Cancer Chemother Pharmacol (2004) 53: 357–360
DOI 10.1007/s00280-003-0735-4

CLINICAL TRIAL REPORT

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Phase I trial of the antifolate ZD9331 in combination with cisplatin in patients with refractory solid malignancies

Received: 21 July 2003 / Accepted: 3 October 2003 / Published online: 13 January 2004

Abstract *Purpose*: To determine the maximum tolerated dose and dose-limiting toxicities (DLTs) of ZD9331 in combination with cisplatin in patients with refractory solid tumors and to describe any preliminary antitumor activity associated with this regimen. *Materials and methods*: Patients received combination therapy with ZD9331 as a 30-min infusion on days 1 and 8 of a 21-day cycle at doses of 100 or 130 mg/m^2, followed by cisplatin at 50 or 75 mg/m^2 as a 30- to 60-min infusion on day 1 only. *Results*: A total of 16 patients received 59 cycles of ZD9331 and cisplatin. Patients were enrolled at three dose levels: ZD9331/cisplatin 100/50 ($n=3$), 130/50 ($n=9$), 130/75 ($n=4$). DLTs at 130/75 included thrombocytopenia, neutropenia, fatigue, nausea, vomiting and stomatitis. Among 15 evaluable patients, 2 showed a partial response (patients with mesothelioma and head and neck cancer) and 6 showed stable disease (for a median of 5.5 cycles). *Conclusions*: ZD9331 in combination with cisplatin was well tolerated at a dose of 130/50 mg/m^2 after establishing the principal DLTs of neutropenia and thrombocytopenia. The combination shows evidence of antitumor activity in a pretreated population.

Keywords ZD9331 · Cisplatin · Antifolate · Folylpolyglutamate synthetase

This work was supported by a grant from AstraZeneca.

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Introduction

ZD9331 is a novel antifolate that inhibits thymidylate synthase (TS), an endogenous enzyme responsible for catalyzing the reductive methylation of deoxyuridine monophosphate (dUMP) to thymidine monophosphate. TS inhibition prevents the formation of thymidine nucleotides essential for DNA synthesis and repair [1]. Moreover, TS inhibition, by blocking uridine deoxynucleotide conversion to thymidine, results in expansion of 2′-deoxyuridine 5′-triphosphate (dUTP) pools, which has been shown to cause the inappropriate incorporation of uridine nucleotides into DNA [8]. The subsequent removal of these nucleotides results in DNA strand breaks, which may contribute to cell death. 5-Fluorouracil (5-FU), which has been used in cancer treatment for more than 40 years, has been the model for TS inhibition as an anticancer strategy. In the last several years, alternative TS inhibitors have been developed in an effort to minimize toxicity and overcome mechanisms of 5-FU resistance. It has been hypothesized that some 5-FU toxicity could be attributed to putative 5-FU–RNA interactions, which could be avoided by more targeted agents [3]. Similarly, many novel TS inhibitors in development have different means of activation and transport than 5-FU, perhaps offering opportunities for activity in 5-FU-resistant tumors [9, 10, 17].

ZD9331 is an antifolate that does not require polyglutamation and is, therefore, designed to be active against cells deficient in folylpolyglutamate synthetase (FPGS). FPGS is an intracellular enzyme that adds glutamate residues to naturally occurring folates to promote their intracellular retention and to enhance their affinity for enzymes in their function as cofactors. The enzyme also catalyzes the polyglutamylation of many investigational antifolates, such as raltitrexed (Tomudex), which in turn contributes to their activity [10]. Indeed, ZD9331 has been shown to be active against FGPS-deficient cells resistant to raltitrexed [9].

Several published phase I studies of ZD9331 as a single agent have demonstrated that the drug has a manageable toxicity profile and may have promising antitumor activity [4, 5, 7, 12, 13, 16]. In three published studies, myelosuppression, namely thrombocytopenia and neutropenic fever, emerged as the dose-limiting toxicities (DLTs) [4, 7, 13], although stomatitis and skin rash have also been observed [4, 13].

