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Original Paper

Raltitrexed (Tomudex™) in Combination with 5-Fluorouracil for the Treatment of Patients with Advanced Colorectal Cancer: Preliminary Results from Phase I Clinical Trials

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The potential of raltitrexed (Tomudex™) in combination with 5-fluorouracil (5-FU) as treatment for advanced colorectal cancer has been investigated in two phase I clinical trials. In the first study, raltitrexed was combined with bolus 5-FU; patients received raltitrexed as a 15-min infusion followed 24 h later by bolus 5-FU every 3 weeks. In the second study, 5-FU was administered as a weekly 24-h infusion for 5 weeks of a 6-week cycle and raltitrexed was given 15-min prior to 5-FU on days 8 and 29. The recommended dose for bolus 5-FU in combination with raltitrexed is likely to be 1200 mg/m^2 as dose-limiting toxicity (DLT) of febrile neutropenia was observed at 1350 mg/m^2, but escalation of raltitrexed above the dose used for single-agent use (3.0 mg/m^2) continues. In the raltitrexed/infusional 5-FU study, dose escalation is also still continuing, but only in men as no DLT has been observed in men; 2 of 3 female patients had DLT of myelosuppression and diarrhoea at raltitrexed 3.0 mg/m^2 and infusional 5-FU 2400 mg/m^2. Raltitrexed had a significant effect on the pharmacokinetics of 5-FU irrespective of 5-FU regimen. Preliminary response data is encouraging with 53% of patients receiving raltitrexed/infusional 5-FU showing a partial response. In addition, significant disease stabilisation was observed in patients receiving raltitrexed combined with bolus 5-FU who had previously failed 5-FU therapy. Recruitment has recently commenced in two studies in which raltitrexed is combined with oral derivatives of 5-FU. In conclusion, preliminary data from these phase I studies indicate that the combination of raltitrexed and 5-FU is well tolerated and has encouraging clinical activity. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: raltitrexed, 5-fluorouracil, chemotherapy, colorectal cancer, tegafur-uracil
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INTRODUCTION

AT PRESENT, standard chemotherapy for advanced colorectal cancer involves combinations of 5-fluorouracil (5-FU) with leucovorin (LV). Studies have shown that objective response rates are equivalent for raltitrexed (Tomudex™) monotherapy and 5-FU/LV-based regimens; typically between 15 and 20% [1]. Combining raltitrexed with 5-FU regimens offers the opportunity to further improve this efficacy.

The rationale for combining raltitrexed and 5-FU is multifactorial. Raltitrexed and 5-FU have both been shown to be active as single agents in the treatment of advanced colorectal cancer [1–4]. Both agents inhibit thymidylate synthase (TS), an essential enzyme in the *de novo* synthesis of DNA, but via different mechanisms and with different binding sites. In addition, raltitrexed is a more specific inhibitor of the enzyme than 5-FU. Raltitrexed is converted within target cells into polyglutamate forms which are potent inhibitors of TS [5]. The mechanism of action of 5-FU is more complex; the metabolite 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) inhibits TS but 5-FU can also be cytotoxic following metabolism to 5-fluorouridine-5′-triphosphate (FUTP) or 5-fluoro-2′-deoxyuridine-5′-triphosphate (FdUTP) which can be incorporated into RNA and DNA, respectively [6].

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Another important consideration when combining agents is their respective toxicity profiles. Both raltitrexed and 5-FU cause dose-limiting toxicities (DLTs) of myelosuppression and diarrhoea. However, raltitrexed is associated with a lower incidence of severe leucopenia and mucositis than 5-FU but a higher incidence of anaemia and raised liver transaminases [1, 7]. The myelosuppressive effects of 5-FU can be reduced by giving it as a continuous infusion rather than a bolus [8]. Therefore, the combination of raltitrexed with a continuous infusion of 5-FU may maximise effectiveness whilst minimising toxicity as higher doses of 5-FU can be given via this regimen compared with the bolus administration. A recent meta-analysis has shown a statistically higher response rate coupled with lower toxicity for infusional 5-FU compared with bolus 5-FU [9]. The cytotoxicity of bolus and infusional 5-FU are thought to be due to different mechanisms; bolus 5-FU is believed to be more RNA-directed than infusional 5-FU which is thought to act more through inhibition of TS [10].

