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Systemic treatment of colorectal cancer

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Abstract

Palliative and adjuvant treatment for colorectal cancer has been, until recently, largely dependent on 5-fluorouracil (5-FU)-based chemotherapy. Oral fluoropyrimidines have been evaluated in the advanced disease setting and they appear to be as effective as 5-FU, but are safer and more convenient for most patients. Irinotecan and oxaliplatin are new cytotoxic agents, which are active in 5-FU-resistant disease, but which may also be combined with 5-FU as initial therapy in advanced disease. Initial combination therapy leads to improved response rates and more prolonged progression-free survival compared with 5-FU monotherapy. Standard regimens for adjuvant therapy usually involve 6 months of chemotherapy using 5-FU and folinic acid. Recent trials of capecitabine, oxaliplatin and irinotecan in the adjuvant setting are ongoing, or have recently completed accrual, and may lead to a change in future clinical practice. Biological therapies are playing an increasing role in the management of colorectal cancer. Farnesyl transferase inhibition, inhibition of the epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF) are undergoing evaluation in advanced disease. In the adjuvant setting, both passive and active immunotherapeutic approaches have been studied. In addition, a large trial will evaluate the role of cyclo-oxygenase(COX)-2 inhibitors as adjuvant therapy. Further research is required in order to define the optimal sequence and combination of these different cytotoxic and biological therapies, in order to secure the best possible outcome for various subgroups of patients with both early and advanced stage colorectal cancer.

Keywords: Systemic treatment; Colorectal cancer; 5-Fluorouracil; Chemotherapy

1. Introduction

Colorectal cancer is a leading cause of cancer morbidity and mortality. Over the past decade, it has become accepted that palliative chemotherapy improves survival and quality of life for patients with metastatic colorectal cancer. Importantly, the early use of chemotherapy in asymptomatic patients prolongs both symptom-free survival and overall survival [1]. Chemotherapy is also being increasingly used in the adjuvant setting. Until recently, the only agent with significant activity in this disease was 5-fluorouracil (5-FU).

2. Systemic treatment of metastatic disease

2.1. Fluorouracil

The paucity of other agents with significant activity resulted in an extensive study of mechanisms to improve the activity and reduce the toxicity of 5-FU. Thus, biochemical modulation of bolus regimens of 5-FU with either folinic acid or methotrexate improved the activity of 5-FU compared with 5-FU alone [2,3] (Fig. 1). The activity and convenience of the combination of bolus 5-FU and folinic acid led to the acceptance of this regimen as a common reference treatment for advanced colorectal cancer. One of the most frequently administered bolus regimens is the 'Mayo Clinic regimen' where 5-FU and folinic acid are administered for 5 days every 4 weeks [4].

2.2. Schedules of fluorouracil administration

However, the activity and toxicity of 5-FU is dependent on the schedule of administration. Thus, the dose-limiting toxicity of bolus schedules are diarrhoea and myelosuppression. A meta-analysis of six randomised trials showed that protracted intravenous infusion regimens of 5-FU achieved superior response rates (22% versus 14%; $P = 0.0002$) with a small, but statistically significant, increase in survival (12.1 months versus 11.3

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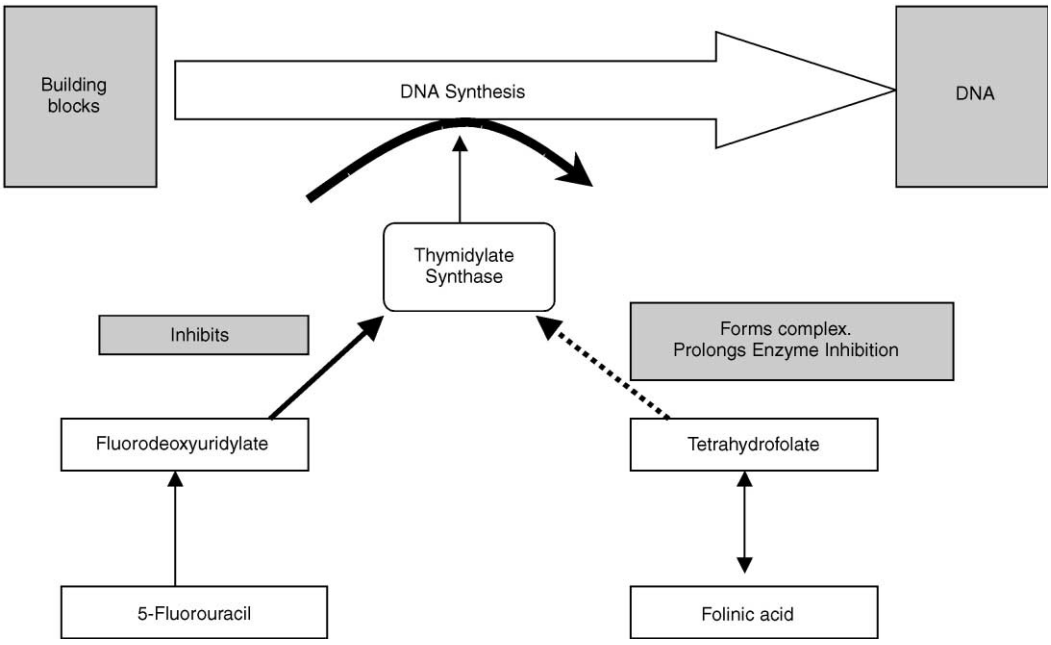

Fig. 1. Intracellular metabolism and mechanism of action of 5-FU and folinic acid.

months; $P = 0.04$) [5]. The superiority of infusion schedules of 5-FU compared with bolus regimens is also suggested by evidence of objective responses in patients who have progressed after bolus 5-FU regimens [6]. The use of infusion schedules of 5-FU results in an altered, but usually more manageable toxicity profile, with minimal myelosuppression, but with increased skin toxicity [5]. Thus, the dose-limiting toxicities for 5-FU infusion are diarrhoea, stomatitis and hand–foot syndrome.

The relatively modest gains in terms of response rates and survival have to be weighed against the increased complexity of treatment using infused schedules of 5-FU. Therefore, despite evidence of superior activity, widespread acceptance of the use of infusion schedules of 5-FU has been limited by the costs and inconvenience of the requirement for prolonged intravenous access and ambulatory pumps to allow delivery of treatment. As a result, there is geographical variation in clinical practice for the first-line management of colorectal cancer with some clinicians using infusion schedules and some preferring bolus schedules. The daily administration of oral 5-FU or an oral 5-FU prodrug simulates a protracted venous infusion (PVI) of 5-FU, and potentially provides an infusion schedule without the costs and inconvenience of prolonged intravenous access and ambulatory pumps. However, the oral bioavailability of 5-FU itself is extremely variable, partly as a consequence of variable metabolism by the enzyme dihydropyrimidine dehydrogenase (DPD) in the intestine. Thus, the approaches taken to overcome this involve the additional administration of inhibitors of DPD or alternatively the administration of 5-FU prodrugs, which are converted to active cytotoxic agents after absorption. The oral agents which have recently been evaluated in clinical trials include capecitabine, uracil ftorafur (UFT) and eniluracil with oral 5-FU.

