuvant chemotherapy regimens for breast cancer based on early clinical or ultrasound response. Between the split course of radiation, cetuximab or bevacizumab can be given as the tumor will be overexpressing VEGF and EGFR in response to the radiation. These biological modifiers act as an environmental primer for the subsequent resumption of chemoradiation. Although there is a tendency to add more drugs together to the radiation, it would seem wiser to use radiosensitizing chemotherapy drugs sequentially with the radiation to allow full dosage of each individual drug and maximal effect. When the first drug has killed a population of tumor cells in combination with the radiation, the second drug can then attempt to kill the remaining tumor cells using a different mechanism. In this sequential manner, the two drugs are not competing, not reducing the dose, or causing synergistic toxicities. On completion of radiation therapy, adjuvant chemotherapy such as FOLFOX should be given in systemic doses during the time interval to surgery to decrease the chance of developing systemic metastasis.

Conclusion

The neoadjuvant chemoradiation approach has proved to be successful in many disease sites including rectal cancer. However, we have hit a plateau in the pCR to treatment, which is lower than 30%. The current approach, coming from the medical oncology community, is to keep the radiation dose constant and add multiple, new chemotherapy agents taken from the metastatic setting to the definitive regimen. This has resulted in unimpressive clinical responses and often in more toxicity. To move forward, we may have to reexamine the known chemosensitizers other than 5-FU that have been clinically discarded without a thorough evaluation of timing and drug delivery. These agents should be given sequentially to each other but concurrently with radiation to allow maximal dosing and sensitization. Greater sensitization can also be achieved with a higher radiation dose per fraction and total dose using newer, conformal delivery techniques, such as intensity-modulated radiation therapy. Biological agents seem to have a role in priming the tumor for chemoradiation and should be used neoadjuvantly or adjuvantly to the definitive course of irradiation. As in anal cancer, the goal is to move the pCR to above 50% so that surgery slowly becomes an adjuvant treatment that may eventually be avoided altogether. If we persist in our current approach to rectal cancer, we are likely to remain clinically frustrated. Real change requires a new mental approach, a paradigm shift. Sir Francis Bacon quotes

> When you wish to achieve results that have not been achieved before, it is an unwise fancy to think that they can be achieved by using methods that have been used before.

