

# Phase I Trial and Pharmacokinetic Study of Raltitrexed in Children with Recurrent or Refractory Leukemia: A Pediatric Oncology Group Study

Terzah M. Horton,<sup>3</sup> Susan M. Blaney,<sup>3</sup>  
Anne-Marie Langevin,<sup>1</sup> John Kuhn,<sup>1</sup>  
Barton Kamen,<sup>4</sup> Stacey L. Berg,<sup>3</sup> Mark Bernstein,<sup>5</sup>  
and Steven Weitman<sup>1,2</sup>

<sup>1</sup>University of Texas Health Science Center; <sup>2</sup>ILEX Oncology, San Antonio, Texas; <sup>3</sup>Texas Children's Cancer Center/Baylor College of Medicine, Houston, Texas; <sup>4</sup>University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey; and <sup>5</sup>University of Montreal, Montreal, Quebec, Canada

## ABSTRACT

**Purpose:** To evaluate the toxicity, antileukemic activity, and pharmacology of raltitrexed administered weekly for 3 weeks to patients with refractory or recurrent leukemia.

**Experimental Design:** Raltitrexed was administered as a 15-minute infusion for 3 consecutive weeks every 5 weeks, at doses ranging from 1.3 to 2.8 mg/m<sup>2</sup>. The first course was used to determine the dose-limiting toxicities and maximum tolerated dose. Correlative studies included an assessment of raltitrexed pharmacokinetics and measurement of plasma 2'-deoxyuridine concentrations, a surrogate measure of thymidylate synthase inhibition.

**Results:** Twenty-one children (18 evaluable) with refractory leukemia received 25 courses of raltitrexed. The dose-limiting toxicity was reversible elevation in liver transaminases at the 2.8-mg/m<sup>2</sup> dose level and the maximum tolerated dose was 2.1 mg/m<sup>2</sup> per dose. Pharmacokinetics were best characterized by a two-compartment model with a clearance of 139 mL/min/m<sup>2</sup> (8.3 L/h/m<sup>2</sup>), a 2.4-L volume of distribution, an initial half-life ( $t_{1/2\alpha}$ ) of 6 minutes, and a terminal half-life ( $t_{1/2\beta}$ ) of 45 minutes. There were three objective responses.

**Conclusions:** Raltitrexed was well tolerated when administered as a single agent to children with recurrent or refractory leukemia. We observed preliminary evidence of antileukemia activity using this weekly dosing schedule and these observations support further evaluation of raltitrexed in this population.

Received 8/18/04; revised 11/23/04; accepted 12/2/04.

**Grant support:** Cancer Therapy Evaluation Programs Division Cancer Treatment/National Cancer Institute (Bethesda, MD) grants U01CA57745 and 5P30CA54174.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** Terzah Horton, Baylor College of Medicine, 6621 Fannin, MC 3-3320, Houston, TX 77030. Fax: 832-825-4276; E-mail: tmhorton@txccc.org.

©2005 American Association for Cancer Research.

## INTRODUCTION

Raltitrexed (Tomudex, ZD1694, NSC 639186) is a quinazolinone-based folate analogue that selectively inhibits thymidylate synthase. Thymidylate synthase, which catalyzes the conversion of dUMP to dTMP, is the rate-limiting enzyme in pyrimidine *de novo* deoxynucleotide biosynthesis. Inhibition of thymidylate synthase results in depletion of dTMP and subsequent inhibition of DNA synthesis. Overabundant dUMP is phosphorylated and misincorporated into DNA, resulting in DNA damage and cell death. Raltitrexed undergoes rapid and extensive intracellular polyglutamation, which prevents drug efflux and enhances its cytotoxic effect (1). The preclinical and clinical development of raltitrexed have been previously reviewed (2, 3).

Raltitrexed is currently approved in Europe for the treatment of colorectal cancer and has been combined with other agents such as irinotecan or oxaliplatin for patients with this malignancy (4). Other solid malignant tumors are also sensitive to raltitrexed either alone or in combination with other cytotoxic agents or irradiation. In a phase 2 study of raltitrexed, cisplatin, and 5-fluorouracil administered to adults with newly diagnosed, locally advanced, or metastatic head and neck cancer, a 67% response rate was reported (5). Whereas the use of this agent in the treatment of solid tumors has generated much interest, few clinical studies have examined raltitrexed for use in leukemia.

Several preclinical studies suggest that raltitrexed may have activity against acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) (6–9). Longo et al. showed that raltitrexed was more potent than methotrexate against ALL and AML cell lines (10). Raltitrexed also significantly improved survival when drug-treated mice were compared with vehicle-challenged mice inoculated with human NALM-6 B-lineage ALL cells (11). *In situ* studies using preB-ALL, T-ALL, and AML cells from children at diagnosis, as well as with preB-ALL cells from patients with recurrent disease who had been previously treated with methotrexate, confirm that raltitrexed is a potent inhibitor of thymidylate synthase and suggest that raltitrexed may be an active agent for the treatment of leukemia (8). Raltitrexed may also circumvent methotrexate resistance induced by dihydrofolate reductase amplification (12–14).