Preclinical studies have also pointed to the possible advantages of combining these agents. Exposure of colon carcinoma cells to raltitrexed for 24 h followed by 5-FU resulted in synergistic cytotoxicity [11, 12]. This synergy was shown to be schedule dependent (the reverse sequence was either additive or antagonistic) and time dependent (greater synergy when 5-FU was given over 4 h as compared with a 5-day exposure). It has also been shown that there is an increased affinity of TS for FdUMP, the active metabolite of 5-FU, in the presence of raltitrexed polyglutamates [13]. Furthermore, pre-treatment of cells with raltitrexed has been shown to increase the incorporation of FUTP into RNA [11]. Raltitrexed may also be able to inhibit dihydropyrimidine dehydrogenase (DPD) activity [14], which could have effects on the antitumour activity of 5-FU as well as on clinical toxicity.

Clinical trials are currently underway to determine the maximum tolerated doses of raltitrexed and 5-FU in combination and to make preliminary evaluations of the efficacy of the combination. This paper will review the current status of two phase I trials of raltitrexed in combination with 5-FU and will briefly outline two further studies in which raltitrexed is being combined with oral derivatives of 5-FU.

PATIENTS AND METHODS

Two phase I studies evaluating the combination of raltitrexed with 5-FU have been undertaken in centres in Germany and the U.S.A. The US study investigated raltitrexed in combination with bolus 5-FU [15], whereas the German study was designed to evaluate the toxicity and efficacy of raltitrexed in combination with infusional 5-FU [16].

Raltitrexed/bolus 5-FU study

Raltitrexed was administered as a 15-min infusion followed 24 h later by a bolus injection of 5-FU. This was repeated on a 3-weekly cycle. 3 patients were entered at each dose level; the planned dose escalation scheme is shown in Figure 1. The recommended doses for raltitrexed and bolus 5-FU were actually determined (including protocol amendments) by first fixing the dose of 5-FU at 900 mg/m^2 and escalating the dose of raltitrexed from 0.5 to 3.0 mg/m^2. As no DLT was observed by raltitrexed 3.0 mg/m^2 then this dose was fixed and the 5-FU dose was escalated until DLT at this fixed dose of raltitrexed was found. The dose of 5-FU was then lowered by one dose level and was fixed at this level. The raltitrexed dose was then escalated from 3.0 mg/m^2 until the toxic dose was achieved; the recommended dose (equivalent to the maximum tolerated dose, MTD) was the dose below the toxic dose.

DLT were defined according to modified WHO criteria as neutropenic fever with a neutrophil count of $< 500/mm^3$ plus fever $> 38.3°C$; grade 4 haematological toxicities of neutropenia or thrombocytopenia that persist for > 7 days; grade 4 non-haematological toxicities of mucositis or diarrhoea that develop despite antidiarrhoeal prophylaxis; failure of thrombocytopenia, neutropenia or any of the non-haematological toxicities to recover within 21 days after causing a dose delay.

Analysis of 5-FU pharmacokinetic parameters was performed on patients at each dose level. Objective responses were defined according to the decrease in the bidimensional measurement of evaluable lesions: complete response—100% decrease; partial response $> 50\%$ but $< 100\%$ decrease; minor response $> 25\%$ but $< 50\%$ decrease. Evaluations for response were obtained after 3 cycles or 9 weeks of study, whichever came first (dependent on whether there were treatment delays).

Raltitrexed/infusional 5-FU study

5-FU was administered weekly for 5 weeks of a 6-week cycle (includes 1 week of rest from treatment) as a 24-h infusion. Raltitrexed was administered on days 8 and 29 as a 15-min infusion followed 15 min later by the infusion of 5-FU. The raltitrexed dose was initially fixed at 2.6 mg/m^2 and 5-FU was escalated from 1200 mg/m^2 to 2400 mg/m^2 in 400 mg/m^2 increments. If the MTD at this fixed 2.6 mg/m^2 dose of raltitrexed did not occur, then the raltitrexed dose was increased to 3.0 mg/m^2 and escalation of 5-FU continued.