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References

1 ACS Cancer Facts and Figures 2010. *http://www.cancer.org/research/cancerfactsfigures/cancerfactsfigures/cancer-facts-and-figures-2010.*
2 Meyer JE, Narang T, Schnoll-Sussman FH, Pochapin MB, Christos PJ, Sherr DL. Increasing incidence of rectal cancer in patients aged younger than 40 years: an analysis of the surveillance, epidemiology, and end results database. *Cancer* 2010; **116**:4354–4359.
3 Wolmark N, Wieand HS, Hyams DM, Colangelo L, Dimitrov NV, Romond EH, *et al.* Randomized trial of postoperative adjuvant chemotherapy with or without radiotherapy for carcinoma of the rectum: National Surgical Adjuvant Breast and Bowel Project Protocol R-02. *J Natl Cancer Inst* 2000; **92**:388–396.
4 Rich T, Gunderson LL, Lew R, Galdibini JJ, Cohen AM, Donaldson G. Patterns of recurrence of rectal cancer after potentially curative surgery. *Cancer* 1983; **52**:1317–1329.
5 Peeters KC, Marijnen CA, Nagtegaal ID, Kranenbarg EK, Putter H, Wiggers T, *et al.* The TME trial after a median follow-up of 6 years: increased local control but no survival benefit in irradiated patients with resectable rectal carcinoma. *Ann Surg* 2007; **246**:693–701.
6 Colorectal Cancer Collaborative Group. Adjuvant radiotherapy for rectal cancer: a systematic overview of 8507 patients from 22 randomised trials. *Lancet* 2001; **358**:1291–1304.
7 NIH consensus conference. Adjuvant therapy for patients with colon and rectal cancer. *JAMA* 1990; **264**:1444–1450.
8 O'Connell MJ, Martenson JA, Wieand HS, Krook JE, Macdonald JS, Haller DG, *et al.* Improving adjuvant therapy for rectal cancer by combining protracted-infusion fluorouracil with radiation therapy after curative surgery. *N Engl J Med* 1994; **331**:502–507.
9 Douglass HO Jr, Moertel CG, Mayer RJ, Thomas PR, Lindblad AS, Mittleman A, *et al.* Survival after postoperative combination treatment of rectal cancer. *N Engl J Med* 1986; **315**:1294–1295.
10 Krook JE, Moertel CG, Gunderson LL, Wieand HS, Collins RT, Beart RW, *et al.* Effective surgical adjuvant therapy for high-risk rectal carcinoma. *N Engl J Med* 1991; **324**:709–715.
11 Kusters M, Marijnen CA, Van de Velde CJ, Rutten HJ, Lahaye MJ, Kim JH, *et al.* Patterns of local recurrence in rectal cancer: a study of the Dutch TME trial. *Eur J Surg Oncol* 2010; **36**:470–476.
12 Smalley SR, Benedetti JK, Williamson SK, Robertson JM, Estes NC, Maher T, *et al.* Phase III trial of fluorouracil-based chemotherapy regimens plus radiotherapy in postoperative adjuvant rectal cancer: GI INT 0144. *J Clin Oncol* 2006; **24**:3542–3547.
13 Tepper JE, O'Connell M, Niedzwiecki D, Hollis DR, Benson AB III, Cummings B, *et al.* Adjuvant therapy in rectal cancer: analysis of stage, sex, and local control – final report of intergroup 0114. *J Clin Oncol* 2002; **20**:1744–1750.
14 Mohiuddin M, Marks G. High dose preoperative irradiation for cancer of the rectum, 1976–1988. *Int J Radiat Oncol Biol Phys* 1991; **20**:37–43.
15 Janjan NA, Crane CN, Feig BW, Cleary K, Dubrow R, Curley SA, *et al.* Prospective trial of preoperative concomitant boost radiotherapy with continuous infusion 5-fluorouracil for locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 2000; **47**:713–718.
16 Mendenhall WM, Million RR, Bland KI, Pfaff WW, Copeland EM III. Initially unresectable rectal adenocarcinoma treated with preoperative irradiation and surgery. *Ann Surg* 1987; **205**:41–44.
17 Hartley A, Ho KF, McConkey C, Geh JI. Pathological complete response following pre-operative chemoradiotherapy in rectal cancer: analysis of phase II/III trials. *Br J Radiol* 2005; **78**:934–938.

18 Sauer R, Becker H, Hohenberger W, Rodel C, Wittekind C, Fietkau R, *et al.* Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med* 2004; **351**:1731–1740.

19 Tepper JE, Goldberg RM. An embarrassment of riches: neoadjuvant therapy of rectal cancer. *J Clin Oncol* 2005; **23**:1339–1341.

20 Vestermark LW, Jacobsen A, Qvortrup C, Hansen F, Bisgaard C, Baatrup G, *et al.* Long-term results of a phase II trial of high-dose radiotherapy (60 Gy) and UFT/l-leucovorin in patients with non-resectable locally advanced rectal cancer (LARC). *Acta Oncol* 2008; **47**:428–433.

21 Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, *et al.*; Irinotecan Study Group. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2000; **343**:905–914.

22 Giacchetti S, Perpoint B, Zidani R, Le Bail N, Faggiuolo R, Focan C, *et al.* Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracil-leucovorin as first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2000; **18**:136–147.

23 Gerard JP, Azria D, Gourgou-Bourgade S, Martel-Laffay I, Hennequin C, Etienne PL, *et al.* Comparison of two neoadjuvant chemoradiotherapy regimens for locally advanced rectal cancer: results of the phase III trial ACCORD 12/0405-Prodige 2. *J Clin Oncol* 2010; **28**:1638–1644.

