Phase I Study of an Oral Formulation of ZD9331 Administered Daily for 28 Days

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Purpose: **To define the maximum-tolerated dose and dose-limiting toxicities (DLTs) of an oral formulation of ZD9331, a novel thymidylate synthase inhibitor that is not a substrate for folylpolyglutamate synthase.**

Patients and Methods: **Patients had Cancer and Leukemia Group B performance status ≤ 2 and refractory solid tumors. Initially, patients received ZD9331 daily for 2 weeks, with the duration of treatment escalated to a maximum of 4 weeks, followed by a 2-week rest period. Once the maximum-tolerated duration of treatment was determined, the dose of ZD9331 was increased until DLT occurred.**

Results: **Fifty-five patients were enrolled at eight dose levels. The DLTs were thrombocytopenia and neutropenia. At 3 mg/d, two of 19 patients developed DLT; one patient had grade 3 thrombocytopenia and grade 4 neutropenia, and the other patient had grade 3 thrombocytopenia only. Anemia was common, with a median hemoglobin nadir of 75% of baseline, before recovery or transfusion. The apparent oral clearance of ZD9331 was 11.6 ± 6.3 mL/min. Dose-limiting myelosuppression was associated with both an increased 24-hour ZD9331 concentration and blood urea nitrogen.**

Conclusion: **The recommended phase II dose on this schedule is 3 mg/d for 4 weeks, followed by a 2-week rest period. ZD9331 seems to have a manageable toxicity profile, although it should be used with caution in patients with renal impairment.**

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THYMIDYLATE SYNTHASE catalyzes conversion of deoxyuridine monophosphate to thymidylate and is the only de novo source of thymidylate for DNA synthesis.[1] The central role of thymidylate synthase in nucleotide synthesis has led to the development of several folate analogs that inhibit thymidylate synthase. CB3717, the first antifolate to specifically target thymidylate synthase, was abandoned because of its unpredictable nephrotoxicity,[2] which was thought to be due to its low water solubility.[3] Because CB3717 had significant antitumor activity, the Cancer Research Campaign Center for Cancer Therapeutics developed antifolates with improved water solubility.

This research led to the development of raltitrexed (Tomudex; ZD1694; AstraZeneca, Macclesfield, UK), which had improved water solubility and decreased nephrotoxicity in animal models.[3] Subsequent clinical studies demonstrated activity of ZD1694 in colorectal cancer and led to its approval in Australia, Canada, and Europe for treatment of metastatic colorectal cancer.[4] In a later study in the United States, patients with metastatic colorectal cancer were randomly assigned to either fluorouracil and leucovorin or ZD1694; a slight survival advantage was found for fluorouracil and leucovorin.[5] On the basis of this study, ZD1694 was not developed further in the United States. ZD1694 required polyglutamation by folylpolyglutamate synthase to effectively inhibit thymidylate synthase.[6] In vitro studies have shown that resistance to antifolates can occur by cancer cells decreasing accumulation of polyglutamated antifolates.[7,8] In addition, variability in polyglutamation may be a factor in the interpatient variability of antifolate pharmacokinetics.

ZD9331 was developed to overcome decreased polyglutamation as a mechanism of resistance to antifolates and have more predictable pharmacokinetics and less interpatient variability than ZD1694. Preclinical studies demonstrated that ZD9331 was active against lymphoid and leukemia cell lines and small-cell lung, gastric, and colorectal cancer xenografts.[9] ZD9331 was more active when given by a protracted schedule than an intermittent schedule. In vitro studies using L5178Y TK−/− mouse lymphoma cell lines demonstrated that exposure to 10 μmol/L of ZD9331 for 4 hours inhibited colony formation by 80%, whereas exposure to 0.1 μmol/L of ZD9331 for 24 hours inhibited colony formation by 99.96%. In vivo studies also showed that ZD9331 had superior activity administered on a protracted schedule. Mice were implanted with L5178Y TK −/− cells and treated with ZD9331; nine of 16 mice treated with 100 mg/kg of ZD9331 given by continuous infusion for 7 days were cured, whereas none of the mice treated with 100 mg/kg of ZD9331 given by intraperitoneal injection were cured.[10] Preclinical pharmacokinetic studies showed good oral bioavailability: 30% to 60% in rats at 6 mg/m^2 and 80% in dogs at 2 mg/m^2.

