Phase I dose-escalation trial of ZD9331 in Japanese patients with refractory, solid malignancies

Wasaburo Koizumi[a], Keisuke Aiba[b], Tsuneo Sasaki[c], Atsushi Sato[d] and Noboru Horikoshi[b]

Background **ZD9331 is a novel thymidylate synthase inhibitor that, unlike some other antifolates, does not require polyglutamation for activity. This phase I dose-escalation trial investigated the tolerability, efficacy and pharmacokinetics of ZD9331 in Japanese patients with refractory, solid malignancies.**

Patients and methods **The mean age of patients was 57.6 years, and the most common primary tumours were gastric and colorectal cancer. Most patients had received prior chemotherapy and/or surgery. ZD9331 (69, 108 and 130 mg/m^2/day) was administered as a 30-min i.v. infusion on days 1 and 8 of a 3-week cycle.**

Results **A total of 18 patients received ZD9331 treatment; six at each dose level. Patients received a median of 2 cycles of treatment. ZD9331 demonstrated some antitumour activity, with one-third of patients showing no significant change in tumour size. ZD9331 was associated with non-dose-dependent myelosuppression, and dose-limiting toxicity was observed in one patient given 69 mg/m^2/day and one patient given 130 mg/m^2/day. The maximum plasma concentration and total area under the concentration–time curve increased with ZD9331 dose, whereas other pharmacokinetic parameters remained constant and independent of dose. Pharmacokinetic parameters were comparable following day 1 and 8 doses, with no accumulation of ZD9331 following the second dose.**

Conclusion **ZD9331 has a manageable toxicity profile and shows some evidence of activity in Japanese patients with refractory, solid malignancies. The pharmacokinetic profile of ZD9331 in Japanese patients is similar to that observed in Western patients.** ***Anti-Cancer Drugs*** **14 (suppl 1):S1–S5**

Anti-Cancer Drugs 2003, **14 (suppl 1)**:S1–S5

Keywords: Japanese, patients, pharmacokinetics, tolerability, tumours, ZD9331

[a]Kitasato University East Hospital, Kanagawa, Japan, [b]Japanese Foundation for Cancer Research, Cancer Chemotherapy Centre, Toshima-ku, Tokyo, Japan, [c]Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo, Japan and [d]Toyosu Hospital, Showa University School of Medicine, Kohtoh-ku, Tokyo, Japan.

Correspondence to W. Koizumi, Department of Gastroenterology/Medical Oncology, Kitasato University East Hospital, 2-1-1 Asamizodai Sagamihara, Kanagawa 228-8520, Japan.
Tel: +81 427 48 9111; fax: +81 427 48 5120;
e-mail: koizumi@med.kitasato-u.ac.jp

Introduction

Thymidylate synthase (TS) is an essential enzyme for DNA replication and cell growth, and is the only *de novo* source of thymine nucleotide precursors for DNA synthesis [1]. TS is a chemotherapeutic target for conventional anticancer drugs, e.g. 5-fluorouracil and, more recently, for folate-based TS inhibitors such as ZD9331 [2]. ZD9331 is a novel, direct-acting TS inhibitor that is transported into cells via a saturable, carrier-mediated system [reduced folate carrier (RFC)]. RFC proteins are believed to be overexpressed in certain cancer cells [3] and thus ZD9331 may selectively target tumour cells. Many antifolate drugs require polyglutamation by the enzyme folylpolyglutamate synthetase (FPGS) for cell penetration and retention [4], whereas, in contrast to some other folate analogues, ZD9331 does not require polyglutamation by FPGS for activity [5]. Some cancer cells display antifolate drug resistance due to underexpression of FPGS; therefore, ZD9331 may also be active in these tumour types. [6].

