PERGAMON

European Journal of Cancer 37 (2001) 2302–2309

European Journal of Cancer

www.ejconline.com

Current Perspective

Recent advances in the management of colorectal cancer

E. Van Cutsem[a,*], M. Dicato[b], J. Wils[c]

[a]*Department of Internal Medicine, University Hospital Gosthuisberg, Herestraat 49, BE-3000 Leuven, Belgium*
[b]*Luxembourg Medical Centre, Luxembourg*
[c]*Laurentius Hospital, Roermond, The Netherlands*

Received 6 February 2001; received in revised form 12 July 2001; accepted 12 July 2001

1. Introduction

After several decades of modest progress in the management of colorectal cancer, the recent identification of colorectal cancer genes, increased understanding of colorectal carcinogenesis, the introduction of new chemotherapeutic agents and the identification of new biological targets have all led to substantial improvements in survival and quality of life (QoL) of patients. This progress has also raised hopes for further improvements in the near future. This paper reviews the current 'state of the art' in colorectal cancer management and the impact of recent innovations.

2. Molecular biology, prevention and screening of colorectal cancer

2.1. Molecular mechanisms and chemoprevention of colorectal cancer

The importance of the adenoma–carcinoma sequence in the development of colorectal cancer is well established and recent research has helped to further elucidate the mechanisms involved in the pathogenesis of this disease. Results of recent studies suggest that the *adenomatous polyposis coli* (*APC*) gene may well be the ultimate 'gatekeeper' of colonic epithelial cell proliferation. Mutations in the *APC* gene, which controls the Wnt signal transduction pathway through regulation of β-catenin expression [1,2], may result in unregulated β-catenin expression with subsequent tumorigenic effects [3]. Improved understanding of the molecular processes has revealed new targets for chemoprevention, and potentially suppression or reversal of the carcinogenic process.

Among the most extensively evaluated chemopreventive strategies for colorectal cancer are the non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs have been shown to cause reduction and regression of polyps in familial adenomatous polyposis (FAP) [4], but have no consistent effect on duodenal neoplasia. The long-term gastro-intestinal side-effects of these agents have also raised concerns. Another approach involving disruption of cyclo-oxygenase (COX)-2 activity appears more promising, particularly as most colorectal adenomas and cancers overexpress the *COX-2* gene [5]. In animal models of COX-2 overexpression, tumour growth can be blocked through inhibition of COX-2 [6]. More recently, the selective COX-2 inhibitor celecoxib has been shown to significantly decrease polyps in FAP [7], resulting in approval of celecoxib by the United States Food and Drug Administration for the management of rectal polyps in patients with the FAP syndrome. This and other COX-2 inhibitors (rofecoxib) in clinical development may also have potential in the secondary prevention of sporadic adenoma recurrence after adenoma, polyp or tumour removal. These agents are particularly attractive because of their good safety profile.

2.2. Screening

Screening for colorectal cancer has numerous ethical implications, and the psychosocial issues and problems of confidentiality for patients and family members must always be considered when conducting screening tests. Counselling is an important component of the process

* Corresponding author. Tel.: +32-16-344225; fax: +32-16-344419.
E-mail address: eric.vancutsem@uz.kuleuven.ac.be (E. Van Cutsem).

PII: S0959-8049(01)00249-0

and a multiprofessional approach should be adopted when dealing with hereditary colon cancer. In families with the FAP-syndrome or the Hereditary Non-Polyposis Colorectal Cancer Syndrome (HNPCC), the use of DNA tests looking for *APC*-mutations or for the responsible mutations in one of the mutated DNA-mismatch repair genes (*hMLH1*, *hMSH2*, *hMSH6*) has dramatically changed the screening policy.

Several different techniques, including faecal occult blood testing (FOBT), sigmoidoscopy, double-contrast barium enema and colonoscopy are currently used either alone or together when screening for colorectal cancer. The choice of technique is usually based on a number of different criteria, including complexity, potential effectiveness, evidence of effectiveness and the risks associated with the procedure. Continuing research in this field has resulted in the development of new screening techniques. Among these, one of the most promising is virtual colonoscopy. Virtual colonoscopy uses spiral or multi-slice computed tomography imaging or magnetic resonance imaging of the colon following bowel preparation and air distention [8]. For smaller adenomas, this method produces a higher false-negative rate than colonoscopy and, consequently, cannot yet be recommended for routine use outside clinical trials. However, as data become available from more centres and more studies, it will be exciting to see how use of this technique evolves. It can be expected that virtual colonoscopy will have a place in the screening of colon cancer.

