Oxaliplatin as a radiosensitizing agent in rectal cancer

Sergio Huerta and John Hrom

The efficacy of oxaliplatin monotherapy against several solid tumors and its relative lack of nephrotoxicity and myelosupression, coupled with results of the Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer trial, led to a great deal of interest for the implementation of this chemotherapeutic agent in the preoperative setting for the management of adenocarcinoma of the rectum. Despite limited in-vitro and in-vivo data with regard to the radiosensitizing properties of oxaliplatin in rectal cancer, it rapidly entered phase I–III clinical trials. This study reviews the results of these trials and the current status of oxaliplatin as a radiosensitizing agent in the neoadjuvant management of rectal cancer. ***Anti-Cancer Drugs*** **22:317–323 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Platinum-based chemotherapeutic agents are broadly used in a variety of solid tumors including lung, genitourinary system, and colon malignancies. Although the efficacy of some of these compounds has been well established (e.g. cisplatin in testicular cancer), widespread clinical use of platinum-based agents was limited by substantial toxic side effects and the development of chemoresistance. To overcome these limitations more than 3000 analogs have been synthesized, of which around 30 have entered clinical trials. However, only a handful are used in clinical practice [1].

Oxaliplatin is a platinum-based compound in the alkylating family of anticancer agents (Fig. 1) [1]. Although oxaliplatin was discovered 34 years ago, it gained Food and Drug Administration approval only in the past 8 years for the management of advanced colon cancers. As with other platinum-based compounds used in cancer treatment, oxaliplatin brings about its antitumor activity by interfering with DNA synthesis owing to the formation of adducts between the drug and the double-stranded structure of DNA [1]. Intra-strand links occur between two contiguous guanine or two neighboring adenine and guanine adducts (Fig. 2) [2]. Although oxaliplatin shares the same mode of action as other platinum compounds, it differs from its analogs in its variability in mechanisms of resistance [3]. Oxaliplatin causes fewer intrastrand adducts, but it provides equal DNA cytotoxicity. The adducts formed by oxaliplatin are less well recognized by the DNA repair complex than those formed by cisplatin and carboplatin [3]. Platinum agents including oxaliplatin can thus synergize in combination with radiation in the killing of cells.

This study gives an overview of the current status of oxaliplatin in combination with other chemotherapeutic agents and radiation for the treatment of rectal cancer in a neoadjuvant setting.

Preclinical studies

Oxaliplatin in colon cancer

Data from preclinical studies related to the effects of oxaliplatin in colorectal cancer are surprisingly limited. In our studies, we have shown *in vitro* that SW620 colorectal cancer cells were more resistant to oxaliplatin-mediated apoptosis compared with SW480 colorectal cancer cells at baseline (control) and after 24-h treatment with oxaliplatin (500 μg) (20 vs. 49%, respectively). Caspase-3 activity was 14 and 34% in SW620 versus SW480 cells, respectively. Expression of Bax, Bcl-2, Bcl-xL, and anti-apoptosis-inducing factor (AIF) was similar in both cell lines. However, immunohistochemistry showed a marked increase in staining in the SW480 cell line with AIF antibody. These findings suggested that oxaliplatin-mediated resistance to apoptosis in metastatic colon cancer might be mediated by AIF and that this regulation is unlikely to be affected by individual gene expression of Bax, Bcl-2, or Bcl-xL [4].

Oxaliplatin led to G0–G1 arrest in p53 wild-type colorectal cancer cells exposed to the drug for 2 h. In contrast, the same study showed that colorectal cancer-p53-null cells experienced S-phase arrest. Colorectal cancer cells with a p53 wild-type status were sensitive to oxaliplatin, whereas most p53 null cells were resistant to the drug [5]. Other investigators have shown *in vitro* that oxaliplatin caused G0–G1 cell-cycle arrest in HCT-116 p53 wild-type cells, whereas it caused p21 degradation and G2–M arrest in its knockout counterpart [6].

Oxaliplatin and ionizing radiation *in vitro*

The first study that documented the effect of oxaliplatin combined with radiation was in an abstract in which the