2.3. Oral fluoropyrimidines

2.3.1. Capecitabine

Capecitabine is a fluoropyrimidine carbamate which is rapidly absorbed via the oral route and which offers several advantages over 5-FU. Capecitabine and its intermediate metabolites are not cytotoxic. They become effective only after they have been rapidly converted to 5-FU which is dependent on the enzyme thymidine phosphorylase (TP) [7]. The activity of TP has been found to be significantly higher in a number of different epithelial tumours compared with normal tissue [7]. In addition, the administration of either chemotherapy or radiotherapy has been shown to increase TP activity in tumour tissue without affecting TP activity in normal tissue [8]. Thus, the use of capecitabine allows the preferential activation of cytotoxic metabolites in tumour tissue. This may potentially lead to greater efficacy and reduced toxicity compared with 5-FU alone. Evidence that tumour selective conversion to 5-FU actually occurs in clinical practice has been observed by assessing the levels of 5-FU in tumour tissue and normal tissue after 5–7 days of prior treatment with capecitabine. The levels of 5-FU were on average 3.2 times higher in tumour tissue than in normal tissue ($P = 0.002$) [9].

The efficacy of various capecitabine regimens were evaluated in a randomised phase II study, which demonstrated similar response rates for both continuous or intermittent capecitabine with or without folinic acid [10]. The use of folinic acid did not improve efficacy, but led to increased toxicity especially skin toxicity in the form of hand–foot syndrome. Intermittent monotherapy had a slightly increased toxicity profile, but time to disease progression was superior in this group and hence this regimen was chosen for further clinical development. The difference in time to disease progression in this study was not statistically significant and may have arisen due to the small numbers of patients included in this study.

Capecitabine has now been evaluated in two phase III randomised clinical trials comparing the effects with standard first-line treatment using a monthly regimen of bolus 5-FU and folinic acid (Mayo Clinic regimen) [11]. Patients were randomised to treatment with either capecitabine 1250 mg/m^2 twice daily (days 1–14, every (q) 3 weeks) or Mayo Clinic regimen. The use of capecitabine resulted in superior response rates compared with the Mayo Clinic regimen (25.7% versus 16.7%, $P < 0.0002$) and led to similar times to disease progression with similar overall survival times. Overall toxicity was lower for capecitabine, with only hand–foot toxicity occurring more frequently in the capecitabine treated group. In addition, hospitalisation for severe toxicity was lower for patients treated with capecitabine. Thus, oral capecitabine is a safe, tolerable and effective alternative to bolus 5-FU modulated by folinic acid.

2.3.2. UFT

UFT is composed of uracil which acts as an inhibitor of DPD and ftorafur which is a 5-FU prodrug and which is converted to 5-FU by the enzyme cytochrome P450. The efficacy of UFT was determined in various phase II studies and activity was improved with the addition of folinic acid as a biochemical modulator [12]. Two large phase III studies have compared the use of UFT (300 mg/m^2/day) and folinic acid (days 1–28, q5 weekly) with the Mayo Clinic regimen 5-FU in advanced colorectal cancer [13,14]. Both of these studies concluded that UFT and folinic acid had a similar activity to bolus 5-FU and folinic acid as response rates, and overall survival times were not significantly different in both arms of each trial, although time to disease progression was inferior using UFT in one study. However, overall toxicity was less in the UFT and folinic acid arm, with less febrile neutropenia and mucositis. In one of the trials, the Mayo Clinic control arm was modified to involve treatment every 5 weeks instead of every 4 weeks, which has been criticised as a non-standard, dose-reduced control therapy [14]. Nevertheless, oral UFT appears to have comparable activity with bolus 5-FU modulated by folinic acid with less toxicity.

2.3.3. Eniluracil and oral 5-FU

Eniluracil is a potent inactivator of DPD, and since this enzyme accounts for much of the interpatient variability in the metabolism of 5-FU, it is a logical agent for novel therapeutic strategies. Clinical studies have demonstrated that eniluracil results in complete inactivation of DPD enzyme activity [15]. An attractive feature of eniluracil therapy in combination with 5-FU is the potential to prevent the formation of catabolites of 5-FU which may contribute to toxicity or interfere with cytotoxicity. In addition, increased expression of DPD activity has been suggested as a possible cause of 5-FU resistance. Two randomised trials of eniluracil plus oral 5-FU versus Mayo Clinic regimen in patients with advanced colorectal cancer have completed accrual, and preliminary results have been published in abstract form. In one of these studies, overall survival was statistically inferior [16]. Since there are now two oral fluoropyrimidines which have both recently been licensed in Europe for the treatment of metastatic colorectal cancer, it is unlikely that further strategies using eniluracil will be pursued in the immediate future.

2.4. Rationale for combination chemotherapy

The use of the new oral fluoropyrimidines will allow the administration of chronic oral schedules of fluoropyrimidines simulating PVI 5-FU. However, approximately 30% of patients with metastatic colorectal cancer show primary resistance to fluoropyrimidines and secondary resistance will develop in most of the remaining patients. Although higher levels of expression of TP may allow higher intratumoral levels of 5-FU after the administration of capecitabine, it is unlikely that this will overcome most cases of 5-FU resistance. Goldie and Coldman developed models of tumour growth and response to chemotherapy in the early 1980s. They predicted that the results of chemotherapy would be improved by the use of concurrent or alternating drug schedules provided that the individual agents were independently active and non-cross-resistant [17]. Whilst combination chemotherapy has been widely used in a number of different tumour types, it was not possible to apply this approach in colorectal cancer because colorectal cancer showed primary resistance to the majority of chemotherapeutic agents tested. However, in recent years evidence has emerged for the efficacy of mitomycin C, irinotecan (CPT11) and oxaliplatin in advanced colorectal cancer.

2.4.1. Mitomycin C

The activity of mitomycin-C (MMC) as second-line therapy has largely been evaluated in phase II studies which have shown significant response rates for the combination of 5-FU and MMC in patients who were resistant to 5-FU [10]. However, the use of MMC alone

in the same group of patients appeared less active, indicating a requirement for synergism between 5-FU and MMC.

The activity of MMC as first-line treatment in advanced colorectal cancer has been evaluated in a randomised phase III study comparing the addition of MMC to PVI 5-FU versus PVI 5-FU alone [18]. The MMC combination therapy showed an impressive 54% response rate which was superior to 5-FU alone. Failure-free survival and global quality of life after 6 months were superior in the combination arm, although overall survival was similar. A further randomised phase III study confirmed that the combination of PVI 5-FU plus MMC resulted in response rates of 40% with a median overall survival time of 17.6 months [19]. These results demonstrate significant activity for MMC and in view of its modest toxicity profile, it could be easily incorporated into combination regimens including other agents.

2.4.2. Irinotecan

CPT11 is a topoisomerase I inhibitor which was initially shown to have activity as second-line therapy in patients with 5-FU-resistant disease. Two randomised phase III trials demonstrated superior survival and improvement in tumour-related symptoms, such as pain, for patients who received CPT11 after progression with 5-FU compared with best supportive care or 5-FU infusion [6,20]. Survival without deterioration of performance status or weight loss of greater than 5% were superior in patients receiving irinotecan in one study, and there was a trend towards improvement of these parameters in the other study. Thus, two independent randomised phase III studies have reached broadly similar conclusions.

