Phase I trial of sequential raltitrexed followed by bolus 5-fluorouracil in patients with advanced colorectal cancer

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Our objective was to determine the maximum tolerated dose (MTD) of sequential raltitrexed (Tomudex) and 5-fluorouracil (5-FU) by bolus administration every 3 weeks in patients with advanced colorectal cancer (aCRC) and appendiceal adenocarcinoma. This phase I dose-escalation study was carried out in three stages: (1) 5-FU fixed at 900 mg/m^2, raltitrexed escalated from 0.5 to 3.0 mg/m^2, (2) raltitrexed fixed at 3.0 mg/m^2, 5-FU escalated from 900 mg/m^2 until dose-limiting toxicity (DLT) and (3) 5-FU fixed at the dose level below DLT, raltitrexed escalated from 3.0 mg/m^2 until MTD. Seventy-one patients with measurable disease were enrolled. No DLTs were observed during stage 1 of treatment. At a fixed dose of raltitrexed 3.0 mg/m^2, DLT developed when 5-FU was increased to 1350 mg/m^2 (stage 2). When 5-FU was fixed at 1200 mg/m^2 and raltitrexed was increased to 6.0 mg/m^2 (stage 3), DLT was dose limiting. The recommended doses for further study are 5.5 mg/m^2 ralitrexed and 1200 mg/m^2 5-FU. Of the 69 patients evaluated for efficacy, one had a complete response (8.0 months) and five had partial responses (5.1–11.6 months). Thirty patients had stable disease for 5 or more cycles of therapy (mean time to progression: 3.6 months). Median survival was 11.7 months. We conclude that raltitrexed can be combined with bolus 5-FU, at raltitrexed doses that are higher than the recommended single-agent dose of 3.0 mg/m^2, with manageable toxicity. This combination shows encouraging activity, and survival appears promising in the pre-treated aCRC patient population. Further clinical trials are warranted. *Anti-Cancer Drugs* 15:219–227 © 2004 Lippincott Williams & Wilkins.

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Introduction

Colorectal cancer (CRC) is the fourth most common form of cancer worldwide. It affects both men and women in similar proportions, and its incidence has been rapidly increasing since 1975 [1]. CRC is the second leading cause of death in the US and approximately 500 000 people worldwide die as a result of this malignancy every year [2,3].

Chemotherapy is commonly used to treat cases of metastatic or advanced CRC (aCRC) for which surgical removal is not sufficient. Amongst the various chemotherapeutic options, 5-fluorouracil (5-FU), alone or in combination with a modulating agent such as leucovorin (LV), has been the mainstay of treatment for the last 40 years. Nevertheless, only approximately 20% of patients have an objective response (OR) to such treatment (a measurable tumor decrease of more than 50%) [4].

The primary target of 5-FU is the enzyme thymidylate synthase (TS), which catalyzes the synthesis of thymidine monophosphate (TMP) from deoxyuridine monophosphate (dUMP). TMP is a precursor of thymidine triphosphate (TTP), which is essential for DNA replication and repair. 5-FU is metabolically inactive and for it to inhibit TS, it must be converted to fluorodeoxyuridine monophosphate (F-dUMP), which is able to bind covalently to the dUMP binding site of TS and eventually halt the production of TTP. However, 5-FU is also converted via several other enzymatic pathways to other metabolites such as fluorouridine triphosphate (FUTP) and fluorodeoxyuridine triphosphate (F-dUTP), which interfere with RNA and protein synthesis (Fig. 1) [5].

In order to function properly, TS needs the cofactor tetrahydrofolate. Raltitrexed ('Tomudex'), an inhibitor of TS, blocks the folate-binding site of TS, specifically inhibiting the activity of this enzyme. Raltitrexed undergoes intracellular polyglutamation and this increases the potency of the drug as well as its intracellular retention time, which allows a less frequent dosing schedule (every 3 weeks). Raltitrexed has shown efficacy and tolerability as a single anti-tumor agent in previous clinical trials in patients with CRC. In this setting, raltitrexed has shown similar objective responses, overall survival and time to progression to those achieved with 5-FU/LV [6–8].

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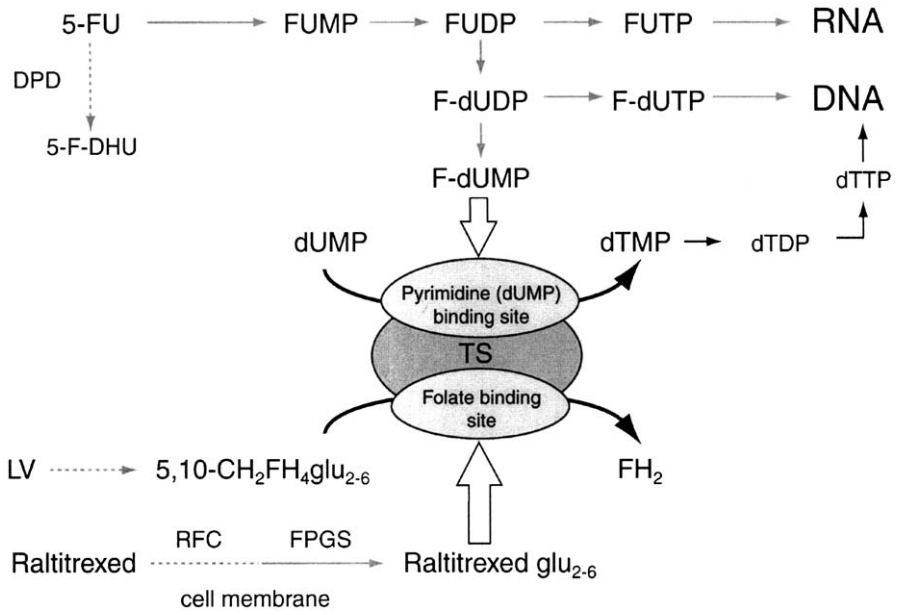
Fig. 1



Mechanism of action of raltitrexed and 5-FU.