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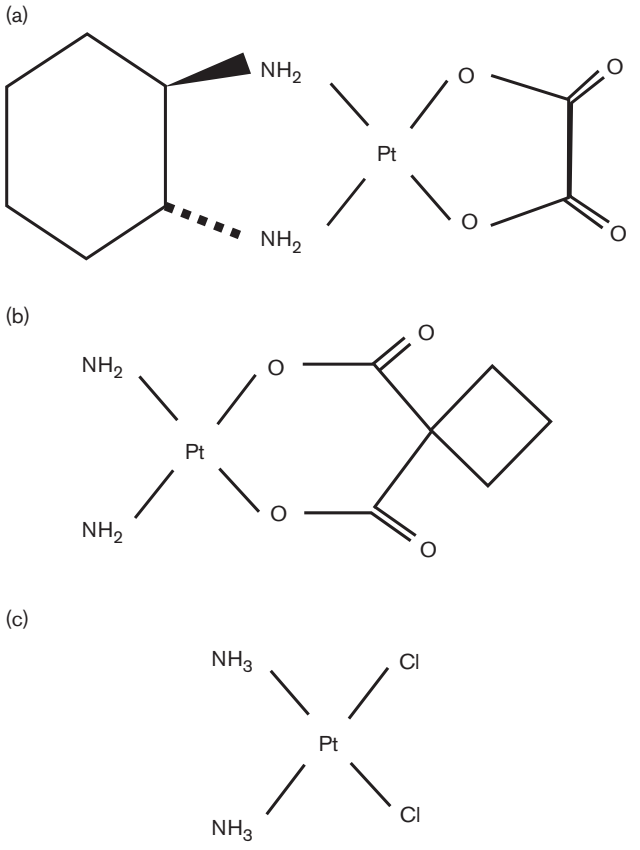
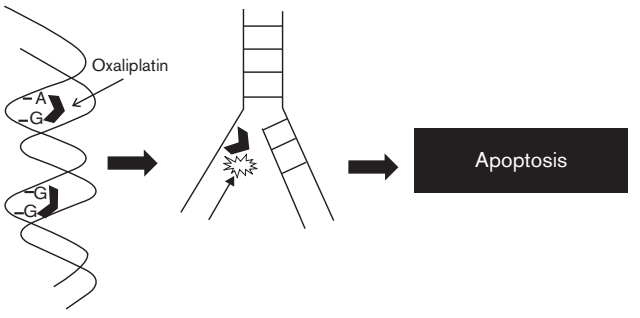
Fig. 1



Three Food and Drug Administration-approved platinum-based compounds: (a) oxaliplatin, (b) carboplatin, and (c) cisplatin.

Fig. 2



Mechanism of action of oxaliplatin: adducts between the drug and the double-stranded structure of DNA. Intrastrand links occur between two contiguous guanine or two neighboring adenine and guanine adducts. DNA replication is interrupted, leading to apoptosis.

researchers showed in-vitro and in-vivo activity of oxaliplatin as a radiosensitizer [7]. Available evidence of the radiosensitizing properties of oxaliplatin in the preclinical setting was summarized by Hermann *et al.* [8]. In a study by Magne *et al.* [9], oxaliplatin sensitized both p53 wild-type (SW403) and p53-mutated (WiDR) cells to the effects of ionizing radiation. However, when 5-fluorouracil (5-FU)/folinic acid was added to oxaliplatin and ionizing radiation in different sequencing combinations, there seemed to be antagonistic interactions in the WiDR line and synergy in SW403 cells. Another study documented the importance of the period of incubation with oxaliplatin in a colorectal cancer cell line exposed to ionizing radiation and 5-FU. The researchers concluded that a longer oxaliplatin exposure in S1 colorectal cancer cells led to increased radiosensitivity [10].

Oxaliplatin and ionizing radiation *in vivo*

Data on the effects of oxaliplatin in combination with ionizing radiation *in vivo* are also substantially limited. Hess and Blackstock [7] first documented the in-vivo activity of oxaliplatin combined with ionizing radiation in HT-29 colon cancer xenografts. The results of this study were in disagreement with the findings by Folkvord *et al.* who found no changes in tumor growth in HT-29 xenografts treated with oxaliplatin, capacitabine, and irradiation [11].

In a separate study, delayed tumor growth was observed in a xenograft model with mammary adenocarcinoma xenografts treated with oxaliplatin (6–14 mg/kg, intraperitoneally) and irradiation ($2\,\text{Gy} \times 10$ days). Different sequences and time intervals did not influence the results of this study, [12].

Even though there was limited in-vitro and in-vivo evidence of oxaliplatin as a radiosensitizer, it rapidly entered phase I–III clinical trials for the management of adenocarcinoma of the rectum in the neoadjuvant setting. Clinical studies have shown that oxaliplatin monotherapy was effective against several solid tumors including ovarian, melanoma, giloma, and colon cancer [13]. In addition, the toxicity observed with other platinum-based agents was substantially less with oxaliplatin. Patients treated with oxaliplatin did not experience the limiting nephrotoxicity compared with individuals subjected to cisplatin [14]. Similarly, oxaliplatin-treated patients had less myelosuppression compared with patients receiving carboplatin-based therapy [15].