24 Aschele C, Pinto C, Cordio S, Rosati G, Tagliagambe A, Artale S, *et al.* Preoperative fluorouracil (FU)-based chemoradiation with and without weekly oxaliplatin in locally advanced rectal cancer: pathologic response analysis of the Studio Terapia Adiuvante Retto (STAR)-01 randomized phase III trial. *J Clin Oncol* 2009; **27** (**Suppl; abstr CRA4008**).

25 Weiss C, Arnold D, Dellas K, Liersch T, Hipp M, Fietkau R, *et al.* Preoperative radiotherapy of advanced rectal cancer with capecitabine and oxaliplatin with or without cetuximab: a pooled analysis of three prospective phase I–II trials. *Int J Radiat Oncol Biol Phys* 2010; **78**:472–478.

26 Willett CG, Duda DG, Di Tomaso E, Boucher Y, Ancukiewicz M, Sahani DV, *et al.* Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. *J Clin Oncol* 2009; **27**:3020–3026.

27 Zlobec I, Vuong T, Compton CC, Lugli A, Michel RP, Hayashi S, Jass JR. Combined analysis of VEGF and EGFR predicts complete tumour response in rectal cancer treated with preoperative radiotherapy. *Br J Cancer* 2008; **98**:450–456.

28 Horisberger K, Treschl A, Mai S, Barreto-Miranda M, Kienle P, Strobel P, *et al.* Cetuximab in combination with capecitabine, irinotecan, and radiotherapy for patients with locally advanced rectal cancer: results of a Phase II MARGIT trial. *Int J Radiat Oncol Biol Phys* 2009; **74**: 1487–1493.

29 Bertolini F, Chiara S, Bengala C, Antognoni P, Dealis C, Zironi S, *et al.* Neoadjuvant treatment with single-agent cetuximab followed by 5-FU, cetuximab, and pelvic radiotherapy: a phase II study in locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 2009; **73**:466–472.

30 NCCN Rectal Guidelines 2010. *http://www.nccn.org/professionals/physician_gls/PDF/rectal.pdf*.

31 Romanus D, Weiser MR, Skibber JM, Ter Veer A, Niland JC, Wilson JL, *et al.* Concordance with NCCN Colorectal Cancer Guidelines and ASCO/NCCN Quality Measures: an NCCN institutional analysis. *J Natl Compr Canc Netw* 2009; **7**:895–904.

32 Gerard JP, Chapet O, Nemoz C, Hartweig J, Romestaing P, Coquard R, *et al.* Improved sphincter preservation in low rectal cancer with high-dose preoperative radiotherapy: the lyon R96-02 randomized trial. *J Clin Oncol* 2004; **22**:2404–2409.

33 Wiltshire KL, Ward IG, Swallow C, Oza AM, Cummings B, Pond GR, *et al.* Preoperative radiation with concurrent chemotherapy for resectable rectal cancer: effect of dose escalation on pathologic complete response, local recurrence-free survival, disease-free survival, and overall survival. *Int J Radiat Oncol Biol Phys* 2006; **64**:709–716.

34 Movsas B, Hanlon AL, Lanciano R, Scher RM, Weiner LM, Sigurdson ER, *et al.* Phase I dose escalating trial of hyperfractionated pre-operative chemoradiation for locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 1998; **42**:43–50.

35 Mohiuddin M, Ahmed MM. Critical issues in the evolving management of rectal cancer. *Semin Oncol* 1997;**24**:732–744.

36 Mohiuddin M, Lingareddy V, Rakinic J, Marks G. Reirradiation for rectal cancer and surgical resection after ultra high doses. *Int J Radiat Oncol Biol Phys* 1993; **27**:1159–1163.

37 Wang Y, Cummings B, Catton P, Dawson L, Kim J, Ringash J, *et al.* Primary radical external beam radiotherapy of rectal adenocarcinoma: long term outcome of 271 patients. *Radiother Oncol* 2005; **77**:126–132.

38 Derdel J, Mohiuddin M, Kramer S, Marks G. Is dose/time fractionation important in treating rectal cancer? *Int J Radiat Oncol Biol Phys* 1985; **11**:579–582.