Raltitrexed (3.0 mg/m^2) is currently approved as monotherapy for the first-line treatment of aCRC in over 40 countries worldwide.

Therefore, although raltitrexed and 5-FU both inhibit TS, they have different mechanisms of action and consequently incompletely overlapping toxicity profiles [9]. In particular, bolus 5-FU is believed to be directed towards RNA, whereas raltitrexed acts through direct inhibition of TS [10]. Combining raltitrexed with 5-FU may augment the efficacy of 5-FU in a manner similar to the methotrexate/5-FU combination [11,12]. *In vitro* studies have shown that raltitrexed followed by 5-FU results in synergistic cytotoxicity in human colon tumor (HCT)-8 cells [13]. Pre-treatment with raltitrexed also increases the incorporation of FUTP into RNA. Other authors have shown improved response rates in patients with CRC using the combination of raltitrexed and 5-FU with either infusional 5-FU or oral 5-FU pro-drugs [14–16].

The primary aim of this phase I trial was to determine the maximum tolerated dose (MTD) of sequential raltitrexed and bolus 5-FU in patients with aCRC. It is hoped that the results from this study will ultimately allow further clinical research into such a drug combination.

Methods

Patient selection

Male or non-pregnant female patients with aCRC and appendiceal adenocarcinomas were eligible for the study if they were aged $\geq$ 18 years, had a WHO performance status ≤ 2 and ≥ 4 weeks had elapsed since the last administration of chemotherapy (≥ 8 weeks if treated with nitrosoureas or mitomycin C). Patients may have received prior adjuvant therapy or treatment for metastatic disease (5-FU-based regimen and/or irinotecan). Exclusion criteria included severe recurrent infection or recent surgery (<4 weeks), inadequate renalfunction (serum creatinine clearance < 65 ml/min), insufficient hepatic function [bilirubin > 1.5 mg/dl, or serum glutamic-oxaloacetic transaminase (SGOT) $> 2.5 \times$ upper limit of normal (ULN), or $> 5 \times$ ULN for subjects with known liver metastases], impaired hematologic function [absolute neutrophil count (ANC) $< 2000/\text{mm}^3$, platelets $< 100\,000/\text{mm}^3$]; concomitant anticancer therapy or folate supplements, brain metastases or prior radiotherapy to $> 30\%$ of bone marrow.

All patients gave written informed consent prior to participation. The study received Institutional Review Board approval and was performed in adherence with the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines.

Treatment

Raltitrexed was supplied by AstraZeneca as a lyophilized product that was reconstituted with 4 ml of water for injections to produce a 0.5 mg/ml. isotonic solution. This solution was diluted further with 50 ml of 5% dextrose or 0.9% saline and administered as a short i.v. infusion over 15 min 5-FU was commercially available and was supplied

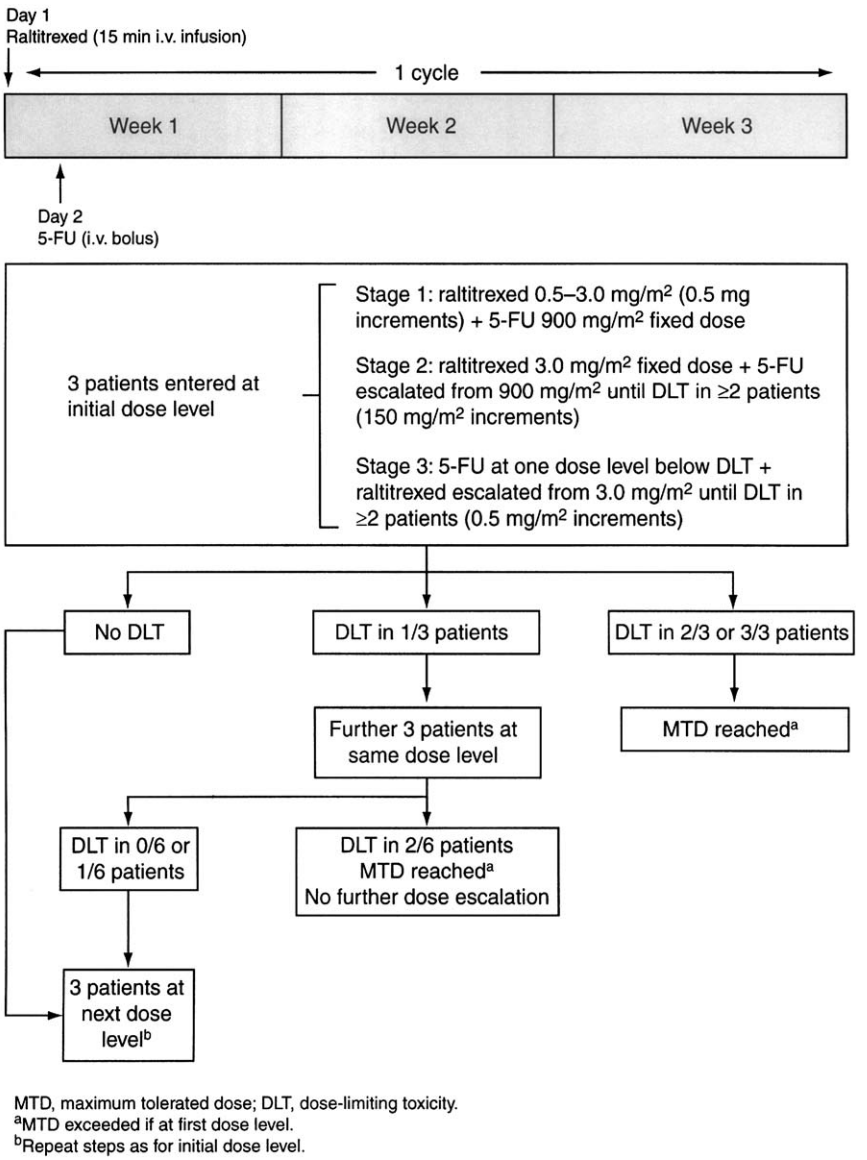
as 500 mg/10-cm^3 vials, administered undiluted by rapid i.v. injection 24 h after raltitrexed infusion. This treatment was repeated once every 3 weeks.