3. The management of rectal cancer

In Europe, the standard surgical treatment for rectal cancer is total mesorectal excision (TME). Studies in Europe and the USA have demonstrated that pre- or postoperative radiotherapy can improve outcomes for patients with resectable rectal cancer compared with surgery alone. In Europe, there has been a greater enthusiasm for the preoperative administration of radiotherapy. Two types of preoperative radiotherapy are used: 25 Gy in five fractions (Swedish regimen) and 45 Gy in 1.8 Gy fractions 4–6 weeks before surgery (long regimen). Potential advantages of the long schedule include an increased likelihood of tumour regression and the increased feasibility of performing sphincter-saving surgery. The Swedish Rectal Cancer Trial [9], the CR-02 study by the Medical Research Council (MRC) in the UK, as well as the Dutch TME trial showed that rates of local recurrence are reduced in patients with resectable rectal cancer receiving preoperative radiotherapy. Furthermore, in the Swedish trial, the 5-year survival rate was significantly superior in the preoperative radiotherapy arm compared with patients receiving surgery alone (58 versus 48%, respectively; $P = 0.004$). Another Swedish study showed that preoperative radiotherapy achieves more effective local control at a lower dose compared with postoperative radiotherapy [10]. In clinical trials, radiotherapy alone in the postoperative setting, which is the predominant approach used in the USA, increases local control, but has failed to show a convincing benefit in terms of survival. Furthermore, preoperative radiotherapy produces less morbidity than postoperative radiotherapy, with higher rates of compliance. The results of a number of controlled, randomised trials have demonstrated that adjuvant 5-fluorouracil (5-FU)-based chemotherapy administered with postoperative radiotherapy achieves a significant reduction in rates of local recurrence and improved overall survival compared with surgery alone or surgery plus postoperative radiotherapy [11–13]. The use of chemoradiation is attractive, as it simultaneously combines systemic treatment with a locoregional treatment. In addition, 5-FU is an effective radiosensitiser.

Several approaches to improving the efficacy of chemoradiation with 5-FU have been investigated, including the co-administration of agents such as leucovorin (LV), levamisole or nitrosourea. Most of these strategies have failed to significantly improve outcome compared with standard chemoradiation. However, in a large, randomised trial comparing bolus and infused 5-FU regimens in 660 patients with TNM stage II or III rectal cancer, both overall and disease-free survival were significantly improved by administering 5-FU as an infusion for the duration of radiotherapy compared with bolus 5-FU administration [14]. In addition, the rate of development of distant metastases was significantly decreased with infused versus bolus 5-FU. The safety profiles of infused and bolus 5-FU regimens were similar, except for a higher incidence of grade 3/4 diarrhoea and a lower incidence of grade 3/4 leucopenia with the infused regimen. A recently published study by the National Surgical Adjuvant Breast and Bowel Project (NSABP) (protocol R-02) confirmed that at a 5-year follow-up, postoperative radiotherapy plus 5-FU/LV achieves a statistically significant reduction in the rate of local relapse from 13 to 8% ($P = 0.02$) compared with chemotherapy alone [15]. However, in this study the incidence of distant relapse and overall survival rates were not significantly improved by the addition of radiotherapy to chemotherapy.

It is anticipated that a number of ongoing studies will help to further define the optimal regimen for the treatment of rectal cancer. A Dutch phase III trial compared TME alone versus preoperative radiotherapy (25 Gy in five fractions) immediately prior to the TME. Preliminary results have indicated a significantly lower local relapse rate for patients treated with preoperative radiotherapy followed by TME compared with surgery alone [16]. Another trial, which has been initiated in

Germany, is investigating preoperative versus postoperative radiotherapy (long regimen), both with concurrent and adjuvant 5-FU. The results of an ongoing European Organization of Research and Treatment of Cancer (EORTC/FFCD (Fondation Francaise de Canceroologie Digestive)) phase III trial should determine whether the addition of chemotherapy (5-FU/LV) to preoperative radiotherapy provides a clinical benefit.

3.1. Anal sphincter preservation

Another important consideration when assessing the merits of adjuvant radiotherapy in the treatment of rectal cancer is the impact on sphincter preservation and QoL. In many of the trials evaluating radiotherapy immediately prior to surgery, no improvement in the rate of sphincter preservation was observed. In a French, randomised study comparing surgery either immediately ($\leqslant$2 weeks) after radiotherapy or $>$5 weeks after completion of radiotherapy in a total of 201 patients with rectal cancer, a long interval between preoperative irradiation and surgery was associated with a significantly improved tumour response and pathological tumour downstaging [17]. Furthermore, a delay between irradiation and surgery appeared to increase the rate of sphincter preservation, a difference that was even more pronounced in patients with low-lying tumours ($\leqslant$5 cm above the anal verge). However, despite these benefits, there was no significant difference in 5-year survival rates. These investigators are currently evaluating contact X-ray or preoperative external beam radiotherapy (EBRT) with concomitant boost, for further tumour downstaging and possibly even total sterilisation of the tumour. Chemoradiation represents an alternative, potentially effective technique for tumour downstaging to achieve sphincter preservation. Therefore, the feasibility of the new drugs raltitrexed, oxaliplatin (+5-FU) and irinotecan (+5-FU) is actually being investigated in combination with radiotherapy in rectal cancer to try to achieve higher response rates.

