A Phase I Trial of ZD9331, a Water-Soluble, Nonpolyglutamatable, Thymidylate Synthase Inhibitor

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ABSTRACT

Purpose: ZD9331 is a novel, direct-acting antifolate cytotoxic that does not require polyglutamation for activity, and is a specific thymidylate synthase inhibitor. This Phase I trial aimed to determine the maximum tolerated dose of ZD9331, given as a 30-min i.v. infusion on days 1 and 8 of a 21-day cycle. Pharmacokinetic parameters and tumor response were also assessed.

Experimental Design: A total of 71 patients, with a range of solid malignancies and refractory to standard therapies (44% had received ≥3 prior chemotherapy regimens), were treated. The most common malignancies were colorectal cancer (35% of patients) and ovarian cancer (31%). ZD9331 was escalated from 4.8 mg/m²/day.

Results: Dose-limiting toxicity occurred at 162.5 mg/m² ZD9331, with grade 4 thrombocytopenia, grade 4 neutropenia lasting ≥7 days, and grade 3 nonhematologic toxicity. Plasma clearance of ZD9331 was slow and dose-dependent; however, ZD9331 pharmacokinetics were nonlinear. Pharmacodynamics of ZD9331 were determined by measurement of plasma deoxyuridine, which increased at all of the dose levels; dose-related increases in plasma deoxyuridine were significant (P = 0.003) on day 5. Stable disease was observed in 37% of patients; 23% of ovarian cancer patients had a ≥50% reduction in CA125 levels.

Conclusions: The maximum tolerated dose of this schedule was 130 mg/m². The toxicity profile at this dose was acceptable, with 7 of 28 patients treated developing grade 3/4 neutropenia and thrombocytopenia, 2 grade 4 diarrhea, and 2 grade 3/4 rash. This schedule was convenient and demonstrated activity in extensively pretreated patients; therefore, this is the recommended dose for study in Phase II trials.

INTRODUCTION

TS2 catalyzes the methylation of dUMP to TMP and is, thus, a key enzyme in the synthesis of nucleotides for DNA synthesis. Because the specific inhibition of TS2 may be expected to inhibit DNA synthesis selectively, the development of TS inhibitors has been an area of much interest in drug development. TS is the target enzyme for 5-fluorodeoxyuridine monophosphate, the active metabolite of 5-FU (6), although 5-FU is known to have other biochemical effects such as incorporation into nucleic acids, and is also the substrate for extensive in vivo catabolism. In recent years a number of folate-based inhibitors that act directly on TS have been investigated. The most successful of these is raltitrexed ("Tomudex"), which is licensed in a number of countries for the treatment of colorectal cancer (1). Structures of 5-FU, raltitrexed, and ZD9331 are shown in Fig. 1.

It is generally believed that it is necessary to expose tumor cells to antimetabolites for a number of days to inhibit proliferation over at least one cell cycle and, thus, achieve a cytotoxic effect. Folate-based TS inhibitors, such as raltitrexed, that are capable of forming intracellular polyglutamates can achieve this because of the retention of the polyglutamates within the cell. It has been proposed that the formation of polyglutamates also contributes to the therapeutic efficacy of such drugs, on account of the higher activity of FPGS in the tumor compared with the normal tissues (2). However, the activity of FPGS is high in the liver and may be high in bone marrow stem cells (3); therefore, the formation of polyglutamate stores may contribute to the sporadic toxicity seen with many classical antifolates. In addition, some instances of drug resistance may be because of reduced levels of, or mutant, FPGS in the tumor cell. These observations suggest that there may be a role for a compound that is not the subject of polyglutamation, both because it might increase the predictability of the toxicity, and because it might

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2 The abbreviations used are: TS, thymidylate synthase; 5-FU, 5-fluouracil; FPGS, folypolyglutamate synthetase; MTD, maximum tolerated dose; ULN, upper limit of normal; DLT, dose-limiting toxicity; AUCcycle, area under the curve for the treatment cycle; ZD9331, (2S)-2-{(2,7-dimethyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl)-N-[prop-2-ynyl]amino} benzamido)-4-(tetrazol-5-yl)butric acid.

3 Tomudex is a trade mark of the AstraZeneca group of companies.
Phase I Study of Thymidylate Synthase Inhibitor ZD9331

PATIENTS AND METHODS

Patient Eligibility. Before entry each patient underwent assessment with a full history, clinical examination, radiological staging, and laboratory evaluation. Eligibility criteria included: histological/cytological confirmation of a solid malignant tumor refractory to standard therapies; WHO performance status ≤2; aged ≥18 years; life expectancy >12 weeks; and written informed consent from the patient to participate in the trial. Approval from the relevant local ethical committees was obtained for each center before recruitment into the trial.