Probably the most pronounced enthusiasm for the rapid inclusion of oxaliplatin in the management of rectal cancer in the preoperative setting was the results from the Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer trial that established the use of oxaliplatin as part of the FOLFOX4 (oxaliplatin, leucovorin, and 5-fluorouracil) regimen as standard treatment in the adjuvant setting for locally advanced colon cancer after surgical resection. In this trial, patients with stage II or stage III colon cancer underwent surgical resection and

were randomly assigned to either infusional 5-FU with leucovorin (FL) or FOLFOX4 for 12 cycles. This trial initially showed a disease-free survival advantage of 78.2% at 3 years in the FOLFOX4 arm as compared with 72.9% in the FL group ($P = 0.002$) [16]. Final analysis carried out at 6 years showed an overall survival (OS) advantage with OS rates of 78.5 and 76.0% in the FOLFOX4 and FL groups, respectively ($P = 0.046$) [17]. Although this study provided important therapeutic options in the management of colon cancer, it has also documented oxaliplatin-associated toxicity when combined with fluorouracil and leucovorin, compared with fluorouracil and leucovorin alone. It is noteworthy that although the toxicity of oxaliplatin-based therapies in the management of solid tumors is less than that with cisplatin and carboplatin, its toxicity profile is higher than that of fluoropyrimidine-based therapy alone. This is an important aspect in considering the risks and benefits associated with oxaliplatin in the preoperative management of rectal cancer. Significant differences in grade III and grade IV toxicities associated with these two therapies reported in the Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer trial are shown in Fig. 3 [16].

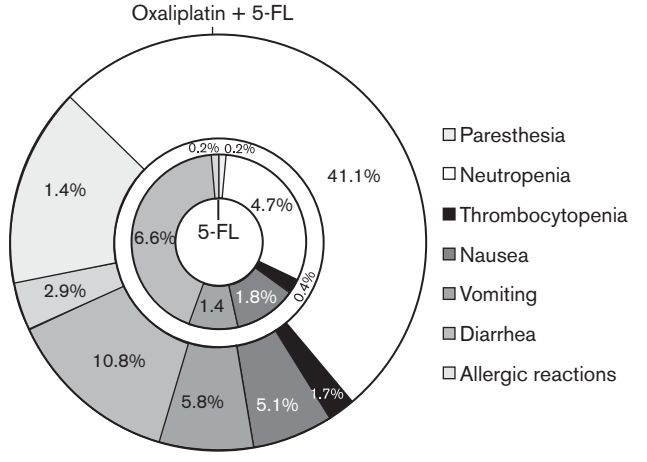
Clinical studies

Several phase I and II studies have been conducted to assess the addition of oxaliplatin to other chemotherapy agents in combination with radiation. In these trials, oxaliplatin is typically combined with continuous infusion of 5-FU or its oral pyrimidine analog.

Phase I/II studies

The first phase I trial was published in 2001. In a single-institution phase I study, 17 patients with various stages of rectal cancer were treated with escalating doses of oxaliplatin at 80, 100, and 130 mg/m^2 with concomitant 45 Gy radiation therapy (RT) and 5-FU with L-folinic acid. Only one patient had treatment failure at a dose of 80 mg/m^2. This study did not arrive at a maximum tolerated dose (MTD). The researchers recommended a dose of 130 mg/m^2 for phase II trials [18].

Fig. 3



Drug-related toxicity from the Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer trial: 5-flurouracil (5-FU, small circle) vs. oxaliplatin plus 5-FU (larger circle).

After a period of 2 years, the feasibility and efficacy of a different phase I/II protocol (xeloda and oxaliplatin) was assessed in 32 patients with rectal cancer [19]. In this study, patients were subjected to 50.4 Gy RT, capacitabine at 825 mg/m^2, and 10 mg/m^2 incremental doses of oxaliplatin starting at 50 mg/m^2. Dose-limiting toxicity (DLT) was at 60 mg/m^2. Of all the patients, 55% achieved a tumor depth downstaging; pathological complete response (pCR) was 19%. Sphincter-preserving low anterior resection (LAR) was achieved in 36% of patients who had tumors of size greater than or equal to 2 cm above the dentate line. The rate of postoperative complications in this trial was 39%.

Subsequent to these trials, a different study evaluated raltitrexed (3 mg/m^2) and escalating doses of oxaliplatin (60–130 mg/m^2) combined with RT (50 Gy) in 48 patients in a phase I/II trial [20]. This study also did not reach an MTD. Of all the patients in this cohort, 93% underwent a sphincter-preserving LAR. There was a 67% rate of tumor downstaging and a 77% nodal downstaging with a pCR of 57% [20].