Previous studies have investigated the tolerability and pharmacokinetics of ZD9331. Phase I studies of treatment refractory patients with a range of solid tumours indicated that the principal dose-limiting toxicities (DLTs) for ZD9331 were myelosuppression and skin rash [7–10]. In phase I studies of Western patients, where ZD9331 was administered as a 30-min infusion for 5 consecutive days, every 3 weeks, clearance of ZD9331 was non-linear and did not correlate with body surface area. A fixed dose of 25 mg/m^2/day was well tolerated and a minor response was observed in one patient with colorectal cancer treated with 12 mg/m^2/day ZD9331 [7]. In patients receiving ZD9331 as a continuous infusion for 5 days, DLTs were observed in three of six patients at the 8 mg/m^2/day dose level. Maximum plasma concentration (C_{max}) and area under the concentration–time curve (AUC) increased with dose; clearance (CL) was predominantly renal and dose-dependent. Furthermore, evidence of some antitumour activity against breast and ovarian cancers was observed [8]. ZD9331 is also

0959-4973

available in an oral formulation and phase I studies recommend a daily oral dose of 3 mg/day for 28 days followed by a 14-day rest period [9].

This paper summarises the main tolerability, efficacy and pharmacokinetic data from a phase I study of i.v. ZD9331 in Japanese patients with refractory solid malignancies. In addition, a secondary objective of this study was to compare pharmacokinetic parameters of ZD9331 in Japanese patients with data from a similar phase I Western study [11,12].

Patients and methods

Study design

This was a multicentre, non-randomised, collaborative, open study employing a central registration method.

Patients

Adult Japanese patients with histologically or cytologically confirmed solid tumours resistant to standard therapy, or for whom no appropriate treatment was available, were entered into this phase I, dose-escalating study, provided they had a life expectancy of ≥3 months and a WHO performance status of 0–2. Patients were also required to present with adequate haematological, renal and hepatic function. Patients were excluded if they had received anticancer therapy (including treatment with other investigational anticancer drugs) within 4 weeks of the study, or extensive radiotherapy or chemotherapy with mitomycin C or nitrosoureas. Patients taking folic acid supplements within 48 h of treatment, presenting with brain metastasis or other severe complications (e.g. active double cancer) were also excluded.

The study was conducted in agreement with the principles of Good Clinical Practice according to the Declaration of Helsinki, approved by the local ethics committee and written informed consent was obtained from each patient. Criteria for patient withdrawal included non-compliance, the occurrence of serious adverse events (AEs) or complications, disease progression (PD) and failure to meet re-treatment criteria.

Treatment

Four different dose levels of ZD9331 were originally planned (69, 108, 130 and 162.5 mg/m^2/day). Patients were administered ZD9331 as a 30-min i.v. infusion on days 1 and 8 of a 3-week treatment cycle. Patients met the following re-treatment criteria before each subsequent cycle of treatment: white blood cell count ≥3500/mm^3, neutrophil count ≥2000/mm^3, platelets ≥10^5/mm^3, total bilirubin ≤1.25 × upper limit of reference range (ULRR), glutamic-oxaloacetic transaminase (GOT) or glutamic-pyruvic transaminase (GTP) ≤2.5 × ULRR (5 × ULRR in the presence of liver metastases) and serum creatinine ≤1.25 × ULRR.

DLT was evaluated after the first cycle of treatment at each dose level. Three patients were to be included at each dose level and three additional patients recruited to the same dose level if DLT was observed in one or more out of three patients. If DLT was observed in three patients, there was no further treatment at that dose level. If no DLT was observed, three patients were recruited to the next dose level. Intra-patient dose-escalation was not permitted. DLT was defined as: grade 4 neutropenia with fever or grade 4 neutropenia without fever of 7 days or longer duration (including grade 4 neutropenia with risk of potential infection, as judged by study investigators); grade 4 thrombocytopenia; grade 3 or 4 non-haematological toxicity not improved by symptomatic therapy (excluding transient and reversible elevations of GOT or GPT). If re-treatment criteria were not met or if grade 3 or 4 treatment-related neutropenia or thrombocytopenia, or grade 2, 3 or 4 treatment-related non-haematological toxicity (diarrhoea, stomatitis, mucositis or rash) was observed in a patient at any time during the first week of dosing in a cycle, the second dose of treatment was withheld.

