Cancer Chemother Pharmacol (2009) 63:945–952
DOI 10.1007/s00280-008-0795-6

CLINICAL TRIAL REPORT

A multi-centre dose-escalation and pharmacokinetic study of diflomotecan in patients with advanced malignancy

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Received: 20 February 2008 / Accepted: 7 July 2008 / Published online: 25 July 2008

Abstract
Purpose Diflomotecan, a homocamptothecin, targets DNA topoisomerase I. Previous clinical trials have demonstrated a variable degree of dose limiting toxicity. The purpose of this study was to further evaluate the safety and pharmacokinetic profile of a range of diflomotecan doses administered intravenously.
Methods Patients with advanced solid malignant tumours, refractory to standard therapies, with adequate haematologic, renal and hepatic function, received diflomotecan administered as a 20 min intravenous infusion every 21 days. Cohorts of six patients were recruited sequentially to one of three fixed starting dose groups—2, 4, or 7 mg, with drug administered by fixed-dose rather than dosing by body surface area. Pharmacokinetic analyses were performed on serial blood samples taken over the first 24 h after diflomotecan administration (cycles 1 and 2). Cytochrome P450 3A4 (CYP3A4) activity was determined by an erythromycin breath test (EBT) prior to diflomotecan administration in cycles 1 and 2.
Results Thirteen patients, were treated with a starting dose of either 2 mg ($n = 8$) or 4 mg ($n = 5$) of diflomotecan. Dose limiting toxicities (DLTs) were observed in 1 patient in the 2 mg starting dose level (grade 4 neutropenia which lasted for 8 days), and in 2 of 5 patients enrolled at the 4 mg starting dose level (grade 4 neutropenia for 11 days; grade 4 neutropenia leading to withdrawal from the study), and no further dose escalation was performed. Pharmacokinetic analyses revealed a less than dose-proportional increase in diflomotecan and for the two metabolites BN80942 and P-20, with a magnitude of P-20 exposure similar to the parent drug. There was a high inter-patient variability in diflomotecan exposure similar to that observed with other camptothecin derivatives. One minor response was observed in a patient with oesophageal cancer.
Conclusions Diflomotecan administered as a 20-min intravenous infusion 3-weekly is characterised by a variable pharmacokinetic profile. Alternative oral dosing schedules of diflomotecan have been shown to display a more predictable PK/PD and safety profile and should be selected for further evaluation in Phase II clinical trials.

Keywords Topoisomerase-1 · Homocamptothecins · Diflomotecan · Pharmacokinetics · CYP3A4

This study was financially supported by Hoffmann-La Roche Inc.

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Introduction

Camptothecin is a plant alkaloid [1]. It targets topoisomerase I [2, 3] which is critical for cell growth and proliferation and is over-expressed in certain tumour types, including colorectal and cervical cancers [4, 5]. This makes it an attractive target for the development of anti-cancer agents. Clinical development of camptothecin has been compromised by its sometimes severe and unpredictable toxicity, particularly haemorrhagic cystitis [6, 7]. Subsequently, a number of structural analogues have been synthesised with greater water solubility and lower toxicities, including irinotecan and topotecan and these are widely used in clinical oncology practice [8, 9]. Homocamptothecins (hCPTs) are distinguished from camptothecin-based topoisomerase I inhibitors by their seven-membered β-hydroxylactone E-ring instead of the six-membered α-hydroxylactone. They have several distinct pharmacologic advantages compared to camptothecin including a greater ability to induce DNA cleavage mediated by topoisomerase I [10, 11] and a greater ability to stabilize topoisomerase I-DNA cleavage complexes [11].

The first homocamptothecin to enter clinical trials was diflomotecan. It has superior IC_{50} values compared to topotecan and SN-38 (the active metabolite of irinotecan) in a number of cancer cell lines in vitro [10, 12, 13], and also displays potent anti-tumour activity in vivo in mouse models [10]. Diflomotecan metabolism results in the formation of two main metabolites (BN80942 and P-20). The proposed metabolic pathway of diflomotecan is drug hydrolyzation to give the inactive open lactone form (BN80942) or drug hydroxylation to give P-20 via an intermediate compound. However, the P-20 metabolite is considered biologically inactive in the context of topoisomerase-mediated toxicity.