Because of its *in vitro* and *in situ* antileukemic activity, the Pediatric Oncology Group (POG) conducted a phase 1 study of raltitrexed in pediatric patients with recurrent or refractory leukemia. This study complemented an earlier phase 1 study of raltitrexed in pediatric patients with refractory solid tumors (POG 9379; ref. 15) and is the first trial of raltitrexed in either adults or children with leukemia. In addition to evaluating the pharmacokinetic disposition of raltitrexed, we measured the accumulation of plasma 2'-deoxyuridine, a dephosphorylated by-product of dUMP (16), which may serve as a surrogate marker of thymidylate synthase inhibition (17).

This study used a weekly dosing strategy to maximize the effects of raltitrexed in patients with leukemia. Eighteen of the

21 pediatric patients with solid tumors enrolled in the pilot study POG 9379 recovered from the myelosuppressive toxicity of raltitrexed in <7 days after a dose of 4.2 mg/m<sup>2</sup> administered every 3 weeks (15). In preclinical studies, Takemura et al. showed a rapid regrowth of leukemic cells after a single-dose exposure to raltitrexed (6). Smith et al. also noted recovery of cell growth occurred within several days after raltitrexed-induced cytotoxicity (18). Based on this preclinical and pilot clinical data, we administered raltitrexed using a weekly dosing schedule, with a starting dose of raltitrexed (1.3 mg/m<sup>2</sup>) beginning at one-third the tolerated dose in the pediatric pilot study that had used an every-3-week dosing schedule.

The objectives of this study were (a) to establish the toxicities and maximum tolerated dose of raltitrexed administered as a weekly infusion for 3 weeks beginning every 5 weeks, (b) to establish the pharmacokinetic profile of raltitrexed in children, (c) to evaluate the relationship between 2'-deoxyuridine plasma concentrations and raltitrexed toxicity, and (d) to document any raltitrexed antileukemic activity.

## PATIENTS AND METHODS

**Patient Selection.** Patients ≤21 years of age with histologically or cytologically documented leukemia (>25% lymphoblasts in a bone marrow aspirate or biopsy) refractory to conventional treatment were eligible for this trial. Patients with active central nervous system leukemia (CNS-3) were not eligible. Other eligibility criteria included (a) a life expectancy of ≥8 weeks, (b) a Karnofsky or Lansky score of ≥50%, (c) adequate liver function [bilirubin level ≤1.5 mg/dL and alanine aminotransferase (ALT) level <5 times upper limits of normal], and (d) adequate renal function (serum creatinine level normal for age or glomerular filtration rate ≥70 mL/min per 1.73 m<sup>2</sup>). Patients could not receive any other anticancer agents or be on any other study during the course of therapy and had to have recovered from the toxicity of all previous chemotherapy. Patients must not have received myelosuppressive chemotherapy during the preceding 2 weeks (6 weeks for prior nitrosurea), biological agents during the preceding 7 days, or a bone marrow transplant within the preceding 6 months. At least 2 weeks must have elapsed from receipt of local palliative radiation or 6 months from substantial radiation including total body irradiation. In addition, patients could not have had significant third-space fluid collections or have been taking nonsteroidal anti-inflammatory agents. Informed consent was obtained from the patient or his or her legal guardian before entering onto the study in accordance with National Cancer Institute (Bethesda, MD) and institutional review board policies.

**Drug Administration and Study Design.** Raltitrexed was supplied by the National Cancer Institute. The product was reconstituted to a 0.5 mg/mL stock solution and further diluted with 0.9% saline or 5% dextrose solution to a final concentration between 2 and 200 µg/mL. The raltitrexed starting dose was 1.3 mg/m<sup>2</sup> per dose administered as a 15-minute infusion weekly for 3 weeks beginning every 5 weeks. Each weekly dose was approximately one-third of the pediatric maximum tolerated dose after drug administration once every 3 weeks, so that the dose administered over the entire 3-week interval was approximately the same as the maximum tolerated dose determined in the pilot

study of raltitrexed in solid tumor patients (POG 9379). Dose escalation proceeded in 30% increments for four dose levels (1.3, 1.6, 2.1, and 2.8 mg/m<sup>2</sup> per dose). No inpatient dose escalation was permitted.

Adverse events were graded according to the National Cancer Institute common toxicity criteria (CTC version 2.0). Dose-limiting toxicities were defined as any grade 3 or 4 nonhematologic toxicity with the specific exclusion of grade 3 nausea and vomiting, grade 3 transaminase elevations that returned to grade ≤1 before the next treatment course, grade 3 fever, or grade 3 infection. Hematologic dose-limiting toxicity was defined as a duration of bone marrow aplasia >5 weeks from the first treatment day. Aplasia was defined as a failure to recover a peripheral absolute neutrophil count >500/µL and platelets >20,000/µL due to bone marrow aplasia, not malignant infiltration.