The results of these studies led to the use of combinations of CPT11 and 5-FU as initial therapy for metastatic colorectal cancer, and the results of two randomised trials have been reported. The first study evaluated the addition of CPT11 to an infused 5-FU and folinic acid regimen [21]. 387 patients were randomised in the study. The addition of CPT11 resulted in a superior response rates (49% versus 31%; $P<0.001$), progression-free survival (6.7 versus 4.4 months; $P<0.001$) and overall survival (17.4 versus 14.1 months; $P=0.03$). Toxicity was increased in the combination arm, with more patients suffering grade 3/4 diarrhoea, asthenia and neutropenia than in the 5-FU and folinic acid monotherapy arm. However, quality of life scores were similar in each arm and time to deterioration of quality of life was longer in the combination arm. The second study compared the use of CPT11 with 5-FU and folinic acid administered weekly for 4 weeks every 6 weeks with Mayo clinic regimen and CPT11 monotherapy [22]. The use of the CPT11 and 5-FU combination treatment resulted in a superior response rate (50% versus 38%; $P<0.001$), progression-free survival (7.0 versus 4.3 months; $P<0.004$) and overall survival times (14.8 versus 12.6 months; $P=0.04$). The CPT11 combination arm resulted in more diarrhoea, but less mucositis and neutropenia than the Mayo Clinic regimen and the decline in quality of life was less in the CPT11 combination arm. The results of both independent trials are thus broadly similar with both demonstrating improved response rates, prolongation of time to progression, and improvement in overall survival for the combination treatment compared with 5-FU alone. The improvement in overall survival, which was statistically significant in both of these randomised studies, is an important issue. Amongst the patients who initially received 5-FU monotherapy, 58 and 70% of patients received second-line treatment in each of the two studies.

2.4.3. Oxaliplatin

Oxaliplatin is a third generation platinum compound. *In vitro* studies suggested synergism between oxaliplatin and 5-FU and much of the clinical development of oxaliplatin has relied upon study of the combination of oxaliplatin and 5-FU. Unlike CPT11, the use of oxaliplatin as second-line therapy has not been evaluated in randomised clinical trials. However, a number of phase II studies do suggest significant activity in this context [23,24]. In contrast, the use of oxaliplatin and 5-FU as initial therapy for advanced colorectal cancer has been studied in two randomised trials. The first study compared the addition of oxaliplatin to chronomodulated 5-FU with chronomodulated 5-FU alone [25]. The combination arm achieved superior response rates (53% versus 16%; $P<0.0001$), progression-free survival (8.7 months versus 6.1 months; $P=0.048$), but there was no difference in overall survival. A second study compared the combination of oxaliplatin and the 2-weekly schedule of 5-FU by bolus and infusion (de Gramont regimen) with the de Gramont regimen alone [26]. Response rates (50.0% versus 21.9%) and progression-free survival (9.0 months versus 6.2 months; $P=0.0001$) were superior in the combination arm, but overall survival was not significantly different ($P=0.12$). Both of these trials have shown a significant progression-free survival benefit with oxaliplatin, but no overall survival advantage. Since it is likely that patients have few symptoms prior to tumour progression, progression-free survival would probably represent an adequate surrogate marker of the true clinical benefit derived from oxaliplatin.

2.5. Differences between the combination regimens

Thus, the randomised trials show an overall survival benefit for the initial use of combination treatment using CPT11 and 5-FU, but not for the combination of oxaliplatin and 5-FU. The interpretation of these results, however, is complicated by the use of different

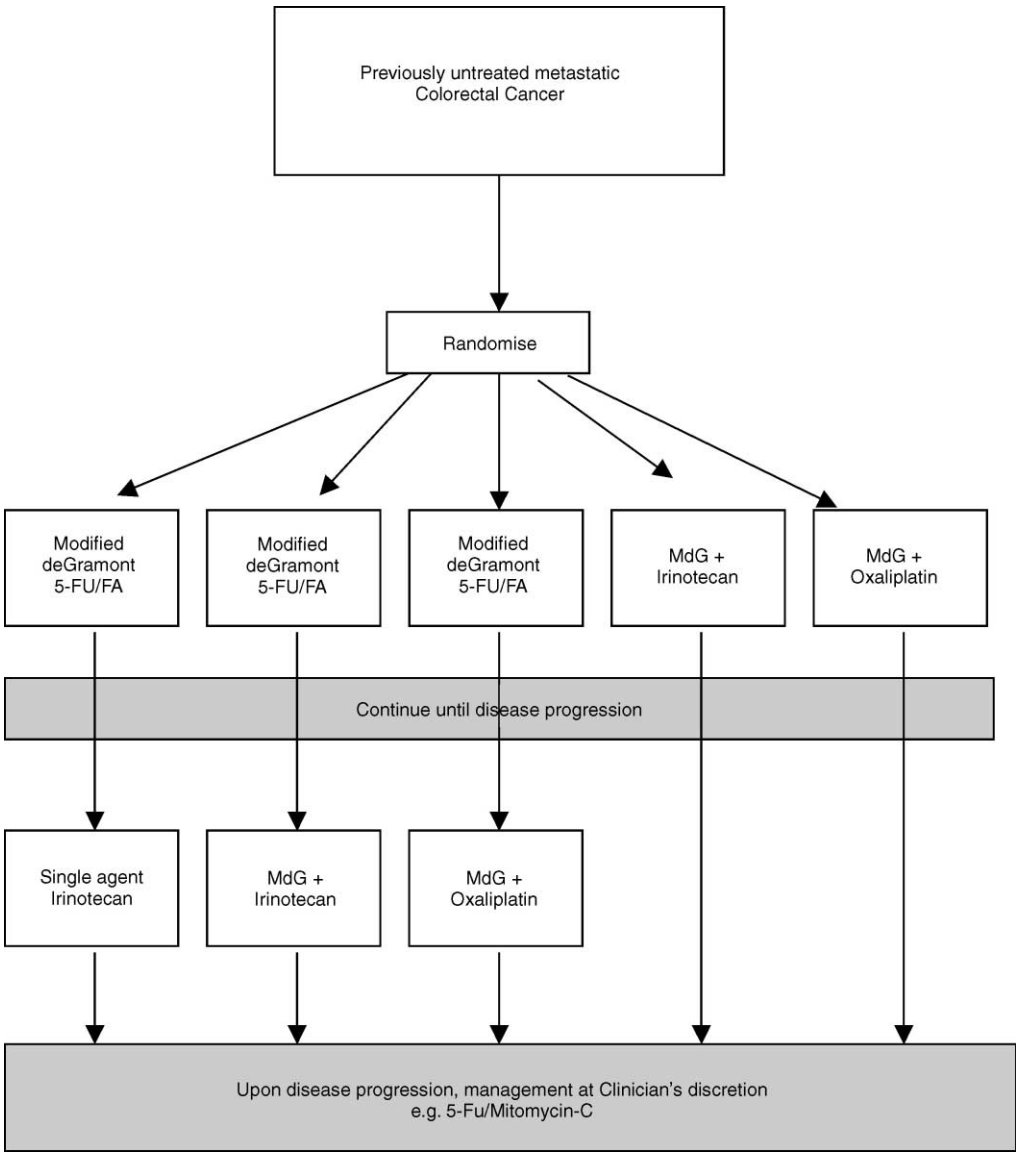

Fig. 2. Schematic representation of the FOCUS/CR08 trial for metastatic colorectal cancer.

control regimens and cross-over to second-line therapies. The control arm was a bolus 5-FU regimen in one of the CPT11 studies, but was an infusion schedule in the oxaliplatin studies. In addition, a large proportion of patients in the 5-FU/FA arm in all trials crossed over to oxaliplatin salvage or received irinotecan. The heterogeneity of posttrial treatment complicates the interpretation of the true survival advantage attributable to oxaliplatin. The best way to eliminate this bias is to control the second-line treatment patients receive and such a trial has recently commenced for oxaliplatin.

2.6. Sequential or combination therapy

The fact that a number of agents have demonstrable efficacy in advanced colorectal cancer raises the important issue of what constitutes standard care for this disease and how the agents active in this disease should be combined. Whilst one use of the new agents may be to apply them sequentially, the fact that initial therapy using CPT11 and 5-FU achieved superior overall survival suggests that this may not be the optimal approach. Tracking studies in the United States suggest that around 30% of patients who receive 5-FU monotherapy will not receive second-line chemotherapy. These may well represent patients whose tumours are 5-FU-resistant and who develop tumour progression with 5-FU monotherapy, thereby becoming too unwell to receive second-line treatment. However, these patients may have fared much better had they received CPT11 or oxaliplatin as part of their initial treatment.

