Anti-Cancer Drugs 1997, **8** (suppl 2):S5–S9

'Tomudex' (raltitrexed) development: preclinical, phase I and II studies

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Raltitrexed ('Tomudex', formerly ZD1694) is a new active drug for advanced colorectal cancer, an area that has been without new drugs for over 40 years. It has a convenient dosing schedule and a potential for lower toxicity which represent important advantages over existing treatments. Advanced colorectal cancer is currently treated with 5-fluorouracil, generally in combination with other agents such as leucovorin. This leads to complex dosing schedules with increased activity but potentially serious toxicity. Raltitrexed is a novel cytotoxic agent, rationally designed to inhibit a specific molecular target, thymidylate synthase. In contrast to other current agents, raltitrexed inhibits thymidylate synthase directly, specifically and non-competitively, which may lead to an improved toxicity profile. It is retained within cells as polyglutamate metabolites, allowing a more convenient dosing schedule than for 5-fluorouracil. Phase I and pharmacokinetic studies established the optimum dosing schedule as 3 mg/m², administered in a single 15-min intravenous infusion once every 3 weeks. In a phase II study in 177 patients with advanced colorectal cancer, this dose produced a response rate of 26% and median survival of 11.2 months. The safety profile was acceptable, the main adverse events being myelosuppression, gastrointestinal toxicity, asthenia and transient asymptomatic increases in liver transaminases without evidence of any other liver dysfunction. Activity of raltitrexed has also been observed in a range of other solid tumours, including breast, pancreatic, non-small-cell lung and refractory ovarian cancer.

Keywords: Advanced colorectal cancer, efficacy, palliation, raltitrexed, safety, 'Tomudex'

Introduction

Thymidylate synthase is a key enzyme in the replication of DNA, and is a target enzyme (directly or indirectly) for 5-fluorouracil, methotrexate and raltitrexed ('Tomudex', formerly ZD1694). Using tetrahydrofolate as a methyl donor, thymidylate synthase converts deoxyuridine monophosphate (dUMP) into thymidine monophosphate (TMP), which is then converted by other enzymes into thymidine triphosphate, a key substrate for DNA synthesis (Fig. 1).

5-Fluorouracil inhibits thymidylate synthase indirectly, since it is metabolized within the cell to 5-fluorodeoxyuridine monophosphate (FdUMP). This compound competes with dUMP for the substrate-binding site on thymidylate synthase, thereby blocking synthesis of TMP. This results in the accumulation of dUMP. The concentration of dUMP increases and may ultimately be high enough to compete successfully with FdUMP and overcome the enzyme inhibition. 5-Fluorouracil is also converted to other metabolites such as 5-fluorouridine triphosphate and 5-fluorodeoxyuridine triphosphate, which may be incorporated into RNA and DNA, contributing not only to antitumour activity but also producing a variety of toxic effects [1]. The addition of leucovorin to 5-fluorouracil increases the concentration of 5,10-methylenetetrahydrofolate, thus stabilizing the inactive FdUMP–thymidylate synthase–5, 10-methylenetetrahydro-folate complex.

Methotrexate inhibits dihydrofolate reductase, thereby reducing intracellular levels of tetrahydrofolates. These are required for pyrimidine synthesis, including thymidine, 5,10-methylenetetrahydrofolate acting as the methyl donor for thymidylate synthase. Reduced folates are also required by other crucial enzymes, including those responsible for purine synthesis, and this may lead to additional unwanted effects [2].

By contrast, raltitrexed inhibits thymidylate synthase directly and specifically, without requiring modulation by a second agent. It is an analogue of the tetrahydrofolate cofactor, so it cannot be incorporated into nucleic acids and the inhibition cannot be competitively overcome by accumulation of dUMP [3]. Unlike methotrexate, it does not affect purine synthesis [3]. Therefore, raltitrexed has the potential for an improved toxicity profile compared with the older agents.

