Pergamon

European Journal of Cancer, Vol. 34, No. 7, pp. 987–991, 1998

Printed in Great Britain
0959-8049/98 $19.00+0.00

PII: S0959-8049(98)00017-3

Original Paper

Single Agent Infusional 5-Fluorouracil is not Effective Second-line Therapy After Raltitrexed (Tomudex®) in Advanced Colorectal Cancer

D.C. Farrugia, A.R. Norman and D. Cunningham

Cancer Research Campaign, Section of Medicine and The GI Unit, The Institute of Cancer Research and The Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, U.K.

Raltitrexed (Tomudex®) is currently licensed for first-line treatment of advanced colorectal cancer. We evaluated 101 patients treated with raltitrexed whose data were collected prospectively, in order to study the outcome of second-line treatments used after this drug. Of 98 evaluable patients, 50 received second-line treatments, the commonest being 5-fluorouracil (5-FU)-based therapy (22 patients with 20 evaluable) and mitomycin-c (MMC) (22 patients with 18 evaluable). Only 1 response was seen in a patient treated with 5-FU and MMC and none following other treatments. This patient was not evaluable for outcome of raltitrexed treatment, having stopped after two courses. Patients who had responded to raltitrexed and later progressed off treatment were more likely to be offered second-line 5-FU, but despite the earlier sensitivity to thymidylate synthase inhibition, response rates were minimal. Underlying mechanisms for this lack of activity and proposals for future studies are discussed.

Key words: raltitrexed, 5-fluorouracil, chemotherapy, colorectal cancer
Eur J Cancer, Vol. 34, No. 7, pp. 987–991, 1998

INTRODUCTION

RALTITREXED (TOMUDEX®) is a novel folate-based thymidylate synthase (TS) inhibitor licensed for the treatment of advanced colorectal cancer. It inactivates TS by binding to the folate-binding site in competition with the natural co-factor 5,10-methylene tetrahydrofolate, thus inhibiting the sole pathway for *de novo* synthesis of dTMP (thymidylate) from dUMP (deoxyuridine monophosphate). dTMP is phosphorylated to TTP (triphosphate) and exclusively incorporated into DNA. TTP deficiency results in DNA strand breaks leading to cell death.

Raltitrexed is actively transported into cells predominantly via the reduced folate carrier (RFC) which is also utilised by natural reduced folates, leucovorin and other classical antifolates, such as methotrexate (MTX). Intracellularly, raltitrexed is a substrate for folylpolyglutamyl synthetase (FPGS) which attaches up to five glutamate residues to the parent molecule. This process of polyglutamation leads to intracellular retention of drug, as well as increasing its potency as a TS inhibitor around 60-fold [1].

Correspondence to D. Cunningham.
Received 28 Aug. 1997; revised 5 Dec. 1997; accepted 8 Jan. 1998.

In phase II evaluation, raltitrexed gave objective response rates (ORR) of 26% in both advanced colorectal and breast cancer, with ORR of 12 and 9% in pancreatic and non-small cell lung cancer, respectively [2]. Dose limiting toxicities included lethargy, diarrhoea, myelosuppression and reversible elevations in hepatic transaminases [2]. The first phase III evaluation was carried out in 439 patients with chemo-naive advanced colorectal cancer where raltitrexed was compared with a 4-weekly schedule of 5-fluorouracil (5-FU) (425 mg/m^2) and folinic acid (FA, 20 mg/m^2). Raltitrexed gave an ORR of 19.3% versus 16.7% with 5-FU. Event-free and overall survival, as well as quality of life scores, were comparable in both arms, while raltitrexed was associated with less toxicity, in particular, leucopenia and mucositis [3].

Raltitrexed is now licensed in a number of countries as first-line treatment for advanced colorectal cancer. Its convenient dosage schedule coupled with comparable response rates to modulated 5-FU at the cost of less toxicity should contribute to its establishment among standard treatments for this disease. As its use becomes more widespread, the issue will undoubtedly arise of suitable second-line chemotherapy for those patients who fail to respond or progress shortly after therapy with this agent. It is generally thought

that 5-FU is unlikely to be effective as second-line therapy after treatment with TS inhibitors such as raltitrexed. *In vitro* evidence suggests that acquired resistance to raltitrexed may be associated with sustained TS overexpression through gene amplification [4], a mechanism which is also recognised in acquired resistance to 5-FU [5]. However, there is evidence that 5-FU does not only inhibit TS through ternary complex formation of its anabolite FdUMP with the enzyme, but that other anabolites of 5-FU, such as FUMP, are formed which can be incorporated into RNA [6]. There is also increasing evidence that the mode of action of 5-FU at the target level is influenced by its schedule of administration, as well as the addition of modulating agents [7]. Thus, bolus administration may favour RNA incorporation, while prolonged exposure, as in the case of protracted venous infusion (p.v.i.) and the addition of FA, favour TS inhibition.