Several Phase I studies have been performed in which diflomotecan has been administered by either the intravenous or oral routes in various schedules, including as a single 20-min intravenous infusion at 21-day intervals[12] a 20-min infusion once-daily for 5 days every 3 weeks [13] and orally once-daily for 5 days every 3 weeks [21]. Diflomotecan showed a variable degree of toxicity in these studies with DLTs seen at the 5 and 6 mg/m^2 dose level when given intravenously once every 3 weeks [15]—recommended dose was 4 mg/m^2—and with a recommended dose of 0.27 mg/day when given orally once-daily for 5 days every 3 weeks[14]. In addition, non-compartmental pharmacokinetic (PK) analysis revealed clearance values that varied by sixfold and population PK analysis revealed an inter-individual variability in clearance of 66%. No clinically significant covariates were identified as having a significant effect on the variability of PK parameters in a population analysis of the combined data from the completed Phase I studies [15]. This study was therefore undertaken to further evaluate the safety and pharmacokinetic profile of a broad range of diflomotecan doses by one of the routes and schedules that was to be used for subsequent Phase II studies to better characterise the factors that might be predictive of diflomotecan toxicity.

Patients and methods

Study design

This was a non-randomised, multi-centre, sequential-group, dose escalation study of the tolerability and pharmacokinetics of diflomotecan in patients with advanced solid malignancies. The primary objective was to describe the pharmacokinetics of diflomotecan and its two main metabolites when a fixed dose of diflomotecan was administered intravenously every 3 weeks to this patient population, and to determine if parameters known to be related to drug metabolism and clearance could explain the inter-patient variability in diflomotecan PK parameters. Secondary objectives included the toxicity and safety profile of different doses of diflomotecan in this patient population, and objective tumour responses in those patients with measurable disease as determined by the RECIST criteria [16].

Patients and eligibility criteria

Eligible patients were those with a histological or cytological confirmed advanced solid malignant tumour that was refractory to standard therapies or for which no standard therapy existed. All patients were aged 18–70 years, had ECOG performance status $\leq$1, and had adequate renal (calculated creatinine clearance $\geq$60 mL/min), hepatic [bilirubin $\leq 1.5 \times$ upper limit of normal (ULN); transaminases, alkaline phosphatase, and γGT $\leq 2 \times$ ULN or $\leq 5 \times$ ULN in the presence of hepatic metastases], and haematologic (absolute neutrophil count [ANC] $\geq 1.5 \times 10^9$/L; platelet count $\geq 100 \times 10^9$/L) function. Patients could have received up to three prior regimens of cytotoxic chemotherapy including adjuvant treatment and a camptothecin, and an unlimited number of other non-cytotoxic systemic anti-cancer therapies (e.g. hormonal therapy, immuno-therapy) but patients who had received prior therapy with mitomycin-C or a nitrosurea were excluded. All patients were at least 3 weeks from any prior systemic anti-cancer therapy or radiotherapy. Patients with central nervous system (CNS) metastases were eligible if they had received prior radiotherapy to the site(s) of CNS metastatic disease, had been on a stable dose of glucocorticoids for at least 4 weeks, and had no overt evidence of neurologic deficit. Patients were excluded if they had received radio-

therapy to greater than 30% of their active bone marrow, or if they had received prior bone marrow or peripheral blood progenitor cell transplants. Other exclusion criteria included patients who were known to be HIV-positive, patients who were pregnant or of child-bearing potential and unwilling to use an acceptable method of birth control, hypersensitivity to any of the components of diflomotecan, and any other unstable concurrent medical condition. This study was approved by the Research Ethics Committees of all participating institutions, and all patients gave written, informed, consent prior to any study-related procedures being performed.

Administration of diflomotecan

Diflomotecan was administered by intravenous infusion over 20 min every 21 days. Body surface area did not account for the inter-patient variability of the PK parameters of diflomotecan in the initial studies, and this has also been observed in other studies of camptothecins [17]. Consequently, fixed-dosing rather than dosing by body surface area was used in this study. The recommended dose of diflomotecan was 4 mg/m^2 when administered intravenously in a previous Phase 1 study using a 21-day schedule [15]. However, a dose of 2 mg/m^2 given intravenously every 3 weeks had resulted in a DLT in one patient. Consequently, a cautious starting dose of 2 mg fixed dose was selected for this study as this was approximately 40% lower than the dose (2 mg/m^2) which had previously resulted in one DLT [18].