No prophylactic treatment for vomiting, diarrhea or stomatitis was administered for at least the first treatment dose. However, full supportive care measures and symptomatic treatment for chemotherapy-associated toxicity were given on the first signs of toxicity. Imodium (two caplets) was given if subjects presented with a loose bowel movement, and treatment (one caplet every 2 h) was continued until patients were free from diarrhea for 12 h or more. Subjects with neutropenic fever (ANC $< 500/mm^3$ plus fever $\geq 38.3^{\circ}C$) could receive granulocyte colony stimulating factor (G-CSF) if it was considered necessary.

Study design and dose-escalation schedule

This was an open-label, non-comparative, phase I trial of raltitrexed in combination with 5-FU according to a three-stage dose escalation (Fig. 2): (1) 5-FU fixed at 900 mg/m^2, raltitrexed increased from 0.5 to 3.0 mg/m^2 in increments of 0.5 mg/m^2, (2) raltitrexed fixed at 3.0 mg/m^2 (the standard single-agent dose), 5-FU escalated from 900 mg/m^2 in increments of 150 mg/m^2 until dose-limiting toxicity (DLT; see definition below) in two or more patients and (3) 5-FU fixed at the dose level below DLT in at least two of three or two of six patients, raltitrexed

Fig. 2



MTD, maximum tolerated dose; DLT, dose-limiting toxicity.
[a]MTD exceeded if at first dose level.
[b]Repeat steps as for initial dose level.

Dose-escalation schedule.

escalated from 3.0 mg/m^2 in increments of 0.5 mg/m^2 until MTD was defined (the dose level immediately preceding the dose level in which two or more patients have DLT).

At least three patients were entered at each dose level. If one of three patients had DLT within the first cycle, a further three patients were entered at the same dose. The MTD was exceeded if at least two of three or two of six patients had DLT within the first cycle.

The next cycle of treatment was delayed for a maximum of 21 days in the presence of any grade drug toxicity, with the exception of grade 1 neutropenia. Treatment was continued for an ANC $\geq 1500/mm^3$. If the toxicity had not resolved within 21 days, DLT was reached. However, further treatment could be continued at the next lowest dose level once toxicity had resolved and if clinically appropriate. Patients with stable or responding disease continued treatment for as long as they were benefiting from therapy, unless they had DLT.

An additional 15 patients were entered at the recommended dose (RD) of 5-FU and raltitrexed (the RD was defined as the same dose level as the MTD).

DLT was defined as drug-related WHO grade 4 neutropenia or thrombocytopenia of more than 7 days duration, grade 4 neutropenia with fever $\geq 38.3^{\circ}C$, grade 4 mucositis, dermatitis or diarrhea (despite anti-diarrheal prophylaxis), or failure of thrombocytopenia, neutropenia or non-hematologic toxicity to resolve within 21 days (neutropenia to resolve to grade 1 or less).

Assessments

Tolerability

Clinical assessments including a physical examination and performance score evaluation were performed within 1 week before starting treatment, on each day of raltitrexed administration, and 3 weeks after the final dose of raltitrexed. Blood samples (5–10 ml) were collected for biochemical and hematologic analyses. Biochemical tests, including serum creatinine, urea and bilirubin, were performed within 1 week before each dose of raltitrexed (initial dose 3.5 mg/m^2) and 3 weeks after the final dose of raltitrexed. Hematologic tests (hemoglobin, white cell and platelet counts) were performed within 1 week before each administration of raltitrexed, on the day of treatment and weekly during treatment. Laboratory results were used to determine whether the patient had reached DLT or was ready for another course.

Adverse events (AEs) were assessed according to WHO criteria [17] and patients were followed for 3 weeks after the final cycle of treatment for any new AEs.

Pharmacokinetics

Blood (5 ml) and urine samples (5–10 ml) were collected during the first course of treatment for pharmacokinetic analyses of 5-FU metabolites. Blood samples were drawn prior to 5-FU administration, and at 15 and 55 min post-administration. Using a limited sampling strategy, 5-FU levels were characterized by the methods of Bierman *et al.* [18] and Moore *et al.* [19].