At least three patients were treated at each dose level. If one of three patients entered at any dose level experienced a dose-limiting toxicity during the first course of therapy, three additional patients were entered at that dose level. If two of two to six patients had dose limiting toxicity at any dose level, the maximum tolerated dose was exceeded, and three more patients were treated at the next lower dose level. The maximum tolerated dose was defined as the dose immediately below the dose level at which two patients of a cohort (of two to six patients) experienced dose-limiting toxicity during the first course. Patients without evidence of progressive disease could continue to receive raltitrexed as long as they had complete recovery from all nonhematologic toxicity. If patients were benefiting from treatment, but experienced a dose-limiting toxicity, subsequent treatment courses were given at the next lower dose level. Physical examination, complete blood count, electrolytes, creatinine, bilirubin, and ALT were done weekly.

**Evaluation of Response.** Assessment of bone marrow involvement was done before course two. Complete response was defined as <5% bone marrow blasts, partial response was defined as 5 to 25% bone marrow blasts, and no response was defined as >25% blasts. Patients showing no response were removed from study. For patients demonstrating a complete marrow response, bone marrow aspirates were repeated every three courses or as clinically indicated. Patients demonstrating a partial marrow response had a marrow aspirate repeated before course 3, then as clinically indicated.

**Pharmacology Studies Sample Collection.** Blood samples (3-5 mL) for raltitrexed analysis were collected into heparinized tubes (in an alternative i.v. access site whenever possible) before the infusion and at the end of the 15-minute infusion. Samples were also collected at 15 minutes, 30 minutes, 1, 2, 4, 8, and 24 hours after the end of the infusion. Pre- and postinfusion samples were collected on days 8 and 15. Blood samples for 2'-deoxyuridine measurements were collected before the raltitrexed infusion and at 1, 8, 24, and 48 hours post infusion. Pre- and postdose samples for assessment of the 2'-deoxyuridine concentration were also obtained on days 8 and 15. The samples were immediately centrifuged for 5 minutes at 2500 rpm at 4°C and the plasma frozen. Samples were stored at -70°C until analysis.

**Analysis of Raltitrexed.** The analytic standard for raltitrexed was obtained from the Pharmaceutical Management

Table 1 Patient characteristics for evaluable patients (n = 18)

Age (y)	
Median	11
Range	1-20
Sex	
Male/Female	10/8
Diagnosis	
ALL	9
AML	8
JCML	1
Prior therapy	
Chemotherapy only	11
Median no. prior regimens	3
Range	1-6
BMT	1
BMT with TBI or CSI	6
No. of courses per patient	
Median	1
Range	1-4

Abbreviations: TBI, total body irradiation; JCML, juvenile chronic myelogenous leukemia; CSI, craniospinal irradiation.

Branch, National Cancer Institute. The internal standard, 4-nitroacetanilide, was obtained from Sigma-Aldrich (Milwaukee, WI). Twenty-five microliters (100 ng) of internal standard were added to 250  $\mu$ L plasma followed by 250  $\mu$ L of 0.1 mol/L sodium acetate (pH 4). The mixture was vortexed and applied to a preconditioned C<sub>2</sub> solid-phase extraction cartridge (100 ng, 1 mL, United Chemical Technologies, Bristol, PA). The sample cartridge was preconditioned with two 1-mL aliquots of methanol/water/0.1 mol/L sodium acetate (pH 4.0; 1:1:1 v/v/v). Immediately after the addition of the sample mixture, the column was washed with 1 mL of high-performance liquid chromatography (HPLC) water (pH 4.0). The adsorbed raltitrexed was eluted with 1.5 mL 90% methanol and dried under a gentle stream of nitrogen at 40°C. The extraction efficiency of raltitrexed from plasma was 95%. The residue was reconstituted with 200  $\mu$ L mobile phase, centrifuged, and filtered before HPLC analysis.

Raltitrexed ( $M_r$  458.5) was quantitated using a Waters HPLC system (Waters Corp, Milford, MA) with UV detection at 350 nm. Chromatographic data were collected and stored using Waters Maxima chromatography data collection software. The mobile phase consisted of methanol/water/0.5 mol/L sodium acetate and triethylamine (28.4:64.3:7.2:0.1) adjusted to pH 4 with glacial acetic acid. The reconstituted solution (150  $\mu$ L) was injected onto a Spheriosorb C<sub>6</sub> column (150  $\times$  4.6 mm, 3  $\mu$ m). With the mobile phase flow rate of 0.8 mL/min, the internal standard eluted at 10 minutes and raltitrexed at 27 minutes. Seven-point calibration curves were constructed by using 1/x weighted least squares linear regression of peak area ratios. Calibration curves were linear ( $r^2 = 0.99$ ) over the respective concentration range of 5 ng/mL (10.9 nmol/L) to 2,500 ng/mL (5.45  $\mu$ mol/L). The intra-/interassay coefficient of variation at the low (20 ng/mL) and high (1,500 ng/mL) end of the curve were 10.6/9.1% and 1.1%/1.3%, respectively.