39 Guckenberger M, Wulf J, Thalheimer A, Wehner D, Thiede A, Muller G, *et al.* Prospective phase II study of preoperative short-course radiotherapy for rectal cancer with twice daily fractions of 2.9 Gy to a total dose of 29 Gy – long-term results. *Radiat Oncol* 2009; **4**:67.

40 Myerson R, Zobeiri I, Birnbaum E, Dietz D, Fleshman J, Kodner I, *et al.* Early results from a phase I/II radiation dose-escalation study with concurrent amifostine and infusional 5-fluorouracil chemotherapy for preoperative treatment of unresectable or locally recurrent rectal carcinoma. *Semin Oncol* 2002; **29**:29–33.

41 Haveman J, Castro Kreder N, Rodermond HM, van Bree C, Franken NA, Stalpers LJ, *et al.* Cellular response of x-ray sensitive hamster mutant cell lines to gemcitabine, cisplatin and 5-fluorouracil. *Oncol Rep* 2004; **12**:187–192.

42 Mohiuddin M, Chendil D, Dey S, Alcock RA, Regine W, Mohiuddin M, *et al.* Influence of p53 status on radiation and 5-flourouracil synergy in pancreatic cancer cells. *Anticancer Res* 2002; **22**:825–830.

43 Mohiuddin M, Winter K, Mitchell E, Hanna N, Yuen A, Nichols C, *et al.* Randomized phase II study of neoadjuvant combined-modality chemoradiation for distal rectal cancer: Radiation Therapy Oncology Group Trial 0012. *J Clin Oncol* 2006; **24**:650–655.

44 Wong SJ, Winter K, Meropol NJ, Anne R, Kachnic LA, Rashid A, *et al.* RTOG 0247: a randomized phase II study of neoadjuvant capecitabine and irinotecan versus capecitabine and oxaliplatin with concurrent radiation therapy for locally advanced rectal cancer (abstra 4021). *J Clin Oncol* 2008; **26**.

45 Blackstock AW, Kwock L, Branch C, Zeman EM, Tepper JE. Tumor retention of 5-fluorouracil following irradiation observed using 19F nuclear magnetic resonance spectroscopy. *Int J Radiat Oncol Biol Phys* 1996; **36**:641–648.

46 Vallerga AK, Zarling DA, Kinsella TJ. New radiosensitizing regimens, drugs, prodrugs, and candidates. *Clin Adv Hematol Oncol* 2004; **2**:793–805.

47 Patel A, Puthillath A, Yang G, Fakih MG. Neoadjuvant chemoradiation for rectal cancer: is more better? *Oncology (Williston Park)* 2008; **22**:814, 826; discussion 826, 828–831, 836.

48 Flatmark K, Ree AH. Radiosensitizing drugs: lessons to be learned from the oxaliplatin story. *J Clin Oncol* 2010; **28**:e577, e578; author reply e581–e583.

49 Rodel C, Sauer R. Integration of novel agents into combined-modality treatment for rectal cancer patients. *Strahlenther Onkol* 2007; **183**:227–235.

50 Chan AK, Wong AO, Langevin J, Jenken D, Heine J, Buie D, Johnson DR. Preoperative chemotherapy and pelvic radiation for tethered or fixed rectal cancer: a phase II dose escalation study. *Int J Radiat Oncol Biol Phys* 2000; **48**:843–856.

51 Plunkett W, Huang P, Gandhi V. Preclinical characteristics of gemcitabine. *Anticancer Drugs* 1995; **6** (**Suppl 6**):7–13.

52 Plunkett W, Huang P, Xu YZ, Heinemann V, Grunewald R, Gandhi V. Gemcitabine: metabolism, mechanisms of action, and self-potentiation. *Semin Oncol* 1995; **22**:3–10.

53 Crane CH, Varadhachary G, Pisters PW, Evans DB, Wolff RA. Future chemoradiation strategies in pancreatic cancer. *Semin Oncol* 2007; **34**:335–346.