In 2005, another group conducted a phase I study in patients with T3–T4, metastatic, and recurrent rectal cancer to determine the MTD of eight escalating doses of both oxaliplatin and continuous infusion of 5-FU. DLT occurred at the last dose (oxaliplatin 80 mg/kg^2 and 5-FU 225 mg/kg^2 concurrent with 45 Gy ionizing radiation). At a lower dose (oxaliplatin 60 mg/kg^2 and 5-FU 225 mg/kg^2), two patients had grade 3 diarrhea and this was the recommended dose by the researchers for phase II/III studies [21].

The same year, a phase I–II study was conducted to evaluate the recommended dose and clinical activity of oxaliplatin in combination with 5-FU and RT in 46 patients with recurrent or stage II/III rectal cancer. In the phase I portion of this study, the investigators established the recommended dose of oxaliplatin at 60 mg/m^2 and 5-FU at 225 mg/m^2/day in conjunction with 50.4 Gy RT. In this study, downstaging occurred in 84% of the patients, with a rate of 28% pCR [22].

The results of the phase I/II Colorectal Clinical Oncology Group study were also published in 2005 [23]. This study included treatment with escalating doses of oxaliplatin (85, 130,150, and 170 mg/m^2) with leucovorin (20 mg/m^2), 5-FU (350 mg/m^2), and 45 Gy RT. The follow-up period for these patients was 41 months. The MTD with this regimen was 150 mg/m^2 of oxaliplatin. The secondary aims

of this study were to determine the rates of pCR, resectability, and local recurrence, and late morbidity. pCR was 7%, downstaging was achieved in 30% of patients, circumferential resection was possible in 81% of cases. There was a 27% rate of relapse (one had local disease and five had distant metastasis) in 22 patients who had R0 operations. Severe late effects were uncommon [23].

The results of the Cancer and Leukemia Group B (protocol 89901) phase I/II study were available in 2006. In this study the researchers assessed the MTD of oxaliplatin in patients with T3–T4 tumors receiving continuous infusion of 5-FU (200 mg/m^2), irradiation (50.4 Gy), and escalating oxaliplatin treatment from 30 to 60 mg/m^2. A secondary objective of this study was to determine pCR. In the phase I portion of the study, MTD was established at 60 mg/m^2. Compliance was 56%. The toxicity profile was 67% for grades 3–4 diarrhea, 17% for grade 3 neutropenia, and 6% for grade 3 trhombocytopenia. Eight patients who completed the phase II portion of the study experienced a pCR [24].

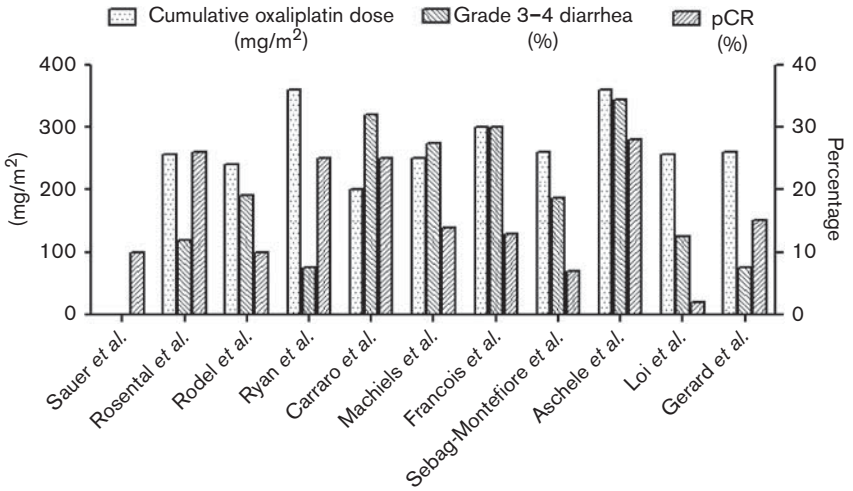
The Easter Cooperative Oncology Group study (E-1297) published the results of their phase I study in 2008. This study evaluated 21 patients with T3–T4 rectal cancer who were subjected to RT (50.4 Gy), infused with 5-FU (200 mg/m^2), and varying doses of oxaliplatin (55, 75, or 85 mg/m^2). The primary objectives of this study were to determine the MTD for oxaliplatin and DLTs. This study found no DLTs and the highest dose was well tolerated. Five of the 21 patients had a pCR. The researchers recommended a 85 mg/m^2 dose of oxaliplatin every 2 weeks for phase II studies [25]. In their analysis, the researchers of this study also included a number of published studies in which oxaliplatin had been used in RT. Compliance was lower and grades 3–4 diarrhea more pronounced with higher cumulative doses of oxaliplatin (Fig. 4) [22–32].