Cost and concerns regarding toxicity may, however, mean that it is not always feasible to offer combination therapy to all patients as initial treatment. There are

two approaches which may facilitate patient selection for initial combination treatment. Retrospective subgroup analysis suggested that the greatest impact of combination therapy using CPT11 on survival was seen in patients who were less than 65 years old, with good performance status, normal levels of lactate dehydrogenase, one site of metastatic disease and who had not received adjuvant therapy. Thus, selection of patients with these clinical parameters may provide one method of selecting patients for initial combination therapy, although the validity of this method of case selection needs to be determined prospectively. The use of these criteria would be expected to select patients who are likely to be able to tolerate a more toxic treatment and in whom the tumour load is not extensive. This group may also include a subgroup of patients who are potential candidates for metastasectomy and the use of a regimen which would result in higher response rates would be expected to be particularly appropriate in this subgroup.

2.7. Current randomised trials involving combination and sequential chemotherapy

Current randomised trials are comparing different possible sequences of agents for patients with advanced colorectal cancer. One study has been reported in preliminary form which compares initial combination therapy using 5-FU and CPT11 followed on progression by 5-FU and oxaliplatin versus the reverse sequence. The response rates to the initial treatment were high (greater than 60%), but more mature results are awaited [27]. Another study compares initial treatment with oxaliplatin and 5-FU with 5-FU alone. All patients who are sufficiently fit for second-line therapy are stipulated to receive CPT11. A further study compares three different first-line combination therapies, 5-FU plus CPT11, 5-FU plus oxaliplatin and oxaliplatin plus CPT11. Finally, the Medical Research Council is coordinating a five arm trial; three arms are 5-FU monotherapy, one arms is 5-FU plus CPT11 combination therapy and one arm is 5-FU plus oxaliplatin. Second-line therapy is stipulated for three of the arms (Fig. 2). Mature results from these studies will not be available for some time, but they will provide valuable information regarding the use of different combination treatments.

2.8. Molecular markers predicting fluorouracil resistance

An alternative approach to guide clinical decisions regarding the use of chemotherapy depends on the use of molecular markers to predict resistance or sensitivity to individual agents. The identification of tumours which are likely to be resistant to 5-FU has been the focus of intense investigation. The advantages of this approach are that it allows specific chemotherapy to be targeted to patients who are most likely to derive benefit from this treatment. In turn, patients whose tumours are likely to be resistant to treatment are not exposed to potential toxicity from a therapy which is unlikely to provide clinical benefit. Moreover, alternate treatments such as oxaliplatin and CPT11 can be used instead of 5-FU for those patients who are likely to be resistant to 5-FU.

The level of expression of thymidylate synthase (TS), the key target enzyme of 5-FU activity, has been shown to be an important indicator of 5-FU resistance [28]. Thus, tumours expressing high levels of TS were resistant to 5-FU. The prediction of 5-FU sensitivity in low TS level tumours could be further stratified by the level of expression of other enzymes implicated in 5-FU metabolism, DPD and TP [29,30]. Thus, tumours which had low levels of TS, as well as TP and DPD, showed very high response rates to 5-FU.

There are no molecular markers currently available which will allow the identification of patients destined to respond to oxaliplatin or CPT11. The expression of high levels of mRNA encoding the DNA repair gene *ERCC1* has been associated with cisplatin resistance in a variety of tumour types, but it has not been established whether expression levels correlate with resistance to oxaliplatin in advanced colorectal cancer [31].

Whilst the use of these markers may play an important role in future clinical practice, there remain difficulties associated with their routine use. Firstly, accurate determination of enzyme levels has depended on quantitative assessment of mRNA levels, which is a procedure that could not be applied by most routine histopathology laboratories. Moreover, the use of immunohistochemistry to assess varying levels of protein expression may not be sufficiently accurate to allow response prediction, although in some reported cases it was satisfactory [32,33]. A further problem with this approach is that the TS levels may not correlate identically between the primary tumour and sites of metastatic disease [34]. Thus, it may prove necessary to repeat tumour biopsies.

2.9. Novel combination regimens

The availability of a number of agents with activity in colorectal cancer allows the formulation of novel combination therapies. The lower toxicity profile associated with the oral fluoropyrimidines provides a strong rationale for their inclusion in combination therapy which is more toxic than monotherapy. Current studies are evaluating the activity of combination regimens comprising irinotecan or oxaliplatin with oral fluoropyrimidines such as capecitabine or UFT. In addition, the combination of oxaliplatin and CPT11 is being evaluated and even three drug regimens comprising a fluoropyrimidine, CPT11 and oxaliplatin may be feasible for

selected patients. The non-overlapping toxicity profiles of MMC, oxaliplatin and 5-FU suggests that this combination also merits further study.

3. Principles of adjuvant therapy

Although over 80% of colorectal carcinomas are macroscopically resectable at the time of diagnosis, 50% of patients subsequently relapse with metastatic disease. This is due to the presence of micrometastasis. Adjuvant treatment aims to eliminate residual tumour cells and increase the proportion of patients achieving long-term disease-free intervals and overall survival benefit. Instituting treatment soon after surgery may have a number of advantages. The tumour burden is low and the likelihood of development of chemotherapy-resistant cell clones smaller. Surgical resection of the primary tumour may have a stimulatory effect on residual cells, increasing the proportion undergoing cell division and therefore increasing their susceptibility to cytotoxic agents [35]. The development of new agents and an increasing knowledge of the pathogenesis of colorectal cancer are opening up an ever increasing array of possible targets that may significantly impact on treatment in the adjuvant setting in the future.

4. Cytotoxic chemotherapy in the adjuvant setting

Randomised phase III trials of 5-FU and folinic acid versus follow-up in the adjuvant setting have consistently shown an absolute survival advantage for patients with Dukes' C Colon Cancer of 5–6% at 5 years, which represents a 25–35% reduction in the risk of dying from colorectal cancer [36–38]. International consensus has accepted 5-FU/folinic acid bolus regimes such as the Mayo regime as the standard of care, provided the risk of toxicity is not considered too great for the individual.

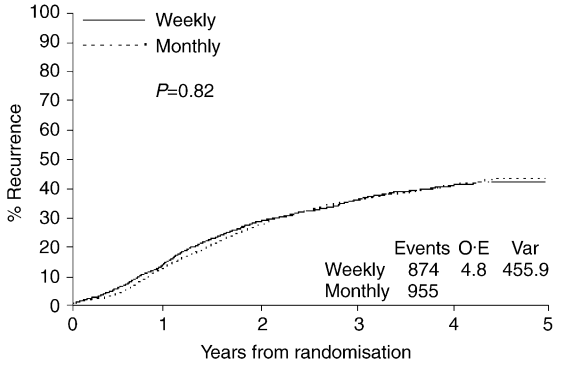

Fig. 3. 5-year recurrence risk of patients enrolled to Quasar, weekly versus 4-weekly schedule ($n=4927$). O, observed; E, expected; Var, variance.

Outstanding questions in the adjuvant setting relate to dosing schedules and duration of treatment. Levamisole was historically thought to add benefit as a non-specific stimulator of immune function, but recent evidence has shown conclusively that it does not contribute anything to survival [39]. Six months of treatment with 5-FU and folinic acid has been shown to be as effective as 12 months [40,41]. Low-dose folinic acid (20 mg/m^2) is as effective as high-dose (200 mg/m^2) [39]. Although bolus regimes of 370–425 mg/m^2 of 5-FU daily for 5 days every 4 weeks are commonly used, a recent non-randomised comparison carried out by the Quasar collaborative group suggested that 30 weekly doses of 370 mg/m^2 is as effective and less toxic than six cycles of bolus 370 mg/m^2 for 5 days every 28 days [42] (Fig. 3). Diarrhoea, stomatitis and neutropenia were significantly reduced in the weekly regime, and patients were less likely to require a dose reduction.