These preclinical studies demonstrating increased activity with prolonged exposure to ZD9331 and good oral bioavailabil-

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ity prompted this phase I study of oral ZD9331 with prolonged administration. Because the intravenous formulation of ZD9331 had demonstrated that clearance was independent of body-surface area,[11] this oral formulation study used fixed dosing rather than dosing that was based on body-surface area.

PATIENTS AND METHODS

Patient enrollment began in April 1998, and the study was closed in February 2000. Eligible patients had a solid tumor that was refractory to standard therapies or for which no standard therapy existed; Cancer and Leukemia Group B performance status ≤ 2; age ≥ 18 years; a life expectancy ≥ 12 weeks; a baseline platelet count ≥ 100×10^9/L; a total WBC count ≥ 3.5×10^9/L or an absolute neutrophil count ≥ 2×10^9/L; serum bilirubin concentration ≤ 1.25 times the upper limit of normal; ALT and AST ≤ 2.5 times the upper limit of normal in the absence of liver metastases and ≤ 5 times the upper limit of normal if liver metastases were present; serum creatinine ≤ 1.25 times the upper limit of normal; no severe or uncontrolled systemic disease, such as uncompensated respiratory or cardiac conditions; no use of folate-containing vitamin supplements; and no chlorambucil, mitomycin, or nitrosoureas for more than 6 months total duration.

Treatment Plan

Patients took ZD9331 as a single daily dose, between 8 and 10 AM (either 30 minutes before food or 2 hours after). The initial patient was treated with ZD9331 0.5 mg/d given for 14 days, followed by a 14-day rest period. Because no toxicity was observed, the next patient was treated for 21 days, followed by a 2-week rest period. The treatment period was increased to 28 days followed by a 2-week rest period for the next patient. The treatment period then remained at 28 days for the duration of the study. The dose of ZD9331 was doubled until the development of any grade of drug-related toxicity, with a minimum of one patient per dose level. Subsequent dose escalations followed a modified Fibonacci scheme starting at the 67% dose-escalation level, with a minimum of three patients enrolled per dose level. If any patient developed dose-limiting toxicity (DLT), three additional patients were enrolled at that dose level. At least 12 patients were to be enrolled at the recommended phase II dose, which was anticipated to be the dose at which less than one third of patients experienced DLT in the first cycle.

DLT was defined as grade 4 neutropenia of any duration with fever, grade 4 neutropenia without fever for at least 7 days, or grade 4 thrombocytopenia. Patients stopped treatment if they developed grade 2 neutropenia or thrombocytopenia while still taking ZD9331, and this was considered a DLT. Grade 3 or 4 nonhematologic toxicity that was not ameliorated by symptomatic directed therapy (with the exception of reversible elevations of AST or ALT) was also considered a DLT.

Chemistry and hematologic measurements were repeated weekly during the first two cycles and within 3 days of starting the next cycle. Tumor measurements were repeated every two cycles.

Patients remained on study until there was evidence of tumor progression, unacceptable toxicity, or until the patient or investigator felt continuing treatment with ZD9331 was not in the patient's interest. Patients who experienced DLT but who were clinically benefiting from ZD9331 could continue to receive treatment at the investigator's discretion at a reduced dose. The University of Chicago institutional review board approved the protocol and the consent form. Written informed consent was obtained from all patients.

Pharmacokinetics

Samples for pharmacokinetic analysis were collected on day 1 pretreatment and at 1, 2, 4, 6, 8, and 24 hours after starting treatment. Samples were also taken at predose and 1, 2, 4, and 6 hours postdose 1 week before the end of dosing, although these results are not presented here. The AstraZeneca Safety of Medicines Laboratory determined the plasma levels of ZD9331 (AstraZeneca) using a high-performance liquid chromatography–mass spectrometry–mass spectrometry assay as previously described.[8]

A noncompartmental approach was used to characterize the pharmacokinetic parameters of ZD9331. The pharmacokinetic software used to analyze the plasma concentrations was WinNonlin (version 2.1; Pharsight Corp, Cary, NC). The area under the concentration-time curve (AUC) was calculated to the 24-hour sampling point (after the first dose) using the linear trapezoidal rule. The terminal half-life was estimated from the terminal part of the log concentration-time curve. The apparent oral clearance (Cl/F) was calculated as dose divided by AUC. Creatinine clearance was estimated using the formula of Cockcroft and Gault.[12]

Table 1. Patient Characteristics

		No. of Patients (n = 55)
Female/male		36:19
Age, years		
Median	58	
Range	33-79	
CALGB performance status		
0		23
1		29
2		3
Prior treatment		
Chemotherapy only		36
Chemotherapy and radiation		19
Chemotherapy regimens		
Median	2	
Range	1-8	
Cancer diagnosis		
Colorectal		31
Ovarian		6
Endometrial		2
Esophagus		2
Hepatocellular		2
Others		12

Abbreviation: CALGB, Cancer and Leukemia Group B.