54 Pisters PW, Abbruzzese JL, Janjan NA, Cleary KR, Charnsangavej C, Goswitz MS, *et al.* Rapid-fractionation preoperative chemoradiation, pancreaticoduodenectomy, and intraoperative radiation therapy for resectable pancreatic adenocarcinoma. *J Clin Oncol* 1998; **16**: 3843–3850.

55 Wolff RA, Evans DB, Gravel DM, Lenzi R, Pisters PW, Lee JE, *et al.* Phase I trial of gemcitabine combined with radiation for the treatment of locally advanced pancreatic adenocarcinoma. *Clin Cancer Res* 2001; **7**: 2246–2253.

56 Li CP, Chao Y, Chi KH, Chan WK, Teng HC, Lee RC, *et al.* Concurrent chemoradiotherapy treatment of locally advanced pancreatic cancer: gemcitabine versus 5-fluorouracil, a randomized controlled study. *Int J Radiat Oncol Biol Phys* 2003; **57**:98–104.

57 Blackstock AW, Tepper JE, Niedwiecki D, Hollis DR, Mayer RJ, Tempero MA. Cancer and leukemia group B (CALGB) 89805: phase II chemoradiation trial using gemcitabine in patients with locoregional adenocarcinoma of the pancreas. *Int J Gastrointest Cancer* 2003; **34**:107–116.

58 McGinn CJ, Zalupski MM, Shureiqi I, Robertson JM, Eckhauser FE, Smith DC, *et al.* Phase I trial of radiation dose escalation with concurrent weekly full-dose gemcitabine in patients with advanced pancreatic cancer. *J Clin Oncol* 2001; **19**:4202–4208.

59 Small W Jr, Berlin J, Freedman GM, Lawrence T, Talamonti MS, Mulcahy MF, *et al.* Full-dose gemcitabine with concurrent radiation therapy in patients