In addition, new developments in surgical techniques, including nerve-sparing surgery, colonic pouch operations and advances in anal sphincter restorative surgery, are all playing an increasingly important role in the management of rectal cancer as more emphasis is placed on QoL.

4. New chemotherapeutic agents for advanced colorectal cancer

4.1. Thymidylate synthase inhibitors

Thymidylate synthase (TS) catalyses the rate-limiting step in DNA synthesis and represents a key target for cytotoxic drugs. Although 5-FU has maintained a key role in the treatment of colorectal cancer for more than 40 years, many attempts have been made to improve the therapeutic benefit of this agent. In addition, several new agents that inhibit TS have been developed. In contrast to the antimetabolites 5-FU and methotrexate, which achieve cytotoxicity through indirect inhibition of TS, new agents have been developed that inhibit TS directly, such as raltitrexed, a quinazoline folate analogue. Another agent in clinical development, multitargeted antifolate (MTA), is a direct inhibitor of TS, but also inhibits TS indirectly through dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyltransferase (GARFT). Of the novel TS inhibitors, raltitrexed has undergone the most extensive phase III evaluation as monotherapy. In three randomised trials, response rates were similar to those achieved with 5-FU/LV, but there was a reduced incidence of stomatitis [18,19]. However, time to disease progression and survival results were disappointing.

More recently, with the introduction of agents such as oxaliplatin and irinotecan that do not rely on TS inhibition, the focus for therapeutic development has generally moved away from the development of novel TS inhibitors. Research interest is now concentrating on the development of regimens combining TS inhibitors and novel agents with different mechanisms of action, such as irinotecan and oxaliplatin. In a randomised study comparing 5-FU/LV in combination with methotrexate, raltitrexed or irinotecan, the response rate with the combination of 5-FU/LV/raltitrexed was similar to that for 5-FU/LV/methotrexate [20]. Combinations of raltitrexed with irinotecan and oxaliplatin are currently being evaluated in phase I and II studies.

4.2. Irinotecan and oxaliplatin

Irinotecan, a topoisomerase I inhibitor, has demonstrated activity in first- and second-line treatment in patients with metastatic colorectal cancer [21]. Following demonstration of a survival benefit in 5-FU-pretreated patients in two phase III trials [22,23], irinotecan monotherapy has become an established second-line treatment for metastatic colorectal cancer.

Recent trials have demonstrated that the addition of irinotecan to either infused or bolus 5-FU/LV regimens as first-line treatment results in significantly superior response rates, time to disease progression and, most importantly, survival [24,25]. In both of the trials, adverse events occurred more frequently when 5-FU/LV was administered with irinotecan, but toxicities (mainly diarrhoea and neutropenia) were generally predictable and manageable, although careful monitoring was required. Both trials confirmed that the addition of irinotecan to 5-FU/LV regimens provided a survival benefit without compromising patients' QoL (global health

status). As a result, irinotecan in combination with 5-FU/LV is now considered by many to be the reference first-line treatment for colorectal cancer. Studies in the adjuvant setting are ongoing with the combination 5-FU/LV/irinotecan (Petacc 3 study).

Oxaliplatin is a third-generation platinum analogue, with activity and toxicity profiles that differ from those of other platinum derivatives, including cisplatin and carboplatin [26]. Oxaliplatin has also been investigated in combination with 5-FU as first-line treatment for colorectal cancer. Although the latest data did not demonstrate a statistically significant survival benefit, the median overall survival was 15 months [27]. The failure of this study to demonstrate a significant survival benefit is probably explained by the fact that it was not designed with survival as the primary endpoint. Furthermore, there was an imbalance between the two treatment groups in baseline concentrations of alkaline phosphatase, a known predictor for survival. Crossover to oxaliplatin in patients whose disease progressed on 5-FU/LV may also have contributed to the failure to demonstrate a significant difference in survival. Two phase III trials have been completed or are ongoing in the adjuvant setting (MOSAIC study, NSABP study C-07), and a study investigating oxaliplatin with 5-FU/LV in the neoadjuvant treatment of patients with resectable liver metastases will be initiated by the EORTC Gastrointestinal Tract Cancer Co-operative Group (GITCCG).