To date, 101 patients have received first-line treatment with raltitrexed for advanced colorectal cancer in our centre, predominantly within the setting of phase II and phase III studies with this agent. We therefore examined our prospective database on these patients to study the outcome of subsequent second-line therapy, in particular whether 5-FU therapy was efficacious in this setting.

PATIENTS AND METHODS

This was a single-institution study in which our database of 101 patients who received first-line treatment with raltitrexed for advanced colorectal cancer was reviewed. 72 patients were treated in the phase II study [8] and 16 patients were treated in the multi-centre phase III studies. 6 were treated in a non-randomised pharmacokinetic study of raltitrexed in patients with hepatic impairment, while another 8 are in an ongoing study of pharmacodynamic markers of response of colorectal cancer to this agent [9]. All of the above studies were approved by the ethics committee.

Untreated advanced disease was an entry requirement for all studies, but patients may have had adjuvant 5-FU chemotherapy after surgery for their primary tumour, provided this was completed 6 months before enrolment. Patients had to be of performance status 0–2 and have at least 12 weeks' life expectancy.

It was hypothesised that 5-FU would be effective in this setting, especially in patients who had shown earlier sensitivity to a TS-directed approach. Additionally, the anti-RNA effects of 5-FU may produce activity in tumours with acquired resistance through TS overexpression. It was also thought that mitomycin-c (MMC), acting via a non-TS mechanism, would also show activity in this setting.

Raltitrexed was given at a dose of 3 mg/m^2 as a 15 min intravenous (i.v.) infusion every 21 days. 5-FU was given either as a p.v.i. (300 mg/m^2/day) by means of a portable programmable pump via an indwelling catheter, or as an i.v. bolus (425 mg/m^2) with 20 mg/m^2 of FA daily for 5 consecutive days every 4 weeks. MMC was given as a bolus i.v. dose of 7 mg/m^2 every 6 weeks for a total of four doses. 3 patients received carmustine (BCNU) (200 mg/m^2 every 6 weeks) in an ongoing study comparing this agent with best supportive care.

The response to second-line therapy was assessed using objective radiological tumour measurements on computed tomography (CT) scanning according to WHO criteria, at 12 week intervals. Patients who showed no benefit stopped chemotherapy and continued supportive care.

Data collection

All data were collected prospectively. At each visit, prior to seeing a physician, patients were interviewed by a clinical nurse specialist using a standard questionnaire in which symptoms and toxicity were graded according to CTC criteria. This information was entered into the unit database. Categorical data were compared using the chi-squared test with Fisher's exact test used where expected frequencies were less than 5. Overall survival was calculated from first treatment date to the date of death, using the product-limit method of Kaplan–Meier and differences in survival were examined using the Log-rank test.

RESULTS

Patient characteristics

Patient characteristics are shown in Table 1. There was a 2:1 male preponderance with most patients having a good performance status. The liver was by far the commonest site of metastatic disease.

Response and survival

98 of 101 patients were evaluable for outcome of therapy with raltitrexed and of these 20 responded giving an ORR of 20% (95% confidence interval (CI): 12.1–27.9). All were partial responses. 3 of 101 were not evaluable, as 2 stopped after only one to two treatments, 1 after an acute myocardial infarction, the other after an upper gastrointestinal bleed. The third had multiple hepatic cysts which made disease response non-assessable.