Statistics

Stata 6 (version 6.0; Stata Corp, College Station, TX) was used to perform the statistical analysis. Pharmacokinetic parameters were examined for possible relationships with the development of myelosuppression using the Student's *t* test. Exploratory analyses (using univariate and multivariate regression) were conducted to assess the possible relationship of various patient characteristics (eg, age, renal function) to ZD9331 clearance. In addition, an exploratory pharmacodynamic analysis was conducted after log transformation of the hematologic parameters. This analysis included an assessment of the relative contributions of variability in peak (maximum concentration; C_{max}) and trough concentrations (24-hour minimum concentration; C_{min}) and the AUC.

RESULTS

Demographics

A total of 55 patients were enrolled, from April 1998 to February 2000. Patient characteristics are outlined in Table 1. Patients were assessable for toxicity if they were able to complete cycle 1 of treatment. Forty-five patients were assessable for toxicity. One patient who developed suspected cellulitis during cycle 1 was withdrawn and therefore was not assessable for toxicity. Two patients died while on study, but the deaths were not considered to be related to ZD9331. One patient

presented to her local hospital in the third week of treatment with acute shortness of breath; at presentation her WBC count was 9.6×10^9/L, creatinine was 0.5 times the upper limit of normal, and blood urea nitrogen (BUN) was 9 mg/dL. Her respiratory status deteriorated, and she died from a presumed pulmonary embolus. At enrollment, she had a creatinine clearance of 81 mL/min and BUN of 13.5 mg/dL. The other patient had a history of recurrent urinary tract infections and was taking nitrofurantoin to prevent recurrent infections. At enrollment, she had a calculated creatinine clearance of 151 mL/min and a BUN of 9 mg/dL. She developed urosepsis in the fifth week of treatment and presented to her local hospital. She progressed to septic shock and her advance directive precluded inotropic support. She died despite maximal support outside of the intensive care unit. The remaining patients not assessable for toxicity either withdrew consent or developed progressive disease, preventing completion of one cycle.

Dose Escalation

A total of 128 cycles were administered, and the median number of cycles completed by each patient was two (range, one to seven cycles). The treatment duration was escalated to 28 days (at a dose of 0.5 mg/d) without the occurrence of DLT. The dose of ZD9331 was then escalated from 0.5 to 5 mg/d. No DLTs occurred until the 5 mg/d dose level, at which two patients developed DLTs. At the prior dose level of 3 mg/d, no significant drug-related toxicity was observed, which led us to examine 4 mg/d. The three initial patients evaluated at 4 mg/d experienced more severe toxicity than patients treated at 5 mg/d. A preliminary analysis suggested that patients who experienced DLT at the 4 and 5 mg/d dose levels had decreased drug clearance and that this was correlated with an elevated BUN. Seven additional patients were enrolled at the 4-mg level to test this hypothesis; three patients had a high BUN, ≥ 20 mg/dL (4.0H), and four patients had a low BUN, less than 20 mg/dL (4.0L). An additional 16 patients were subsequently enrolled at the recommended phase II dose of 3 mg/d to better define the toxicity and pharmacodynamics at this dosage level.

Hematologic Toxicity

The DLT of ZD9331 was myelosuppression, both thrombocytopenia and neutropenia (Table 2). Patients developed thrombocytopenia at a median of 14 days after starting therapy (range, 11 to 14 days). Platelet counts returned to baseline at a median of 10 days (range, 7 to 14 days). Neutropenia occurred at a median of 21 days (range, 11 to 21 days), with a median duration of 7 days before recovery (range, 7 to 11 days). At 3 mg/d, the recommended phase II dose, two of 19 patients developed DLTs; one patient had grade 3 thrombocytopenia and grade 4 neutropenia, and the other patient had grade 3 thrombocytopenia only.

Anemia was a common toxicity at doses ≥ 3 mg/d (Fig 1), but was not dose-limiting. This was managed with erythropoietin and RBC transfusions. Midway through the study, one patient developed anemia associated with a low haptoglobin, elevated bilirubin and lactate dehydrogenase (LDH), and a negative Coombs test. After this index case, all subsequent patients had haptoglobin levels measured along with routine biochemistry. At the 3-mg dose level, five of 16 patients had anemia with undetectable haptoglobin levels. As Fig 2 demonstrates, the decrease in hemoglobin was gradual over several weeks. There was no significant difference in nadir hemoglobin, bilirubin, or LDH between patients with or without low haptoglobin levels.