In vitro studies showed raltitrexed to be a potent inhibitor of the growth of both mouse and human cells in culture, with a median inhibitory concentration (IC_{50}) in the 1–10 nmol/l range [3]. This is equivalent in potency to methotrexate but 94-fold more potent than 5-fluorouracil alone, and 56-fold more potent than 5-fluorouracil modulated with 10 μmol/l leucovorin [3].

Once absorbed into the cell, raltitrexed is rapidly converted into polyglutamate metabolites; 4 h later less than 4% of the intracellular drug pool in cultured cell lines was present as the parent drug [4]. These polyglutamate metabolites are more potent inhibitors of thymidylate

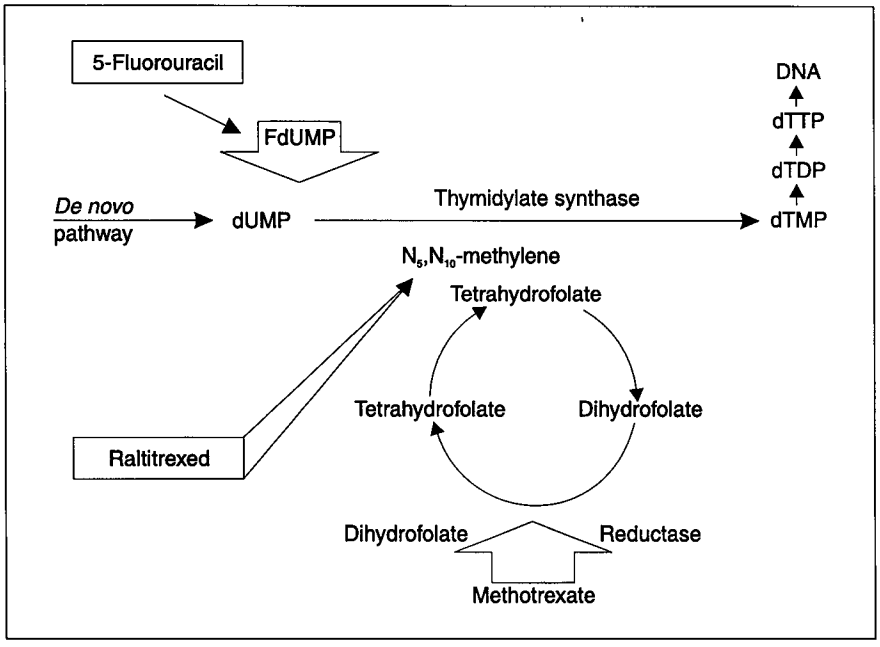

Fig. 1. Function of thymidylate synthase and sites of drug action. FdUMP, 5-fluorodeoxyuridine monophosphate; dUMP, deoxyuridine monophosphate; dTTP, deoxythymidine triphosphate; dTDP, deoxythymidine diphosphate; dTMP, deoxythymidine monophosphate.

synthase than the parent drug [4], and are retained within tissues [5]. Their rapid formation and retention is consistent with the prolonged inhibition of this enzyme that is observed after a brief exposure of cultured cells to raltitrexed, even after the cells are resuspended in drug-free medium [6,7].

After intraperitoneal or intravenous bolus administration to mice, the half-life of raltitrexed in plasma was about 30 min, with a long final elimination phase [8]. Tissue samples recovered 24 h after intraperitoneal injection contained 50- to 100-fold higher concentrations of raltitrexed than plasma, with most of the drug present as polyglutamates [5]. This slow elimination of raltitrexed underlies the infrequent, convenient dosing schedule used in the clinical studies.

Toxicity studies in mice and dogs found that the major toxic effects of raltitrexed were gastrointestinal and haematological [9]. This is to be expected, since thymidylate synthase is not unique to cancer cells and so its inhibition is likely to affect all rapidly dividing cells. Co-administration of thymidine largely prevented the toxic effects, confirming that they were due to thymidylate synthase inhibition and not another non-specific effect. Raltitrexed was free of nephrotoxicity even at high doses [9].

The encouraging results of these preclinical studies led to the initiation of the clinical development programme.