50 patients who progressed during or following treatment with raltitrexed were offered second-line chemotherapy. 9 of these had initially responded to raltitrexed, while the remaining 40 had achieved, at best, disease stabilisation or had progressed and 1 patient was not evaluable for raltitrexed response. 51 patients did not receive second-line therapy. These included 11 responders to raltitrexed, and 38 non-responders and 2 non-evaluable patients. Patients who responded to raltitrexed but did not receive second-line therapy were either of poor performance status ($n = 8$, 5 of whom died within 4 months of relapse), or had stopped raltitrexed following severe toxicity ($n = 2$), or were treated with phase I agents ($n = 1$). The median interval between raltitrexed cessation and the start of second-line treatment was 336 days

Table 1. *Patient characteristics*

Characteristics	Number of patients
Median age (range) (years)	62 (30–81)
Sex (male/female)	67/34
Performance status	
WHO 0,1	79
WHO ≤ 2	22
Primary site ($n = 100$)	
Colon	59
Rectum	41
Metastatic or recurrent disease sites ($n = 101$)*	
Local recurrence	24
Liver	83
Lymph nodes	22
Lung	22
Peritoneum	5
Bone	1

*Multiple sites present in some patients.

(range 139–749 days, $n = 9$) for responders to raltitrexed and 98 days (range 35–694 days, $n = 41$) for non-responders.

44 of 50 patients having second-line treatment were evaluable for response. 22 patients received 5-FU-based therapy, 20 having p.v.i. of 5-FU (combined with MMC in 4 cases), and 2 having bolus 5-FU/FA. 1 patient on p.v.i. and 1 on bolus 5-FU were not evaluable because of early treatment cessation from severe toxicity. Of the 20 evaluable 5-FU treated patients, 19 had p.v.i. 5-FU and 1 had bolus 5-FU/FA, of whom only 1 responded to a combination of p.v.i. 5-FU plus MMC. This was a 69-year-old man who stopped after two courses of raltitrexed because of an acute myocardial infarct and was therefore not evaluable for response. He was then progression free for 18 months after which he commenced 5-FU and MMC, achieving a partial response at the end of 6 months of therapy. A further 8 5-FU-treated patients achieved stable disease for a median duration of 158 days (range 72–275 days) with 2 being progression free at 6 months.

Of 28 patients treated with other second-line agents, 24 were evaluable, 18 of whom had MMC, 3 had BCNU and 3 had other agents. No responses were seen. The 4 non-evaluable patients who received MMC all stopped therapy early because of rapid disease progression.

Table 2 shows the outcome of 75 patients given raltitrexed and second-line therapy, recorded as having progressed on raltitrexed at the time of this analysis. Patients who progressed while on raltitrexed (columns A and B), or stopped because of severe toxicity (column C), were more likely not to be offered second-line treatment (23 of 55, i.e. 42%), compared with those (columns D and E) progressing some time after raltitrexed (6 of 19, i.e. 32%). Of 26 patients who progressed on raltitrexed at first assessment and went on to second-line treatment (column A), 19 (73%) had non-5-FU-based therapy with no responses seen. Of 14 patients who had initially responded or stabilised on raltitrexed and then progressed off treatment (columns D and E), 10 (71%) received 5-FU. Despite having shown earlier sensitivity to a TS inhibitor, there were still no responses to 5-FU in this category.

The median follow-up of all 101 patients from primary (raltitrexed) treatment was 214 days (range 70–1362 days), while the median follow-up from start of second-line therapy ($n = 50$) was 190 days (range 21–231 days). The median survival from raltitrexed therapy was 329 days and 177 days from the start of second-line therapy.

Toxicity

Table 3 shows the incidence of toxicity following raltitrexed, as well as second-line therapy with 5-FU-based regimes. Although the two treatments are not directly

Table 2. Outcome of therapy with raltitrexed and second-line treatments in 75 patients who had progressed at the time of analysis

	Best response to raltitrexed										
Second-line therapy	PD at 1st assessment (A)	PR/SD as best response but PD on Rx (B)		PR/SD but stopped because of toxicity (C)		PR/SD but PD within 3 months of stopping Rx (D)		PR/SD but PD > 3 months from stopping Rx (E)		NE for raltitrexed outcome (F)	Totals
		PR	SD	PR	SD	PR	SD	PR	S		
No second-line therapy	13	1	2	7		1	1	1	3		29
PR on 5-FU										1	1
SD/PD on 5-FU ± MMC	6		1			1	1	4	3		16
NE on 5-FU or stopped for other reasons	1				1						2
SD/PD on MMC	13	1						1	2		17
NE on MMC or stopped for other reasons	4										4
SD/PD on others	2	1	2					1			6
Totals	39	3	5	7	1	2	2	7	8	1	75*

PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; Rx, treatment; 5-FU, 5-fluorouracil; MMC, mitomycin-C.
*Of 101 patients in this study, 75 had progressed at analysis.