Tolerability and efficacy assessments

AEs were recorded continually throughout the study and were assessed according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Objective tumour response was assessed by changes in tumour size according to the Japanese Society for Cancer Therapy's Criteria for Direct Response to Chemotherapy for Solid Cancers [13]. The numbers of complete responses, partial responses and the number of patients experiencing no change or PD was also assessed. Measurable or assessable lesions were examined by techniques including X-ray, computed tomography, magnetic resonance imaging and ultrasound at: (i) baseline before the first cycle of ZD9331, (ii) at the end of each second cycle of ZD9331 and (iii) at the last dosing or withdrawal from treatment.

Pharmacokinetic assessments

Blood samples for pharmacokinetic analyses were collected from each patient during each cycle of therapy; pre-dose (0 h), and at 0.5, 1, 4, 8, 24, 48, 72, 96 and 168 h after both day 1 and day 8 ZD9331 doses. One further blood sample was taken before administration of the first ZD9331 dose of treatment cycle 2 (336 h). Plasma concentrations of ZD9331 were determined using high-performance liquid chromatography tandem mass spectroscopy with detection limits of 1.0 ng/ml [14]. Pharmacokinetic parameters were defined for ZD9331 following the day 1 and day 8 doses. These included C_{max}, area under the plasma concentration–time curve from time 0 to 168 h ($AUC_{0-168\,h}$), terminal half-life ($t_{1/2}$), CL, volume of distribution at steady state (V_{ss}) and accumulation ratio. These parameters were calculated using WinNonlin Professional (version 1.5) non-compartmental analyses software.

Results

Patients

Eighteen patients with refractory solid tumours were recruited into the study. Baseline patient characteristics are shown in Table 1. The most common primary tumours were gastric cancer (six patients, 33%) and colorectal cancer (four patients, 22%), with most patients having received prior chemotherapy (17 patients, 94%) and/or surgery (10 patients, 56%).

Treatment

Eighteen patients received a total of 49 cycles of ZD9331 treatment at three dose levels (69, 108 and 130 mg/m^2/day). Six patients were included at each dose level. The median number of cycles of ZD9331 treatment was 2 cycles/patient (range 1–10). Table 2 summarises the number of cycles of ZD9331 administered to patients at each dose level. Of the 49 cycles administered, the second dose of treatment was withheld in 20 cycles (41%) because patients did not meet the re-treatment criteria.

Tolerability

Overall, the most common AEs following treatment with ZD9331 were myelosuppression (anaemia, 83%; lymphocytopenia, 83%; leucopenia, 67% and neutropenia, 56%) and liver and gastrointestinal toxicities (elevated hepatic transaminases, 89%; nausea, 67% and elevated alkaline phosphatase, 56%). All CTC grade 3/4 AEs are shown in Table 3. None of the patients experienced CTC grade 3 or 4 gastrointestinal, renal or neurological AEs at any dose level of ZD9331. CTC grade 1/2 events occurring in 20% and higher of patients at each dose level of ZD9331 are shown in Table 4. Eleven patients (61%) experienced mild to moderate (grade 1/2) skin rash, but no severe (grade 3/4) events of this type were reported. However, DLT (grade 4 febrile neutropenia and grade 4 thrombocytopenia) was confirmed in one patient receiving ZD9331 69 mg/m^2/day and in one other patient receiving ZD9331 130 mg/m^2/day. Based on these results and data from a study in Western patients [11,12], the incidence of toxicity (especially DLT following the first dose of ZD9331) was predicted to be high at 162.5 mg/m^2/day ZD9331 and therefore treatment at this dose level was not carried out.