The objectives of this phase I study were to explore the tolerability and antitumor activity of ZD9331 in combination with cisplatin, a first generation platinum compound with activity in many human cancers. Cisplatin acts by binding to a free nitrogen atom in purine base pairs to form a stable adduct, which in turn impairs transcription, replication and DNA repair [6]. The DLT of cisplatin is nephrotoxicity while that of ZD9331 is myelosuppression. Thus, cisplatin and ZD9331 have both complementary toxicity profiles and mechanisms of action, making them promising candidates for a combination regimen. Moreover, likely synergy between cisplatin and other antifolate drugs has been shown in both preclinical models and numerous clinical studies [15]. We elected to give ZD9331 on days 1 and 8 of a 21-day cycle and cisplatin on day 1, by analogy to commonly used regimens incorporating methotrexate and other antifolates in combination with cisplatin.

Materials and methods

Study design

This study was a nonrandomized, noncomparative, dose-escalating phase I combination trial of the intravenous formulation of the investigational agent ZD9331, coadministered with intravenous cisplatin in adults with refractory solid tumors. It was designed primarily to assess the DLT and maximum tolerated dose (MTD) of this combination, with a secondary endpoint of describing antitumor activity. The trial was designed and conducted in accordance with the ethical principals of Good Clinical Practice. It was approved by the Institutional Review Board of the Hospital of the University of Pennsylvania. All patients gave written informed consent prior to entering the study.

Study group

To be eligible for the study, patients were required to have a histologically or cytologically confirmed solid malignant tumor refractory to treatment or for which no effective treatment existed. Patients were also required to have measurable disease, a life expectancy of more than 12 weeks, World Health Organization (WHO) performance status of 0 or 1, age ≥18 years and the ability to provide written informed consent [11]. Patients were excluded if they had inadequate bone marrow reserve (neutrophils $<1.5\times10^9$/l, platelets $<100\times10^9$/l), inadequate liver function (total bilirubin ≥1.25 times the upper limit of the reference range (ULRR), alanine aminotransferase or aspartate transferase >2.5 times ULRR or >5 times ULRR in the presence of liver metastasis) or inadequate renal function (creatinine clearance of <80 ml/min). Patients were also excluded if they had had systemic anticancer therapy within 4 weeks of beginning the study (6 weeks for nitrosoureas or mitomycin C), though antiandrogen therapy for hormone-refractory prostate cancer was permitted. Patients were instructed not to use folic acid supplements within 24 h of receiving ZD9331.

Therapy

ZD9331 was given as a 30-min i.v. infusion on days 1 and 8 of a 21-day cycle. Cisplatin was administered after ZD9331 as a 30- to 60-min infusion on day 1. Patients were treated as outpatients at one of three dose levels. The starting doses of ZD9331 and cisplatin were 100 mg/m^2 and 50 mg/m^2, respectively. The second and third dose levels were 130/50 mg/m^2 and 130/75 mg/m^2 for ZD9331 and cisplatin, respectively. Dose escalations were based on the occurrence of DLTs, defined as drug-related adverse events and graded according to the National Cancer Institute Common Toxicity Criteria 2.0. DLTs were defined as any of the following: absolute neutrophil count $<0.5\times10^9$/l associated with fever, absolute neutrophil count $<0.5\times10^9$/l lasting longer than 5 days, platelet count $<25\times10^9$/l, grade 3 or 4 nonhematological toxicity that was not ameliorated by symptomatic measures (except reversible elevations of ALT or AST), inability to administer the day-8 dose of ZD9331, or treatment delay longer than 2 weeks due to toxicity. Only events occurring during the first cycle at a dose level were considered as DLTs. If a DLT was observed in none of three patients at a dose level, then escalation to the next dose level was permitted. If a DLT was observed in one of the first three patients at a dose level, then three additional patients were treated at that level. If two or more patients experienced a DLT at a dose level, then that dose level was deemed the maximum dose level to be tested. If a DLT occurred, repeated cycles could be delayed for up to 14 days (total 35 days between starting cycles). Intrapatient ZD9331 doses could be adjusted for those experiencing a decline in creatinine clearance.