with nonmetastatic pancreatic cancer: a multicenter phase II trial. *J Clin Oncol* 2008; **26**:942–947.
60 Allal AS, Bieri S, Gervaz P, Soravia C, Bernier J, Gertsch P, *et al.* Preoperative concomitant hyperfractionated radiotherapy and gemcitabine for locally advanced rectal cancers: a phase I–II trial. *Cancer J* 2005; **11**:133–139.
61 McMullen KP, Blackstock AW. Chemoradiation with novel agents for rectal cancer. *Clin Colorectal Cancer* 2002; **2**:24–30.
62 Suntharalingam M, Paulus R, Edelman MJ, Krasna M, Burrows W, Gore E, *et al.* RTOG 0229: a phase II trial of neoadjuvant therapy with concurrent chemotherapy and full dose radiotherapy (XRT) followed by resection and consolidative therapy for LA-NSCLC. *Int J Radiat Oncol Biol Phys* 2010; **78**:S111.
63 Herscher LL, Cook J. Taxanes as radiosensitizers for head and neck cancer. *Curr Opin Oncol* 1999; **11**:183–186.
64 Herscher LL, Hahn SM, Kroog G, Pass H, Temeck B, Goldspiel B, *et al.* Phase I study of paclitaxel as a radiation sensitizer in the treatment of mesothelioma and non-small-cell lung cancer. *J Clin Oncol* 1998; **16**:635–641.
65 Hennequin C. Association of taxanes and radiotherapy: preclinical and clinical studies. *Cancer Radiother* 2004; **8** (**Suppl 1**):S95–S105.
66 Pisters PW, Wolff RA, Janjan NA, Cleary KR, Charnsangavej C, Crane CN, *et al.* Preoperative paclitaxel and concurrent rapid-fractionation radiation for resectable pancreatic adenocarcinoma: toxicities, histologic response rates, and event-free outcome. *J Clin Oncol* 2002; **20**:2537–2544.
67 Safran H, Cioffi W, Iannitti D, Mega A, Akerman P. Paclitaxel and concurrent radiation for locally advanced pancreatic carcinoma. *Front Biosci* 1998; **3**:E204–E206.
68 Rich T, Harris J, Abrams R, Erickson B, Doherty M, Paradelo J, *et al.* Phase II study of external irradiation and weekly paclitaxel for nonmetastatic, unresectable pancreatic cancer: RTOG-98-12. *Am J Clin Oncol* 2004; **27**:51–56.
69 Safran H, Gaissert H, Akerman P, Hesketh PJ, Chen MH, Moore T, *et al.* Paclitaxel, cisplatin, and concurrent radiation for esophageal cancer. *Cancer Invest* 2001; **19**:1–7.
70 Rosenthal DI, Lee JH, Sinard R, Yardley DA, Machtay M, Rosen DM, *et al.* Phase I study of paclitaxel given by seven-week continuous infusion concurrent with radiation therapy for locally advanced squamous cell carcinoma of the head and neck. *J Clin Oncol* 2001; **19**:1363–1373.
71 Liebmann J, Cook JA, Fisher J, Teague D, Mitchell JB. *In vitro* studies of Taxol as a radiation sensitizer in human tumor cells. *J Natl Cancer Inst* 1994; **86**:441–446.
72 Coughlin CT, Richmond RC. Biologic and clinical developments of cisplatin combined with radiation: concepts, utility, projections for new trials, and the emergence of carboplatin. *Semin Oncol* 1989; **16**:31–43.
73 Ajani JA, Winter KA, Gunderson LL, Pedersen J, Benson AB III, Thomas CR Jr, *et al.* Fluorouracil, mitomycin, and radiotherapy versus fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. *JAMA* 2008; **299**:1914–1921.
74 Khorana A, Ryan C, Eberly S. EGFR expression and survival in stage II, III and IV colon cancer (abstract 1272). *Proc Am Soc Clin Oncol* 2003; **22**:317.
75 Giralt J, De las Heras M, Cerezo L, Eraso A, Hermosilla E, Velez D, *et al.* The expression of epidermal growth factor receptor results in a worse prognosis for patients with rectal cancer treated with preoperative radiotherapy: a multicenter, retrospective analysis. *Radiother Oncol* 2005; **74**:101–108.
76 Azria D, Bibeau F, Barbier N, Zouhair A, Lemanski C, Rouanet P, *et al.* Prognostic impact of epidermal growth factor receptor (EGFR) expression on loco-regional recurrence after preoperative radiotherapy in rectal cancer. *BMC Cancer* 2005; **5**:62.
77 Huang SM, Harari PM. Epidermal growth factor receptor inhibition in cancer therapy: biology, rationale and preliminary clinical results. *Invest New Drugs* 1999; **17**:259–269.
78 Harari PM, Huang SM. Combining EGFR inhibitors with radiation or chemotherapy: will preclinical studies predict clinical results? *Int J Radiat Oncol Biol Phys* 2004; **58**:976–983.
79 Wu X, Fan Z, Masui H, Rosen N, Mendelsohn J. Apoptosis induced by an anti-epidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin. *J Clin Invest* 1995; **95**:1897–1905.
80 Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, *et al.* Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006; **354**:567–578.