Major exclusion criteria included: inadequate bone marrow function (total white cell count <3.5 × 10⁹/liter, absolute neutrophil count <2.0 × 10⁹/liter, and platelet count <<100 × 10⁹/liter); serum bilirubin >1.25 × ULN; serum creatinine >1.25 × ULN; liver transaminases >2.5 × ULN in the absence of liver metastases or ≥5 × ULN in the presence of liver metastases; any evidence of severe or uncontrolled systemic disease; risk of transmitting HIV, or hepatitis B or C; systemic anticancer therapy within the past 4 weeks (6 weeks for nitrosoureas or mitomycin C); concomitant use of folic acid as a vitamin supplement; extensive radiotherapy within the last 6 weeks; >6 months of therapy within the last 12 months with chlorambucil, mitomycin C, or nitrosoureas; unresolved toxicity from prior anticancer therapy; incomplete recovery from prior surgery; active brain metastases; and pregnancy, breastfeeding, or woman of childbearing potential in whom pregnancy cannot be excluded. Patients were asked to abstain from alcohol 24 h before and after dosing.

Treatment Regimen. ZD9331 was supplied as a sterile isotonic 0.2% w/v aqueous solution in glass vials. The contents of each vial were diluted in 5% dextrose to a final concentration of between 0.0004 and 0.4 mg/ml. The solution was infused via a peripheral vein over 30 min on days 1 and 8 of a 21-day cycle.

The starting dose of ZD9331 was 4.8 mg/m²/day, selected from one of the previous Phase I trials (9). At least 3 patients were treated per dose level, the first patient at each dose level being evaluated for toxicity and pharmacokinetics over one full cycle before a patient was entered at the next dose level. Intrapatient dose escalation was permitted during the study after discussion with the project physician; such a patient was not permitted to be the first patient dosed at the new increased dose level. A two-stage dose escalation schedule was used. For stage 1, the dose was doubled until any drug-related neutropenia or thrombocytopenia, or any drug-related nonhematologic toxicity (excluding nausea, vomiting, alopecia, lethargy, and transient reversible rises in liver transaminases) was seen. For stage 2, the dose was then escalated based on a modified Fibonacci scheme, starting at a dose that was 67% higher than the last dose received in stage 1 and increasing until DLT was reached.

A given dose level was expanded to include an additional 3 patients if >1 of the initial 3 patients at that dose level experienced any of the following: grade 4 neutropenia with fever, grade 4 neutropenia without fever for at least 7 days duration, grade 4 thrombocytopenia, or any drug-related grade 3 or 4 nonhematologic toxicity not ameliorated by symptomatic measures (except transient reversible rises in liver transaminases). DLT was reached if ≥3 patients at a given dose level demonstrated toxicity as above on any cycle of treatment. This is a more aggressive definition than has been more habitually used in Phase I trials. MTD was defined as the dose below DLT that caused acceptable, manageable, and reversible toxicity, and this dose level was additionally expanded.

Before beginning each cycle of treatment, patients were required to meet the eligibility criteria regarding bone marrow,
liver, and kidney function. Dosing could be delayed for up to 3 weeks to allow the patient to meet the criteria, after which, if they had not, the patient was withdrawn from the trial. If any patient experienced grade 3/4 neutropenia or thrombocytopenia, or grade 2–4 nonhematologic toxicity (except nausea, vomiting, alopecia, lethargy, or transient reversible elevations of liver transaminases) during the first week of dosing in a cycle, the second dose (i.e., day 8 dose) of treatment was withheld and the cycle was not completed. The dose of ZD9331 given during the second and subsequent treatment cycles could be reduced if there was evidence of clinical benefit (complete or partial response or stable disease) together with significant toxicity in a previous cycle. If this occurred, the dose could be reduced to a lower dose level, which was given for all of the subsequent cycles.

**Pharmacokinetic and Pharmacodynamic Assessments.** Pharmacokinetic and pharmacodynamic parameters were determined as a secondary objective of the trial. Additional blood samples (3–5 ml) were drawn for this purpose during the first cycle of treatment. Blood samples were withdrawn for analysis before and after the doses on days 1 and 8 (predose, and 0.5, 1, 4, 8, 24, 48, 72, 96, and 168 h).

Plasma concentrations of ZD9331 were measured using liquid chromatography mass spectroscopy by the Drug Metabolism and Pharmacokinetics, Alderley Park, AstraZeneca. The following parameters were derived: \( AUC_{0-168\ h} \) after the first and second dose; and the accumulation ratio assessed from the \( AUC_{0-168\ h} \) after the second dose compared with the \( AUC_{0-168\ h} \) after the first dose. The maximum plasma concentration, the \( T_{1/2} \), plasma clearance, the steady-state volume of distribution, and the AUC cycle were also calculated.

As inhibition of TS leads to a rise in the levels of intracellular dUMP and, hence, plasma dUrd, the latter can be used as a surrogate for TS inhibition. During the trial an assessment of plasma dUrd levels was undertaken according to the method described by Mitchell et al. (10). Blood samples were withdrawn for analysis before and after the doses on days 1 and 8 (predose, and 24, 48, 72, and 96 h).