Table 2. Hematologic Toxicity by Dose Level (fully assessable patients only)

Dose of ZD9331 (mg/d)	Cycle Length (days)	No. of Patients	Grade 3 and 4 Adverse Events (no. of patients)	Dose-Limiting Toxicity (no. of patients)
0.5	14	2	—	—
0.5	21	2	—	—
0.5	28	2	—	—
1.0	28	3	—	—
1.5	28	2	—	—
3.0	28	3	—	—
5.0	28	5	Grade 3 leukopenia (1), grade 3 thrombocytopenia (1), grade 3 neutropenia (1)	2
4.0	28	3	Grade 3 thrombocytopenia (1), grade 3 neutropenia (1), grade 4 neutropenia (1)	2
3.0	28	16	Grade 3 thrombocytopenia (2), grade 3 anemia (1), grade 4 neutropenia (1)	2
4.0H*	28	3	Grade 3 thrombocytopenia (1), grade 2 thrombocytopenia (1)	2
4.0L*	28	4	Grade 4 thrombocytopenia (1), grade 2 nausea and vomiting (1)	2

*4.0H (high) blood urea nitrogen ≥ 20 mg/dL, 4.0L (low) blood urea nitrogen < 20 mg/dL.

Nonhematologic Toxicity

The most frequent toxicities encountered at the 3-mg dose level are listed in Table 3. The most common toxicity was fatigue, which occurred in almost all patients. Nausea was a frequent side effect but was not dose-limiting. Four patients developed an erythematous maculopapular rash, which preceded the development of myelosuppression. Six patients developed elevated AST and ALT while receiving therapy, but the ALT and AST levels returned to baseline during the rest period and did not exceed grade 2 toxicity.

Tumor Response

Patients were evaluated after the first two cycles and every two cycles thereafter. There were no objective minor or major responses observed, but 14 patients had prolonged stabilization of disease. The median duration of stable disease was 12 weeks (range, 12 to 48 weeks). At the 3-mg dose level, five of 19 patients achieved stable disease, including two patients who had prolonged stable disease (36 and 48 weeks). The majority of patients with stable disease had colorectal cancer (n = 10), but two patients with ovarian cancer also had disease stabilization.

Pharmacokinetics

None of the patients with DLT reached the day 21 sampling time. Therefore, to avoid biasing the pharmacokinetic analysis,

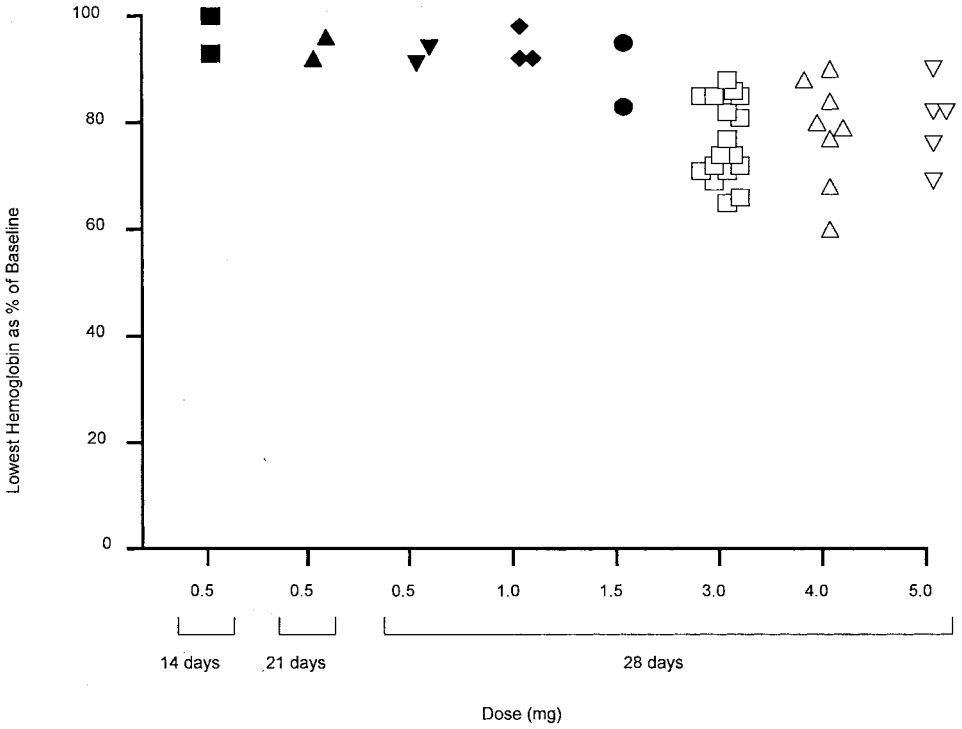

Fig 1. Relationship between dose and development of anemia. Symbols differentiate dose levels.