Phase II studies

The results of the Argentinean phase II Inergrupo Argentino para el Tratamiento de los Tumores Gastrointestinales study were available in 2002. This study included 22 patients with unresectable T3–T4 rectal cancer. In this study, 72% of the patients underwent surgical resection, 75% of whom had a complete resection. Forty-two percent of patients had a sphincter-preserving LAR. The rate of pCR was 25%. Median time of survival was 15.7 months and median OS was 19.5 months. The researchers concluded that oxaliplatin was highly active in this clinical setting and that it was associated with low toxicity. No individual was excluded from the study as a result of oxaliplatin toxicity. One patient died of febrile neutropenia, and diarrhea was the most prevalent severe toxicity. Three patients had local recurrence [26].

The results of the phase I study published by Freyer *et al.* [18] in 2001 was followed by a phase II trial in the Lyon RO-04 study. The dose of oxaliplatin was at 130 mg/m^2 with the same dose of 5-FU and L-folinic acid, but there was an increase in RT from 45 to 50 Gy. The main goal of this trial was to identify the rate of postoperative complications with this regimen. A total of 25 patients underwent LAR, nine of whom had a diverting ostomy and 14 patients had an abdominoperineal resection. In this study, toxicity was minimal and all 40 patients completed the study. Sphincter-preserving operations were possible in over 50% of patients and the pCR rate

Fig. 4



Summary of selected studies that show cumulative dose of oxaliplatin and the rate of grade 3–4-related diarrhea and pathological complete response (pCR). As the cumulative dose of oxaliplatin increases, grade 3–4 diarrhea also increases. The rate of pCR is variable.

was 15%. There were no postoperative deaths, four patients required a second operation to manage complications, one patient had anastomotic fistula, and one had pelvic abscess [27].

In the RadiOxCape phase II study, 42 patients with stage II/III rectal cancer received 50 mg/m^2 of oxaliplatin and an oral capacitibine of 825 mg/m^2 twice daily during RT treatment for a total of 45 Gy. The main endpoint was pCR, which was achieved in five of the 42 patients treated (14%) [29].

Phase III studies

On the basis of the encouraging data of the phase I–II trials, a few phase III studies have further investigated the addition of oxaliplatin to fluoropyrimidine combined with RT. These studies have recently been closed to accrual. The Italian, Studio Terapia Adiuvante Retto-01 trial enrolled 747 patients to receive infusional 5-FU concurrently with RT or the same regimen with weekly addition of oxaliplatin. Preliminary results were published recently indicating a similar pCR rate of 16% in the 5-FU arm as compared with 15% in the arm also receiving oxaliplatin. Grade III/IV toxicity was much higher in the arm that received oxaliplatin, with the majority of these events being diarrhea [33].

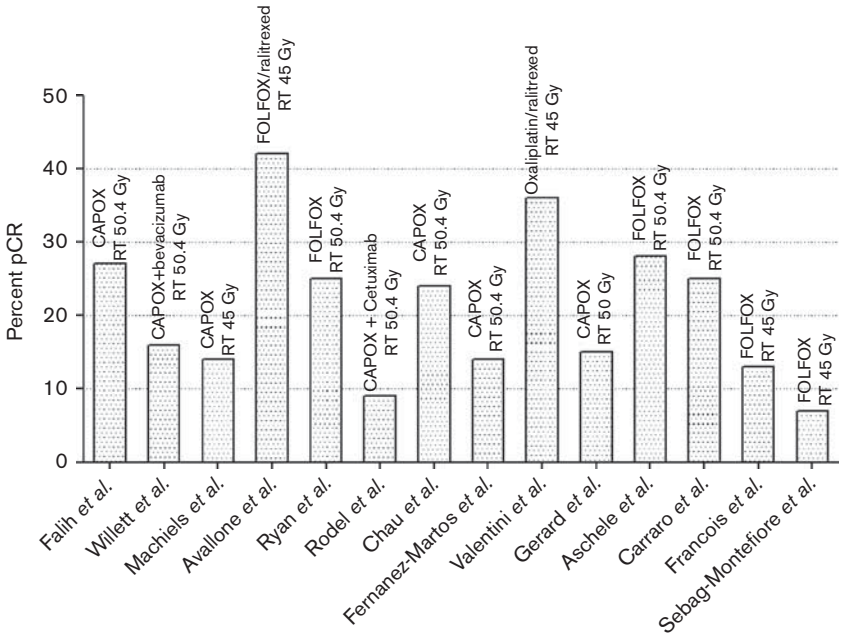
The Action Clinique Coordonnées en Cancérologie Digestive/Partenariat de Recherche en Oncologie Digestive 2 trial enrolled 598 patients to either capecitabine given concurrently with RT or the same regimen with weekly addition of oxaliplatin. Preliminary results from this trial showed a nonsignificant trend toward better pCR rate in the oxaliplatin arm. The pCR rates were 14% in the capecitabine-arm alone compared with a pCR of 19% with the addition of oxaliplatin ($P = 0.11$) [34].