3 patients were entered at each dose level. If no patients experienced DLT, escalation to the next dose could proceed. If 1 or 2 of the patients experienced DLT then an extra 3 patients were recruited; escalation could only proceed if $\leq 2/6$ patients had DLT. If all 3 of the original patients or $\geq 4/6$ patients experienced DLT this was classed as the toxic dose and the recommended dose was designated as two doses below (Figure 2).

DLT was defined according to WHO criteria as grade 3/4 diarrhoea, mucositis or thrombocytopenia; grade 4 (or grade 3 with complication) granulocytopenia; other toxicity grade 2 or higher (excluding nausea/vomiting, alopecia and increases in transaminases).

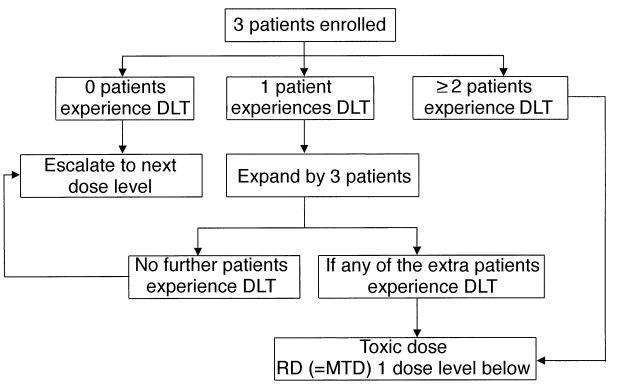


Figure 1. Determination of recommended dose in the raltitrexed/bolus 5-FU study. DLT, dose-limiting toxicity; MTD, maximum tolerated dose.

Analyses of 5-FU pharmacokinetics were conducted at week 1 and 2 of the cycle in order to compare 5-FU pharmacokinetic parameters in the absence and presence of raltitrexed.

Evaluation of objective tumour response, based on UICC criteria using computed tomography, ultrasound and chest X-ray, was performed 3 weeks prior to the study and then repeated every 6 weeks until week 18.

RESULTS

Patients

To date, 67 patients have been recruited: 38 have received bolus 5-FU and 29 infusional 5-FU (Table 1). The majority of patients in the two studies have been male; 60.5% in the raltitrexed/bolus 5-FU study and 69.0% in the raltitrexed/infusional study. The median age of patients receiving raltitrexed/bolus 5-FU was 59 years of age compared with 60 years of age for those patients receiving raltitrexed/infusional 5-FU. The primary tumours were of the colon and rectum in 76 and 24% of patients, respectively, in the raltitrexed/bolus study and 52 and 48%, respectively, in the raltitrexed/infusional 5-FU study. In the study involving bolus 5-FU the majority of patients had been unresponsive to prior therapy with bolus 5-FU, whereas in the study involving infusional 5-FU most patients had not had any previous chemotherapy.

Toxicity

The MTD has not been reached for raltitrexed in combination with bolus 5-FU. No DLT was observed up to raltitrexed 3.0 mg/m^2 and bolus 5-FU 1200 mg/m^2 (Table 2); a fourth patient was entered at this dose whilst awaiting approval of a protocol amendment to increase the raltitrexed dose above 3.0 mg/m^2. DLT of febrile neutropenia was observed in 3 out of 4 patients when raltitrexed and bolus 5-FU were combined at doses of 3.0 mg/m^2 and 1350 mg/m^2, respectively and all 4 patients entered at this dose level had grade 4 neutropenia. 4 patients were entered at this dose level rather than the 3 specified in the protocol because at the time of expansion of the cohort only 1/3 patients were reported to have had neutropenic fever, this was, however, later amended to 2/3. The additional patient recruited at this dose level also experienced neutropenic fever. 2 of these patients also had grade 3 nausea and 1 patient had grade 4 vomiting, despite the administration of anti-emetics to all 3 patients. The 5-FU dose was subsequently reduced to 1200 mg/m^2 and escalation of raltitrexed continued. No patients experienced DLT at 3.5 mg/m^2 raltitrexed; 1 patient had transient grade 4 neutropenia and 2 patients had grade 3 neutropenia but these did not require dose reductions. 6 patients were entered at raltitrexed 4.0 mg/m^2 and 5-FU 1200 mg/m^2 but only 1 patient experienced DLT of febrile neutropenia and grade 4 mucositis. Escalation of raltitrexed at a fixed dose of 5-FU of 1200 mg/m^2 continues.