the pharmacokinetic analysis is limited to the day 1 pharmacokinetic samples that are available for all patients. ZD9331 concentrations were measured in 51 patients on day 1 (Table 4). A sample concentration-time profile of a patient at the 3-mg dose level is shown in Fig 3. Peak concentrations occurred within the first 2 hours, followed by a rapid decline. Some patients who had decreased Cl/F reached a plateau in the terminal part of the concentration-time curve. There was a rebound in concentrations seen at 6 to 8 hours, which may represent enterohepatic circulation. The mean terminal half-life at 3 mg/d was 25 ± 22 hours (range, 9 to 109 hours). The mean half-life is likely an underestimate of the true half-life; only 24-hour data were available on all patients because patients with an estimated long half-life came off study early, before the late pharmacokinetic sample times.

As the dose of ZD9331 increased from 3 to 5 mg (a 67% increase in dose), the mean AUC increased from 2,839 to 3,431 ng/mL · h (a 21% increase), and the mean peak concentrations were 206 ng/mL and 298 ng/mL, respectively (a 45% increase).

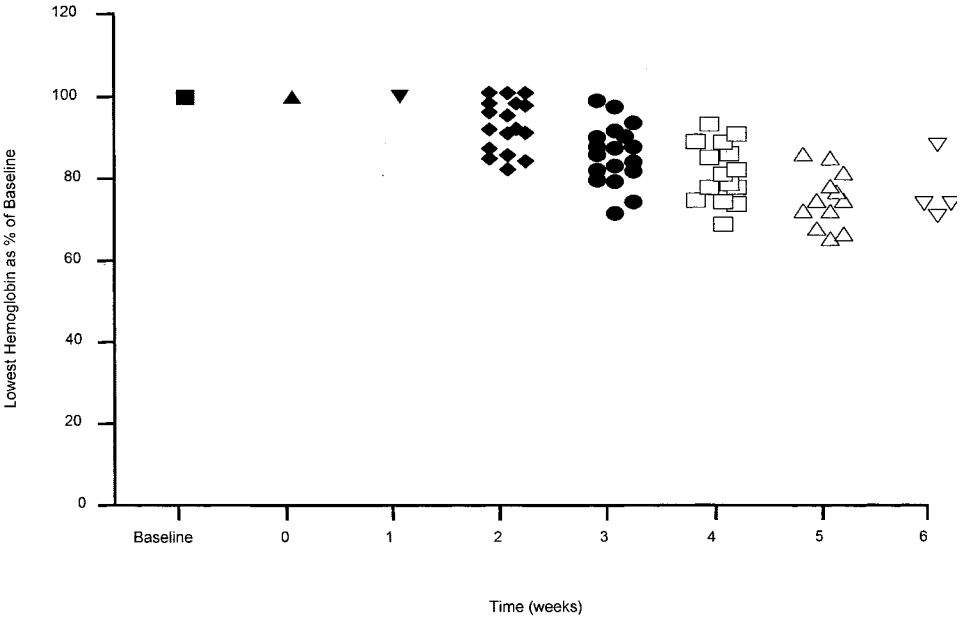

Fig 2. Time course of the development of anemia. Symbols differentiate time points.

Table 3. Common Toxicities at the 3 mg/d Dose Level (n = 19)

	Grade			
	1	2	3	4
Fatigue	8	9	1	
Nausea	14	1	1	
Anorexia	10	5	1	1
Constipation	10			
Alopecia	6	2		
Diarrhea	6	1		
Vomiting	5	1		
Increased AST	5	1		
Dyspnea	5			
Increased ALT	3	2		
Ankle edema	4			
Rash	4			
Mucositis	3			
Skin hyperpigmentation	2			
Fever	1		1	1
Increased bilirubin		1		

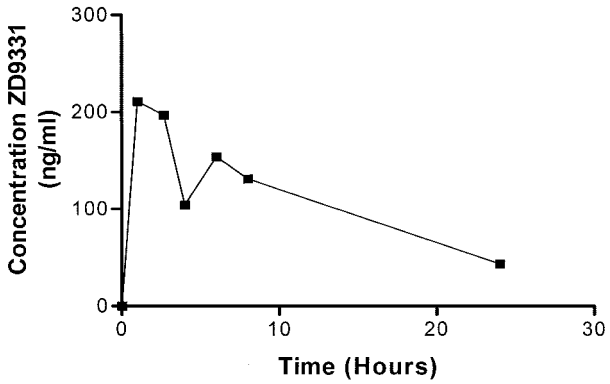

Fig 3. Typical concentration profile of ZD9331.