Most recently, preliminary results have been reported from trials investigating irinotecan plus oxaliplatin in combination with 5-FU/LV [28,29]. Although these trials included relatively small numbers of patients, the results obtained with this combination seem promising. As predicted by the pharmacology of these agents, the main grade 3/4 toxicities were diarrhoea and haematological toxicities. Combination regimens including irinotecan and oxaliplatin have also been investigated as second-line therapy. However, a randomised, phase II trial evaluating second-line irinotecan/5-FU versus oxaliplatin/5-FU versus irinotecan/oxaliplatin in 101 patients failed to identify any significant differences in outcome [30]. A first study on the sequence of combination treatment comparing 5-FU/FA/irinotecan followed by 5-FU/FA/oxaliplatin versus 5-FU/FA/oxaliplatin followed by 5-FU/FA/irinotecan reported a high response rate in first-line treatment for both regimens (56–54%). Both sequences have a long time to progression (TTP) after sequential use (14.4–11.5 months, respectively) and a long median survival (20.4-21.5 months). Toxicity was comparable [31].

4.3. Oral fluoropyrimidines

The oral fluoropyrimidines represent another relatively new class of drugs, and include agents such as capecitabine and UFT (uracil plus tegafur). Capecitabine undergoes a three-stage conversion to 5-FU, with the final step mediated by thymidine phosphorylase [32,33]. As this enzyme is present at significantly higher concentrations in tumour cells compared with healthy tissue, administration of capecitabine results in the generation of 5-FU preferentially at the tumour site [34]. The mechanism of action of UFT and eniluracil relies on inhibition of dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme in the catabolism of 5-FU [35,36]. Uracil and eniluracil compete with 5-FU for the binding site for DPD, thus leaving a higher proportion of 5-FU available for conversion to cytotoxic 5-FU metabolites. DPD inhibition also plays an important role in 5-FU resistance [37].

Both capecitabine monotherapy and UFT/LV have been evaluated extensively in randomised phase III trials. Capecitabine has demonstrated favourable efficacy compared with 5-FU/LV (Mayo Clinic regimen), with a significantly superior response rate in one trial and equivalent survival and time to disease progression [38,39], and has recently received regulatory approval in the USA and Europe as first-line therapy for metastatic colorectal cancer. UFT/LV has demonstrated efficacy equivalent to the Mayo Clinic regimen [40,41], apart from significantly inferior time to disease progression in one of the two phase III studies [40]. UFT/LV combination therapy has been approved in most European countries for the first-line treatment of metastatic colorectal cancer, but not in Germany or the USA. Both of these oral fluoropyrimidines have demonstrated significantly less myelosuppression than the Mayo Clinic regimen.

The combination of eniluracil and 5-FU, however, was inferior to 5-FU/LV in a large randomised phase III study [42]. It is anticipated that the oral fluoropyrimidines may ultimately replace intravenous (i.v.) 5-FU in combination with irinotecan and oxaliplatin. The results of a phase I/II study of irinotecan and capecitabine in previously untreated patients with colorectal cancer, indicate that the combination provides a feasible and convenient alternative to i.v. 5-FU/LV plus irinotecan, and have provided the rationale for randomised phase III studies to evaluate this combination [43]. Similarly, the combination of capecitabine and oxaliplatin has been shown to be a convenient and effective therapy in a phase I study in patients with 5-FU-refractory colorectal cancer [44], and a phase II study is being conducted to investigate capecitabine plus oxaliplatin as first-line treatment. The combination of UFT/LV plus irinotecan or oxaliplatin is also under investigation.

4.4. Combined versus sequential therapy

The introduction of new treatment options in recent years has opened the debate on whether combined or

sequential chemotherapy is more appropriate. In the absence of a randomised trial, there is disagreement on whether the data available provide enough evidence to support combined first-line chemotherapy (5-FU/LV plus either oxaliplatin or irinotecan) for all patients or for a subset of patients. Furthermore, it remains to be determined whether sequential therapy can be as effective as combination therapy and if there are subpopulations of patients that benefit particularly from sequential therapy. Consequently, most oncologists feel that in some patients, an aggressive approach with first-line 5-FU/LV/irinotecan combination therapy is the most appropriate, whereas other patients are more appropriately treated with 5-FU/LV as first-line treatment followed by irinotecan monotherapy or the addition of oxaliplatin only when disease has progressed. It is anticipated that a randomised trial organised by the EORTC to compare sequential and combination therapy will go some way to resolving this issue. In addition, a trial initiated by the Colorectal Cancer Group of the MRC in the UK is ongoing to establish whether sequential therapy can be as effective as combination therapy. A French study comparing the sequence of 5-FU/LV/irinotecan followed by 5-FU/LV/oxaliplatin compared to 5-FU/LV/oxaliplatin followed by 5-FU/LV/irinotecan has shown a long, but similar TTP and survival for both sequences.