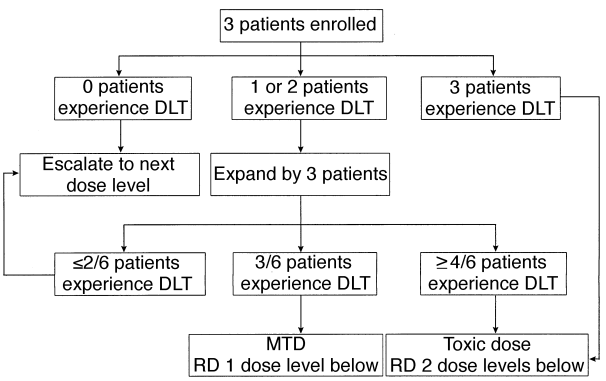


Figure 2. Determination of recommended dose in the raltitrexed/infusional 5-FU study. DLT, dose-limiting toxicity; MTD, maximum tolerated dose.

Table 1. Demographics of patients treated with raltitrexed in combination with bolus or infusional 5-FU

	Raltitrexed/ bolus 5-FU	Raltitrexed/ infusional 5-FU
No. patients	38	29
Men:women	23:15	20:9
Median age (range), years	59 (40–76)	60 (33–77)
Primary tumour		
Colon	29	15
Rectum	9	14
Prior therapy		
None	6	20
Chemotherapy	32*	7†
Radiation	–	2†

*5-FU (bolus). †Adjuvant therapy (stopped at least 6 months before study).

Recruitment is also still continuing in the raltitrexed/infusional 5-FU study, but only for male patients. DLTs, of myelosuppression and diarrhoea were observed in 2/3 female patients at 3.0 mg/m^2 raltitrexed and 2400 mg/m^2 5-FU (Table 3). One patient had grade 4 thrombocytopenia, septicaemia and grade 3 diarrhoea in week 3 and the other patient had grade 4 leucopenia, thrombocytopenia and died from septicaemia in week 6. Interestingly, the 3 male patients recruited at this dose level did not show any DLT and 6 men have now been recruited at the next dose level (raltitrexed 3.0 mg/m^2 and infusional 5-FU 2800 mg/m^2). 3 patients were withdrawn from the study due to adverse events but these were not classified as DLTs: 1 patient died from a gastrointestinal haemorrhage at dose level one, but he was later found to have a long history of gastrointestinal ulcers; 1 patient had a recurrence of rectal cancer and was discontinued; 1 patient had 5-FU-induced angina pectoris at dose level 3 and was discontinued.

Table 2. Dose-limiting toxicity of raltitrexed in combination with bolus 5-FU

Raltitrexed (mg/m^2)	5-FU (mg/m^2)	No. patients entered	Dose-limiting toxicity
0.5	900	3	None
1.0	900	3	None
1.5	900	3	None
2.0	900	3	None
2.5	900	3	None
3.0	900	3	None
3.0	1050	3	None
3.0	1200	4	None
3.0	1350	4	Febrile neutropenia (3 patients)
3.5	1200	3	None
4.0	1200	6	Febrile neutropenia (1 patient)
4.5	1200		(trial ongoing)

Table 3. Dose-limiting toxicity of raltitrexed in combination with infusional 5-FU

Raltitrexed (mg/m^2)	5-FU (mg/m^2)	No. patients entered	Dose-limiting toxicity
2.6	1200	4	None
2.6	1600	3	None
2.6	2000	4	None
2.6	2400	6	None
3.0	2400	6*	G3 thrombocytopenia, septicaemia, G3 diarrhoea in week 3 (1 female) G4 leucopenia, thrombocytopenia, lethal septicaemia in week 6 (1 female)
3.0	2800	6†	Too early

*The 3 male patients had no dose-limiting toxicity. †All male.

Pharmacokinetics

In the raltitrexed/bolus 5-FU study increases in 5-FU C_{max} (Figure 3) and 5-FU area under the curve (AUC) were observed when the raltitrexed dose was raised to 2.5 mg/m^2 compared with 2.0 mg/m^2, with the 5-FU dose fixed at 900 mg/m^2. In the raltitrexed/infusional 5-FU study pharmacokinetics of 5-FU were studied at week 1 (5-FU alone) and week 2 (5-FU plus raltitrexed) of the cycle. Increases in 5-FU C_{max} (Figure 4), AUC and a prolonged terminal half life were observed when 5-FU was given in combination with raltitrexed compared with 5-FU alone.