4.1. Infused fluorouracil therapy in the adjuvant setting

In the adjuvant setting protracted venous infusion (PVI) of 12 weeks of 300 mg/m^2/day 5-FU has been compared with six cycles of 425 mg/m^2 bolus 5-FU with leucovorin 5 days every 4 weeks: patients on PVI therefore got a larger dose of 5-FU over a shorter period of time. 716 patients with Dukes' B (42%) and C (56%) colorectal cancer were accrued, and 697 were randomised. PVI was better tolerated, particularly in terms of grade 3/4 neutropenia (55% versus 3%; $P<0.0001$) and mucositis (20 versus 3%; $P<0.0001$). Median follow-up was 21.9 months. The 5-year recurrence-free survival was marginally better for PVI, with no difference in overall survival, although the number of patients is too small to draw any definite conclusions [43]. A similar study, SWOG 9415, compared an infusional regime of 250 mg/m^2/day of 5-FU for 8 out of 9 weeks for 27 weeks with Mayo-style bolus 5-FU and leucovorin for 26 weeks for adjuvant treatment of Dukes' B and C colon cancer. This was stopped at an interim analysis after accruing 1078 patients because early data suggested that continuing accrual was unlikely to show a difference in terms of disease-free or overall survival. Although toxicity was marginally worse on the bolus arm, more patients stopped early on the infusional arm (73 versus 32 patients) [44]. 905 patients with stage B2 or C colon cancer have been randomised in a trial comparing a de Gramont style regime of 2 consecutive days every 2 weeks (bolus 100 mg/m^2 of L-Leucovorin and 400 mg/m^2 5-FU followed by 600 mg/m^2 5-FU infused over 22 h) with Mayo style bolus 5-FU/FA. Patients were randomised to either 24 or 36 weeks of treatment in both arms. Preliminary data suggests a better toxicity profile for the infusional regime [45]. The

EORTC-40963 trial comparing bolus 5-FU with two different weekly and a fortnightly infusional 5-FU regime is still accruing (see Table 1).

Most metastases develop within the liver, and administration of 5-FU via a catheter into the portal vein should theoretically increase the response rate and reduce systemic toxicity. A meta-analysis of 10 trials suggested a marginal benefit of 4.9% at 5 years [46]. The Adjuvant X-Ray and 5-FU infusion study (AXIS) trial was designed to address this question: mature data is awaited, but it seems unlikely that the benefit will be greater than standard systemic adjuvant therapy [47].

5. Adjuvant therapy of Dukes' B colon cancer and rectal cancer

The role for adjuvant chemotherapy in Dukes' B colon cancer remains controversial. In the IMPACT 2 meta-analysis, data from five trials were pooled for 1016 patients with stage B2 colon cancer [48]. No significant benefit was seen for patients treated with 5-FU/FA over the control arm for 5-year disease-free survival (79% treatment versus 76% controls Hazard Ratio (HR) 0.83 (95% Confidence Interval (CI) 0.72–1.07)) or overall survival (82 versus 80% HR 0.81(95% CI 0.64–1.01). A statistically complex overview of the NSABP trials CO-1 to CO-4 suggested that the percentage risk reductions were similar between Dukes' B and Dukes' C cancers [49]. The overview contained a variety of treatments, including portal vein infusion and the MOF regime (semustine, vincristine and 5-FU), making it difficult to draw definite conclusions from these results. Although one would accept that the proportional reductions in the odds of recurrence or death are similar for Dukes' B and C stage cancer, the absolute survival benefit is likely to be much smaller (2–3%) because the event rate is much lower. There is increasing research in the field of

Table 1
Summary of ongoing phase III trials in the adjuvant treatment of colon cancer

Protocol ID	Description	Treatment arms	Target accrual	Status
EORTC-40963	Stage III colon cancer	I. Bolus 5-FU + LV days 1–5 every 4 weeks for 24 weeks	1600	Active
	Bolus 5-FU with leucovorin versus high dose infusional 5-FU with leucovorin	II. Weekly 5-FU + LV over 24 h 6 weeks every 7 weeks for 21 weeks; or high-dose 5-FU over 48 h weekly for 24 weeks; or 5-FU + LV over 22 h days 1 + 2 every 2 weeks for 24 weeks		
FRE-FNCLCC-ACORD-2, EU-20014	Stage III colon cancer	I. LV over 2 h and 5-FU over 22 h days 1 and 2 every 2 weeks for 24 weeks	400	Active
	Infusional 5-FU and LV with or without irinotecan	II. As above with i.v. irinotecan over 90 min		
CLB-89803 CTSU	Stage III colon cancer	I. Weekly bolus of 500 mg/m^2 LV and 500 mg/m^2 5-FU for 5 out of 8 weeks for 32 weeks	1260 (1263 accrued)	Closed[a]
	Bolus 5-FU and LV with or without bolus irinotecan	II. Weekly bolus of 500 mg/m^2 5FU + 20 mg/m^2 LV + 125 mg/m^2, irinotecan for 4 weeks every 6 weeks for 30 weeks		
PEACC-3–40993	Stage II and III colon cancer	I. LV 200 mg/m^2 over 2 h + 5-FU 400 mg/m^2 bolus + 5-FU 600 mg/m^2 over 22 h days 1 and 2 every 2 weeks for 26 weeks	1794	Active
	Infusional 5-FU and LV with or without irinotecan	II. Irinotecan 180 mg/m^2 plus above every 2 weeks for 26 weeks Alternatively, the AIO regime may be used[b]		
NSABP CO-7	Stage II or III carcinoma of the colon	I. Weekly bolus 5-FU plus LV every 6 weeks out of 8 weeks for 24 weeks	2472	
	Bolus 5-FU and LV with or without oxaliplatin	II. Above plus oxaliplatin bolus on days 1, 15 and 29		
ROCHE-M66001	Stage III colon cancer	I. Mayo regime bolus LV + 5-FU for 5 days every 4 weeks for 26 weeks	1990	Active
	Bolus 5-FU + LV versus oral capcitabine	II. 2500 mg/m^2/day capcitabine orally for 14 days every 3 weeks for 24 weeks		

EORTC, European Organization for Research and Treatment of Cancer; NSABP, National Surgical Adjuvant Breast and Bowel Project; FNCLCC, Federation Nationale des Centres de Lutte Contre le Cancer; CALGB-89803, Cancer and Leukemia Group B; PETACC-3-40993, Pan-European Trials in Adjuvant Colon Cancer.

[a] Currently under review due to an excess of deaths in the irinotecan arm.

[b] 5-FU 500 mg/m^2 over 2 h + 5-FU 2000 mg/m^2 over 24 h on days 1, 8, 15, 22, 29, 36 with or without irinotecan 80 mg/m^2 repeated every 50 days for 26 weeks.

prognostic or predictive factors and a range of pathological features and molecular markers have been assessed which may identify patients at higher risk, but none is yet robust enough for standard clinical practice [50].