4.5. Adjuvant therapy for stage II and III colorectal cancer

Weekly or monthly bolus 5-FU/LV is currently considered the standard adjuvant treatment for Dukes' C colon cancer. Several studies are exploring other infusion schedules but although promising, these regimens are inconvenient for patients. Adjuvant treatment for stage II disease is controversial and treatment decisions should probably be made on an individual patient basis using molecular tumour markers and prognostic indicators wherever possible. The incorporation of new agents such as irinotecan or oxaliplatin into treatment regimens may enable further improvements in adjuvant therapy, and trials assessing new combinations have been initiated in Europe and the USA. In the future, the oral fluoropyrimidines may play a role in adjuvant therapy, possibly in combination with other drugs.

4.6. New biological agents and markers

Although cytotoxic agents will probably remain the cornerstone of treatment of advanced cancer, the rapid growth in the number of biological agents with novel targets should provide interesting new strategies for the treatment of colorectal cancer. Among the most promising of these are agents that inhibit farnesyl transferase, vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR), as well as inhibitors of angiogenesis.

Ras oncogenes are mutated in more than 40% of colorectal cancers and mutation leads to constitutive activation of ras [45]. Association of ras with the inner surface plasma membrane is facilitated by farnesyl protein transferase (FPT). A variety of farnesyl transferase inhibitors are currently in clinical development for therapy of colorectal cancer. SCH66336 has demonstrated significant antitumour activity in preclinical studies [46] and phase II studies are currently in progress. R115777 [47], another farnesyl transferase inhibitor, has been studied in the third-line treatment of advanced colorectal cancer compared with Best Supportive Care. Results are awaited.

Vascular endothelial growth factor (VEGF) is another promising target, as it is an important angiogenic factor in colon cancer [48,49]. Furthermore, increased VEGF expression is associated with a poor prognosis and increased risk of metastasis in patients with primary colorectal cancer [48]. Several strategies for inhibition of VEGF in colorectal cancer cells have been evaluated in preclinical studies and include VEGF antisense, monoclonal antibodies and specific small molecule inhibitors [50–52]. The role of monoclonal antibodies against epidermal growth factor receptor (EGFR) in colorectal cancer therapy has also been evaluated in preclinical studies [50]. It has been shown that Cetuximab (IMC-C225), a monoclonal antibody against the EGFR, can produce major objective responses (response rate (RR): 22%) in association with irinotecan in patients with EGFR-positive irinotecan-refractory colorectal cancer, with acceptable toxicity [53].

Improved understanding of tumour biology and the molecular phenotype of colorectal cancer has also helped to identify ways of improving therapy. Several biological markers have been shown to be accurate predictors of outcome and/or response to treatment in colorectal cancer. Of these TS and p53 have been the most extensively evaluated. TS activity correlates directly with response to 5-FU in advanced disease [54,55] and in one study, TS was identified as a predictor of survival and disease-free survival [56]. Two ongoing clinical trials are investigating this relationship further. The prognostic significance of p53 is more uncertain, although an interaction between p53 and TS levels has been observed [57].

Allele loss and microsatellite instability (MSI), a disorder of DNA mismatch repair, are also important in determining prognosis in colorectal cancer. Allele loss is widespread in colorectal cancer and several studies have demonstrated the clinical significance of allele loss of 17p and 18q, which are highly relevant in defining prognosis [58,59]. Recent results have identified MSI as an additional important prognostic marker [60]. MSI-positive patients were found to have a better prognosis

than MSI-negative patients in stage III disease ($P = 0.02$), supporting previous studies. The investigators found that among patients who received adjuvant 5-FU/levamisole, the 5-year survival rate was 96% in MSI-positive patients compared with 37% in MSI-negative patients ($P = 0.0007$). However, MSI-positive patients who did not receive chemotherapy had only a small survival benefit. The survival advantage was most pronounced in females and in tumours of the proximal/transverse colon. As 15% of all patients were MSI-positive, it has been suggested that most of the benefit of adjuvant chemotherapy is derived from MSI-positive patients. If this is true, MSI is a very powerful new molecular marker and therefore prospective trials should be conducted to establish the predictive value of MSI.