Efficacy

The total tumor burden was assessed within 3 weeks before starting treatment by abdominal/pelvic computerized axial tomography (CAT) scans and chest X-rays (CXR). Objective disease assessments were performed every 9 weeks during treatment and defined according to the Union Internationale Contre le Cancer (UICC) criteria. Since CAT scans were obtained every 9 weeks, confirmatory CAT scans were not required to assess response.

Results

Patients

From April 1996 to September 1999, a total of 71 patients with measurable colorectal and appendiceal adenocarcinoma were enrolled in this trial at the Memorial Sloan-Kettering Cancer Center, New York. Three patients were evaluable for toxicity, but not for response, because of withdrawal from the study after one cycle of therapy. One patient withdrew for toxicity (neutropenic fever), another decided she no longer wanted to receive an investigational drug and the third elected to receive another investigational agent in a cancer center that was geographically closer to home. Almost all patients (97%) had CRC (76% colon cancer and 21% rectal cancer). Two patients (3%) had appendiceal cancer. The mean Karnofsky performance status was 90 (range 70–90). The majority of patients (90%) had received prior 5-FU-based regimens either as adjuvant therapy or as treatment for advanced disease (Table 1). This included 17 patients who had only received chemotherapy as adjuvant treatment.

Tolerability

In the first phase of the study, 5-FU was fixed at 900 mg/m^2 and raltitrexed was escalated from 0.5 to 3.0 mg/m^2 in six successive cohorts (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/m^2). When raltitrexed reached 3.0 mg/m^2 (the target dose), there were no DLTs reported at these dose levels. During the second phase of the trial, raltitrexed was fixed at 3.0 mg/m^2 and 5-FU was dose escalated starting at 1050 mg/m^2. At 5-FU doses of 1050 and 1200 mg/m^2, there were no DLTs. However, when 5-FU was escalated to 1350 mg/m^2, dose-limiting febrile neutropenia occurred in three patients (Table 2). For the second cycle of combination therapy, each of these patients had 5-FU reduced by one cohort to 1200 mg/m^2 without developing recurrent neutropenic fever. One patient received 5

cycles at this reduced dose of 5-FU without developing subsequent DLT.

In the third drug escalation phase, 5-FU was fixed at the RD and MTD of 1200 mg/m^2. Raltitrexed was escalated in successive cohorts by incremental doses of 0.5 mg/m^2 starting at 3.5 mg/m^2. One DLT (febrile neutropenia) was seen in six patients treated with raltitrexed doses of 4.0 and 5.0 mg/m^2 (Table 2). One patient also developed grade 4 mucositis and withdrew consent after the first episode of neutropenia; one patient was re-treated with a reduced raltitrexed dose of 4.5 mg/m^2 and developed recurrent neutropenic fever despite this dose reduction. DLT (dose-limiting neutropenia of more than 7 days duration) was observed in two of six patients when raltitrexed was escalated from 5.5 to 6.0 mg/m^2. Therefore, based on this dose escalation study, the MTD/RD on a 3-weekly schedule was to be raltitrexed and 5-FU at 5.5 and 1200 mg/m^2, respectively.

Table 1 Patient characteristics

	Patients (*N*=71)
Males/females (*N*)	41/30
Age (years)	
mean	61
range	40–78
Race [*N* (%)]	
white	61 (86)
black	5 (7)
Asian	3 (4)
Hispanic	2 (3)
Primary tumor site [*N* (%)]	
colon	54 (76)
rectal	15 (21)
appendix	2 (3)
Median baseline Karnofsky performance status (range)	90 (70–90)
Prior treatment [*N* (%)]	
none	7 (10)
5-FU-based adjuvant only	17 (24)
5-FU-based and irinotecan for advanced disease	47 (66)[a]

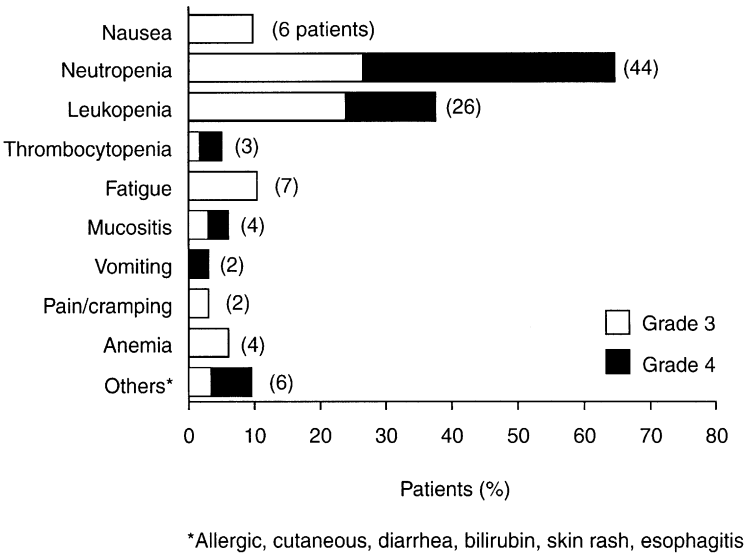
[a]Includes 38 patients who were previously treated in the adjuvant setting.