81 Lievre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, *et al.* KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006; **66**:3992–3995.
82 Milas L, Mason K, Hunter N, Petersen S, Yamakawa M, Ang K, *et al. In vivo* enhancement of tumor radioresponse by C225 antiepidermal growth factor receptor antibody. *Clin Cancer Res* 2000; **6**:701–708.
83 Glynne-Jones R, Mawdsley S, Harrison M. Cetuximab and chemoradiation for rectal cancer – is the water getting muddy? *Acta Oncol* 2010; **49**:278–286.
84 Bengala C, Bettelli S, Bertolini F, Sartori G, Fontana A, Malavasi N, *et al.* Prognostic role of EGFR gene copy number and KRAS mutation in patients with locally advanced rectal cancer treated with preoperative chemoradiotherapy. *Br J Cancer* 2010; **103**:1019–1024.
85 Maughan TS, Adams R, Smith CG, Seymour MT, Wilson RH, Meade AM, *et al.* Oxaliplatin and fluoropyrimidine chemotherapy plus or minus cetuximab: the effect of infusional 5-FU or capecitabine on the outcomes of the MRC COIN trial in advanced colorectal cancer (ACRC) (abstr 402). *J Clin Oncol* 2010.
86 Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, *et al.* Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**:1408–1417.
87 Bokemeyer C, Bondarenko I, Hartmann JT, De Braud FG, Volovat C, Nippgen J, *et al.* KRAS status and efficacy of first-line treatment of patients with metastatic colorectal cancer (mCRC) with FOLFOX with or without cetuximab: the OPUS experience. *J Clin Oncol* 2008; **26** (**15 Suppl**):4000.
88 Van Cutsem E, Nowacki M, Lang I, Cascinu S, Shchepotin I, Maurel J, *et al.* Randomized phase III study of irinotecan and 5-FU/FA with or without cetuximab in the first-line treatment of patients with metastatic colorectal cancer (mCRC): the CRYSTAL trial. *J Clin Oncol* 2007; **25**:4000.
89 Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med* 2001; **7**:987–989.
90 Ferrara N. The role of VEGF in the regulation of physiological and pathological angiogenesis. *EXS* 2005; **94**:209–231.
91 Nor JE, Christensen J, Mooney DJ, Polverini PJ. Vascular endothelial growth factor (VEGF)-mediated angiogenesis is associated with enhanced endothelial cell survival and induction of Bcl-2 expression. *Am J Pathol* 1999; **154**:375–384.
92 Cascinu S, Graziano F, Catalano V, Staccioli MP, Rossi MC, Baldelli AM, *et al.* An analysis of p53, BAX and vascular endothelial growth factor expression in node-positive rectal cancer. Relationships with tumour recurrence and event-free survival of patients treated with adjuvant chemoradiation. *Br J Cancer* 2002; **86**:744–749.
93 Cao Y, Tan A, Gao F, Liu L, Liao C, Mo Z. A meta-analysis of randomized controlled trials comparing chemotherapy plus bevacizumab with chemotherapy alone in metastatic colorectal cancer. *Int J Colorectal Dis* 2009; **24**:677–685.
94 Gorski DH, Beckett MA, Jaskowiak NT, Calvin DP, Mauceri HJ, Salloum RM, *et al.* Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. *Cancer Res* 1999; **59**:3374–3378.
95 Li S, Kim JS, Kim JM, Cho MJ, Yoon WH, Song KS, *et al.* Epidermal growth factor receptor as a prognostic factor in locally advanced rectal-cancer patients treated with preoperative chemoradiation. *Int J Radiat Oncol Biol Phys* 2006; **65**:705–712.
96 Toulany M, Dittmann K, Kruger M, Baumann M, Rodemann HP. Radioresistance of K-Ras mutated human tumor cells is mediated through EGFR-dependent activation of PI3K-AKT pathway. *Radiother Oncol* 2005; **76**:143–150.
97 Bengala C, Bettelli S, Bertolini F. Predictive value of EGFR gene copy number and K-ras mutation for pathological response to preoperative cetuximab, 5FU, and radiation therapy in locally advanced rectal cancer (LARC). *J Clin Oncol* 2008; **26**:4125.
98 Rodel C, Grabenbauer GG, Papadopoulos T, Bigalke M, Gunther K, Schick C, *et al.* Apoptosis as a cellular predictor for histopathologic response to neoadjuvant radiochemotherapy in patients with rectal cancer. *Int J Radiat Oncol Biol Phys* 2002; **52**:294–303.
99 Kuremsky JG, Tepper JE, McLeod HL. Biomarkers for response to neoadjuvant chemoradiation for rectal cancer. *Int J Radiat Oncol Biol Phys* 2009; **74**:673–688.
100 Bertolini F, Bengala C, Losi L, Pagano M, Iachetta F, Dealis C, *et al.* Prognostic and predictive value of baseline and posttreatment molecular marker expression in locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2007; **68**:1455–1461.