The considerable advances being made in so many areas of colorectal cancer biology and treatment are likely to have a major impact on the therapy of colorectal cancer in the future. Not only are we seeing significant improvements in the range of therapies available, but we are also witnessing the more rational use of these agents, enabling each individual patient with colorectal cancer to receive the therapy most suitable for him or her. This is certainly an exciting time for oncologists treating colorectal cancer, and the management of colorectal cancer is set to improve dramatically in the next decade.

References

1. Willert K, Nusse R. β-catenin: a key mediator of Wnt signaling. *Curr Opin Genet Dev* 1998, **8**, 95–102.
2. Su LK, Vogelstein B, Kinzler KW. Association of the APC tumor suppressor protein with catenins. *Science* 1993, **262**, 1734–1737.
3. Smits R, Kielman MF, Breukel C, *et al*. Apc 1638T: a mouse model delineating critical domains of the adenomatous polyposis coli protein involved in tumorgenesis and development. *Genes Devel* 1999, **13**, 1309–1321.
4. Nugent KP, Farmer KC, Spigelman AD, *et al*. Randomized, controlled trial of the effect of sundilac on duodenal and rectal polyposis and cell proliferation in patients with familial adenomatous polyposis. *Br J Surg* 1993, **80**, 1618–1619.
5. Eberhart CE, Coffey RJ, Radhika A, *et al*. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994, **107**, 1183–1188.
6. Oshima M, Dinchuk JE, Kargman SL, *et al*. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 1996, **87**, 803–809.
7. Steinbach G, Lynch PM, Phillips RKS, *et al*. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000, **342**, 1946–1952.
8. Rex DK, Vining D, Kopecky KK. An initial experience with screening for colon polyps using spiral CT with and without CT colography (virtual colonoscopy). *Gastrointest Endosc* 1999, **50**, 437–440.
9. The Swedish Rectal Cancer Trial. Improved survival with preoperative radiotherapy in resectable cancer. *N Engl J Med* 1997, **336**, 980–987.
10. Pahlman L, Glimelius B. Pre- or postoperative radiotherapy in rectal and rectosigmoid carcinoma. Report from a randomized multicenter trial. *Ann Surg* 1990, **211**, 187–195.
11. Gastrointestinal Tumour Study Group—GITSG 7175. Prolongation of disease-free interval in surgically treated rectal carcinoma. *N Engl J Med* 1985, **312**, 1464–1472.
12. Krook JE, Moertel CG, Gundersen LL, *et al*. Effective surgical adjuvant therapy for high risk rectal cancer. *New Engl J Med* 1991, **324**, 709–715.
13. Tveit KM, Guldvog I, Trondsen E, *et al*. Randomised controlled trial of post operative radiotherapy and short term time scheduled 5-fluorouracil against surgery alone in the treatment of Duke's B and C rectal cancer. Norwegian Adjuvant Rectal Cancer Project Group. *Br J Surg* 1997, **84**, 1130–1135.
14. O'Connell MJ, Martensen JA, Wieand HS, *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.
15. Wolmark N, Wieand HS, Hyams DM, *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.
16. Kapiteijn E, Kranenbarg EK, Steup WH, *et al*. Total mesorectal excision (TME) with or without preoperative radiotherapy in the treatment of primary rectal cancer. Prospective randomised trial with standard operative and histopathological techniques. Dutch ColoRectal Cancer Group. *Eur J Surg* 1999, **165**, 410–420.
17. Francois Y, Nemoz CJ, Baulieux J, *et al*. Influence of the interval between preoperative radiation therapy and surgery on downstaging and on the rate of sphincter-sparing surgery for rectal cancer: the Lyons R90-01 randomized trial. *J Clin Oncol* 1999, **17**, 2396–2402.
18. Cocconi G, Cunningham D, Van Cutsem E, *et al*. Open, randomized, multicenter trial of raltitrexed versus fluorouracil plus high-dose leucovorin in patients with advanced colorectal cancer. *J Clin Oncol* 1998, **16**, 2943–2952.
19. Cunningham D, Zalcberg JR, Rath U, *et al*. 'Tomudex' (ZD1694): results of a randomised trial in advanced colorectal cancer demonstrate efficacy and reduced mucositis and leucopenia. The 'Tomudex' Colorectal Cancer Study Group. *Eur J Cancer* 1995, **31A**, 1945–1954.
20. Comella P, De Vita F, Mancarella S, *et al*. Biweekly irinotecan or raltitrexed plus 6S-leucovorin and bolus 5-fluorouracil in advanced colorectal carcinoma: a Southern Italy Cooperative Oncology Group phase II-III randomized trial. *Ann Oncol* 2000, **11**, 1323–1333.
21. Van Cutsem E, Cunningham D, Ten Bokkel Huinink WW, *et al*. Clinical activity and benefit of Irinotecan (CPT-11) in patients with colorectal cancer truly resistant to 5-Fluorouracil (5-FU). *Eur J Cancer* 1999, **35**, 54–59.
22. Cunningham D, Pyrhönen S, James RD, *et al*. Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. *Lancet* 1998, **352**, 1413–1418.
23. Rougier P, Van Cutsem E, Bajetta E, *et al*. Randomised trial of irinotecan versus fluorouracil by continuous infusion after fluorouracil failure in patients with metastatic colorectal cancer. *Lancet* 1998, **352**, 1407–1412.
24. Douillard JY, Cunningham D, Roth AD, *et al*. Irinotecan combined with fluorouracil compared to fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000, **355**, 1041–1047.
25. Saltz LB, Cox JV, Blanke C, *et al*. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2000, **343**, 905–915.