Activity

All patients were evaluated for tumour response during this study. No complete or partial responses were observed following treatment with ZD9331; however, no change was observed in six patients and PD in 12 patients. At all three dose levels no change was observed, and included one patient with lung carcinoma and one patient with myxoma in the 69 mg/m^2/day group; two patients with gastric cancer and one patient with oesophageal cancer in the 108 mg/m^2/day group; and one patient with colorectal cancer in the 130 mg/m^2/day group. The patient with myxoma in the 69 mg/m^2/day dose group received 10 cycles of treatment and the patient with gastric cancer in the 108 mg/m^2/day dose group received 8 cycles of treatment.

Table 1 Baseline characteristics of patients

	No. of patients ($n = 18$)
Males/females (*n*)	9/9
Mean age [years (range)]	57.6 (33–70)
Prior therapy (*n*)	
chemotherapy	17
surgery	10
radiotherapy	2
hormonal therapy	1
other	1
none	1
Performance status (*n*)	
0	7
1	8
2	3
Primary tumour site (*n*)	
gastric	6
colorectal	4
breast	1
other	7
Site of metastases (*n*)	
lymphoid	9
liver	8
lung	5
bone	4
other	9

Table 2 Patient exposure to ZD9331

ZD9331 dose (mg/m^2/day)	No. of patients	No. of cycles of treatment			
		Median	Maximum	Minimum	Total
69	6	3	10	1	22
108	6	2	8	1	17
130	6	2	2	1	10

Table 3 All CTC grade 3/4 AEs

	Dose level ZD9331 (mg/m^2/day)					
	69[a]		108		130[a]	
Grade	3	4	3	4	3	4
Haematological (*n*)						
anaemia	0	0	3	0	2	0
thrombocytopenia	0	1	0	0	0	1
neutropenia	0	1	2	0	0	1
lymphocytopenia	1	3	4	2	1	4
leucopenia	0	1	1	0	0	1
Non-haematological (*n*)						
bilirubin elevation	0	2	0	0	0	1
hepatic transaminase	4	0	0	0	1	0
alkaline phosphatase	3	0	0	0	2	0
pulmonary	0	0	0	1	0	0

[a]DLT of grade 4 neutropenia and grade 4 thrombocytopenia confirmed in one patient.

Pharmacokinetics

The mean plasma concentrations of ZD9331 following administration on day 1 and day 8 at each dose level are shown in Fig. 1. At the end of the 30-min infusion of dose 1 (day 1), ZD9331 plasma levels declined