Optimal adjuvant treatment for rectal cancer remains uncertain and there is a wide geographical variation in the management of this disease. Concerns about the risk of local recurrence have ensured that pre- or post-operative radiotherapy is frequently a component of adjuvant therapy. However, randomised studies have shown superior disease-free and overall survival using the combination of adjuvant postoperative chemotherapy combined with radiotherapy compared with surgical treatment alone [51,52]. Preoperative chemoradiation is a potentially promising approach as it may improve the likelihood of sphincter-sparing surgery, as well as modify the acute and late toxicity profile. The effects of pre-operative treatment are being evaluated in current randomised studies [53].

6. Chemotherapy in older patients

The role of adjuvant chemotherapy in patients over the age of 70 years has yet to be established. Although octogenarians have been entered into randomised trials, the numbers have been too small to draw meaningful conclusions. Patients over the age of seventy are more likely to experience side-effects, particularly grade 3/4 neutropenia and mucositis, and therefore the risks may outweigh the benefits in patients who are older or physiologically unfit [54].

Patients with an uncertain indication for chemotherapy such as Dukes, B colon cancer, rectal cancer and the elderly are being randomised to receive either adjuvant chemotherapy or no adjuvant chemotherapy within the QUASAR1 trial. This trial has accrued almost 2500 patients and is due to close in the near future (Fig. 4).

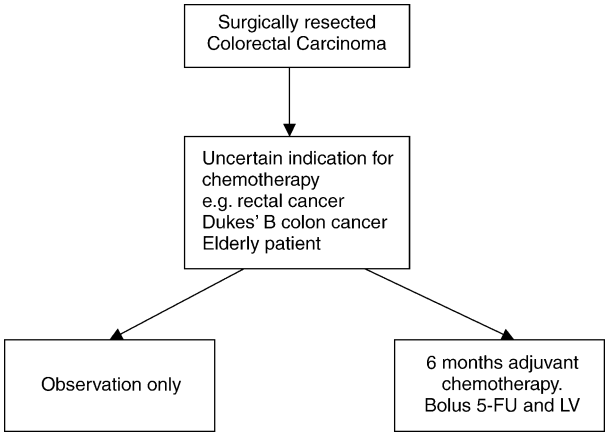

Fig. 4. The Quasar 1 Trial.

7. Newer drugs in the adjuvant setting

Newer agents such as oral fluoropyrimidines, irinotecan and oxaliplatin have been shown to be active in metastatic disease. Phase III trials are now accruing to see if these agents have advantages in the adjuvant setting (Table 1). Capecitabine is being compared with bolus Mayo style 5-FU, the primary objective being to show that it has at least an equivalent effect on disease-free survival [55]. Toxicity and the effect on quality of life are important secondary endpoints: In the metastatic setting, febrile neutropenia and stomatitis are less common, but hand–foot syndrome is frequently a problem. The NSABP C-06 trial randomised 1530 patients to either weekly bolus 5-FU and LV or oral UFT and LV for 4 out of 5 weeks for 35 weeks [56]. The final analysis will be performed in 2003.

Irinotecan and oxaliplatin are being added to the 5-FU regimes to assess whether they improve disease-free survival rates. One trial comparing a weekly bolus 5-FU regime with or without 125 mg/m^2 irinotecan, as described in the metastatic setting by Saltz and colleagues has completed accrual, but preliminary results suggest an unacceptably high death rate in the irinotecan arm (14 of 635, versus 5 of 628 in the 5-FU/LV alone arm, $P = 0.06$) [57]. The causes of death were diverse and included pulmonary embolus (PE) ($n = 3$) and aspiration ($n = 3$). The PETACC-3 trial involves giving infusional 5-FU with or without irinotecan every 14 days, and further data is needed to establish the relative safely and benefit of irinotecan in the adjuvant setting.

8. Future directions in colorectal cancer therapy

Much of the basic cancer research during the era of molecular biology has focused on the dissection of molecular pathways resulting in tumorigenesis. The rationale for this approach is that it allows the identification of pathways whose disruption may provide a novel therapeutic approach to treatment of that tumour. One of the most impressive examples of this process has been demonstrated in chronic myeloid leukaemia (CML). In this disease, a frequent pathological mutation involves the constitutive activation of the abl tyrosine kinase due to the translocation of the *bcr* and *abl* genes. The generation of an orally administered, highly specific inhibitor of the abl tyrosine kinase has provided a novel therapeutic agent which has been demonstrated to have activity in both the chronic phase of CML as well as during transformation [58].

There are two phases in the application of a similar process to colorectal cancer. Firstly, it is important to identify genetic pathways whose dysregulation plays an important role in the pathogenesis of colorectal cancer. Secondly, pharmacological compounds which are cap-

able of interfering with these pathways must be identified. There are several different methods of disrupting biochemical pathways which can involve classic pharmacological drugs, antibodies directed at cell surface receptors and gene therapy including both gene replacement and antisense strategies.

8.1. Gatekeeper function of APC in colorectal tumorigenesis

In the case of colorectal cancer, it has become apparent that there are two fundamental pathways involving co-operating mutations of a number of genes which ultimately lead to the development of colorectal tumours. The most frequent mechanism is initiated by the development of somatic mutations in the tumour suppressor gene adenomatous polyposis coli (*Apc*) [59]. A less frequent mechanism is initiated by mutation of DNA repair genes which result in tumours characterised by microsatellite instability which arise as a result of mutations affecting repetitive DNA sequences. The importance of mutations affecting *Apc* was initially described based on the study of the patterns of germline mutations in the familial syndrome, familial adenomatous polyposis coli, and the patterns of somatic mutations in sporadic human colorectal cancers [60]. The effects of mutation of *Apc* have now been tested directly *in vivo* by the generation of predetermined mutations in mice through gene targeting. Study of the biochemical pathways downstream of APC has shown that a key event in colorectal tumorigenesis involves increased levels of the cytoplasmic signalling protein β-catenin. β-Catenin can translocate to the nucleus and in association with the DNA binding domain of proteins of the T-cell factor/lymphoid enhancer factor family lead to increased transcription of a number of target genes. This enhanced transcriptional activity is frequently the result of attenuated cytoplasmic degradation of β-catenin most commonly due to mutations in APC or less frequently in the N-terminus of β-catenin. The importance of mutations of APC has led to this gene being described as a 'gatekeeper' gene for colorectal tumorigenesis. In the presence of truncated APC, colorectal epithelial cells may acquire other co-operating mutations including mutations affecting *Smad-4, ras* and *TP53* which contribute to the development of tumours. However, in the absence of a mutation affecting the APC/β-catenin pathway, the presence of mutations affecting these other genes do not usually result in tumours.

8.2. Targeting the APC/β-catenin pathway

The pivotal importance of APC/β-catenin as a gatekeeper for colorectal tumorigenesis makes this pathway an attractive target for novel therapeutic approaches. For example the levels of β-catenin could be reduced using antisense strategies, or the domain of APC responsible for regulating cytoplasmic β-catenin levels could be replaced using gene therapy approaches. Although these novel methods have been greeted with some enthusiasm, they remain at an early developmental stage. Antisense strategies are designed to specifically reduce the levels of particular target mRNA species. However, the efficacy of this method to reduce the expression levels of the target mRNA is highly variable [61]. In addition, although antisense strategies should theoretically be specific, non-sequence specific activity has certainly been demonstrated [62]. The extent to which this process impacts on the efficacy or toxicity of antisense treatment is difficult to judge because many reports assess the expression of a limited number of mRNA species before and after treatment. The potential specificity of the effects of antisense treatment need further prospective evaluation, preferably using techniques allowing the assessment of expression levels of multiple genes simultaneously, such as microarray technology. Gene therapy is also a potentially promising approach for the future. However, it would appear to be most logical to concentrate most initial efforts with this technique on well-defined single genetic disorders such as cystic fibrosis. The inability of gene therapy to correct these diseases where the gene defect is clearly defined does not augur well for the treatment of cancer, where multiple and variable co-operating genetic defects occur [63].