Phase I studies

Phase I dose-finding and tolerability studies were undertaken in Europe [10] and North America [11].

European study

The European study [10] was carried out at two centres in the United Kingdom and the Netherlands. A total of 61 patients were recruited, with a range of refractory solid tumours including colon (26%), ovary (18%), head and neck (8%), mesothelioma (8%), melanoma (7%), sarcoma (7%) and breast (5%). Raltitrexed was administered as a 15-min intravenous infusion once every 3 weeks.

The trial followed a conventional modified Fibonacci dose-escalation design. The first patients were treated with a starting dose of 0.1 mg/m^2; if no dose-limiting toxicity was observed 1 week after the second dose of raltitrexed, subsequent patients entering the trial were given the next highest dose. At least three patients were treated at each dose level, and dose escalation continued in this manner until the maximum tolerated dose was reached. Dose escalation for individual patients was not permitted. The patients were continued on raltitrexed at the same dose for up to six courses if no tumour progression was observed, or longer if symptomatic improvement or tumour regression was seen.

A total of 161 courses of raltitrexed were administered to the 61 patients. No significant toxic effects were

observed at doses below 1.6 mg/m^2. Four patients received eight courses of 1.6 mg/m^2, 10 patients received 24 courses of 2.6 mg/m^2, 23 patients received 76 courses of 3.0 mg/m^2 and six patients received nine courses of 3.5 mg/m^2. Dose-related gastrointestinal toxicity and myelosuppression were observed at doses above 1.6 mg/m^2. Reversible disturbances in liver function (mainly increases in transaminases) also occurred at doses of 1.6 mg/m^2 and above, although they were generally resolved with continued treatment. No drug-related nephrotoxicity was observed.

The maximum tolerated dose was 3.5 mg/m^2, at which the dose-limiting toxicity of severe malaise (anorexia, nausea and asthenia) occurred in four out of six patients (67%). This dose also produced World Health Organization (WHO) grade 3 or 4 neutropenia in two patients (33%), one of whom also developed thrombocytopenia.

The preceding dose, 3.0 mg/m^2, was generally better tolerated, and no patient experienced the severe malaise seen at the higher dose. Leucopenia occurred in 14 out of 23 (61%) patients, reaching WHO grade 3 or 4 in five patients (22%). Two patients developed grade 4 neutropenia. Thrombocytopenia was observed in six patients (26%), but was severe (grade 3 or 4) in only two patients (9%). Anaemia occurred in 16 patients (70%), although only three required blood transfusions. Diarrhoea occurred in 14 out of 23 patients (61%), reaching grade 3 in four patients and grade 4 in two. A further patient receiving raltitrexed at 3.5 mg/m^2 developed grade 3 diarrhoea, and of these seven patients with severe diarrhoea, five required hospitalization and four died. At 3.0 mg/m^2 nausea occurred in 16 out of 23 patients (70%) receiving 3.0 mg/m^2 raltitrexed, reaching grade 3 in four patients. No grade 4 nausea was observed. Severe (grade 3 or 4) vomiting occurred in four patients (17%), and mild to moderate vomiting (grades 1 or 2) in a further 10 patients (43%). Mucositis developed in 11 patients (48%), but was never worse than grade 2.

Six of the 23 patients (26%) treated with raltitrexed at 3.0 mg/m^2 developed grade 3 or 4 abnormalities in liver function, predominantly an increase in transaminase levels. These effects did not necessitate delaying or reducing the dose of raltitrexed, and liver function returned to normal upon cessation of treatment (except in patients with progressive hepatic malignancy).

Eight patients receiving raltitrexed 3.0 mg/m^2 developed a rash of varying clinical severity, and five patients developed transient fever.

Renal function was followed up in 29 patients in the trial. Six showed evidence of decreased renal clearance, but the patients had either progressive disease causing debilitation (three patients), or disease progression leading to ureteric compression (three patients). There was no evidence of drug-related nephrotoxicity.