Endpoints and statistical methods

Upon enrollment in the study, patients underwent a complete medical history and physical examination. A laboratory assessment was also performed that included a complete blood cell count, a comprehensive metabolic panel, liver function tests, urinalysis and if appropriate tumor markers (e.g. CA-125, PSA, CEA). An ECG was also performed. Additional laboratory samples were obtained before each dose on day 1 and day 8 and again on day 15, upon withdrawal from the study and 30 days following the last day of dosing in the final cycle of treatment. Tumor evaluations were performed at screening and then after every subsequent two cycles during treatment and upon withdrawal from trial therapy, according to revised (May 1999) World Health Organization (WHO) definitions (RECIST Criteria). Patients were removed from the study upon progression of their disease.

The original sample size determination conformed to the standard 3–6 rule, whereby continuous toxicity assessments detect the occurrence of more than one DLT in the first three patients enrolled per dose level and if present, prevent an additional three patients from being enrolled.

Results

Patients

A total of 16 patients were enrolled in the study, including 9 men and 7 women. Their median age was 56 years (range 23–71 years). All patients were either asymptomatic or minimally limited by their disease (PS 1). Patients had received a median of 3.5 prior chemotherapy regimens. The most common tumor type was mesothelioma (five), followed by colorectal (four), lung (two), head and neck (two), breast (one), osteosarcoma (one) and prostate (one) cancers. The total number of assessable courses was 59 and the median number of cycles was three (range one to nine).

Dose-limiting toxicity

Patients were enrolled at three combination dose levels: (1) ZD9331 at 100 mg/m^2 and cisplatin 50 mg/m^2 (three patients); (2) ZD9331 at 130 mg/m^2 and cisplatin 50 mg/m^2 (nine patients); (3) ZD9331 at 130 mg/m^2 and cisplatin 75 mg/m^2 (four patients). No DLTs were observed at the first two dose levels. At the third dose level, three of four patients experienced grade 3 and 4 neutropenia (Table 1). One patient experienced grade 4 neutropenia requiring hospitalization, a second patient experienced grade 4 neutropenia requiring treatment delay and a third patient demonstrated grade 3 neutropenia requiring treatment delay. Because of these DLTs, the 130/50 mg/m^2 ZD9331/cisplatin cohort was expanded to include six additional patients, for a total of nine patients treated at that dose level. At the expanded dose level, a single patient with colorectal cancer, who had previously been treated extensively with four commercially available chemotherapy agents and two investigational drugs, experienced grade 4 neutropenia and thrombocytopenia in cycle 1. There did not appear to be significant cumulative neutropenia in subsequent cycles (data not shown).

Though this study required an ANC of at least 1.5×10^9/l upon enrollment, the patient population was heavily pretreated with a median number of prior chemotherapy regimens of 3.5. Prior exposure to a taxane or platinum derivative did not correlate with the degree of neutropenia, though this study was not powered to detect such a relationship (data not shown). Moreover, these toxicities were seen in all cycles of therapy, and there was no clear trend of cumulative toxicity.

Other toxicities of note included an episode of grade 3 stomatitis in a patient at the first dose level and an instance of grade 2 stomatitis at the second dose level (Table 2). There was little additional gastrointestinal toxicity. One patient experienced grade 1 hearing loss in her sixth cycle at the first dose level.

Antitumor activity

Of the 16 patients, 15 were assessable for tumor response. A patient with mesothelioma previously treated with two courses each of gemcitabine/cisplatin, gemcitabine alone, and Adriamycin experienced a partial response at the first dose level. This patient remained on therapy for nine cycles. A second patient at the second dose level with head and neck cancer, previously treated with carboplatin/Taxol and radiation therapy, also demonstrated a partial response and received five cycles of treatment.

A total of six patients experienced stabilization of their disease: one patient at the first dose level, two at the second dose level and three at the third dose level. These patients had the following diagnoses: non-small-cell lung cancer, mesothelioma, colorectal cancer (two), breast cancer and prostate cancer. Patients with stable disease remained on therapy for a median of 5.5 cycles (range 4–6).