Urine was collected at the MTD up to 48 h after the first dose in cycle 1. The volume of urine was measured and an aliquot retained for analysis of ZD9331 concentrations.

**Toxicity Assessment.** Toxicity assessment was graded according to the NCI-CTC Version 2.0. Routine prophylactic antiemetics were not used.

**Efficacy Assessment.** Although this was a Phase I study, tumor lesions were assessed for response where possible. Objective antitumor activity was assessed every second cycle using standard response criteria based on Union Internationale Contre le Cancer/WHO criteria. A complete response was defined as no clinical, radiological, or biochemical evidence of residual lesions for >4 weeks. A partial response was defined as no evidence of disease progression with a >50% reduction in the sum of the products of the two largest perpendicular diameters of all of the measurable marker lesions maintained for >4 weeks. Progressive disease was defined as the appearance of a new lesion or a >25% increase in an existing lesion. Stable disease was defined as neither an objective response nor progression. Patients showing no evidence of disease progression continued treatment until withdrawal criteria were met.

**RESULTS**

**Patient Characteristics.** Patient characteristics are summarized in Table 1. A total of 74 patients were recruited of whom 3 did not receive treatment because of an acute, unexpected clinical deterioration between registration and first dosing. The remaining 71 patients, 40.8% of whom were male, received treatment. The mean age was 55 years (range, 34–75 years). The majority of patients had a performance status of 1 on entry. Patients with many solid tumor types were recruited into the trial, with a predominance of colorectal (35%) and ovarian (31%) carcinomas. All of the patients, except 2 patients with mesothelioma and 1 patient with gastric carcinoma, had received ≥1 prior chemotherapy regimen (Table 1).

**Dose Escalation.** A summary of the dose levels evaluated for toxicity and patient numbers treated is given in Table 2. The number of patients demonstrating defined DLT at each dose level is also given. DLT was first seen in patients dosed at 32 mg/m²/day, and appeared as neutropenia, thrombocytopenia, diarrhea, and skin rash. At two dose levels clinical decisions were taken to expand the dose level despite protocol-defined DLT having not been seen. The 69 mg/m² level was expanded to 6 patients because the first patient showed a transient NCI-CTC grade 4 myelosuppression, and a precautionary approach was taken in view of the toxicity seen at the previous dose level.
The 108 mg/m² level was expanded after the second patient to be entered suffered from an acute myocardial infarction 24 h after his first dose of drug. No additional evidence of cardiac toxicity was observed during the trial suggesting that this event was nondrug-related.

The dose at which DLT occurred was identified as 162.5 mg/m²/day. From an assessment of the toxicities seen during the escalation to DLT, MTD was set at 130 mg/m²/day, and this dose level was expanded to a total of 25 patients. In addition, 1 patient was dose escalated to this level, and 2 were dose reduced giving a total of 28 patients receiving ≥1 course at this dose. This gave adequate toxicity data to recommend a Phase II dose with the most frequent NCI-CTC grade 3/4 toxicities being neutropenia (7 patients), neutropenia (7 patients), vomiting (5 patients), skin rash (3 patients), and anemia (3 patients). Overall, ZD9331 130 mg/m² was shown to be well tolerated, and myelosuppression was generally manageable and short-lived.

Four patients had a dose escalation during the trial. Dose reduction was recommended only in patients showing prolonged or profound myelosuppression. A dose reduction was necessary in 12 patients, only 2 of whom required a reduction of >25% of starting dose to continue treatment. Dosing was delayed in 15 patients because of toxicity and in 12 patients for personal reasons. The day 8 dose was omitted in 45 of the 157 courses given. This was because of toxicity in 22 courses, disease progression, or personal reasons in 16 courses, and planned dose omission to avoid toxicity in 7 cases.

Hematologic Toxicity. Hematologic toxicity was predominantly neutropenia and thrombocytopenia (Table 3). Grade 3/4 neutropenia (duration, 10–31 days) was observed in 19 patients, of whom 3 patients experienced neutropenic fever [lasting for 2 days (2 patients) and 10 days (1 patient)]. DLT was not always seen after the first cycle at a given dose level and was occasionally seen after several cycles had been administered without toxicity [after the second cycle (2 patients) and fourth cycle (1 patient)]. Sporadic instances of grade 3/4 hematologic toxicity were seen at the lower dose levels in some patients; these levels were expanded and minimal toxicity seen in the subsequent patients. Seventy-one patients received up to 11 cycles of treatment with no evidence of cumulative bone marrow toxicity, 35 patients receiving 1–2 cycles, 25 patients receiving 2–4 cycles, and 11 patients receiving >4 cycles of treatment.