The pharmacokinetics of raltitrexed were investigated during this study. The area under the curve (AUC) and maximum plasma concentration (C_{max}) varied approximately linearly with dose. Considerable interpatient variability was observed, but there was no apparent relationship between pharmacokinetic parameters and the occurrence of toxic effects [10]. Plasma concentrations peaked during or shortly after the infusion, and then declined in a tri-exponential manner. The half-life (β) varied from 0.8 to 3 h, apparently unrelated to the dose, and a long terminal elimination phase was apparent (as predicted from the animal studies), with the half-life (γ) ranging from 8.2 to 105 h, again independently of dose [10]. At all doses above 0.6 mg/m^2 raltitrexed was still detectable in plasma immediately before the second dose; however, no accumulation was observed after repeated dosing [10].

From radiolabelling studies, the major route of raltitrexed elimination appeared to be by urinary excretion of the parent drug [12]; therefore, the pharmacokinetics of raltitrexed were studied in a group of patients with renal impairment. Eight patients with mild to moderate renal impairment (glomerular filtration rate of 25–65 ml/min) exhibited a significantly prolonged half-life (γ) and a twofold greater AUC than patients with normal renal function [12]. Renally impaired patients also experienced more adverse events than those with normal renal function. Accordingly it is recommended that patients with a creatinine clearance of 25–65 ml/min should be given 50% of the recommended dose every 4 weeks and those with a creatinine clearance of <25 ml/min should not be treated with raltitrexed.

Although the major route of elimination of raltitrexed appears to be renal, some excretion occurred in the faeces (approximately 15%). Therefore, it was considered important to study the pharmacokinetics of the drug in patients with hepatic dysfunction. Hepatic dysfunction was defined as total bilirubin 1.25–3 times the upper limit of normal, with aspartate aminotransferase or alanine aminotransferase 3–10 times the upper limit of normal. These patients were compared with a group of patients with normal hepatic function. The differences in the pharmacokinetic profiles between patients with mild to moderate hepatic impairment and those with normal hepatic function were small (less than 25% reduction in plasma clearance) and were not considered likely to be of clinical relevance. A comparative review of the safety profiles between the two groups did not provide clear evidence of a difference in patient tolerance to raltitrexed. Therefore, there did not appear to be sufficient justification to recommend a dose reduction for raltitrexed when prescribed to patients with mild to moderate hepatic impairment. However, these patients have a poor prognosis and should be carefully assessed before being treated with chemotherapy.

Table 1. Grade 3 and 4 haematological and non-haematological adverse events in the phase II colorectal cancer study (irrespective of causality)

Adverse event	% (n = 177)
Transient leucopenia	15
Asthenia	12
Severe nausea/vomiting	11
Asymptomatic reversible increases in liver transaminases	10
Severe diarrhoea	10
Other toxicities (including mucositis, skin reactions, alopecia)	≤2

Asthenia as an adverse event was defined as severe (not gradeable by the World Health Organization system).

Table 2. Efficacy results from phase II studies of raltitrexed in solid tumours (from [14])

Tumour type	No. of patients	Objective response rate (%)	95% confidence interval (%)
Colorectal cancer	177	26	19–33
Breast cancer	46	26	14–42
Ovarian cancer	31	7	1–23
Non-small-cell lung cancer	22	9	1–29
Pancreatic cancer	42	12	Not available
Gastric cancer	33	0	0–11
Hepatoma	33	0	0–13
Small-cell lung cancer	21	0	0–19

Only 176 of the total of 177 patients with colorectal cancer were evaluable for efficacy. Some patients with non-small-cell lung cancer were given raltitrexed at 4.0 mg/m².

North American study

The North American dose-finding study [11] looked at doses of raltitrexed ranging from 0.6 to 4.5 mg/m². The majority of patients in the study had colorectal cancer and the maximum tolerated dose was 4.5 mg/m². Dose-limiting toxicity manifested as asthenia and/or neutropenia occurred in five out of nine patients (56%) at the 4.5 mg/m² dose and in three out of 11 patients (27%) at the 4.0 mg/m² dose. Other grade 3 toxicities observed were liver dysfunction (9% of treatment cycles) and nausea and vomiting (2% of cycles). Mucositis and diarrhoea were mild, occurring at grade 2 in only four patients and there were no treatment-related deaths.