Objective response

Overall, 37 patients have been evaluated for response in the raltitrexed/bolus 5-FU study, across all the dose levels. In total, 1 patient had a complete response, 3 patients had a partial response; 3 of these patients had previously failed 5-FU therapy. 17 (46%) patients had stable disease (SD), including 1 patient who had a minimal response; 13 of these patients had received prior 5-FU therapy.

17 patients were evaluable for response in the infusional 5-FU study at the four highest dose levels (raltitrexed/5-FU, mg/m^2): 2.6/2000, 3 patients; 2.6/2400, 6 patients; 3.0/2400, 6 patients; and 3.0/2800, 2 patients. 9 patients (53%) achieved at least a partial response (PR) and (35%) had SD.

Phase I combination study with oral derivatives of 5-FU

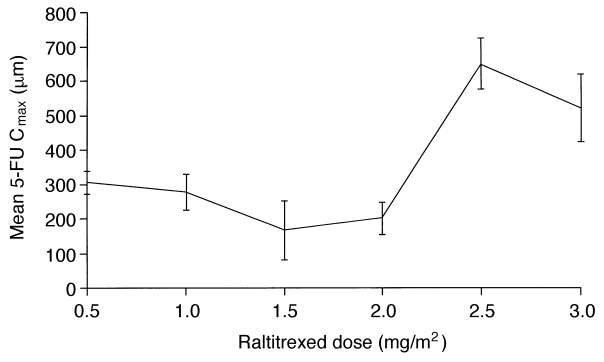
A dose-escalating pilot study of raltitrexed in combination with tegafur-uracil (UFT) in patients with advanced colorectal cancer is scheduled to begin in the autumn of 1998 at centres in Spain. Raltitrexed will be administered as a 15-min infusion on days 1 and 22 of a 6-week cycle, and UFT will be given orally every day for 28 days. Raltitrexed will be escalated from 2.0 mg/m^2 to 3.5 mg/m^2 and UFT will be escalated from 200 mg/m^2 to 350 mg/m^2. The MTD and DLT are defined as for the raltitrexed/5-FU infusional study. Once the MTD has been achieved a further 20–30 patients will be enrolled at the recommended dose level. It is expected that 20–50 patients will be recruited in a 9-month period. This second phase will be conducted to assess efficacy and further assess tolerability at the chosen dose levels.

A further study is ongoing in Finland (study director P. Österlund), in which raltitrexed is combined with 1-hexylcarbomoyl-5-fluorouracil (HCFU), an oral derivative of 5-FU. Raltitrexed is being given as a 15-min infusion on day 1 followed by oral HCFU on days 2–14 of a 3-week cycle. Raltitrexed is being escalated from 1.5 mg/m^2 and HCFU from 300 mg/m^2. To date, 16 patients have been recruited and escalation has proceeded to raltitrexed 3.0 mg/m^2 and HCFU 300 mg/m^2.

CONCLUSIONS

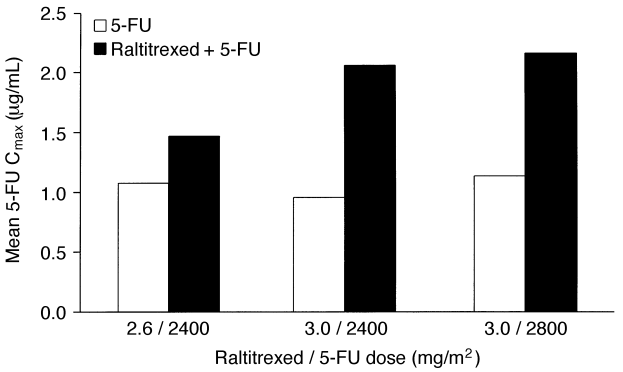
The data from the two ongoing phase I trials, combining raltitrexed with either bolus 5-FU or infusional 5-FU, support the preclinical rationale for combining these agents. In both studies, raltitrexed has been combined with 5-FU at doses which have been shown to be effective for the single agents whilst still maintaining acceptable toxicity.