In total, 319 cycles of treatment with raltitrexed and 5-FU were administered in this clinical trial (mean 4.5 cycles, range 1–15 cycles). The most common grade 3/4 AEs were short-lived, non-dose-limiting neutropenia, which occurred in 44 (64%) patients and was not related to dose (Fig. 3). Nevertheless, dose-limiting febrile neutropenia did account for several of the respective DLTs, but none of these were life threatening and all of these patients recovered completely. The two patients with prolonged neutropenia with 6.0 mg/m^2 of raltitrexed

Table 2 DLTs during the first cycle

Cohort	Dose raltitrexed/5-FU (mg/m^2)	Patients (*N*)	DLT (*N*)	Adverse event
9	3.0/1350	4	3	febrile neutropenia
11	4.0/1200	6	1	febrile neutropenia and grade 4 mucositis
13	5.0/1200	6	1	febrile neutropenia
15	6.0/1200	6	2	grade 4 neutropenia >7 days duration

Fig. 3



Total incidence of grade 3/4 adverse events (highest grade in each cycle).

and 1200 mg/m^2 5-FU recovered completely without significant sequelae. Other common grade 3/4 AEs were leukopenia in 26 (38%) patients and fatigue in seven (10%) patients, none of which appeared to be related to renal insufficiency.

In order to gain more experience with the planned RD, an additional 15 patients were treated at 5.5 mg/m^2 raltitrexed and 1200 mg/m^2 5-FU. In this expanded cohort, we observed only one DLT: neutropenic fever with the first cycle of therapy. This patient received 6 subsequent cycles of raltitrexed at a reduced dose of 5.0 mg/m^2 with 1200 mg/m^2 5-FU without DLT. There were also three cases of transient grade 4 neutropenia (without fever) following the first cycle of combination therapy. The first patient was re-treated without dose modification, according to the protocol. This had been proven safe on all prior patients. This patient subsequently developed neutropenic fever and dehydration which required hospitalization. Even though her white blood cells were recovering, she had a cardiac arrest requiring intubation. The family elected against further intervention and the patient died on study. This patient had been heavily pre-treated with four prior regimens. Of the other two patients with transient grade 4 neutropenia in this expanded cohort, one had the raltitrexed dose reduced to 5.0 mg/m^2 with 1200 mg/m^2 5-FU. This patient received this dose of raltitrexed in this combination for an additional 4 cycles without subsequent grade 4 neutropenia or fever. The other patient continued on the full dose of raltitrexed without dose-limiting neutropenia on the subsequent 4 cycles. Three additional patients developed grade 4 neutropenia, but without fever, on cycle 2 of the combination therapy. Two of these patients were re-treated with a reduced raltitrexed dose of 5.0 mg/m^2 without subsequent grade 4 neutropenia. The third patient progressed before a third cycle could be administered.

Pharmacokinetics

Pharmacokinetic analysis demonstrated significant increases in 5-FU exposure at raltitrexed doses of 2.5 mg/m^2 or higher (Table 3). With the raltitrexed dose increased from 2.0 to 2.5 mg/m^2 and with the 5-FU dose fixed at 900 mg/m^2, the mean maximum observed plasma concentration (C_{max}) for 5-FU significantly increased from 213 ± 24 to 648 ± 74 μM ($p < 0.007$) and the mean area under the plasma concentration–time curve (AUC) significantly increased from 6794 ± 1167 to 19 593 ± 6074 μM/min ($p < 0.05$). When raltitrexed was increased from 2.5 to 3.0 mg/m^2, with 5-FU still fixed at 900 mg/m^2, the C_{max} and AUC for 5-FU did not increase any further, although the levels still remained significantly greater than that observed with raltitrexed at 2.0 mg/m^2 and 5-FU at 900 mg/m^2 ($p < 0.02$ for C_{max} and $p < 0.05$ for AUC). With higher doses of 5-FU (1050–1200 mg/m^2) and raltitrexed (3.5–6.0 mg/m^2), the 5-FU C_{max} and AUC were not significantly higher than when the raltitrexed dose was increased from 2.0 to 2.5 mg/m^2 and 5-FU was 900 mg/m^2. Raltitrexed levels were not obtained as part of this study, as the methodology for the determination of raltitrexed had not been formulated at the time of this clinical trial.

Efficacy

Sixty-eight patients were evaluable for efficacy assessments. Of these, six (9%) patients had an objective response (OR) [one complete response (CR) and five partial responses (PR)] to treatment (mean time to tumor progression 7.3 months, range 5.1–11.6 months). One patient who experienced a PR had lung metastases and had completed adjuvant 5-FU 3 months before starting the raltitrexed plus 5-FU combination (Table 4). Of the remaining patients with PRs, one patient had liver metastases and had not received prior chemotherapy, and three patients had lung, liver and peritoneal metastases, and had received either neoadjuvant or adjuvant 5-FU

Table 3 Pharmacokinetics of 5-FU

Cohort	Dose raltitrexed/5-FU (mg/m^2)	Patients (*N*)	Mean (SD) 5-FU C_{max} (μM)	Mean (SD) 5-FU AUC (μM/min)
1	0.5/900	3	306 (32)	10498 (1119)
2	1.0/900	3	278 (52)	9176 (611)
3	1.5/900	3	166 (86)	5362 (2591)
4	2.0/900	3	213 (24)	6794 (1167)
5	2.5/900	3	648 (74)[a]	19593 (6074)[b]
6	3.0/900	3	521 (97)	16360 (1452)
7	3.0/1050	3	667 (116)	20979 (6046)
8	3.0/1200	4	737 (129)	20770 (5178)
9	3.0/1350	4	834 (77)	20377 (1063)
10	3.5/1200	3	686 (169)	22801 (8269)
11	4.0/1200	6	654 (118)	16880 (3628)
12	4.5/1200	3	743 (177)	20748 (7539)
13	5.0/1200	5	739 (128)	20624 (5654)
14	5.5/1200	10[c]	835 (209)	22471 (6755)
15	6.0/1200	6	624 (334)	17000 (8204)

[a] $p < 0.007$ versus 3.0/900 dose level.
[b] $p < 0.05$ versus 2.0/900 dose level.
[c] Expanded MTD cohort.