Dose-finding

From the results of the European phase I work a dose of 3.0 mg/m² raltitrexed was chosen to take forward into the phase II study. On completion of the North American phase I dose-finding study it appeared that some patients were able to tolerate higher doses of raltitrexed and so the phase III North American study included arms for both the 3 and 4 mg/m² doses.

Phase II trial programme

Colorectal cancer trial

The largest phase II study in patients with advanced colorectal cancer involved 15 centres in Europe, Australia and South Africa [2]. One hundred and seventy-six patients were recruited and included in the efficacy analysis; a further single patient with hepatocellular cancer was recruited in error and included only in the safety analysis. Most patients (over 80%) had liver metastases at entry, and many also had extrahepatic involvement. Patients who had previously undergone chemotherapy for advanced colorectal cancer were excluded, and only 5% had previously received adjuvant chemotherapy (mainly a 5-fluorouracil-based regimen). The drug was administered at a dose of 3 mg/m² once every 3 weeks, as a single 15-min intravenous infusion. A total of 848 courses of raltitrexed were given during the study, and 47% of the patients were given five or more courses.

Four patients of the 176 included in the efficacy analysis (2%) experienced complete remission, 41 patients (23%) experienced partial remission and 86 patients (49%) had stable disease giving an overall response rate (complete or partial remission or stable) of 74%.

The median time to progression was 4.2 months (95% confidence interval 3.2–5.1), and the median survival time was estimated at 11.2 months (95% confidence interval 8.1–13).

Raltitrexed was generally well tolerated, with 82% of patients able to take raltitrexed doses on schedule without requiring a dose reduction or delay. The safety profile was consistent with that observed in the phase I studies: asthenia, transient rises in liver transaminases, gastrointestinal and myelosuppressive effects (Table 1).

A large phase III programme has been completed and reported elsewhere; it is reviewed within this supplement [13–15].

Raltitrexed in other cancers

The activity of raltitrexed has also been investigated in seven further phase II studies of patients with other solid tumours [16]. Table 2 presents brief data on patient numbers and objective response rates. Raltitrexed demonstrated encouraging activity against ovarian, non-small-cell lung, pancreatic and breast cancer. Further studies are under way to confirm its activity in other tumour types [16]. Therefore, at present, raltitrexed is only licensed for use in advanced colorectal cancer.

Conclusions

Raltitrexed is the first new chemotherapeutic agent to show activity against advanced colorectal cancer since the advent of 5-fluorouracil four decades ago. It was rationally designed to inhibit a specific target enzyme, thymidylate synthase, which is crucial to the replication of DNA. Unlike 5-fluorouracil, raltitrexed does not need to be co-administered with a modulating agent, and does not affect RNA synthesis. These attributes may give raltitrexed an improved safety profile and a more convenient dosing schedule compared with current agents.

This potential has been realized in clinical trials. Raltitrexed has demonstrated activity against advanced colorectal cancer and appears to have a favourable toxicity profile. The optimal dose has been established as 3.0 mg/m^2, administered as a convenient single intravenous infusion once every 3 weeks. This simple schedule is in marked contrast to the complex 5-fluorouracil-based combination regimens, some of which require prolonged or frequent hospitalization. Encouraging results have also been reported in other tumour types including ovarian, non-small-cell lung, pancreatic and breast cancer, and confirmatory studies are under way.

Acknowledgements

I acknowledge the work of Ann Jackman and her team, who together with Zeneca scientists identified raltitrexed as the lead candidate for clinical evaluation; Jaap Verweij, Stephen Clarke, Janet Hanwell and Claire Berry for assisting with the phase I trial; and all the physicians who contributed to the phase II trials.

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