Table 4 CTC grade 1/2 AEs occurring in 20% or more of patients

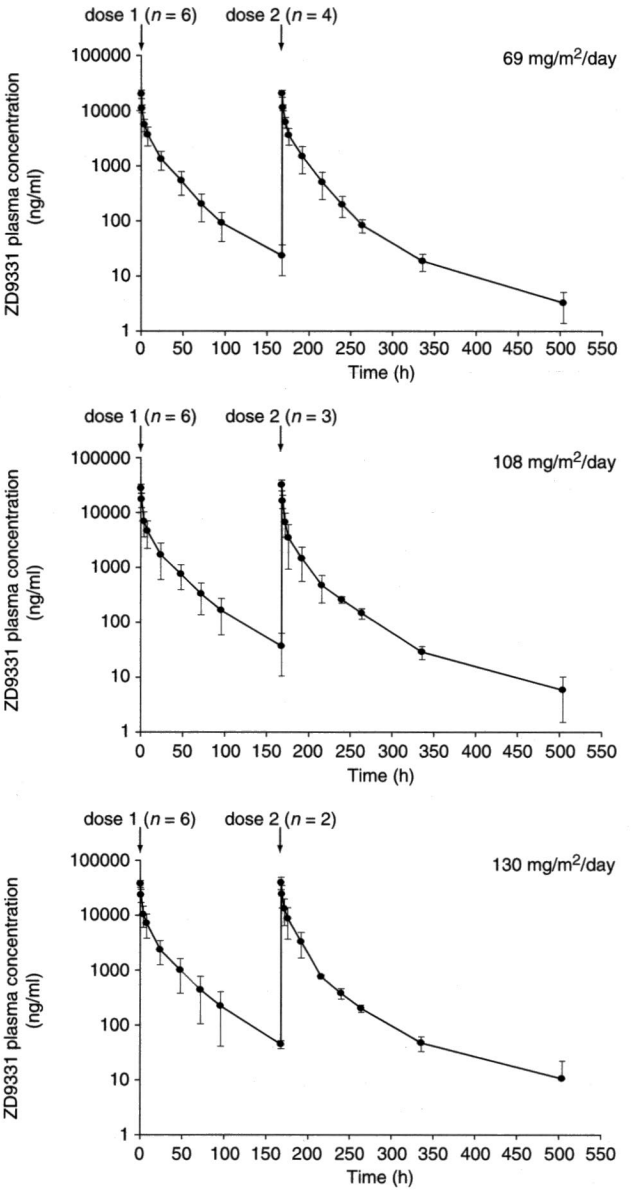
	Dose level ZD9331 (mg/m²/day)					
	69		108		130	
Grade	1	2	1	2	1	2
Haematological (*n*)						
anaemia	0	4	0	3	1	2
thrombocytopenia	1	0	1	1	3	0
neutropenia	3	1	0	0	1	1
leucopenia	1	2	2	2	1	1
Non-haematological (*n*)						
hepatic transaminase	1	0	4	1	3	2
alkaline phosphatase	0	1	2	0	0	2
nausea	2	3	0	3	3	1
vomiting	1	1	1	2	1	1
diarrhoea	2	1	0	1	3	1
proteinuria	2	1	1	1	1	0
haematuria	3	0	2	0	2	1
weight gain/loss	1	0	2	0	0	2
alopecia	3	0	1	0	0	0
fever in absence of infection	0	4	2	1	0	4
skin rash	2	2	2	1	3	1

Table 5 Pharmacokinetic parameters after day 1 dose of ZD9331

	Dose level ZD9331 (mg/m²/day)		
Mean (SD)	69	108	130
$AUC_{0-168\,h}$ (μg/h/ml)	135.0 (38.0)	184.0 (80.8)	263.0 (94.6)
C_{max} (μg/ml)	19.9 (3.4)	27.5 (5.3)	37.3 (5.2)
$t_{1/2}$ (h)	35.5 (7.4)	34.5 (9.8)	39.0 (9.0)
CL (ml/min)	13.0 (3.7)	15.9 (6.0)	14.1 (7.1)
V_{ss} (l)	15.5 (3.8)	22.0 (11.4)	18.0 (7.2)

bi-exponentially, with an initial, more rapid, decline over the first 24 h post-dose and a subsequent slower decrease observed up to day 8. Following the second dose (day 8), the ZD9331 plasma concentration–time profile was triphasic, with ZD9331 detectable until day 15 post-dose. The $t_{1/2}$ was approximately 2 days. The mean pharmacokinetic parameters following the day 1 dose of ZD9331 are shown in Table 5. Exposure (in terms of C_{max} and AUC) generally increased with dose, whereas the pharmacokinetic parameters $t_{1/2}$, CL and V_{ss} were constant and dose-independent. Pharmacokinetic parameters following the day 1 and day 8 doses of ZD9331 were comparable, with similar C_{max} and $AUC_{0-168\,h}$ values. The accumulation ratio, calculated on the basis of $AUC_{0-168\,h}$ values following doses 1 and 2, was approximately 1, suggesting that no obvious accumulation of ZD9331 occurred following administration of the second dose. However, patients showing a high systemic exposure to ZD9331 (in terms of AUC) were more likely to experience a haematological DLT (data not shown).

Fig. 1



Mean plasma concentrations of ZD9331 following administration of 69, 108 and 130 mg/m²/day on day 1 and day 8 in Japanese patients with solid tumours.