Table 4 Objective disease assessment

Cohort	Dose raltitrexed/5-FU (mg/m^2)	Patients (*N*)	OR [*N* (months)]	SD and MR [*N* (months)]	PD (*N*)
1	0.5/900	3	1PR (17.9)[a]	1 (3.5)[b]	1[b]
2	1.0/900	3	0	2 (12.9, 3.8)[b,b]	1[a]
3	1.5/900	3	0	2 (9.8, 5.9)[a,c]	1[c]
4	2.0/900	3	1PR (5.1)[a]	1 (6.7)[c]	1[a]
5	2.5/900	3	0	3 (5.5, 7.4, 3.7)[a,c,c]	0
6	3.0/900	3	1CR (7.8)[b]	0	2[a,a]
7	3.0/1050	3	0	1 (4.4)[a]	2[a,c]
8	3.0/1200	4	0	1 (3.8)[c], 1 MR (3.9)[a]	2[c,c]
9	3.0/1350	4	1PR (6.0)[c]	2 (6.0, 2.5)[c,c]	1[a]
10	3.5/1200	3	0	1 (4.3)[a]	2[a]
11	4.0/1200	5	0	2 (6.4, 0.8)[b,c]	3[c,c,c]
12	4.5/1200	3	0	2 (5.5, 1.8)[a,c]	1c
13	5.0/1200	6	1PR (7.3)[b]	2 (3.3, 1.8)[a,c]	3[c,c,c]
14	5.5/1200	17	1 PR (11.6)[a]	6 (5.1, 5.0, 13.4, 3.0, 2.9, 3.5)[b], 4 MR (3.5, 2.1, 4.0, 4.6)[c]	6[c]
15	6.0/1200	6	0	4 (3.8, 2.7, 4.4, 2.8)[c]	2[a,c]
Total		69	6 (5 PR + 1 CR)	35 (30 SD + 5 MR)	28

OR, objective response; CR, complete response; PR, partial response; MR, minor response (25–50% decrease in the dimensions of all measurable lesions); PD, progressive disease.
[a]Patient previously failed adjuvant 5-FU-based therapy or irinotecan.
[b]Patient received no prior treatment.
[c]Patient previously failed 5-FU-based therapy or irinotecan for advanced disease.

more than 1 year before study entry. The patient with peritoneal metastases represented one of the two patients in the study who had metastatic adenocarcinoma of the appendix. The patient experiencing a CR had liver metastases and had received no prior chemotherapy. This patient remained on study for 8.0 months.

Five (7%) patients, all of whom had liver or lung metastases, experienced minor responses (MRs; mean time to tumor progression 3.6 months, range 2.1–4.6 months). Four of these five patients had progressed on prior 5-FU/LV and irinotecan; the fifth had received adjuvant 5-FU more than 1 year before inclusion on this trial. In addition, 30 (43%) patients showed stable disease (SD) (mean time to tumor progression 5.2 months, range 2.5–13.4 months); this included two patients who remained on the study for more than 1 year (13.0 and 13.4 months, respectively). Neither of these patients had received prior chemotherapy. There was no evidence of a dose–response relationship.

Seventy-one patients were eligible for median survival analysis. Using the drug combination, median survival was 11.7 months [95% confidence interval (CI) 8.9–14.0] (Fig. 4). As of 16 August 2002, six patients from this study remain alive. Median follow-up time for survivors was 25.3 months (range 8.0–71.2 months).