The preliminary results from these phase III studies do not show a substantial response in pCR. It is unclear whether the pCR is a surrogate marker for survival in rectal cancer. Multiple studies do show a correlation with pCR with improvements in DFS and a few even showed an improvement in OS [30,35–37]. However, other studies have shown no benefit with regard to either DFS or OS [32,38]. Given the significant systemic activity of oxaliplatin, there may be a benefit observed with a reduction in distant metastases leading to an improvement in OS, but these data with the current studies are still at large.

Conclusion

Even though only limited preclinical data were available on the efficacy of oxaliplatin as a radiosensitizer in rectal cancer, it rapidly entered phase I–III trials. This enthusiasm has been an extension of the efficacy of this drug in colon cancer. Although DFS and OS should be the cornerstone for the development of novel chemoradiotherapeutic interventions, the rationale for introducing new radiosensitizers in the preoperative setting should stem primarily from the ability of a new combination to decrease tumor load. This goal should

Fig. 5



Phase I–III studies that have reported pathological complete response (pCR). Multiple anticancer agents have been combined with oxaliplatin. The rate of pCR is variable. CAPOX, capecitabine and oxaliplatin; FOLFOX, oxaliplatin, leucovorin, and 5-fluorouracil; RT, radiation therapy.

permit the performance of an intervention that otherwise could not be undertaken (i.e. an LAR vs. an abdominoperineal resection). Moreover, the ability to obtain radial margins, thereby decreasing the rate of locoregional recurrence, should be attainable with a decrease in tumor burden. Although it is not clear whether pCR might serve as a marker for OS and DFS, it is a desirable outcome for a neoadjuvant modality. Figure 5 depicts the rate of pCR in several studies using multiple oxaliplatin-based combinations [19,22,24,29,34,39–45]. Any given combination with oxaliplatin and several agents with activity in colon cancer (i.e. cetuximab or bevacizumab) has not shown substantial advantage in pCR compared with historical pCR rates or fluoropyrimidine-based therapy alone. The fact that pCR has not been drastically altered with oxaliplatin-based therapy and that the potential rate of toxicity compared with fluoropyrimidine-based interventions indicate that oxaliplatin therapy should be used selectively rather than routinely in the preoperative setting. A better understanding of tumor biology might contribute to our ability in selecting a specific cohort of patients who could potentially benefit from preoperative oxlalipatin-based therapy in combination with RT.

References

1 Jung Y, Lippard SJ. Direct cellular responses to platinum-induced DNA damage. *Chem Rev* 2007; **107**:1387–1407.

2 Fink D, Zheng H, Nebel S, Norris PS, Aebi S, Lin TP, *et al.* In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. *Cancer Res* 1997; **57**:1841–1845.

3 Cvitkovic E. Ongoing and unsaid on oxaliplatin: the hope. *Br J Cancer* 1998; **77** (**Suppl 4**):8–11.

4 Huerta S, Anguiano-Hernandez YM, Goulet EJ, Huerta-Yepez S, Livingston EH. Decreased levels of AIF renders resistance to oxaliplatin mediated apoptosis in the progression of colon cancer carcinogenesis beyond caspase-3 activity. *Am J Gastroenterol* 2006; **101**:452.

5 Toscano F, Parmentier B, Fajoui ZE, Estornes Y, Chayvialle JA, Saurin JC, *et al.* p53 dependent and independent sensitivity to oxaliplatin of colon cancer cells. *Biochem Pharmacol* 2007; **74**:392–406.

6 Hata T, Yamamoto H, Ngan CY, Koi M, Takagi A, Damdinsuren B, *et al.* Role of p21waf1/cip1 in effects of oxaliplatin in colorectal cancer cells. *Mol Cancer Ther* 2005; **4**:1585–1594.

7 Hess S, Blackstock W. Oxaliplatin: In vitro and in vivo evidence of its radiation sensitizing activity. Preclinical observations relevant to ongoing clinical trials. *Proc Am Assoc Can Res* 2000; **41**:53a.

8 Hermann RM, Rave-Frank M, Pradier O. Combining radiation with oxaliplatin: a review of experimental results. *Cancer Radiother* 2008; **12**:61–67.

9 Magne N, Fischel JL, Formento P, Etienne MC, Dubreuil A, Marcie S, *et al.* Oxaliplatin-5-fluorouracil and ionizing radiation. Importance of the sequence and influence of p53 status. *Oncology* 2003; **64**: 280–287.