**Analysis of 2-Deoxyuridine.** 2-Deoxyuridine ( $M_r$  228.2) and the internal standard, 5'-fluoro-2-deoxyuridine were obtained from Sigma-Aldrich. The internal standard (30  $\mu$ L, 3  $\mu$ g) was added to 500  $\mu$ L of plasma, vortexed, transferred to a Centrifree Micropartition cartridge (Amicon, Beverley, MA), and centrifuged at 2000  $\times g$  for 10 minutes at 20°C using a Beckman J2-

HS (JA-17 rotor) centrifuge (Fullerton, CA). The ultrafiltrate was subjected to HPLC analysis. The percent recovery for 2'-deoxyuridine and 5'-fluoro-2-deoxyuridine was 96.2% and 90.7%, respectively. 2'-Deoxyuridine was detected using a mobile phase consisting of 0.05% v/v trifluoroacetic acid/water with a flow rate 1.0 mL/min. The ultrafiltrate (80  $\mu$ L) was injected onto a Waters Nova-Pak C<sub>18</sub> column (3.9  $\times$  300 mm, 4  $\mu$ m). UV absorbance for 2'-deoxyuridine was monitored at 261 nm. Retention times for 2'-deoxyuridine and 5'-fluoro-2-deoxyuridine were 11 and 15 minutes, respectively. The calibration curves generated by least squares regression weighted 1/x<sup>2</sup> were linear ( $r^2 = 0.99$ ) from 5 ng/mL (22 nmol/L) to 2,500 ng/mL (11  $\mu$ mol/L). The interassay coefficient of variation for the quality control samples at the low (50 ng/mL) and high (1500 ng/mL) end of the curve were 7.47% and 3.51%, respectively.

**Pharmacokinetic Analysis.** Raltitrexed concentration-time data were modeled using ADAPT II with maximum likelihood estimation (19). Two- and 3-compartment models were fit to the data and the best fit was determined using Akaike's Information Criterion (20). In all cases, the two-compartment model provided the best fit. Clearance (Cl) was determined from the equation  $Cl = K_{10} \times V_c$ ; half-lives for each phase were determined from the equation  $t_{1/2} = \ln 2/\lambda$ , where  $\lambda$  is the disposition rate constant for the phase; and the area under the concentration versus time curve (AUC) was determined from the equation  $AUC = \text{dose}/Cl$  (21).

## RESULTS

A total of 21 patients were entered into the study. Three patients were not evaluable for toxicity due to early progressive disease, which prevented administration of the scheduled three weekly doses of raltitrexed during course 1 of therapy. Characteristics of the 18 evaluable patients are shown in Table 1. Fourteen of the 18 evaluable patients received one complete course of therapy. Two patients received two complete courses, one at dose level 1 (1.3 mg/m<sup>2</sup>) and another at dose level 3 (2.1 mg/m<sup>2</sup>). One patient received three courses of raltitrexed (1.3 mg/m<sup>2</sup>) and another patient received four courses (2.1 mg/m<sup>2</sup>).

**Adverse Events.** Elevations in liver transaminases was the dose-limiting toxicity in patients treated at the 2.8 mg/m<sup>2</sup> dose level (Table 2). Three patients had grade 3 or 4 elevations in liver transaminases during either the first ( $n = 2$ ) or second ( $n = 1$ ) raltitrexed course. A 12-year-old female with refractory ALL

Table 2 Transaminase elevations after raltitrexed

Course 1		Grade			Associated findings
Dose (mg/m <sup>2</sup> )	Number*	2	3	4	
1.3	6	1†	1	0	None
1.6	3	0	1	0	Grade 2 hyperbilirubinemia†
2.1	6	4	2‡	0	Grade 1, 2 hypoalbuminemia Grade 2 hyperbilirubinemia Grade 3 hepatomegaly
2.8	3	0	1†	2	Grade 1 hyperbilirubinemia Grade 2 hyperbilirubinemia† Grade 2 hypoalbuminemia

\*Number of patients evaluable at this dose level.

†Attributed to progressive disease.

‡Occurred during second cycle of raltitrexed.

Table 3 Antileukemia activity of raltitrexed

Age/Sex	Diagnosis	Response	Dose (mg/m <sup>2</sup> )	Prior therapy	Precourse marrow blasts (%)			Comments
					1	2	3	
3/M	AML	PR	1.3	Chemotherapy BMT/TBI	28	14	10	Progressed after third course
12/F	AML	NR	1.3	Chemotherapy*	56	NA	NA†	Skin lesions cleared
11/M	ALL	CR	2.1	Chemotherapy‡	98	4§	1	BMT after 2nd course
11/M	ALL (Philadelphia+)	PR	2.1	BMT/TBI	28	31	7	Progressed after 4th course

Abbreviations: CR, complete response; PR, partial response; NR, no response; NA, not available.

\* 2 prior chemotherapy regimens.

† Noted to have extensive marrow involvement unchanged from initial diagnosis.