Table 3. Toxicity after raltitrexed and second-line 5-fluorouracil (5-FU)-based therapy

	Raltitrexed Incidence (%) ($n = 101$)		5-FU-based second-line therapy Incidence (%) ($n = 22$)	
Toxicity	Grade* 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Diarrhoea	25	8	40	0
Stomatitis	17	1	30	0
Nausea and vomiting	58	6	60	0
Alopecia	9†	6‡	10†	10‡
Infection	17	5	35	0
Neutropenia	18	14	5	10
Thrombocytopenia	7	2	0	10
PPE	2	0	45	10

*WHO grade. †Grade 1 only. ‡Grade 3. PPE, plantar-palmar erythema.

comparable as they were given sequentially to the same subset of patients, a lower incidence of grade 1–2 mucositis and all grades of plantar-palmar erythema were seen with raltitrexed, while there were more cases of grade 3–4 diarrhoea and neutropenia in this group.

DISCUSSION

Resistance of tumour cell lines to both raltitrexed and 5-FU has been associated with TS gene amplification and increased TS protein levels [4, 5]. Cross-resistance to these agents has also been demonstrated in the clinic where, in a phase I study of raltitrexed, the drug was inactive in patients with advanced colorectal cancer previously treated with 5-FU [10]. However, cross-resistance *in vitro* was not complete, so that raltitrexed retained activity in some tumour lines resistant to 5-FU [1, 11].

Although TS overexpression would be expected to confer cross-resistance to 5-FU as well as raltitrexed, Johnston and colleagues [12] showed that human tumour cell lines which acquired raltitrexed resistance through TS overexpression, could still retain sensitivity to 5-FU. Mechanistic studies suggested that, in at least one of these lines, 5-FU was acting through incorporation of its metabolise FUTP into RNA.

In vitro studies in human breast and colon cancer cell lines have shown that resistance to raltitrexed may be multifactorial and not only a consequence of TS overexpression. Other mechanisms which may contribute to raltitrexed resistance include impaired polyglutamation and drug transport [4]. Thus, whereas TS upregulation would be expected to confer at least partial cross-resistance to 5-FU, changes in polyglutamation or transport would not directly affect 5-FU efficacy, unless these changes led to depletion of intracellular folates (which are also polyglutamated) and, hence, compromised formation of ternary complexes between FdUMP, TS and folate co-factor [13]. Therefore, TS may still be a valid target in tumours resistant to raltitrexed through impaired transport or polyglutamation, but not TS overexpression. This coupled with the anti-RNA effects of 5-FU could potentially lead to tumour response to 5-FU-based therapy after failure of raltitrexed.

In this study, we report that of 20 evaluable patients treated with 5-FU-based second-line therapy, 5 of whom had responded to raltitrexed, there was only 1 response to 5-FU. There were no responses among 16 patients treated with p.v.i. 5-FU alone. Bearing in mind the limitations of such a retrospective review and the small numbers involved, the data suggest that p.v.i. 5-FU is not an active treatment in this setting. The single response occurred following p.v.i. 5-FU and MMC, a combination which is currently standard first-line therapy for advanced colorectal cancer in this unit, following improved response rates and longer progression-free survival versus p.v.i. 5-FU alone [14]. Only 4 patients received this combination as second-line therapy after raltitrexed and more patients are being treated in order to evaluate its efficacy in this setting.

8 of 20 evaluable 5-FU-treated patients had disease stabilisation for a median of 5.3 months, with one quarter of these remaining progression free at 6 months. This is comparable to data reported by Rougier and associates [15] in a study of the novel topoisomerase I inhibitor CPT-11 in pretreated colorectal cancer, in which 28.6% of patients achieved stable disease, with 31.6% progression free at 6 months. This parameter is, however, influenced by the indolent nature of these tumours. As can be seen from Table 2, 5-FU was more likely to be offered to patients who progressed some time after cessation of raltitrexed, raising the possibility that these included inherently slow growing tumours.

While TS overexpression following raltitrexed treatment would be expected to confer resistance to p.v.i. 5-FU, a schedule which is predominantly TS directed, 5-FU by bolus injection may partly overcome such resistance by also targeting RNA. This hypothesis remains to be tested.