Nonhematologic Toxicity. Nonhematologic DLTs consisted of grade 3/4 diarrhea, rash, and (in 1 case) myocardial infarction. All of the drug-related NCI-CTC grade 3/4 nonhematologic toxicity is summarized in Table 4. In addition, grade 2 rash and rises in liver transaminases are shown. The most frequent nonhematologic event was asthenia.

Nausea and vomiting were generally mild despite the omission of routine prophylactic antiemetics. The majority of grade 3/4 emesis observed was considered to be disease-related, reflecting the preponderance of ovarian carcinoma and intra-abdominal disease.

Nonspecific fatigue was reported by almost all of the patients, with 2 patients requiring a dose reduction for disabling lethargy. A grade 2 maculopapular truncal rash was seen in a number of patients over a range of dose levels. This rash was more severe and generalized in 3 patients at 130 mg/m², and prevented repeat dosing in 2 patients.

Transient rises in liver transaminases were seen, as predicted from preclinical toxicology. Table 4 illustrates the level of toxicity, including NCI-CTC grades 2–4. Transaminase levels returned to or near baseline before redosing on day 21. In 1 patient who received 9 cycles of treatment, levels did not return to baseline after cycle 6, remaining at grade 1 toxicity until treatment stopped. Neither of the patients at higher doses who

<table>
<thead>
<tr>
<th>Dose (mg/m²/day × 2)</th>
<th>No. patients treated</th>
<th>No. patients with escalation*</th>
<th>No. patients with reductionb</th>
<th>No. patients with DLT</th>
<th>Description of DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>3</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>G4 neutropenia</td>
</tr>
<tr>
<td>9.6</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>G4 neutropenia</td>
</tr>
<tr>
<td>19.2</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>G4 neutropenia</td>
</tr>
<tr>
<td>32</td>
<td>5</td>
<td>—</td>
<td>1</td>
<td>1</td>
<td>G4 neutropenia</td>
</tr>
<tr>
<td>42</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>G4 neutropenia</td>
</tr>
<tr>
<td>55</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>G4 neutropenia</td>
</tr>
<tr>
<td>69</td>
<td>6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>G4 neutropenia</td>
</tr>
<tr>
<td>90</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>1a</td>
<td>G4 neutropenia</td>
</tr>
<tr>
<td>108</td>
<td>9</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>G3 rash</td>
</tr>
<tr>
<td>130(MTD)</td>
<td>25</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>G4 neutropenia</td>
</tr>
<tr>
<td>162.5</td>
<td>5</td>
<td>—</td>
<td>4</td>
<td>3</td>
<td>G3 diarrhea</td>
</tr>
</tbody>
</table>

* Patients may have a dose increase after first cycle.

b Patients may have a dose decrease after first cycle or a dose reduction within a cycle.

c Patients may have a dose increase after first cycle.

d Patient had severe toxicity at a dose of 130 mg/m² and recurrence of toxicity when dose reduced to 90 mg/m² in cycle 2.
received 8 or 11 cycles, respectively, demonstrated any cumulative toxicity. None of the above 3 patients had liver metastases.

There were no treatment-related deaths during this trial. Proposed Dose Omission to Avoid Toxicity. It was noted that patients who developed subsequent severe (NCI-CTC grade 3/4) drug-related hematologic and/or nonhematologic toxicity had experienced a decrease in hematologic indices by day 8, although they had reached the retreatment criteria for receiving the second dose of the cycle. Nadir hematologic values were most commonly observed around day 11.

A comparison was carried out of all hematologic indices on the two treatment dates of every cycle. It was found that a prediction of severe toxicity could be obtained by comparing ANC on day 8 to those on Day 1. There was no similar correlation with hemoglobin or the platelet count.

For cycles in which there was no severe drug-related toxicity the mean ANC day 1/day 8% value was 73.1 (SD 38.0), whereas for cycles associated with toxicity, the mean ANC day 1/day 8% was 42.8 (SD 26.2).

The possibility of using the percentage decrease in the day 8 neutrophil count to predict those patients who would subsequently experience severe toxicity was investigated. A decrease of ≥50% was taken as a practical point to divide the data. Table 5 demonstrates the numbers of individual cycles for which the ANC on day 8 was > or <50% of that on day 1 for that cycle, split into cycles with or without toxicity (any drug-related

Table 3  Hematologic toxicity (worst grade per patient)*

<table>
<thead>
<tr>
<th>Toxicity (NCI-CTC grade)</th>
<th>4.8 (n = 3)</th>
<th>9.6 (n = 4)</th>
<th>19.2 (n = 3)</th>
<th>32 (n = 5)</th>
<th>42 (n = 3)</th>
<th>55 (n = 8)</th>
<th>69 (n = 7)</th>
<th>90 (n = 4)</th>
<th>108 (n = 12)</th>
<th>130 (n = 28)</th>
<th>162.5 (n = 7)</th>
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<tbody>
<tr>
<td>Anemia</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Leukopenia</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Neutropenia</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

* Patients who received >1 dose could be counted in >1 column owing to dose escalation or reduction.