‡ Patient in first relapse after two failed induction attempts and 2 cycles of POG 9423 (PSC-833, etoposide, and mitoxantrone).

§ Hypocellular marrow.

|| Normocellular marrow.

experienced a rapidly increasing ALT (grade 4) and aspartate aminotransferase (AST; grade 3) after the second of the three scheduled doses of raltitrexed during course 1, prompting discontinuation of raltitrexed and early study withdrawal. Elevated transaminases returned to baseline after drug discontinuation. A 12-year-old male with ALL, previously treated with bone marrow transplant (BMT) and total body irradiation, had a grade 3 ALT and grade 2 hyperbilirubinemia after dose 2, course 1 of raltitrexed. These transaminase elevations were also reversible, returning to grade 1 before the patient's death 1 week after completion of course 1 therapy due to rapidly progressive leukemia. Finally, a 20-year-old female with AML who had minimal liver toxicity during her first course of raltitrexed (grade 1 ALT and grade 2 AST) developed a grade 4 ALT, grade 3 AST, and grade 3 GGT after the first dose of raltitrexed during course 2, prompting her removal from study. This patient was hospitalized at the time for a wound infection and acute renal insufficiency thought unrelated to drug. Her ALT gradually returned to normal over the next several weeks.

One patient at dose level 3 (2.1 mg/m<sup>2</sup>) also had grade 3 elevation in ALT and AST; however, these increases were felt to be at least in part related to rapidly progressive disease. Other toxicities attributed to raltitrexed included grade 3 diarrhea in one of six patients treated at the first dose level and drug-induced nausea and vomiting that were well controlled with antiemetics. Grade 3 and 4 adverse events unrelated to drug included fever/infection (9), hypokalemia (2), hypophosphatemia (2), hyperuricemia (1), diarrhea (1), vomiting (1), anorexia (1), abdominal pain (1), and pulmonary infiltrates (1). In addition, 11 of 18 patients experienced some degree of myelosuppression attributed to their underlying disease process.

Three deaths occurred on study. Two deaths (one each at dose levels 1 and 4) were felt by the treating center to be related

to rapidly progressing disease. The third death, which occurred at the first dose level (day 23 of study), was attributed to *E. coli* sepsis. The study cohort was expanded after this adverse event; however, no further infectious adverse events were noted at this dose level.

**Antileukemia Activity.** Four patients benefited from raltitrexed administration as evidenced by either a decrease in bone marrow blast percentage or clearing of cutaneous leukemic infiltrates (Table 3). An 11-year old male with ALL, in first relapse after failing two prior induction regimens, was treated at the maximum tolerated dose (2.1 mg/m<sup>2</sup>) and had a complete response (98% to 4% blasts) after one course of therapy. This patient received a second course of raltitrexed before undergoing a bone marrow transplant and is currently a long-term survivor ( $n = 43$  months). Two patients had a partial response to raltitrexed, a 3-year old male with AML treated at the 1.3 mg/m<sup>2</sup> (bone marrow blast count decreased from 28% to 10% after two courses of therapy) and an 11-year-old male with Philadelphia positive ALL treated at the 2.1 mg/m<sup>2</sup> dose level (bone marrow blast percentage decreased from 28% to 7% after three courses of raltitrexed). Finally, a 12-year old female with AML had clearing of cutaneous leukemic infiltrates and peripheral blasts after one course of raltitrexed (1.3 mg/m<sup>2</sup>). Despite this cutaneous response, however, there was no apparent effect on the bone marrow blast percentage and the patient declined further doses of raltitrexed to pursue other therapy. Fourteen patients treated with raltitrexed had no apparent response to treatment and were removed from the study after one course of therapy.

**Pharmacokinetics/Pharmacodynamics.** Day 1 pharmacokinetic samples were obtained on 19 of 21 patients enrolled on this trial (Table 4). The elimination of raltitrexed from plasma was best described by a two-compartment model with a median initial half-life of 6 minutes, a terminal half-life of 45 minutes,

Table 4 Pharmacokinetic parameters for raltitrexed after a 15-minute infusion

Dose (mg/m <sup>2</sup> )	No. patients	AUC (µg/mL min)	V <sub>d</sub> (L/m <sup>2</sup> )	t <sub>1/2α</sub> (min)	t <sub>1/2β</sub> (min)	Cl (mL/min/m <sup>2</sup> )	Peak concentration (ng/mL)
1.3	7	12 (6.2-17)	3.0 (1.1-4.3)	6 (4-15)	32 (25-170)	107 (77-265)	234 (190-474)
1.6	3	11 (8.2-13.6)	3.1 (1.7-3.3)	6 (6-8)	59 (38-62)	149 (118-196)	310 (218-318)
2.1	7	14.4 (7.5-48.8)	2.8 (1.5-4.2)	7 (3-30)	37 (29-444)	139 (43-281)	414 (219-763)
2.8	2	20* (16-24.3)	2.3 (2.2-2.6)	6.5 (5-8)	75 (45-105)	145 (115-176)	681 (666-697)
Median (range)		NA	2.8 (1.1-4.3)	6 (3-30)	45 (25-444)	139 (43-281)	NA

Abbreviations: V<sub>D</sub>, volume of distribution; t<sub>1/2α</sub>, initial half-life; t<sub>1/2β</sub>, terminal half-life; Cl, clearance; NA, not applicable.