10 Kjellstrom J, Kjellen E, Johnsson A. In vitro radiosensitization by oxaliplatin and 5-fluorouracil in a human colon cancer cell line. *Acta Oncol* 2005; **44**:687–693.

11 Folkvord S, Flatmark K, Seierstad T, Hansen RA. In vitro and in vivo radiosensitization of colorectal carcinoma cells by oxaliplatin in combination with 5-fluorouracil. *Proceedings of the 10th International Meeting Wolfsberg Meeting on Molecular Radiation Biology/Oncology* 2007; **7**. [Abstract]

12 Cividalli A, Ceciarelli F, Livdi E, Altavista P, Cruciani G, Marchetti P, *et al.* Radiosensitization by oxaliplatin in a mouse adenocarcinoma: influence of treatment schedule. *Int J Radiat Oncol Biol Phys* 2002; **52**:1092–1098.

13 Cvitkovic E, Bekradda M. Oxaliplatin: a new therapeutic option in colorectal cancer. *Semin Oncol* 1999; **26**:647–662.

14 Legallicier B, Leclere C, Monteil C, Elkaz V, Morin JP, Fillastre JP. The cellular toxicity of two antitumoural agents derived from platinum, cisplatinum versus oxaliplatinum, on cultures of tubular proximal cells. *Drugs Exp Clin Res* 1996; **22**:41–50.

15 Christian MC. The current status of new platinum analogs. *Semin Oncol* 1992; **19**:720–733.

16 Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, *et al.* Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* 2004; **350**:2343–2351.

17 Andre T, Boni C, Navarro M, Tabernero J, Hickish T, Topham C, *et al.* Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. *J Clin Oncol* 2009; **27**:3109–3116.

18 Freyer G, Bossard N, Romestaing P, Mornex F, Chapet O, Trillet-Lenoir V, *et al.* Addition of oxaliplatin to continuous fluorouracil, l-folinic acid, and concomitant radiotherapy in rectal cancer: the Lyon R 97-03 phase I trial. *J Clin Oncol* 2001; **19**:2433–2438.

19 Rodel C, Grabenbauer GG, Papadopoulos T, Hohenberger W, Schmoll HJ, Sauer R. Phase I/II trial of capecitabine, oxaliplatin, and radiation for rectal cancer. *J Clin Oncol* 2003; **21**:3098–3104.

20 Gambacorta MA, Valentini V, Coco C, Morganti AG, Smaniotto D, Micciche F, *et al.* Chemoradiation with raltitrexed and oxaliplatin in preoperative treatment of stage II-III resectable rectal cancer: phase I and II studies. *Int J Radiat Oncol Biol Phys* 2004; **60**:139–148.

21 Francois E, Ychou M, Ducreux M, Bertheault-Cvitkovic F, Giovannini M, Conroy T, *et al.* Combined radiotherapy, 5-fluorouracil continuous infusion and weekly oxaliplatin in advanced rectal cancer: a phase I study. *Eur J Cancer* 2005; **41**:2861–2867.

22 Aschele C, Friso ML, Pucciarelli S, Lonardi S, Sartor L, Fabris G, *et al.* A phase I-II study of weekly oxaliplatin, 5-fluorouracil continuous infusion and preoperative radiotherapy in locally advanced rectal cancer. *Ann Oncol* 2005; **16**:1140–1146.

23 Sebag-Montefiore D, Glynne-Jones R, Falk S, Meadows HM, Maughan T. A phase I/II study of oxaliplatin when added to 5-fluorouracil and leucovorin and pelvic radiation in locally advanced rectal cancer: a Colorectal Clinical Oncology Group (CCOG) study. *Br J Cancer* 2005; **93**:993–998.

24 Ryan DP, Niedzwiecki D, Hollis D, Mediema BE, Wadler S, Tepper JE, *et al.* Phase I/II study of preoperative oxaliplatin, fluorouracil, and external-beam radiation therapy in patients with locally advanced rectal cancer: Cancer and Leukemia Group B 89901. *J Clin Oncol* 2006; **24**:2557–2562.

25 Rosenthal DI, Catalano PJ, Haller DG, Landry JC, Sigurdson ER, Spitz FR, *et al.* Phase I study of preoperative radiation therapy with concurrent infusional 5-fluorouracil and oxaliplatin followed by surgery and postoperative 5-fluorouracil plus leucovorin for T3/T4 rectal adenocarcinoma: ECOG E1297. *Int J Radiat Oncol Biol Phys* 2008; **72**:108–113.