The Cl/F, AUC, C_{max}, and C_{min} for each dose level are listed in Table 4. At doses of ≥ 3 mg/d, there was no relationship between dose and the pharmacokinetic parameter AUC or C_{min}. Thus data for the 3-, 4-, and 5-mg dose levels were combined for the purpose of analysis of the determinants of pharmacokinetic variability. At doses ≥ 3 mg/d, there was no significant correlation between AUC and creatinine clearance ($r = 0.14$) but BUN was correlated with AUC ($r = 0.42$; $P = .007$). At doses ≥ 3 mg/d, C_{min} was significantly related to BUN ($r = 0.49$; $P < .001$) but not to creatinine clearance ($r = 0.20$).

Pharmacodynamics

In phase I studies, the large number of dose levels studied can confound analysis of determinants of pharmacodynamic variability.[13] Toxicity is usually correlated with dose administered and therefore dose level. The relationship between dose and AUC will lead to a false-positive association between AUC and toxicity unless the confounding issue of dose is removed. At the 3-, 4-, and 5-mg dose levels, there was no correlation between dose and AUC. The pharmacodynamic analysis was therefore confined to the 3-, 4-, and 5-mg dose levels to eliminate potential confounding by dose level.

Demographic characteristics, pharmacokinetic parameters, and organ function were examined for possible relationships with thrombocytopenia and anemia. In the univariate analysis, significant predictors of log platelet nadir were RBC folate, dose, C_{min}, half-life, AUC, clearance, bilirubin, BUN, and log C_{min} (Table 5). Serum folate, C_{max}, performance status, albumin creatinine clearance, BUN/creatinine ratio, age, and log baseline platelet count were not associated with log platelet nadir. In the multivariate analysis, only log C_{min} and BUN were correlated with log platelet nadir ($r^2 = 0.44$; $P < .001$).

In univariate analysis, significant predictors of log hemoglobin nadir were baseline hemoglobin, dose, C_{min}, bilirubin, albumin, creatinine clearance, and log C_{min}. Serum folate, C_{max} clearance, AUC, performance status, BUN, and BUN/creatinine ratio did not reach statistical significance. In multivariate analysis, significant predictors of log nadir hemoglobin were log baseline hemoglobin, albumin, and log C_{min} ($r^2 = 0.61$; $P < .001$).

AUC (during the first 24 hours), C_{min}, creatinine clearance, and BUN were examined as predictors for dose-limiting myelosuppression (Table 6). BUN levels were significantly higher in patients who developed dose-limiting myelosuppression compared with patients who did not (Student's t test, $P = .04$). In addition, C_{min} was higher in patients who developed dose-limiting myelosuppression than in patients who did not (Student's t test, $P = .03$).

DISCUSSION

The recommended phase II dose for ZD9331 is 3 mg/d for 4 weeks followed by a 2-week rest period. Some patients were able to tolerate ZD9331 doses of 4 and 5 mg/d without toxicity. Because preliminary pharmacokinetic analysis suggested that

Table 4. Pharmacokinetic Parameters

Dose Level (mg/d)	No. of Patients	Cl/F (mL/min)	AUC (ng/mL · h)	C_{min} (ng/mL)	C_{max} (ng/mL)
0.5	6	9.9 ± 4.8	423 ± 125	13 ± 6	32 ± 13
1.0	3	22.1 ± 9.5	414 ± 138	12 ± 4	34 ± 14
1.5	1	13.8	1203	27	96
3.0	23	12.1 ± 8.8	2,839 ± 857	79 ± 35	206 ± 66
4.0	12	10.0 ± 4.5	3,441 ± 1241	110 ± 50	215 ± 58
5.0	6	17.9 ± 8.2	3,431 ± 1045	80 ± 50	298 ± 70

Abbreviations: Cl/F, apparent oral clearance; AUC, area under the curve; C_{min}, minimum concentration; C_{max}, maximum concentration.

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