26. Armand J-P, Boige V, Raymond E, *et al*. Oxaliplatin in colorectal cancer: an overview. *Semin Oncol* 2000, **27**(Suppl. 10), 96–104.
27. De Gramont A, Figer A, Seymour M, *et al*. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000, **18**, 2938–2947.
28. Falcone A, Masi G, Pfanner E, *et al*. Phase I-II study of Irinotecan (CPT-11), Oxaliplatin (LOHP), Leucovorin (LV) and 5-Fluorouracil (5-FU) 48 hours continuous infusion (C.I.) in metastatic colorectal cancer patients. *Proc Am Soc Clin Oncol* 2000, **19**, 297a.
29. Calvo E, Gonzalez-Cao M, Cortes J, *et al*. Combined irinotecan, oxaliplatin, 5-FU in patients (PTS) with metastatic colorectal cancer (MCC). *Proc Am Soc Clin Oncol* 2000, **19**, 259a.
30. Lepille D, Douillard JY, Marre A, *et al*. Essai randomisé de phase II évaluant 3 associations en traitement de seconde ligne du cancer colorectal métastatique: LV5FU2+CPT11 vs LV5FU2+ LOHP vs LOHP+CPT11. *Gastroenterol Clin Biol* 2000, **24**, 183a.
31. Tournigand C, Louvet C, Quinaux E, *et al*. Folfiri followed by FOLFOX versus FOLFOX followed by FOLFIRI in metastatic colorectal cancer: final results of a phase III study. *Proc Am Soc Clin Oncol* 2001, **20**, 494a.
32. Miwa M, Ura M, Nishida M, *et al*. Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. *Eur J Cancer* 1998, **34**, 1274–1281.
33. Van Cutsem E, Findlay M, Osterwalder B, *et al*. Capecitabine, an oral fluoropyrimidine carbamate with substantial activity in advanced colorectal cancer: results of a randomized phase II study. *J Clin Oncol* 2000, **18**, 1337–1345.
34. Schüller J, Cassidy J, Dumont E, *et al*. Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. *Cancer Chemother Pharmacol* 2000, **45**, 291–297.
35. Baccanari DP, Davis ST, Knick VC, *et al*. 5-Ethynyluracil (776C85): a potent modulator of the pharmacokinetics and antitumour efficacy of 5-fluorouracil. *Proc Natl Acad Sci USA* 1993, **90**, 11064–11068.
36. Tuchman M, Ramnaraine ML, O'Dea RF, *et al*. Effects of uridine and thymidine on the degradation of 5-fluorouracil, uracil and thymine by rat liver dihydropyrimidine dehydrogenase. *Cancer Res* 1985, **45**, 5553–5556.
37. Ahmed FY, Johnston SJ, Cassidy J, *et al*. Eniluracil treatment completely inactivates dihydropyrimidine dehydrogenase in colorectal tumours. *J Clin Oncol* 1999, **17**, 2439–2445.
38. Van Cutsem E, Twelves C, Cassidy J, *et al*. Oral capecitabine compared with intravenous 5-fluorouracil plus leucovorin (Mayo Clinic regimen) in patients with metastatic colorectal cancer: results of a large phase III study. *J Clin Oncol*, in press.
39. Hoff PM, Ansari R, Batist G, *et al*. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. *J Clin Oncol* 2001, **19**, 2282–2292.
40. Pazdur R, Douillard JY, Skillings JR, *et al*. Multicenter phase III study of 5-fluorouracil (5-FU) or UFT™ in combination with leucovorin (LV) in patients with metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 1999, **18**, 263a.
41. Carmichael J, Popiela T, Radstone D, *et al*. Randomized comparative study of Orzel® (oral uracil/tegafur) (UFT™) plus LV (LV) versus parenteral 5-fluorouracil (5-FU) plus LV in patients with metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 1999, **18**, 264a.
42. Van Cutsem E, Sorensen J, Cassidy J, *et al*. International phase III study of oral Eniluracil (EU) plus 5-fluorouracil (5-FU) versus intravenous (IV) 5-FU plus leucovorin (LV) in the treatment of advanced colorectal cancer (ACC). *Proc Am Soc Clin Oncol* 2001, **20**, 131a.