Table 4  Nonhematologic toxicity (worst grade per patient; drug-related)

<table>
<thead>
<tr>
<th>Toxicity (NCI-CTC grade)</th>
<th>4.8 (n = 3)</th>
<th>9.6 (n = 4)</th>
<th>19.2 (n = 3)</th>
<th>32 (n = 5)</th>
<th>42 (n = 3)</th>
<th>55 (n = 8)</th>
<th>69 (n = 7)</th>
<th>90 (n = 4)</th>
<th>108 (n = 12)</th>
<th>130 (n = 28)</th>
<th>162.5 (n = 7)</th>
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<tr>
<td>Asthenia</td>
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<td>5</td>
<td>3</td>
<td>8</td>
<td>16</td>
<td>6</td>
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</tr>
<tr>
<td>Diarrhea</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Elevated ALT/AST</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
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<td>2</td>
<td>2</td>
<td>2</td>
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</tr>
<tr>
<td>Nausea</td>
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<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
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<td>1</td>
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</tr>
<tr>
<td>Rash</td>
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<td>1</td>
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</tr>
<tr>
<td>Vomiting</td>
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<td>3</td>
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<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
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</tr>
</tbody>
</table>

* ALT, alanine aminotransferase; AST, aspartate aminotransferase.
NCI-CTC grade 3/4 toxicity, excluding transient, asymptomatic rises in liver transaminases). Patients having >50% reduction in ANC on day 8 had a 39% (21 of 54 cycles) chance of going on to develop severe toxicity, whereas those with <50% reduction had a 10% (10 of 103 cycles) chance of toxicity during that cycle ($\chi^2 = 17; P < 0.001$).

By using this method, the day 8 dose would have been withheld (and toxicity possibly avoided) in 70% (21 of 31) of cycles with toxicity, and in 21% (33 of 157) of cycles a dose would have been omitted where it could safely have been given.

As a result of this analysis, a decision was made to define a 50% decrease in the ANC on day 8 as a criterion for omitting the day 8 dose during the MTD expansion phase of the trial. A total of 60 courses were given at the MTD, and in 21 of these the day 8 dose was omitted. This was for disease progression or personal reasons in 10 courses, and because of unresolved hematologic toxicity in 6 cases. For 44 courses the patients were fit to receive the day 8 dose, but this was omitted in 5 patients because of a 50% fall in ANC. This led to an actual dose intensity of 123 mg/m$^2$ compared with the planned 130 mg/m$^2$. A 5.4% dose intensity reduction in an attempt to improve the safety profile of the drug.

**Pharmacokinetic Data.** Mean plasma pharmacokinetic parameters are shown in Table 6. Plasma levels of ZD9331 were assessed on the first cycle of treatment in the majority of patients (68 patients). Low levels of ZD9331 were detectable in the plasma before the second dose of the cycle (Day 8), but no appreciable accumulation of the drug was observed. Clearance of ZD9331 from the plasma was slow and dose-dependent; there was a 3-fold increase from a mean of 7 ml/min at the lowest dose (4.8 mg/m$^2$) to 20 ml/min at the MTD (130 mg/m$^2$). Renal clearance was found to be an important route of elimination; however, the rate of clearance was low compared with overall glomerular filtration rate and increased with dose. This observation is interesting and is under investigation in ongoing trials. The percentage of drug cleared by the kidneys may be quite variable. ZD9331 has a low volume of distribution (mean steady-state volume of distribution 25 l), which was independent of dose. The $T_{1/2}$ was long with a mean value for dose 1 of 32.2 h at the MTD (130 mg/m$^2$). There was no clear correlation between exposure of ZD9331 (as assessed by AUC$_{cycle}$) and either toxicity or best overall objective response.

**Pharmacodynamic Data.** Plasma dUrd measurements were made on 66 patients during the first cycle (4.8–162.5 mg/m$^2$). Most of these patients had full sampling on days 2–5 (98% of samples available and successfully processed). The majority (80%) also had samples taken on day 8 (pre second dose). Day 8 sampling was not always carried out if the second dose was omitted. Additional samples were taken on days 9–11 (92%) from those patients who had received the second dose. In some patients, other samples were available for measurement (commonly, days 12, 15, and 22).

The results are generally the mean of duplicate plasma extraction and high-performance liquid chromatography analysis. However, duplicate plasma extractions were not always possible because of limited quantities of plasma.