26 Carraro S, Roca EL, Cartelli C, Rafailovici L, Castillo OS, Wasserman E, *et al.* Radiochemotherapy with short daily infusion of low-dose oxaliplatin, leucovorin, and 5-FU in T3-T4 unresectable rectal cancer: a phase II IATTGI study. *Int J Radiat Oncol Biol Phys* 2002; **54**:397–402.

27 Gerard JP, Chapet O, Nemoz C, Romestaing P, Mornex F, Coquard R, *et al.* Preoperative concurrent chemoradiotherapy in locally advanced rectal cancer with high-dose radiation and oxaliplatin-containing regimen: the Lyon R0-04 phase II trial. *J Clin Oncol* 2003; **21**:1119–1124.

28 Loi S, Ngan SY, Hicks RJ, Mukesh B, Mitchell P, Michael M, *et al.* Oxaliplatin combined with infusional 5-fluorouracil and concomitant radiotherapy in inoperable and metastatic rectal cancer: a phase I trial. *Br J Cancer* 2005; **92**:655–661.

29 Machiels JP, Duck L, Honhon B, Coster B, Coche JC, Scalliet P, *et al.* Phase II study of preoperative oxaliplatin, capecitabine and external beam radiotherapy in patients with rectal cancer: the RadiOxCape study. *Ann Oncol* 2005; **16**:1898–1905.

30 Rodel C, Martus P, Papadoupolos T, Fuzesi L, Klimpfinger M, Fietkau R, *et al.* Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. *J Clin Oncol* 2005; **23**:8688–8696.

31 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.

32 Gerard JP, Conroy T, Bonnetain F, Bouche O, Chapet O, Closon-Dejardin MT, *et al.* Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. *J Clin Oncol* 2006; **24**:4620–4625.

33 Aschele C, Friso ML, Pucciarelli S, Lonardi S, Sartor L, Fabris G, *et al.* A phase I-II study of weekly oxaliplatin, 5-fluorouracil continuous infusion and preoperative radiotherapy in locally advanced rectal cancer. *Ann Oncol* 2005; **16**:1140–1146.

34 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.
35 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.
36 Janjan NA, Abbruzzese J, Pazdur R, Khoo VS, Cleary K, Dubrow R, *et al.* Prognostic implications of response to preoperative infusional chemoradiation in locally advanced rectal cancer. *Radiother Oncol* 1999; **51**:153–160.
37 Janjan NA, Crane C, Feig BW, Cleary K, Dubrow R, Curley S, *et al.* Improved overall survival among responders to preoperative chemoradiation for locally advanced rectal cancer. *Am J Clin Oncol* 2001; **24**:107–112.
38 Bosset JF, Collette L, Calais G, Mineur L, Maingon P, Radosevic-Jelic L, *et al.* Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med* 2006; **355**:1114–1123.
39 Avallone A, Delrio P, Guida C, Tatangelo F, Petrillo A, Marone P, *et al.* Biweekly oxaliplatin, raltitrexed, 5-fluorouracil and folinic acid combination chemotherapy during preoperative radiation therapy for locally advanced rectal cancer: a phase I-II study. *Br J Cancer* 2006; **94**:1809–1815.
40 Chau I, Brown G, Cunningham D, Tait D, Wotherspoon A, Norman AR, *et al.* Neoadjuvant capecitabine and oxaliplatin followed by synchronous chemoradiation and total mesorectal excision in magnetic resonance imaging-defined poor-risk rectal cancer. *J Clin Oncol* 2006; **24**:668–674.
41 Fakih MG, Rajput A, Yang GY, Pendyala L, Toth K, Smith JL, *et al.* A Phase I study of weekly intravenous oxaliplatin in combination with oral daily capecitabine and radiation therapy in the neoadjuvant treatment of rectal adenocarcinoma. *Int J Radiat Oncol Biol Phys* 2006; **65**:1462–1470.
42 Minsky BD, Roedel C, Valentini V. Combined modality therapy for rectal cancer. *Cancer J* 2010; **16**:253–261.
43 Rodel C, Engenhart-Cabillic R, Hess C, Reese T, Flentje M, Neubauer A, *et al.* Phase II study on pre-operative radiochemotherapy and adjuvant chemotherapy with Capecitabin and Oxaliplatin in rectal cancer in UICC stages II and III. *Strahlenther Onkol* 2005; **181**:56.
44 Rodel C, Arnold D, Hipp M, Liersch T, Dellas K, Iesalnieks I, *et al.* Phase I-II trial of cetuximab, capecitabine, oxaliplatin, and radiotherapy as preoperative treatment in rectal cancer. *Int J Radiat Oncol Biol Phys* 2008; **70**: 1081–1086.
45 Willett CG, Duda DG, Di TE, 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.