The majority of patients treated with MMC and BCNU had progressed during raltitrexed therapy and the lack of response seen with these second-line agents is in keeping with the general lack of responsiveness seen in such tumours. However, other agents and combinations are under development which show promising activity in 5-FU refractory disease and these may also have a role in raltitrexed-treated patients. These include the topoisomerase I inhibitor irinotecan (CPT-11) [15], the combination of infusional 5-FU and the newer platinum analogue oxaliplatin [16] and combinations of 5-FU, leucovorin and trimetrexate [17]. Trimetrexate, a new methotrexate analogue, may be a useful agent in raltitrexed-resistant disease as it does not utilise the RFC for uptake, and is not polyglutamated, thus potentially circumventing resistance via downregulation of these mechanisms.

Until such treatments are evaluated in this setting, patients progressing after raltitrexed therapy and who require further palliative chemotherapy, should not be treated with infusional 5-FU alone, but may stand a better chance of benefit from bolus 5-FU therapy or 5-FU combinations with non-TS targeting agents.

1. Jackman AL, Farrugia DC, Gibson W, *et al.* ZD1694 (Tomudex): a new thymidylate synthase inhibitor with activity in colorectal cancer. *Eur J Cancer* 1995, **31**A(7/8), 1277–1282.
2. Cunningham D, Zalcberg JR, Rath U, *et al.* Final results of a randomised trial comparing Tomudex® (raltitrexed) with 5-fluorouracil plus leucovorin in advanced colorectal cancer. *Ann Oncol* 1996, **7**, 961–965.
3. Cunningham D, Zalcberg J, Smith I, *et al.* "Tomudex" (ZD 1694): a novel thymidylate synthase inhibitor with clinical antitumour activity in a range of solid tumours. *Ann Oncol* 1996, **7**, 179–182.
4. Drake JC, Allegra CJ, Moran RG, Johnston PG. Resistance to Tomudex (ZD1694): multifactorial in human breast and colon carcinoma cell lines. *Biochem Pharmacol* 1996, **51**, 1349–1355.
5. Peters GJ, Van der Wilt CL, Triest B van, *et al.* Thymidylate synthase and drug resistance. *Eur J Cancer* 1995, **31**A(7/8), 1299–1305.
6. 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, **51**, 1855–1864.
7. Sobrero AF, Aschele C, Bertino JR. Fluorouracil in colorectal cancer—a tale of two drugs: implications for biochemical modulation. *J Clin Oncol* 1997, **15**(1), 368–381.
8. 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**(3), 716–721.
9. Farrugia D, Cunningham D, Danenberg P, *et al.* A pharmacodynamic study of the thymidylate synthase inhibitor Tomudex in advanced colorectal cancer (abstract). *Proc Am Assoc Cancer Res* 1997, **38**, 615.
10. Sorensen JM, Jordan E, Grem JL , *et al.* Phase I trial of ZD1694 (Tomudex), a direct inhibitor of thymidylate synthase. *Ann Oncol* 1994, **5**(Suppl. 5), 132.

11. 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, **31**A(5), S30.
12. Johnston PG, Behan KA, Allegra CJ, Drake JC. Fluorouracil: active in ZD1694 (Tomudex)-resistant cell lines with markedly elevated thymidylate synthase levels. *J Natl Cancer Inst* 1995, **87**(20), 1558–1559.
13. Wang FS, Achele C, Sobrero A, Chang YM, Bertino JR. Decreased folylpolyglutamate synthase expression: a novel mechanism of fluorouracil resistance. *Cancer Res* 1993, **53**, 3677–3680.
14. Ross P, Norman A, Cunningham D, *et al.* A prospective randomised trial of protracted venous infusion (PVI) 5-FU with or without mitomycin C in advanced colorectal cancer. *Br J Cancer* 1997, **76**(1), 32.
15. Rougier P, Bugat R, Douillard JY, *et al.* Phase II study of irinotecan in the treatment of advanced colorectal cancer in chemotherapy-naive patients and patients pretreated with fluorouracil-based chemotherapy. *J Clin Oncol* 1997, **15**(1), 251–260.
16. Bertheault-Cvitkovic F, Jami A, Ithzaki M, *et al.* Biweekly intensified ambulatory chronomodulated chemotherapy with oxaliplatin, fluorouracil, and leucovorin in patients with metastatic colorectal cancer. *J Clin Oncol* 1996, **14**(11), 2950–2958.
17. Conti JA, Kemeny N, Seiter K, *et al.* Trial of sequential trimetrexate, fluorouracil, and high-dose leucovorin in previously treated patients with gastrointestinal carcinoma. *J Clin Oncol* 1994, **12**(4), 695–700.