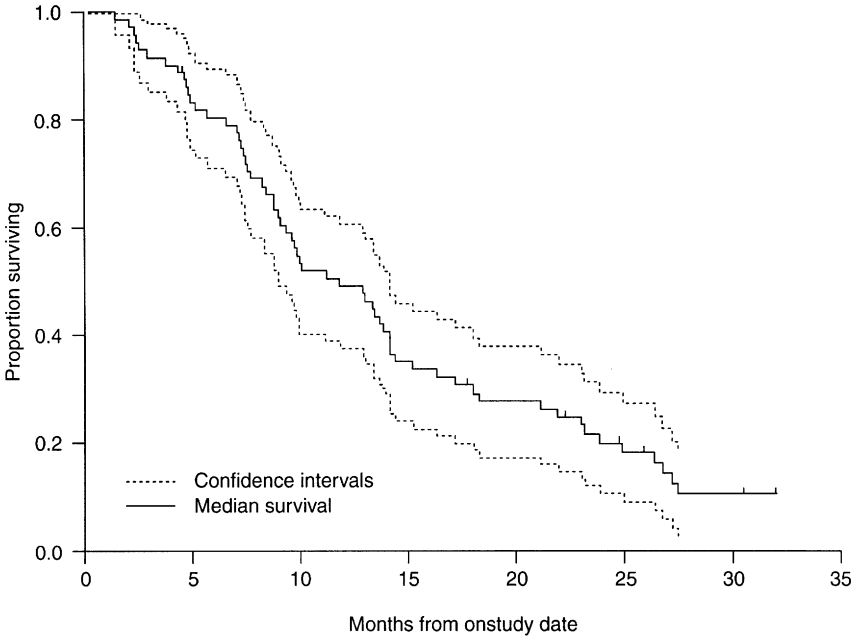
Discussion

aCRC remains a difficult disease to treat and, in order to maximize the benefits of the current drug portfolio, effective drug combinations are still being sought. *In vitro* studies using 5-FU followed by raltitrexed have resulted in synergistic cytotoxicity in human colon tumor cell lines; this suggests that this effect might also occur *in vivo* [13]. This preliminary study was undertaken to determine the MTD of raltitrexed and 5-FU in combination, in order that future clinical trials may use the regimen that provides the greatest efficacy to tolerability ratio. Based on the DLTs observed at each dose level, we had planned to recommend raltitrexed and 5-FU at 5.5 and 1200 mg/m^2, respectively. We therefore expanded this cohort to examine toxicity further. By the definitions of the protocol, we only had one DLT in this expanded patient population (i.e. neutropenic fever on cycle 1). However, we also had three patients who developed grade 4 neutropenia without fever on cycle 1 and an additional three patients on cycle 2. Many of these patients went on to receive a reduced raltitrexed dose of 5.0 mg/m^2 without significant hematologic toxicity. In view of the multiple, safe doses administered in this expanded cohort with raltitrexed at 5.0 mg/m^2 and 5-FU at 1200 mg/m^2, we would recommend these respective doses of raltitrexed and 5-FU for subsequent phase II trials.

Raltitrexed could be combined with 5-FU, at higher doses than the recommended single-agent dose of 3.0 mg/m^2, with manageable toxicity. However, the reason for this is unclear. The results suggest that the standard dose of raltitrexed is either too low or that the combination of raltitrexed and 5-FU somehow reduces the toxicity of 5-FU. A similar observation was made when raltitrexed was combined with irinotecan [20].

The toxicity profile (neutropenia, leukopenia, fatigue) observed in this study was as expected for these two chemotherapeutic agents [21]. Although neutropenia was common, it was of short duration (less than 7 days), not

Fig. 4



Kaplan–Meier survival curve. Tick mark (|) indicates lost follow-up.

associated with fever and did not require growth factor support. The most severe toxicity was seen in a patient who had been heavily pre-treated, suggesting that patients with poor bone marrow reserve should not be treated with the recommended doses of raltitrexed and 5-FU.

The reason for the increase in 5-FU C_{max} and AUC observed above the raltitrexed dose of 2.0 mg/m^2 is unclear. A similar increase in 5-FU was reported in a second trial in which raltitrexed was combined with 5-FU and administered as a 24-h infusion [9]. This also appeared to be associated with a threshold raltitrexed level (2.5 mg/m^2), above which the pharmacokinetics for 5-FU did not increase appreciably. Raltitrexed is predominantly excreted renally and biliary [22]. This is in contrast to 5-FU where less than 10% is excreted renally and most is cleared through metabolic pathways, including inactivation by dihydropyrimidine dehydrogenase (DPD) [23]. 5-FU and its metabolites then undergo biliary excretion or enterohepatic recirculation. All patients who entered this study were required to have normal renal and biliary function, which was carefully monitored during the course of the study. Since we did not measure raltitrexed levels in the plasma it is not possible to comment on the raltitrexed pharmacology or how this could be affecting the 5-FU. However, it does suggest that above a certain dose (2.0 mg/m^2), raltitrexed may be able to decrease 5-FU clearance (resulting in an increase in AUC) by mechanisms which are currently unclear. This may be at the level of biliary excretion or raltitrexed may also be able to inhibit DPD activity [24], which could account for the increase in 5-FU pharmacokinetics observed in this study. This hypothesis will require further testing in subsequent clinical trials.

Several CRs or PRs were obtained and a substantial proportion of patients showed disease stabilization. This included two patients who had SD for more than 1 year. Although these favorable effects were most often observed in patients who had received no prior chemotherapy, one patient with a PR had only recently completed adjuvant therapy before developing metastatic disease. However, the clinical activity of this combination appears most active in chemonaive patients. Preliminary results of a phase II study of raltitrexed combined with carmofur, an oral 5-FU pro-drug, as first-line treatment in patients with metastatic CRC, showed a response rate of 42% (eight of 19 patients) [15]. However, prior failure on 5-FU did not exclude either a response or SD to the raltitrexed and 5-FU combination. In addition, the median survival of 11.7 months is comparable with other second-line regimens (after 5-FU failure), including irinotecan (9.2–10.8 months) [25,26] and oxaliplatin/5-FU (9.6 months) [27].

These data show that raltitrexed can be safely administered in combination with bolus 5-FU and the clinical activity observed indicates that this is a promising drug combination, meriting further evaluation in phase II clinical trials. Although there are theoretical reasons for

the use of bolus 5-FU with raltitrexed, future consideration is likely to be given to combining raltitrexed with protracted 46-h infusions of 5-FU, which has become part of standard therapy.