All of the patients had elevated dUrd in their plasma at some time after their first dose. The mean dUrd levels measured on days 2, 5, 8, and 9 are shown in Fig. 2. It is clear that the most marked elevations occurred on day 2 (1 day after dosing on day 1; Fig. 2A) and on day 9 (1 day after dosing on day 8; Fig. 2D), although the levels remained above baseline before receiving the second dose on day 8 (Fig. 2C). Visual inspection of the curves in Fig. 2 suggests that there is a dose-related increase in the dUrd elevation particularly at the lower doses. However, linear regression analyses only reached significance for the day 5 samples (Fig. 2B). Results were not significantly different when the analysis was restricted to doses in the range 4.8–42 mg/m$^2$ (data not shown). Within each dose level the data spread is wide and the number of patients variable; therefore, it is difficult to correlate dose with percentage increase in dUrd. Furthermore, the percentage increase in dUrd is partly related to the pretreatment dUrd; therefore, it is generally higher when pretreatment levels are low.

At doses of 4.8 and 9.6 mg/m$^2$, day 5 plasma dUrd was at or below the pretreatment values, whereas above this dose, the majority of patients still had elevated dUrd at this time (Fig. 2B). By day 8, plasma dUrd was always lower than the peak level, but in some patients it was still above pretreatment levels (Fig. 2C). This was not apparently related to dose. Twenty-four h after the second dose (day 9), plasma dUrd was again raised in most patients (Fig. 2D). In some patients this was more apparent on days 10 or 11 (data not shown). Only 3 treated patients did not have plasma dUrd levels above their day 8 level (although still elevated above pretreatment). One of these was at the 130 mg/m$^2$ dose level and also had a very small increase in plasma dUrd after the first dose.

Three dose levels are illustrated more fully in Fig. 3. At the 130 mg/m$^2$ dose, only those who received ZD9331 on both days 1 and 8 are included ($n = 3–12$ per time point; for later time points plasma was not always available for analysis). Notably, the dUrd elevation at the 4.8 mg/m$^2$ dose (3 patients) was of relatively short duration after each dose (~48 h).

Taken together, these data suggest that the level and duration of dUrd elevation did increase with dose. Without more data at the lower dose levels, it is difficult to assess whether a threshold level of dUrd is reached. There is some suggestion that at ~32 mg/m$^2$ and above, a consistent pattern of dUrd elevation is observed.

These data confirm that ZD9331 TS inhibition occurs, at least to some extent, at all of the dose levels investigated (4.8–162.5 mg/m$^2$). However, the duration of TS inhibition is probably of longer duration at the higher doses. The dUrd measured is largely the product of TS inhibition in normal proliferating tissues, and it will be interesting to correlate tox-

**Table 5** Number of cycles with percentage fall in neutrophils on day 8 of > or <50% compared with day 1 versus observed toxicity$^a$

<table>
<thead>
<tr>
<th></th>
<th>&gt;50% decrease</th>
<th>&lt;50% decrease</th>
<th>Total no. cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles with no toxicity</td>
<td>33</td>
<td>93</td>
<td>126</td>
</tr>
<tr>
<td>Cycles with toxicity$^b$</td>
<td>21</td>
<td>10</td>
<td>31</td>
</tr>
</tbody>
</table>

$^a$ Data from all except 2 patients, patients with a >50% reduction in neutrophil count had a greater probability of developing toxicity compared with those with a lesser decrease ($P < 0.001$).

$^b$ Patients who developed any drug-related NCI-CTC grade 3 or 4 toxicity (excluding transient, asymptomatic rises in liver transaminases).
icity with dUrd levels. Data presented elsewhere have also correlated plasma ZD9331 levels with plasma dUrd (11).

**Antitumor Activity.** Of the 71 patients who received treatment, 39 with marker lesions were evaluable for tumor response and had received multiple cycles. Marker lesions were identified and measured in two dimensions on the appropriate radiological images by an independent radiologist. Twenty-six (37%) patients had stable disease for 42 days during the trial. Documented stable disease for 4 cycles of treatment (3 months) was observed in 10 patients including: a patient with ovarian cancer (complete response also observed in nonmarker lesion) who had previously progressed through carboplatin and paclitaxel treatment and a patient with metastatic malignant melanoma who received a total of 10 cycles of treatment (49% reduction in lung and right scapular metastases). This patient, who had been treated previously with temozolomide and immuno-therapy, remained well and did not require treatment for progressive disease for an additional 9 months. A 63% reduction in tumor size was observed in the marker lesions of a patient with liver metastases from colorectal cancer; this was maintained over 6 cycles of treatment. This patient had previously received 5-FU with mitomycin-C and irinotecan. All of the other patients with colorectal cancer showing disease stabilization had been treated previously with 5-FU and/or raltitrexed using one or more established schedules, and were considered resistant to these agents.

Fifty-seven patients had evaluable disease with nonmarker lesions alone or in addition to their evaluable disease. Four patients with evaluable disease only had prolonged disease stabilization on treatment.