\*Average of two determinations.

and a clearance of 139 mL/min/m<sup>2</sup>. In most patients, there was a rapid decline in raltitrexed plasma concentrations by 4 hours to below the limit of detection of the assay (Fig. 1). Although there was moderate interpatient variability in the raltitrexed pharmacokinetic parameters, plasma exposure seemed to increase linearly with dose over the limited dose range studied and there was no evidence of dose-dependent clearance.

2'-Deoxyuridine, a surrogate marker of thymidylate synthase inhibition, was also quantitated after raltitrexed administration (Table 5). The average increase in 2'-deoxyuridine after the first dose of raltitrexed was 3.7-fold from baseline. 2'-Deoxyuridine was not detectable before the days 8 and 15 doses of raltitrexed. Peak levels determined after days 8 and 15 raltitrexed were 1.5-fold and 2.6-fold greater than baseline, respectively.

## DISCUSSION

The dose-limiting toxicity of raltitrexed administered weekly for 3 consecutive weeks in children with recurrent or refractory leukemia was hepatotoxicity, manifest as reversible elevations in serum transaminases. In adults, raltitrexed has primarily been administered on a once every 3 week dosing schedule to patients with advanced colorectal cancer. The dose-limiting toxicity of raltitrexed in adults with solid tumors were asthenia, myelosuppression, and gastrointestinal toxicities (22, 23). Nevertheless, grade 3 or 4 hepatotoxicity has been observed in 3% to 24% of adults with solid tumors after raltitrexed administration (3, 24, 22). Fulminant hepatic necrosis, an apparently rare and idiosyncratic adverse event, has also been reported in two adults after raltitrexed administration (25). Other grade 3 adverse events after raltitrexed included fatigue, diarrhea, and myelosuppression (3). In children with refractory or recurrent solid tumors, 30 (62%) of 48 patients experienced mild liver transaminase elevations and 4 patients experienced grade 3 or 4 ALT elevations that seemed unrelated to dose (15). Overall, 12 (66%) of 18 pediatric patients in this phase 1 study had abnormalities in serum transaminases, of which 10 (83%) of 12 were attributed to at least possibly to raltitrexed (Table 2). Although 6 of 10 patients had isolated elevations in transaminases, 4 of 10 patients had additional evidence of mild liver dysfunction, including hypoalbuminemia (2), hyperbilirubinemia (2), and hepatomegaly (1).

The significance of liver transaminase elevations after raltitrexed administration has been addressed in several studies. Cunningham et al. (3) observed transaminase elevations in six raltitrexed phase 2 and 3 trials. He noted that these elevations were reversible, returning to baseline in subsequent courses without dose reduction and were not associated with significant liver

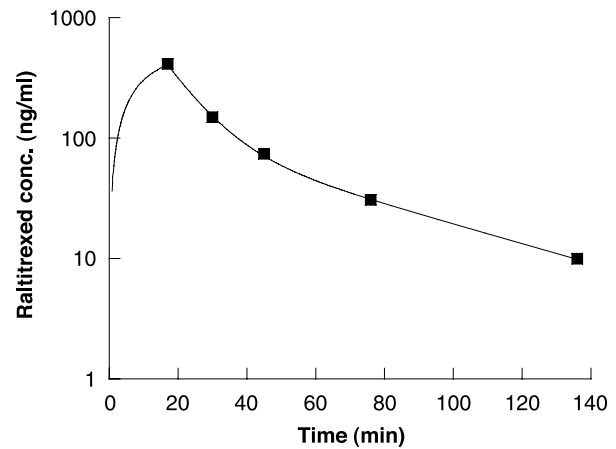


Fig. 1 Plasma concentrations of raltitrexed (ng/mL) in a representative patient at the maximum tolerated dose (2.1 mg/m<sup>2</sup>) after a 15-minute raltitrexed infusion at the doses indicated. Points, observed data; line, modeled fit of the observed data.

pathology. However, raltitrexed was associated with significant liver toxicity in other studies, including death from fulminant hepatic necrosis (25, 26). Liver toxicity correlated with elevated baseline transaminases and high cumulative raltitrexed dose in these studies (26). In our study, grade 3 or 4 transaminase elevations attributed to raltitrexed were associated with additional evidence of mild liver dysfunction in four patients. Unlike the studies in adults, transaminase elevations in this study were an early event, occurring within the first cycle in all but one patient.

Because raltitrexed is rapidly polyglutamated after cell entry (1), it is possible that polyglutamated drug might accumulate using a weekly administration schedule. Because only a few patients received more than two courses of raltitrexed, it is difficult to assess cumulative toxicity in this patient population. However, one patient received four courses of raltitrexed at the maximum tolerated dose (2.1 mg/m<sup>2</sup>) and did not have any evidence of cumulative drug-related toxicity.