References

1 Boyle P, Langman JS. ABC of colorectal cancer: epidemiology. *Br Med J* 2000; **321**:805–808.
2 Miller BA, Kolonel LN, Bernstein L, *et al.* (editors). *Racial/Ethnic Patterns of Cancer in the United States 1988-1992*. NIH publ. no. 96-4104. Bethesda, MD: National Cancer Institute; 1996.
3 World Health Report. *Fighting Disease, Fostering Development*. Geneva: WHO; 1997.
4 Vincent M, Labianca R, Harper P. Which 5-fluorouracil regimen? The great debate. *Anticancer Drugs* 1999; **10**:337–354.
5 Jackman AL, Judson IR. The new generation of thymidylate synthase inhibitors in clinical study. *Exp Opin Invest Drugs* 1996; **5**:719–736.
6 Cunningham D, Zalcberg JR, Rath U, *et al.* Final results of a randomized trial comparing 'Tomudex' (raltitrexed) with 5-fluorouracil plus leucovorin in advanced colorectal cancer.'Tomudex' Colorectal Cancer Study Group. *Ann Oncol* 1996; **7**:961–965.
7 Pazdur R, Vincent M. Raltitrexed (Tomudex) versus 5-fluorouracil and leucovorin (5-FU + LV) in patients with advanced colorectal cancer (ACC): results of a randomized, multicenter, North American trial. *Proc Am Soc Clin Oncol* 1997; **16**:228a (abstr).
8 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. Tomudex Colorectal Cancer Study Group. *J Clin Oncol* 1998; **16**:2943–2952.
9 Schwartz GK, Harstrick A, González Barón M. Raltitrexed (Tomudex) in combination with 5-fluorouracil for the treatment of patients with advanced colorectal cancer: preliminary results from phase I clinical trials. *Eur J Cancer* 1999; **35**(**suppl 1**):S9–S13.
10 Aschele C, Sobrero A, Faderan MA, Bertino JR. Novel mechanism 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 Abad A, Garcia P, Gravalos P, *et al.* Sequential methotrexate, 5-fluorouracil (5-FU), and high dose leucovorin versus 5-FU and high dose leucovorin versus 5-FU alone for advanced colorectal cancer. *Cancer* 1995; **75**: 1238–1244.
12 Blijham G, Wagener T, Wils J, *et al.* Modulation of high-dose infusional fluorouracil by low-dose methotrexate in patients with advanced or metastatic colorectal cancer: final results of a randomized European Organization for Research and Treatment of Cancer Study. *J Clin Oncol* 1996; **14**:2266–2273.
13 Longo GS, Izzo J, Chang YM, *et al.* Pretreatment of colon carcinoma cells with Tomudex enhances 5-fluorouracil cytotoxicity. *Clin Cancer Res* 1998; **4**:469–473.
14 Mayer S, Vanhoefer U, Hilger R, *et al.* Extended Phase I study of raltitrexed and infusional 5-FU in patients with metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 2000; **19**:1169 (abstr).
15 Österlund P, Elomaa I, Virkkunen P, *et al.* A Phase II study of raltitrexed ('Tomudex') combined with carmofur in metastatic CRC. *Proc Am Soc Clin Oncol* 2000; **19**:282a (abstr).
16 Vazquez Estevez S, Vieitez JM, Garcia AP, *et al.* Raltitrexed combined with UFT: a phase I/II dose escalation study in metastatic colorectal cancer. *Ann Oncol* 2000; **11**(**suppl 4**):52 (abstr 229).
17 WHO. *WHO Handbook for Reporting Results of Cancer Treatment*. WHO Offset Publication 48. Geneva: WHO; 1979.
18 Bierman JA, Lingeman H, Reeuwijk HJ, *et al.* Reversed-phase ion-pair analysis of 5-fluorouracil in pig bile, liver homogenate, plasma and urine after administration by isolated liver perfusion. *J Pharm Biomed Anal* 1989; **7**:611–617.
19 Moore MJ, Bunting P, Yuan S, Thiessen JJ. Development and validation of a limited sampling strategy for 5-fluorouracil given by bolus intravenous administration. *Ther Drug Monit* 1993; **15**:394–399.
20 Stevenson JP, Redlinger M, Kluijtmans LA, *et al.* Phase I clinical and pharmacogenetic trial of irinotecan and raltitrexed administered every 21 days to patients with cancer. *J Clin Oncol* 2001; **19**:4081–4087.
21 Zalcberg J. Overview of the tolerability of 'Tomudex' (raltitrexed): collective clinical experience in advanced colorectal cancer. *Anticancer Drugs* 1997; **8**(**suppl 2**):17–22.
22 Touroutoglou N, Pazdur R. Thymidylate synthase inhibitors. *Clin Cancer Res* 1996; **2**:227–243.
23 Lu A, Zhang R, Diasio RB. Purification and characterization of dihydropyrimidine dehydrogenase from human liver. *J Biol Chem* 1992; **267**:17102–17109.
24 Yan JM, Lu ZH, Shao LN, *et al.* The effect of 'Tomudex' on dihydropyrimidine dehydrogenase activity. *Proc Am Ass Cancer Res* 1997; **38**:615 (abstr 4133).
25 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.
26 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.
27 Brienza S, Bensmaine MA, Soulie P, *et al.* Oxaliplatin added to 5-fluorouracil-based therapy (5-FU ± FA) in the treatment of 5-FU-pretreated patients with advanced colorectal carcinoma (ACRC): results from the European compassionate-use program. *Ann Oncol* 1999; **10**: 1311–1316.