The recommended dose for bolus 5-FU in combination with raltitrexed is likely to be 1200 mg/m^2 as DLT of febrile neutropenia was observed at 1350 mg/m^2. Dose escalation of raltitrexed, above the recommended dose of 3.0 mg/m^2 for a single agent, appears to be possible and is currently being investigated. The reason why higher doses of raltitrexed appear to be possible in combination than for single agent use is at present unclear. Interestingly, in the first phase I study of raltitrexed as a single agent, conducted in the U.S.A., the recommended dose for further study was 4.0 mg/m^2 [17]. It is also possible that the definition of the MTD was more stringent in studies of raltitrexed as a single agent than the definition



3—4 patients per dose level

Figure 3. Mean 5-FU C_{max} at different doses of raltitrexed in the raltitrexed/bolus 5-FU study. The 5-FU dose was fixed at 900 mg/m^2.



2—6 patients per dose level

Figure 4. Effect of raltitrexed on 5-FU C_{max} in the raltitrexed/infusional 5-FU study. The C_{max} values are shown for week 1 (5-FU alone; non-shaded) and week 2 (5-FU+raltitrexed, ■) of the cycle.

used in this study. In terms of the 5-FU dose it is difficult to extrapolate from single agent regimens; bolus 5-FU is commonly given as 425 mg/m^2 per day with 20 mg/m^2 of folinic acid per day, for 5 days every 4 weeks [18].

For raltitrexed in combination with infusional 5-FU, the recommended doses are likely to be 2.6 mg/m^2 for raltitrexed and 2400 mg/m^2 for 5-FU in women. However, as no men experienced DLT at 3.0/2400 mg/m^2 raltitrexed/infusional 5-FU the possibility of a gender difference is being investigated with recruitment of men continuing at raltitrexed and infusional 5-FU doses of 3.0 mg/m^2 and 2800 mg/m^2, respectively. The likely recommended dose of infusional 5-FU combined with raltitrexed is comparable with infusional doses of 5-FU modulated with folinic acid. The recommended dose of 5-FU, administered as weekly 24-h infusion every 6 weeks and modulated with folinic acid, is 2600 mg/m^2 [19].

Preliminary objective response data indicates that this combination does have clinical activity, with 53% of patients who received raltitrexed in combination with infusional 5-FU at doses of 2.6 mg/m^2 and 2000 mg/m^2, respectively and above, showing a partial response. Clinical activity was also observed with the bolus 5-FU/raltitrexed combination in patients who had previously failed 5-FU therapy. In both studies, raltitrexed had a significant effect on the pharmacokinetics of 5-FU, increasing the AUC and C_{max} at doses above 2.5 mg/m^2. This will require further evaluation.

Studies combining raltitrexed with two oral derivatives of 5-FU, UFT and HCFU, are now underway. UFT is a non-specific inhibitor of TS with a similar mechanism of action to 5-FU. The clinical effects of UFT are due to tegafur, a 5-FU prodrug which is metabolised to 5-FU by cytochrome P450 in the liver, thymidine phosphorylase in tumour tissue and by spontaneous degradation; uracil competitively inhibits the degradation of 5-FU by inhibition of dihydropyrimidine dehydrogenase, resulting in higher 5-FU levels remaining in the tumour cells [20]. HCFU is also a 5-FU prodrug and has shown similar response rates to other 5-FU regimens in advanced colorectal cancer [21]. The recommended dose of HCFU as a single agent is 300–600 mg/m^2. Patients are currently being recruited at doses of both raltitrexed and HCFU that are recommended for single agent use, i.e. 3.0 mg/m^2 raltitrexed and 300 mg/m^2 HCFU.

In conclusion, the combination of raltitrexed and 5-FU has shown promising clinical activity, whilst maintaining acceptable toxicity, and should be further investigated in phase II clinical trials.