The pharmacokinetic behavior of raltitrexed in children was similar to that described in adults, although the initial half-life ( $t_{1/2\alpha}$ ) in this study was shorter than that reported in adult studies (6 versus 100 minutes; ref. 1). In our two-compartment model, the terminal half-life ( $t_{1/2\beta}$ ) was 45 minutes, which is similar to the  $t_{1/2\beta}$  reported in adults and children (1, 15). Studies using more sensitive assays have also identified a prolonged terminal phase ( $t_{1/2\gamma}$ ) of 96 to 260 hours (1, 15). The clearance in this study (8.3 L/h/m<sup>2</sup>), was more rapid than that reported in adults (2.4-3 L/h/m<sup>2</sup>; ref. 1) and pediatrics (3.5L/h/m<sup>2</sup>; ref. 15).

Table 5 Plasma 2'-deoxyuridine concentrations after raltitrexed administration

Dose (mg/m <sup>2</sup> )	Baseline (ng/mL)*	Day 1 peak concentration (ng/mL)†	Day 8 peak concentration (ng/mL)†	Day 15 peak concentration (ng/mL)†
1.3 (n = 7)	13.0 (<5‡-83)	38.7 (15-216)	17.4 (7.6-49)	19.8 (<5‡-83)
1.6 (n = 3)	8.9 (<5‡-13)	33.3 (18-67)	13.5 (7.4-20)	18.5 (15-44)
2.1 (n = 7)	5.0 (<5‡-28)	34.1 (12-58)	13.0 (<5‡-19)	5.6 (<5‡-9)

NOTE. 2'-Deoxyuridine median values for groups with at least three patients.

\*Median plasma concentration before raltitrexed administration (range in parentheses).

†Plasma concentration immediately after raltitrexed infusion.

‡Below limit of HPLC assay detection (5 ng/mL).

We also examined the plasma levels of 2'-deoxyuridine, a surrogate marker for thymidylate synthase inhibition. Increased plasma 2'-deoxyuridine levels after weekly administration of raltitrexed might indicate polyglutamated drug accumulation. However, levels of 2'-deoxyuridine were undetectable before day 8 and 15 administration of raltitrexed (Table 5). Interestingly, day 8 and 15 peak levels were also lower than day 1 peak levels, an observation noted in several adult studies (17). This effect was also noted in a mouse model (27) and in preclinical studies using leukemic cell lines (17). Mechanisms of leukemic cell resistance to raltitrexed have been examined *in vitro* and have included reduced raltitrexed transport (6, 10), reduced raltitrexed polyglutamation by folylpolyglutamate synthetase (11, 14), and inhibition of thymidylate synthase gene expression (28, 29).

In summary, raltitrexed administered as an i.v. infusion weekly for 3 weeks beginning every 5 weeks was well tolerated in children with recurrent or refractory leukemia. The recommended dose for further evaluation in children with recurrent or refractory leukemia is 2.1 mg/m<sup>2</sup> per dose. The primary adverse event was elevated serum transaminase levels and mild liver dysfunction above the maximum tolerated dose that were clinically asymptomatic. Although not designed to determine efficacy, there were three objective responses in this phase 1 trial. Further evaluation of raltitrexed in this high-risk population should be considered.