**Table 6** Mean plasma pharmacokinetic parameters after a 30-min infusion of ZD9331 given once weekly for 2 weeks every 3 weeks

<table>
<thead>
<tr>
<th>Dose (mg/m²/day × 2)</th>
<th>No. patients evaluated</th>
<th>AUC&lt;sub&gt;cycle&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; (µg/h/ml)</th>
<th>Clearance (ml/min)</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>V&lt;sub&gt;ss&lt;/sub&gt; (l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First dose</td>
<td>Second dose</td>
<td>First dose</td>
<td>Second dose</td>
</tr>
<tr>
<td>4.8</td>
<td>3</td>
<td>39.7</td>
<td>7.37</td>
<td>6.59</td>
<td>31.3</td>
</tr>
<tr>
<td>9.6</td>
<td>3</td>
<td>66.9</td>
<td>8.47</td>
<td>8.35</td>
<td>30.4</td>
</tr>
<tr>
<td>19.2</td>
<td>3</td>
<td>107</td>
<td>8.87</td>
<td>9.80</td>
<td>30.7</td>
</tr>
<tr>
<td>32.0</td>
<td>5</td>
<td>161</td>
<td>12.3</td>
<td>8.78</td>
<td>30.9</td>
</tr>
<tr>
<td>42.0</td>
<td>3</td>
<td>176</td>
<td>13.8</td>
<td>15.8</td>
<td>31.0</td>
</tr>
<tr>
<td>55.0</td>
<td>6</td>
<td>165</td>
<td>17.2</td>
<td>17.6</td>
<td>31.1</td>
</tr>
<tr>
<td>69.0</td>
<td>6</td>
<td>322</td>
<td>15.6</td>
<td>12.0</td>
<td>31.2</td>
</tr>
<tr>
<td>90.0</td>
<td>3</td>
<td>312</td>
<td>18.3</td>
<td>17.4</td>
<td>31.3</td>
</tr>
<tr>
<td>108.0</td>
<td>7</td>
<td>342</td>
<td>18.1</td>
<td>18.7</td>
<td>31.4</td>
</tr>
<tr>
<td>130.0</td>
<td>18</td>
<td>411</td>
<td>18.6</td>
<td>23.3</td>
<td>31.5</td>
</tr>
<tr>
<td>162.5</td>
<td>5</td>
<td>595</td>
<td>17.0</td>
<td>17.6</td>
<td>31.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Geometric mean.

**Fig. 2** Mean levels of dUrd for day 2, 5, 8, and 9 (A–D, respectively); bars, ± SE. Linear regression of percentage elevation with respect to dose showed: (A) \( r = 0.56, P = 0.08 \); (B) \( r = 0.80, P = 0.003 \); (C) \( r = 0.24, P = 0.47 \); and (D) \( r = 0.19, P = 0.58 \).
DISCUSSION

Four Phase I studies using various schedules of i.v. ZD9331 have been conducted. This study determined the MTD of ZD9331, given as a 30-min infusion on days 1 and 8 of a 21-day cycle, to be 130 mg/m². Three of 5 patients entered at the next dose level tested, 162.5 mg/m², had DLT, principally myelosuppression. This is consistent with results from the study by Rees et al. (8) in which patients received ZD9331 as a 5-day continuous infusion, every 21 days; DLT was neutropenia, thrombocytopenia, and diarrhea. Myelosuppression was also the main DLT in the study by Goh et al. (7) in which patients received ZD9331 as a 30-min infusion on 5 consecutive days every 21 days.

In the design of the reported trial an aggressive definition of DLT was used with the toxic dose being reached if ≥3 of 6 patients at a given dose level demonstrated the defined toxicity during any cycle of treatment. However, many agents in common use (e.g., docetaxel) are frequently dosed at levels that induce grade 3/4 toxicity in ~40% of patients, indicating that there is an increasing acceptance of strongly myelosuppressive protocols. It could be argued that failure to reach retreatment hematologic indices on day 8 could have been included in the definition of DLT. This was not considered during design of the protocol, and, therefore, during the conduct of the study an attempt was made to account for the unexpected interpatient variability in the degree of myelosuppression and tolerance of the drug over many dose levels. Grade 4 toxicities were seen at

Figure 3 PlasmadUrd levels over time for dose levels 4.8, 42, and 130 mg/m²; bars, ±SD.
dose levels ranging from 32 mg/m² to 130 mg/m², but not with sufficient frequency to meet the criteria for DLT.

In the present study, the 130 mg/m² dose level was expanded (n = 28) to assess the tolerability of this schedule of ZD9331. The most frequent NCI-CTC grade 3/4 adverse events at this dose level included: thrombocytopenia (7 patients), neutropenia (7 patients), leukopenia (6 patients), vomiting (5 patients), skin rash (3 patients), and anemia (3 patients). Overall, ZD9331 130 mg/m² was shown to be well tolerated; myelosuppression was generally manageable and short-lived. Seven patients experienced grade 2–4 skin rash, which is in line with results from other i.v. Phase I trials of ZD9331 (7, 8) and also the preliminary results of a Phase I trial of oral ZD9331 in which skin rash was a principal DLT (12).