Discussion

The combination of ZD9331, a novel antifolate TS inhibitor, and cisplatin was studied in 16 patients with advanced cancer. Myelosuppression was observed in most patients, and neutropenia was dose limiting

Table 1 Hematological toxicity during cycle 1

Dose level	No. of patients	Neutropenia grade					Thrombocytopenia grade				
		0	1	2	3	4	0	1	2	3	4
1	3	1	1	–	1	–	1	1	–	1	–
2	9	5	–	1	2	1	3	3	–	2	1
3	4	1	–	–	1	2	1	3	–	–	–

Table 2 Selected nonhematological toxicities (selected based on clinical significance and frequency)

Toxicity	No. (%) of courses				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Stomatitis	51 (86)	1 (2)	5 (8)	2 (3)	–
Fatigue	24 (41)	19 (32)	12 (20)	3 (5)	1 (2)
Nausea	40 (68)	16 (27)	–	3 (5)	–
Vomiting	46 (78)	8 (14)	1 (2)	4 (7)	–
Diarrhea	54 (92)	3 (5)	–	2 (3)	–
Constipation	53 (90)	5 (8)	1 (2)	–	–
Hearing loss	55 (93)	4 (7)	–	–	–
Altered taste	58 (98)	1 (2)	–	–	–
Hypomagnesemia	12 (20)	27 (46)	12 (20)	4 (7)	4 (7)
Hypokalemia	39 (66)	17 (29)	–	2 (3)	1 (2)
Transaminase elevation	39 (66)	14 (24)	3 (5)	3 (5)	–
Increased creatinine	55 (93)	1 (2)	3 (5)	–	–
Increased total bilirubin	55 (93)	3 (5)	–	1 (2)	–
Rash	59 (100)	–	–	–	–

(Table 1). Four patients were treated at the third dose level of ZD9331 130 mg/m^2 and cisplatin 75 mg/m^2 with two instances of grade 4 neutropenia, one resulting in hospitalization and the other in treatment delay. This dose level was not well tolerated, mainly because of delayed recovery of marrow function in patients with either grade 3 or 4 toxicity. Protracted myelosuppression has been observed with another folate analogue TS inhibitor, pemetrexed disodium (Alimta/LY231514), and is likely to be a class effect for these compounds [2, 14]. Therefore, for this combination, the recommended phase II doses are ZD9331 130 mg/m^2 on days 1 and 8, and cisplatin 50 mg/m^2 on day 1. Nine patients were treated at this dose level, and while myelosuppression was observed, it was tolerable, with recovery occurring promptly. Given that cisplatin does not usually cause marrow toxicity and that dose levels 2 and 3 differed only by the cisplatin dose administered, a drug-drug interaction causing myelosuppression is possible.

The toxicity profile of this combination regimen is similar to those found in the three published single-agent trials. Goh et al. gave ZD9331 as a short daily i.v. bolus for 5 days every 3 weeks [7]. This study of 74 patients (67 assessable) determined a MTD of 16 mg/m^2 per day, with myelosuppression as the DLT. Pharmacokinetic studies in this trial showed a poor correlation between area under the curve (AUC) drug concentration and body surface area, leading to the recommendation of a fixed daily dose of 25 mg/day. In a recent phase I study of oral ZD9331 given once or twice daily for 5, 7 or 10 days every 21 days at doses ranging from 2.5 to 40 mg in 42 patients [4], myelosuppression and rash emerged as the DLTs, dictating an oral dose of 20 mg daily for five consecutive days every 3 weeks. Rash was not observed in our study (Table 2). Most recently, Rees et al. have reported results in 45 patients given ZD9331 as a 5-day i.v. infusion every 3 weeks at doses ranging between 0.125 and 8.0 mg/m^2 per day. Grade 4 thrombocytopenia was dose limiting at 8 mg/m^2 per day, and 6 mg/m^2 per day was deemed the MTD. Other drug-related toxicities included skin and gastrointestinal toxicity, lethargy, and transaminase elevation.

In summary, we demonstrated that a combination regimen of ZD9331 at 130 mg/m^2 on days 1 and 8, and cisplatin at 50 mg/m^2 on day 1, given every 21 days, has a manageable toxicity profile, with neutropenia and thrombocytopenia being the most frequent adverse events. Moreover, this regimen had promising antitumor activity in many tumor types, including mesothelioma, which is also sensitive to pemetrexed disodium (Alimta/LY231514). We believe that further development of this combination is warranted.

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