## REFERENCES

- Clarke SJ, Beale PJ, Rivory LP. Clinical and preclinical pharmacokinetics of raltitrexed. *Clin Pharmacokinet* 2000;39:429–43.
- Blackledge G. New developments in cancer treatment with the novel thymidylate synthase inhibitor raltitrexed ('Tomudex'). *Br J Cancer* 1998;77:29–37.
- Cunningham D, Zalcberg J, Maroun J, et al. Efficacy, tolerability and management of raltitrexed (Tomudex) monotherapy in patients with advanced colorectal cancer. A review of phase II/III trials. *Eur J Cancer* 2002;38:478–86.
- Custem EV. Future developments with "Tomudex" (raltitrexed). *Anticancer Drugs* 1997;8:S33–8.
- Caponigro F, Rosati G, De Rosa P, et al. Cisplatin raltitrexed, levofofolic acid and 5-fluorouracil in locally advanced or metastatic squamous cell carcinoma of the head and neck: a phase II randomized study. *Oncology* 2002;63:232–8.
- Takemura Y, Gibson W, Kimbell R, Kobayashi H, Miyachi H, Jackman AL. Cellular pharmacokinetics of ZD1694 in cultured human leukaemia cells sensitive, or made resistant, to this drug. *J Cancer Res Clin Oncol* 1996;122:109–17.
- Takemura Y, Kobayashi H, Miyachi H. Raltitrexed, a novel folate-based thymidylate synthase inhibitor, for the treatment of acute leukemia: is this drug active against acute myelogenous leukemia? *Int J Hematol* 2000;72:112–4.
- Mauritz R, Bekken MW, Rots MG, et al. *Ex vivo* activity of methotrexate versus novel antifolate inhibitors of dihydrofolate reductase and thymidylate synthase against childhood leukemia cells. *Clin Cancer Res* 1998;4:2399–410.
- Sakoff JA, Ackland SP. Thymidylate synthase inhibition induces S-phase arrest, biphasic mitochondrial alterations and caspase-dependent apoptosis in leukaemia cells. *Cancer Chemother Pharmacol* 2000;46:477–87.
- Longo GSA, Gorlick R, Tong WP, Ercikan E, Bertino JR. Disparate affinities of antifolates for folylpolyglutamate synthetase from human leukemia cells. *Blood* 1997;90:1241–5.
- Ek O, Reaman GH, Crankshaw DL, Chelstrom LM, Myers DE, Uckun FM. Combined therapeutic efficacy of the thymidylate synthase inhibitor ZD1694 (TOMUDEX) and the immunotoxin B43 (anti-CD19)-PAP in a SCID mouse model of human B-lineage acute lymphoblastic leukemia. *Leukemia and Lymphoma* 1998;28:509–14.
- Rots MG, Pieters R, Peters GJ, et al. Circumvention of methotrexate resistance in childhood leukemia subtypes by rationally designed antifolates. *Blood* 1999;94:3121–8.
- Weigand M, Frei E, Graf N, et al. Mechanisms of resistance to methotrexate in childhood acute lymphoblastic leukemia: circumvention of thymidylate synthase inhibition. *J Cancer Res Clin Oncol* 1999;125:513–9.
- Liani E, Rothen L, Bunni MA, Smith CA, Jansen G, Assaraf YG. Loss of folylpoly-γ-glutamate synthetase activity is a dominant mechanism of resistance to polyglutamylation-dependent novel antifolates in multiple human leukemia sublines. *Int J Cancer* 2003;103:587–99.
- Widemann BC, Balis FM, Reaman GH, et al. Pediatric phase I trial and pharmacokinetic study of raltitrexed (ZD1694, Tomudex). *Proc Am Soc Clin Oncol* 1999;18:2172a.
- Mitchell F, Lynn S, Jackman AL. Modified high-performance liquid chromatography assay for the measurement of 2'-deoxyuridine in human plasma and its application to pharmacodynamic studies of antimetabolite drugs. *J Chromatogr B* 2000;744:351–8.
- Ford HER, Mitchell F, Cunningham D, et al. Patterns of elevation of plasma 2'-deoxyuridine, a surrogate marker of thymidylate synthase (TS) inhibition, after administration of two different schedules of 5-fluorouracil and the specific TS inhibitors raltitrexed (Tomudex) and ZD9331. *Clin Cancer Res* 2002;8:103–9.
- Smith SG, Lehman NL, Moran RG. Cytotoxicity of antifolate inhibitors of thymidylate and purine synthesis to WiDr colonic carcinoma cells. *Cancer Res* 1993;53:5697–706.
- D'Argenio DZ, Schumitzky A. ADAPT II user's guide: pharmacokinetic/pharmacodynamic systems analysis software. Los Angeles: University of Southern California: Biomedical Simulations Resource, 1997.
- Yamaoka K, Nakagawa T, Uno T. Application of Akaike's Information Criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J Pharmacokinet Biopharm* 1978;6:165–75.
- Gibaldi M, Perrier D. Pharmacokinetics. New York: Marcel Dekker, Inc. 1982;2.
- Zalcberg J. Overview of the tolerability of 'Tomudex' (raltitrexed): collective clinical experience in advanced colorectal cancer. *Anticancer Drugs* 1997;8 Suppl 2:S17–22.
- Clarke SJ, Ward J, de Boer M. Phase I study of the new thymidylate synthase inhibitor "Tomudex" (ZD164) in patients with advanced malignancy. *Ann Oncol* 1994;5 Suppl 5A:132.
- Judson IR. 'Tomudex' (raltitrexed) development: preclinical, phase I and II studies. *Anticancer Drugs* 1997;2:S5–9.
- Raderer M, Fiebigler W, Wrba F, Scheithauer W. Fatal liver failure after the administration of raltitrexed for cancer chemotherapy. *Cancer* 2000;89:890–2.
- Massacesi C, Santini D, Rocchi MB, et al. Raltitrexed-induced hepatotoxicity: multivariate analysis of predictive factors. *Anticancer Drugs* 2003;14:533–41.
- Cao S, McGuire JJ, Rustum YM. Antitumor activity of ZD1694 (Tomudex) against human head and neck cancer in nude mouse models: role of dosing schedule and plasma thymidine. *Clin Cancer Res* 1999;5:1925–34.
- Wang W, Marsh S, Cassidy J, McLeod HL. Pharmacogenomic dissection of resistance to thymidylate synthase inhibitors. *Cancer Res* 2001;61:5505–10.
- Chu E, Callender MA, Farrell MP, Schmitz JC. Thymidylate synthase inhibitors as anticancer agents: from bench to bedside. *Cancer Chemother Pharmacol* 2003;52 Suppl 1:S80–9.