Discussion

This study evaluated the tolerability, activity and pharmacokinetics of i.v. ZD9331, a novel TS inhibitor, in Japanese patients with a range of solid refractory malignancies. ZD9331 showed a manageable toxicity profile in this group of patients, with no evidence of grade 3/4 skin rash as reported in studies of Western patients [7,15]. Furthermore, DLTs, evident as grade 3/4 thrombocytopenia and neutropenia, were consistent with the results of other phase I studies [7–9,15]. Although no objective responses were observed in this study, ZD9331 demonstrated some antitumour activity, with one-third of these patients showing no significant change in tumour size under ZD9331 treatment.

Table 6 Pharmacokinetic parameters after day 1 dose of ZD9331 in Japanese and Western studies [11,12]

	Dose level ZD9331 (mg/m²/day)					
	69		108		130	
Mean (SD)	Japanese	Western	Japanese	Western	Japanese	Western
$AUC_{0-168\ h}$ (μg/h/ml)[a]	131.0 (29.3)	132.0 (36.4)	171.0 (34.8)	208.0 (43.0)	247.0 (42.6)	227.0 (57.4)
C_{max} (μg/ml)[a]	19.6 (19.8)	20.0 (12.8)	27.1 (19.7)	27.4 (28.3)	37.0 (13.8)	31.8 (17.8)
$t_{1/2}$ (h)	35.5 (7.4)	33.1 (9.5)	34.5 (9.8)	31.9 (11.3)	39.0 (9.0)	32.2 (7.6)
CL (ml/min)	13.0 (3.7)	15.6 (3.6)	15.9 (6.0)	18.1 (6.6)	14.1 (7.1)	18.6 (9.3)
V_{ss} (l)	15.5 (3.8)	19.6 (4.3)	22.0 (11.4)	24.8 (7.4)	18.0 (7.2)	24.9 (15.9)

[a]Geometric mean, coefficient of variance (%).

Pharmacokinetic data confirmed that ZD9331 plasma levels declined non-linearly and that C_{max} and AUC generally increased with dose, as observed in other studies [7,16]. ZD9331 had a low V_{ss}, as previously reported [8], and no evidence of drug accumulation was found in this study. However, in contrast to the findings of other investigators, the $t_{1/2}$ and CL of ZD9331 were relatively constant and dose independent [7,15,16]. These findings may reflect differences in the treatment regimen between this and other phase I studies in terms of doses, schedule and route of administration.

The pharmacokinetic parameters of ZD9331 administered at identical doses in an identical treatment regimen have also been investigated in a phase I study in Western patients [11,12]. Seventy-one patients with solid tumours refractory to standard therapy and a mean age of 54.6 years (range 34–75) were included in the analysis of safety in this study. When compared, the plasma concentration profiles and pharmacokinetic parameters of ZD9331 in Japanese patients were similar to those of Western patients receiving equivalent doses of ZD9331. $AUC_{0-168\ h}$ and C_{max} increased with dose in the present study and correspond to phase I Western data (Table 6). Both studies confirm that the $t_{1/2}$ of i.v. ZD9331 is long, probably as a result of slow plasma clearance. Furthermore, both studies confirm that patients with a high systemic exposure to ZD9331 are more likely to experience a haematological DLT.

Conclusions

Intravenous ZD9331 has a manageable toxicity profile and shows evidence of some activity in Japanese patients with refractory solid malignancies. The most relevant DLT associated with ZD9331 treatment was myelosuppression, which did not appear to be dose-dependent occurring at both the 69 and 130 mg/m²/day dose levels. Since the incidence of toxicity (DLT) was expected to be high at ZD9331 162.5 mg/m²/day, treatment at this dose level was not carried out. Any future studies in Japanese patients will utilise a ZD9331 dose of 130 mg/m²/day or less. The pharmacokinetic profile of ZD9331 in Japanese patients is similar to that observed in Western patients.