The pharmacokinetics of ZD9331, observed with this once-weekly for 2 weeks and every 3 weeks schedule, correspond with results from the other Phase I i.v. ZD9331 studies. The T₁/₂ was long; T₁/₂ ranged from 30.2 to 65.7 h after the first dose and 33.0 to 88.2 h after the second dose of ZD9331. This compares with 52–135 h observed in the trial evaluating ZD9331 as a single 30-min infusion every 21 days (13) and a T₁/₂ of 35.5–117.0 h when ZD9331 was administered as a 30-min infusion on 5 consecutive days, every 21 days (7). In contrast, nolatrexed, a nonpolyglutamatable TS inhibitor, has a short T₁/₂ (53–193 min), and as a result the infusion time had to be extended from 24 h to 5 days to achieve either antiproliferative toxicity or antitumor responses (14, 15). In this Phase I trial of ZD9331 there was no correlation observed between toxicity and AUC, but this may be because this is not the appropriate parameter to measure. With other antimetabolites the time above a threshold concentration is more likely to determine toxicity, as this correlates better with the duration of target inhibition. It would be useful to carry out prospective studies at a standard dose in the Phase II setting to determine whether correlations are observed between toxicity and drug clearance. The reason for the increased clearance with increasing dose of ZD9331 is unknown; it is possible that reabsorption from the renal tubule is saturated at higher doses or that drug protein binding is saturated at high doses causing increased excretion.

During Phase I studies of nolatrexed the plasma level of dUrd was used as a surrogate for TS inhibition, and it was possible to show that a 5-day infusion was necessary to maintain inhibition of the target enzyme (14, 15). In comparison, in the case of ZD9331, the plasma half-life in humans is sufficient to maintain exposure to the drug for several days, representing a great improvement in convenience for administration. Data on plasma dUrd levels, collected during the present study, show that TS inhibition was maintained for several days after a single dose of ZD9331 (Figs. 2 and 3; Ref. 11). The levels of plasma dUrd only started to approach baseline levels at the time the day-8 dose was due to be given. The prolonged pharmacokinetics of ZD9331 offer a clinical advantage in that repeated doses or infusions are not necessary for clinical effect, but a disadvantage in that variability in the levels may be responsible for the somewhat sporadic myelosuppression seen at various dose levels (perhaps because of interpatient variability in clearance). If future studies demonstrate a correlation between toxicity and plasma dUrd levels one approach to alleviate the interpatient variability in toxicity would be to monitor the plasma levels of dUrd, and to use these to identify patients at risk of toxicity and to omit the day-8 dose in these patients. A second, more pragmatic approach, described herein, uses the percentage of fall in neutrophils from day 1 to day 8 as a pharmacodynamic marker for each patient, allowing the second dose to be withheld if there is a 80% fall. This approach minimizes the occurrence of severe toxicity and is, therefore, a practical approach to dose individualization for susceptible patients.

Preliminary evidence of antitumor activity has been reported in Phase I studies of ZD9331 in a range of tumor types (8, 16). Similarly, in this study encouraging activity was observed in a number of patients; stable disease was observed in 37% of patients. Three of these patients showed some evidence of a response to treatment during the trial; however, none fulfilled the criteria for a partial response. In particular, there are indications that this agent may show useful activity in colorectal carcinoma, reinforcing the important role of antifolate treatment of this disease (16). In 5 patients with ovarian carcinoma a decrease in CA125 was observed; however, it is not clear what the significance is of this rapid fall in CA125, because this was only mirrored by radiological disease response in 1 of these patients, although symptomatic benefit was reported in 3 of the others. A fall in CA125 had been reported previously in a patient with ovarian cancer, who also demonstrated a partial response on a computed tomography scan to ZD9331 administered as a 5-day continuous infusion every 21 days (8). Recent Phase II studies of ZD9331 (130 mg/m² as a 30-min infusion on days 1 and 8, every 21 days) in patients with advanced colorectal or ovarian cancer have reported evidence of antitumor activity (17, 18). In the colorectal cancer trial 2 patients (4.5%) receiving ZD9331 as third-line treatment experienced a partial response, and an additional 21 third-line patients (47.7%) had stable disease (17). Furthermore, 1 patient receiving ZD9331 as eighth-line treatment for ovarian cancer achieved a complete response, and 1 fourth- and 1 fifth-line patient experienced disease stabilization (18).

This schedule appears to offer both convenience and an acceptable safety profile. Therefore, a dose of 130 mg/m² on days 1 and 8, repeated every 3 weeks, is the recommended dose for Phase II studies. Phase II evaluation in several tumor types is ongoing. Because ZD9331 is a direct-acting cytotoxic, evaluation of ZD9331 in combination with other agents is warranted, and such studies are ongoing.

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A Phase I Trial of ZD9331, a Water-Soluble, Nonpolyglutamatable, Thymidylate Synthase Inhibitor

Ruth Plummer, Charlotte Rees, Andrew Hughes, et al.


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