43. Schleucher N, Tewes M, Achterrath W, *et al*. Extended phase I study of capecitabine in combination with a weekly schedule of irinotecan as first line chemotherapy in metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 2001, **20**, 561a.
44. Evans J, Tabernero J, Cassidy J, *et al*. Safety profile and preliminary efficacy of capecitabine (Xeloda®) in combination with oxaliplatin in patients with advanced or metastatic solid tumours: results from a phase I study. *Ann Oncol* 2000; **11** (Suppl. 4), 51 (abstr 222).
45. Bollag G, McCormick F. Regulators and effectors of ras proteins. *Annu Rev Cell Dev Biol* 1991, **7**, 601–632.
46. Lui M, Bryant MS, Chen J, *et al*. Antitumor activity of SCH66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice. *Cancer Res* 1998, **58**, 4947–4956.
47. Skrzat S, Angibaud P, Venet M, *et al*. R115777, a novel imidazole farnesyl transferase inhibitor (FTI) with potent oral antitumor activity. *Proc Am Soc Cancer Res* 1998, **39**, 2170a.
48. Takahashi Y, Kitadai Y, Bucana CD, *et al*. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res* 1995, **55**, 3964–3968.
49. Brown LF, Berse B, Jackman RW, *et al*. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptor in adenocarcinomas of the gastrointestinal tract. *Cancer Res* 1993, **53**, 4727–4735.
50. Cardiello F, Bianco R, Damiano V, *et al*. Antiangiogenic and antitumor activity of anti-epidermal growth factor receptor C225 monoclonal antibody in combination with vascular endothelial growth factor antisense oligonucleotide in human GEO colon cancer cells. *Clin Cancer Res* 2000, **6**, 3739–3747.
51. Kanai T, Konno H, Tanaka T, *et al*. Anti-tumor and anti-metastatic effects of human-vascular-endothelial-growth-factor-neutralizing antibody on human colon and gastric carcinoma xenotransplanted orthotopically into nude mice. *Int J Cancer* 1998, **77**, 933–936.
52. Ellis L, Takahashi Y, Liu W, *et al*. Vascular endothelial growth factor in human colon cancer: biology and therapeutic implications. *Oncologist* 2000, **5**, 11–15.
53. Saltz L Rubin M, Hochster H, *et al*. Cetuximal (IMC-C225) plus irinotecan (CPT-11) is active in CPT-11 refractory colorectal cancer that expresses Epidermal Growth Factor Receptor. *Proc Am Soc Clin Oncol* 2001, **20**, 7a.
54. Peters GJ, Van der Wilt CL, Van Groeningen CJ, *et al*. Thymidylate synthase inhibition after administration of fluorouracil with or without leucovorin in colon cancer patients: implications for treatment with fluorouracil. *J Clin Oncol* 1994, **12**, 2035–2042.
55. Leichman CG, Danenberg PV, Tan M, *et al*. Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted infusion fluorouracil and weekly leucovorin. *J Clin Oncol* 1997, **15**, 3223–3229.
56. Yamachika T, Nakanishi H, Inada KL, *et al*. A new prognostic factor for colorectal carcinoma, thymidylate synthase, and its therapeutic significance. *Cancer (Phila)* 1998, **82**, 70–77.
57. Lenz HJ, Hayashi K, Salonga D, *et al*. p53 point mutations and thymidylate synthase messenger RNA levels in disseminated colorectal cancer: an analysis of response and survival. *Clin Cancer Res* 1998, **4**, 1243–1250.
58. Leggett B, Young J, Buttenshaw R, *et al*. Colorectal carcinomas show frequent allelic loss on the long arm of chromosome 17 with

evidence for a specific target region. *Br J Cancer* 1995, **71**, 1070–1073.

59. Lanza G, Matteuzzi M, Gafa R, *et al*. Chromosome 18q allelic loss and prognosis in stage II and III colon cancer. *Int J Cancer* 1998, **79**, 390–395.
60. Elsaleh H, Joseph DJ, Lacopetta B. Microsatellite instability is a strong molecular predictive marker for sensitivity to adjuvant chemotherapy in Dukes' C colorectal carcinoma. *Proc Am Soc Clin Oncol* 2000, **19**, 238a.