References

1 Danenberg PV, Malli H, Swenson S. Thymidylate synthase inhibitors. *Semin Oncol* 1999; **26**:621–631.
2 Van Triest B, Pinedo HM, Giaccone G, Peters GJ. Downstream molecular determinants of response to 5-fluorouracil and antifolate thymidylate synthase inhibitors. *Ann Oncol* 2000; **11**:385–391.
3 Sirotnak FM. Obligate genetic expression in tumor cells of a fetal membrane property mediating 'folate' transport: biological significance and implications for improved therapy of human cancer. *Cancer Res* 1985; **45**: 3992–4000.
4 Sun X, Cross JA, Bognar AL, *et al.* Folate-binding triggers the activation of folylpolyglutamate synthetase. *J Mol Biol* 2001; **310**:1067–1078.
5 Jackman AL, Kimbell R, Aherne GW, *et al.* Cellular pharmacology and in vivo activity of a new anticancer agent, ZD9331: a water-soluble, nonpolyglutamatable, quinazoline-based inhibitor of thymidylate synthase. *Clin Cancer Res* 1997; **3**:911–921.
6 Marsham PR, Wardleworth JM, Boyle FT, *et al.* Design and synthesis of potent non-polyglutamatable quinazoline antifolate thymidylate synthase inhibitors. *J Med Chem* 1999; **42**:3809–3820.
7 Goh BC, Ratain MJ, Bertucci D, *et al.* Phase I study of ZD9331 on short daily intravenous bolus infusion for 5 days every 3 weeks with fixed dosing recommendations. *J Clin Oncol* 2001; **19**:1476–1484.
8 Rees C, Beale P, Trigo J, *et al.* Phase I trial of ZD9331, a non-polyglutamable thymidylate synthase (TS) inhibitor given as a 5-day continuous infusion every 3 weeks. *Proc Am Soc Clin Oncol* 1999; **18**:171a (abstr 657).
9 Sawyer MB, Ratain MJ, Janisch LA, *et al.* A phase I study of ZD9331 administered orally for up to 28 days every 6 weeks. *Proc Am Soc Clin Oncol* 2000; **19**:236a (abstr 921H).
10 de Jonge MJ, Punt CJ, Sparreboom A, *et al.* Phase I and pharmacologic study of oral ZD9331, a novel nonpolyglutamated thymidylate synthase inhibitor, in adult patients with solid tumors. *J Clin Oncol* 2002; **20**: 1923–1931.
11 Plummer R, Rees C, Judson I, *et al.* Phase I trial of ZD9331 in adult patients with refractory solid malignancies administered by 30-min infusion of days 1 and 8 with the cycle repeated every 3 weeks. *Eur J Cancer* 1999; **35 (suppl 4)**:S285 (abstr 1143).
12 Plummer R, Rees C, Judson I, *et al.* A phase I trial of ZD9331, a water-soluble, non-polyglutamatable, thymidylate synthase inhibitor. *Clin Cancer Res* 2003; **9**:1313–1322.
13 Furue H, Hara Y, Imamura Y, *et al.* The Japan Society for Cancer Therapies Criteria for Direct Response to Chemotherapy for Solid Cancer. *J Jpn Soc Cancer Ther* 1986; **21**:931–942.
14 O'Connor D, Clarke DE, Morrison D, Watt AP. Determination of drug concentrations in plasma by a highly automated, generic and flexible protein precipitation and liquid chromatography/tandem mass spectrometry method applicable to the drug discovery environment. *Rapid Commun Mass Spectrom* 2002; **16**:1065–1071.
15 de Jonge MJ, Punt CJ, Planting AS, *et al.* Oral ZD9331, a non-polyglutamated thymidylate synthase (TS) inhibitor: a phase I and pharmacologic study. *Eur J Cancer* 1999; **35 (suppl 4)**:S29–S291 (abstr 1167).
16 Rowinsky E, Rha SY, Hammond L, *et al.* Absolute bioavailability of single low and high doses of oral ZD9331 in patients with refractory cancer: a phase I study. *AACR-NCI-EORTC* 2000; abstr 423.