1. Cunningham D. Mature results from three large controlled studies with raltitrexed ('Tomudex'). *Br J Cancer* 1998, **77**(Suppl. 2), 15–21.
2. Kemeny N, Lokich JJ, Anderson N, Ahlgren JD. Recent advances in the treatment of advanced colorectal cancer. *Cancer* 1993, **71**, 9–18.
3. Moertel CG. Chemotherapy for colorectal cancer. *N Engl J Med* 1994, **330**, 1136–1142.
4. Zalcberg JR, Cunningham D, Van Cutsem E, *et al.* ZD1694: a novel thymidylate synthase inhibitor with substantial activity in the treatment of patients with advanced colorectal cancer. *J Clin Oncol* 1996, **14**, 716–721.
5. Gunasekara NS, Faulds D. Raltitrexed: a review of its pharmacological properties and clinical efficacy in the management of advanced colorectal cancer. *Drugs* 1998, **55**, 423–435.
6. Diasio RB, Harris BE. Clinical pharmacology of 5-fluorouracil. *Clin Pharmacokinetics* 1989, **16**, 215–237.
7. 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.
8. Lokich JJ, Ahlgren JD, Gullo JJ, Philips JA, Fryer JG. A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: a Mid-Atlantic Oncology Program study. *J Clin Oncol* 1989, **7**, 425–432.
9. Meta-analysis Group in Cancer. Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. *J Clin Oncol* 1998, **16**, 301–308.
10. Aschele C, Sobrero A, Faderan MA, Bertino JR. Novel mechanism(s) of resistance to 5-fluorouracil in human colon cancer (HCT-8) sublines following exposure to two different clinically relevant dose schedules. *Cancer Res* 1992, **52**, 1855–1864.
11. Longo GSA, Izzo J, Chang YM, *et al.* Pretreatment of colon carcinoma cells with Tomudex enhances 5-fluorouracil cytotoxicity. *Clin Cancer Res* 1998, **4**, 469–473.
12. Harstrick A, Schleucher N, Gonzales A, *et al.* Interactions and cross resistance patterns between various schedules of 5-FU and the new, folate-based thymidylate synthase inhibitor 'Tomudex' (D1694). *Eur J Cancer* 1995, **31A**(Suppl. 5), S30.
13. Van der Wilt CL, Pinedo HM, Kuiper CM, Smid K, Peters GJ. Biochemical basis for the combined antiproliferative effect of AG337 or ZD1694 and 5-fluorouracil. *Proc Am Assoc Cancer Res* 1995, **36**, 379 (A2260).
14. Yan JM, Lu ZH, Shao LN, Diasio RB. The effect of 'Tomudex' on dihydropyrimidine dehydrogenase activity. *Proc Am Assoc Cancer Res* 1997, **38** (abstract 4133).
15. Schwartz GK, Bertino JL, Kemeny N, *et al.* Interim results of a phase I trial suggest that 'Tomudex' (raltitrexed) may act synergistically with 5-FU in patients with advanced colorectal cancer (CRC). *Ann Oncol* 1998, **9**(Suppl. 4), 36 (abstract 171P).
16. Harstrick A, Mayer S, Hilger R, *et al.* Combination therapy with infusional 5-FU and 'Tomudex' for patients (pts) with advanced colorectal cancer—a phase I study. *Ann Oncol* 1998, **9**(Suppl. 4), 35 (abstract 170P).
17. Sorensen JM, Jordan E, Grem JL, *et al.* Phase I trial of ZD 1694 (Tomudex), a direct inhibitor of thymidylate synthase. *Ann Oncol* 1994, **5**(Suppl. 5), 132 (A241).
18. Poon MA, O'Connell MJ, Wieand HS, *et al.* Biochemical modulation of fluorouracil with leucovorin: confirmatory evidence of improved therapeutic efficacy in advanced colorectal cancer. *J Clin Oncol* 1991, **9**, 1967–1972.
19. Ardalan B, Chua L, Tian EM, *et al.* A phase II study of weekly 24-hour infusion with high-dose fluorouracil with leucovorin in colorectal carcinoma. *J Clin Oncol* 1991, **9**, 625–630.
20. Takiuchi H, Ajani JA. Uracil-tegafur in gastric carcinoma: a comprehensive review. *J Clin Oncol* 1998, **16**, 2877–2885.
21. Grohn P, Heinonen E, Kumpulainen E, *et al.* Oral carmofur in advanced gastrointestinal cancer. *Am J Clin Oncol* 1990, **13**, 477–479.

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