8.3. Farnesyl transferase inhibitors

Despite the fundamental importance of the APC/β-catenin pathway as the gatekeeper for colorectal tumorigenesis, other current approaches have focused on alternate pathways. One of these pathways involve the ras genes. Three functional *ras* genes (K-*ras*, N-*ras*, H-*ras*) encode K-ras, N-ras and H-ras proteins. The ras proteins are 21 kDa guanine nucleotide binding proteins which play an important role in intracellular signalling. They are important for the generation of mitogenic responses to signalling through growth factor receptors such as epidermal growth factor (EGF) and cytokine receptors.

Ras is synthesised as a soluble and biologically inactive protein which undergoes posttranslational modification before being localised to the inner surface of the membrane. An essential step in the posttranslational processing of Ras involves the addition of a farnesyl group by the enzyme farnesyl transferase. A number of different mutations in colorectal cancers are known to affect the ras protein, and in some cases these may result in the mutant protein constitutively stimulating proliferation. Since farnesylation represents the essential enzymatic step in the process of posttranslational modification, with this step crucial for membrane localisation, inhibition of this step could result in targeted treatment of ras oncogene-dependent tumours [64].

Preclinical studies have shown that farnesyl transferase inhibitors are principally cytostatic rather than cytotoxic, and are thus more likely to cause disease stabilisation rather than tumour regression. Since efficacy in phase II studies is usually based predominantly on response rates, it was judged that the efficacy of these agents would be better evaluated, after phase I studies, in the phase III setting evaluating outcomes such as time to disease progression and survival. On this basis, a multicentre double-blind, placebo controlled phase III trial of the farnesyl transferase inhibitor R115777 for patients with metastatic colorectal cancer who have failed conventional chemotherapy has recently completed accrual.

8.4. Targeting the EGF receptor

The EGF receptor is often overexpressed in a variety of epithelial tumours and may play a role in stimulating tumour proliferation. A study of colorectal neoplasms showed that the level of expression of EGFR was inversely associated with survival [65]. Agents targeted to the EGF receptor include the monoclonal antibody C225 as well as the pharmacological agent 'Iressa' (ZD1839). For reasons that are not entirely clear, agents targeted against the EGFR have modest antitumour activity when administered as a single agent, but display considerable synergism with cytotoxic chemotherapy. Preliminary results of phase I/II trials suggest that C225 combined with chemotherapy has activity in head and neck cancers and colorectal cancers which have become resistant to chemotherapy [66]. Consequently, large phase II studies in the United States and Europe are currently evaluating the effect of C225 plus CPT11 in patients who have progressed after CPT11. Similarly, animal studies using established tumour xenografts treated with Iressa (a pharmacological inhibitor of the EGFR tyrosine kinase) demonstrated synergism between Iressa and conventional chemotherapy [67].

8.5. Targeting VEGF

Vascular endothelial growth factor (VEGF) plays an important role in angiogenesis and is thought to be important in tumour angiogenesis. The expression of VEGF by tumours has been shown to predict disease recurrence after surgery [68]. A randomised phase II study has suggested that the administration of a monoclonal antibody against VEGF in combination with chemotherapy results in improved response rates and time to progression compared with chemotherapy alone [69].

9. Cox-2 inhibition

There is currently considerable interest in the possibility that Non-Steroidal Anti-Inflammatory Drugs (NSAID) may protect against colorectal cancer. Retrospective epidemiological studies suggest a 50% decreased incidence of colorectal cancer in users of aspirin and sulindac [70,71]. Traditional NSAIDs inhibit both cyclo-oxygenase COX-1 and COX-2 (Fig. 5). Evidence suggests that the COX-2 isoform is important in the pathogenesis of colorectal cancer. COX-2 is upregulated in 85% of colorectal carcinomas and 40% of adenomas, but is virtually undetectable in normal colon [72]. Strong COX-2 expression is a marker for poor survival in colorectal cancer [73]. COX-2 inhibitors have been developed which have fewer side-effects than traditional NSAIDs, but retain the ability to induce cell cycle arrest and apoptosis in tumour cell lines [75,76]. They are also thought to be anti-angiogenic in nature [74]. In a murine Min-mouse model, COX-2 is overexpressed [78]. Administration of COX-2 inhibitors suppressed the growth of transplanted tumour cells [77]. COX-2 inhibitors may also prevent progression from adenomatous polyp to invasive carcinoma. In murine models with *Apc* (adenomatous polyposis coli) mutations, deletion of the *COX-2* gene leads to a reduction in the number of polyps [79]. This property was investigated in a human study in which 77 patients with familial adenomatous polyposis were randomised to 6 months of celecoxib (a COX-2 inhibitor) or placebo. 30 patients given 400 mg of celecoxib twice a day had a 28% reduction in the mean number of polyps ($P = 0.003$) and a 30.7% reduction in the sum of the polyp diameter compared with placebo ($P = 0.001$) [80].

The biggest disadvantage of traditional NSAIDs is their upper gastrointestinal (GI) toxicity such as ulceration, bleeding and perforation. The selective nature of COX-2 inhibitors is thought to reduce this risk. Osteoarthritis patients were given 25 mg of Rofecoxib (a COX-2 inhibitor) ($n = 186$), 50 mg Rofecoxib ($n = 178$) or placebo for at least 16 weeks [81]. Endoscopies were

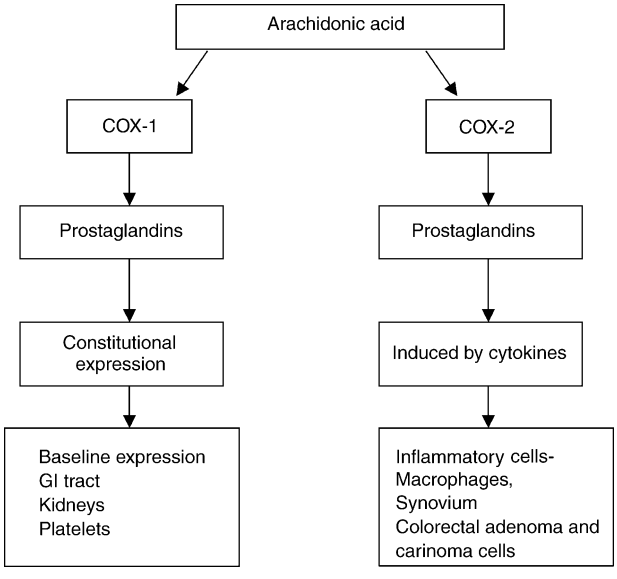

Fig. 5. Role of COX-1 and COX-2 *in vivo*. GI, gastrointestinal.

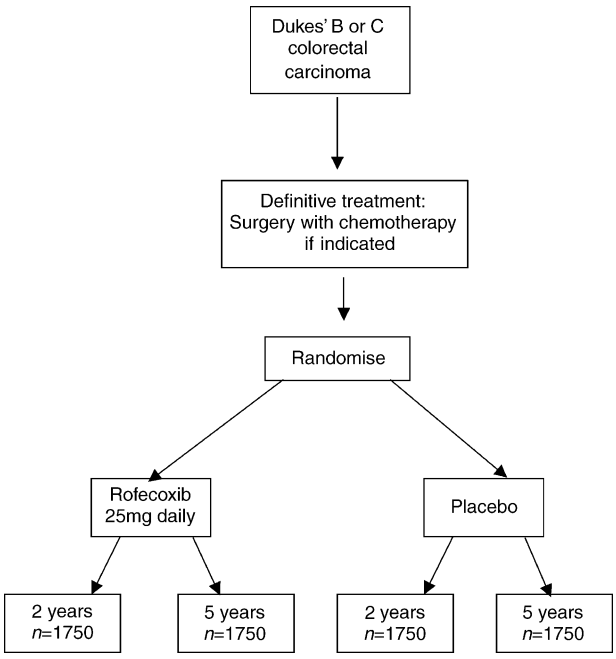

Fig. 6. Schematic representation of the VICTOR trial.

performed at baseline, 6, 12 and 24 weeks. The incidence of ulcer formation greater than 3 mm was statistically equivalent to placebo at 12 weeks ($P=0.001$) and only marginally raised for the 50 mg dose at 24 weeks. A meta-analysis of eight COX-2 inhibitor trials suggests that clinical incidence of peptic ulceration is lower at 12 months for COX-2 inhibitors than other NSAIDs (1.3 versus 1.8%; $P=0.04$) [82].

It is felt that COX-2 inhibitors may have a potential role in the adjuvant setting. VICTOR, a large multicentre phase III trial, aims to randomise 7000 patients with Dukes' B or C disease to 2 or 5 years of Rofecoxib or placebo following surgery, on completion of adjuvant chemotherapy or radiotherapy (Fig. 6).

10. Matrix metalloprotein inhibitors

Matrix metalloproteinases are essential for tumour neo-angiogenesis, invasion and metastasis. Hence metalloproteinase inhibitors may lend themselves better to the adjuvant than the metastatic setting. However, identifying surrogate endpoints for agents which are more likely to be cytostatic than cytotoxic is problematic. Marimastat, a broad spectrum metalloproteinase inhibitor, reduces the rate of rise of carcino-embryonic (CEA) in patients with metastatic colorectal cancer [83]. The evidence for rise in antigen CEA as a surrogate marker is lacking, and large suitably powered randomised trials are required to assess the role of metalloproteinase inhibitors in the adjuvant setting.

11. Immunotherapy

Stimulating the immune system to reject residual tumour cells is an attractive proposition for adjuvant treatment. However, tumour cells frequently develop methods to escape immune surveillance, and therefore isolating appropriate antigens and delivering them in a manner that will trigger one of the many facets of the immune system still presents an enormous challenge. Cytokines and non-specific stimulators of immune function such as Bacillus, Calmette Guerin (BCG) and levimasole do not appear to produce a survival benefit. Three randomised phase III trials have looked at the benefits of active specific immunotherapy in the adjuvant setting (Fig. 7) [84]. Autologous tumour cells were isolated and irradiated and given intradermally along with BCG. In two of the trials, three vaccinations were given at weekly intervals approximately 4 weeks post surgery. In the third trial, patients received a booster injection of tumour cells alone at 6 months. Meta-analysis of the outcomes of the 763 patients randomised suggests no benefit on disease-free or overall survival. There was a trend towards benefit in patients who were stage II who received four vaccinations and who had a response in the form of induration at the vaccine site (Intent to treat disease-free survival Odds Ratio (OR) 2.24, (1.04–4.85) $P=0.041$, overall survival OR = 2.24 (1.07–6.7), $P=0.036$) [85].

Monoclonal antibodies to tumour-associated antigens induce cell death by a variety of mechanisms including

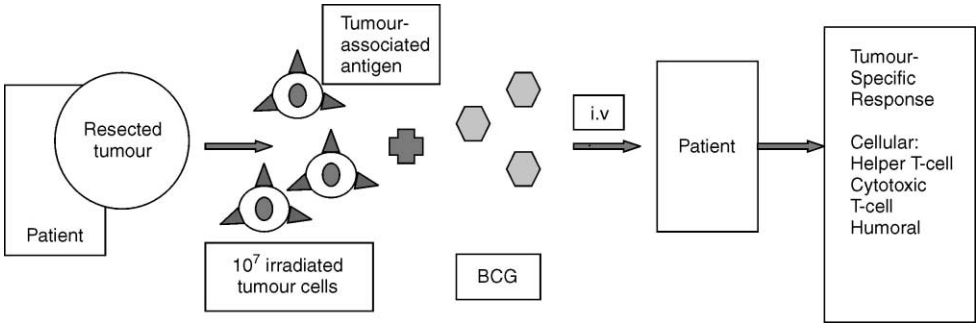

Fig. 7. Active specific immunotherapy. i.v., intravenous; BCG, Bacillus Calmette Guerin.

apoptosis, complement-dependant cytolysis and ADCC (Antibody-dependent cell-mediated cytotoxicity). Edrecolomab, or 17–1A, is an antibody directed against GA733–2, a tumour-associated polypeptide. Recent data suggests that Edrecolomab is inferior to 5-FU/folinic acid as adjuvant treatment of stage III cancer, and adds no additional benefit in combination [86]. A CALGB study of Edrecolomab versus no treatment in stage II disease is still accruing.

An alternative target for colorectal vaccines is CEA, which is a glycoprotein tumour-associated antigen overexpressed in 85% of colorectal carcinomas. CeaVac is an anti-idiotype monoclonal antibody: its variable domain mimics regions of the CEA molecule. Phase II data suggests this stimulates good IgG and helper T-cell responses, and a phase III trial is evaluating the role of Cea Vea in Dukes' C colorectal cancer [87]. The *CEA* gene can be inserted into a viral vector such as vaccinia or avipox. Phase I trials suggest both antibody and cellular responses are generated in patients with advanced colorectal carcinoma [88]. These may be augmented by co-administration of cytokines such as interleukin (IL)-2 or granulocyte macrophage-colony stimulating factor (GM-CSF) [89]. Data from a murine model suggests co-stimulatory molecules normally lacking from tumour cells such as B7 may also augment the response [90]. Dendritic cells have gained a lot of attention in recent years because of their efficiency in presenting antigen to CD4+ T-cells and co-ordinating the immune response. Phase I trials have looked at reinjecting autologous dendritic cells that have been pulsed with CEA peptide. T-Cell responses have been observed, but objective tumour responses are poor in the context of bulky disease [91]. Trials are ongoing in patients with minimal residual disease.

A wide range of other immunological targets have been proposed and a multitude of agents are currently in phase I and II trials. Promising targets include the epidermal growth factor receptor, heat shock proteins and MUC-1. It must be remembered that no two tumour cells are identical and that a universal vaccine may be an unattainable target. However, an increasing understanding of the mechanisms by which tumour cells escape immunological surveillance, an improved knowledge of tumour-associated non-self antigens and increasingly novel ways of presenting antigen, will open up a host of novel therapies for the future. It is also being increasingly recognised that a vaccine that does not produce significant response rates in the metastatic setting may still be of value in the adjuvant setting. Innovative ways of assessing treatment benefits using surrogate markers of immunity, such as the identification of increases in precursor T-cell frequency by enzyme-linked immunospot assay (ELISPOT), will allow more vaccines to progress to clinical trials and enhance progress in the immunotherapy field.

12. Conclusions

- Chemotherapy for advanced colorectal cancer palliates symptoms and increases median survival.
- Adjuvant chemotherapy with 5-FU and folinic acid has proven benefit in Dukes' C colon cancer, with a 5–6% absolute survival advantage at 5 years.
- A definite role for adjuvant chemotherapy in Dukes' B colon cancer and older or less fit patients has not been established.
- Combination regimes including 5-FU, oral fluoropyrimidines, irinotecan and oxaliplatin are being increasingly used in advanced colorectal cancer and are being tested for efficacy and toxicity in the adjuvant setting.
- Novel therapies that specifically target malignant processes are progressing to phase III clinical assessment, but may necessitate the development of new strategies for assessing response.

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