Cohorts of six patients were to be recruited sequentially to one of three fixed starting dose groups—2, 4, and 7 mg. Recruitment to a new dose group could only commence after 6 patients in the prior dose group had completed at least 3 weeks (one cycle) of diflomotecan treatment and if $\leq$1 of 6 patients had experienced a DLT. If $\geq$2 of 6 patients at any one dose level experienced DLT, then no further dose escalation was allowed and an additional 9 patients were to be recruited at the dose level immediately below that which had resulted in DLT in $\geq$2 of 6 patients. Intra-patient dose escalation was utilised to gain additional data on the variability of inter-occasion PK parameters and to ensure that patients who tolerated the treatment with minimal toxicity did not continue to receive a potentially sub-therapeutic dose of diflomotecan. The dose of diflomotecan that was administered in cycle 2 was determined in an individual patient based on the maximum toxicity experienced during cycle 1 (Table 1). The dose of diflomotecan that was administered for the third, and subsequent, cycles of treatment was the same as that administered in cycle 2 unless the criteria for dose modification were met.

Administration of diflomotecan was delayed by up to 14 days for patients in whom toxicities had not resolved to $\leq$grade 1 on the scheduled day of dosing. Patients were withdrawn if toxicities had not resolved to $\leq$grade 1 by day 35 of a treatment cycle. Patients who developed a DLT on the second, or a subsequent, treatment cycle could remain on study with a reduction of the dose of diflomotecan for subsequent treatment cycles to the dose which was administered for cycle 1. Patients who developed a further DLT after a dose modification, or who developed a DLT during cycle 1 of the study, were withdrawn from the study. Diflomotecan was administered every 21 days until the development of DLT, disease progression, or withdrawal from the study due either to the patient's decision or on the advice of the investigators. Patients who were withdrawn for reasons other than drug-related toxicity before completion of one cycle of treatment were replaced to ensure an adequate number of patients were evaluable for DLT within a specific dose group. Patients with stable disease after six cycles of treatment could receive a maximum of four additional cycles, whereas patients with evidence of a partial or complete response after six cycles of treatment could receive a maximum of two additional cycles.

Patient assessments

Baseline assessments were performed prior to entry into the study and included medical history, physical examination, ECOG performance status, vital signs, height, weight, full blood count, serum chemistries (including urea, creatinine, electrolytes, glucose, and liver function tests), pregnancy test (where appropriate), urinalysis, and EKG performed twice (5 min apart), all of which were performed within 7 days of first dosing. Disease assessment by CT scan was performed within 28 days of first dosing. Physical examination, ECOG

Table 1 Dose escalation plan

Starting dose (mg)	If maximum toxicity during cycle 1 was grade 0/1, dose for cycle 2 was (mg)	If maximum toxicity during cycle 1 was grade 2, dose for cycle 2 was (mg)	If grade 3 toxicity (haematologic) occurred during cycle 1, dose for cycle 2 was (mg)
2	4	3	2
4	7	5	4
7	9	9	7

The dose for the second (and subsequent) treatment cycle (s) was based on the toxicities recorded during cycle 1

performance status, and serum chemistries were performed on day 1 of each treatment cycle. Full blood count, weight, vital signs, and toxicity assessments were performed weekly throughout the study. Toxicity was recorded using NCI-CTC (version 3.0). DLT was defined as NCI-CTC grade 4 neutropenia for ≥7 days, febrile neutropenia, grade 4 thrombocytopenia of any duration, grade 3 thrombocytopenia accompanied by haemorrhage, and any ≥grade 3 non-haematologic toxicity other than alopecia or inadequately treated nausea and vomiting. Disease assessment by CT scan was repeated after every two cycles of treatment. Patients were reviewed within 28 days of discontinuing study treatment, and physical examination, vital signs, ECOG performance status, full blood count, serum chemistries, urinalysis, pregnancy test (where appropriate), and EKG were performed and the patient's weight was recorded. Disease evaluation by CT scan was also performed for patients who discontinued study treatment for reasons other than objective disease progression. Patients with any on-going treatment-related toxicities were followed until the toxicities had resolved to ≤grade 1 or were clinically stable. Acute phase reactants (α-1-acid glycoprotein, β-2 microglobulin and C-reactive protein) were measured on day 1 of the first two treatment cycles only. EKGs were performed immediately prior to the diflomotecan infusion, immediately on completion of the infusion, and 4 h after the beginning of the infusion, on day 1 of both cycles 1 and 2 only.

Pharmacokinetic analyses

Blood samples (5 mL) were collected into lithium heparin plastic collection tubes for PK analyses. Samples were taken immediately prior to the diflomotecan infusion, immediately following completion of the infusion, and 1, 2, 4, 8, 12, and 24 h after the end of the infusion, on day 1 of both cycles 1 and 2. Plasma was separated using a refrigerated centrifuge (2,000*g* at 4°C for 15 min) within 30 min of blood collection. The plasma supernatant (1.2 mL) was decanted into each of two polypropylene cryotubes and stored at −70°C until transferred for analysis to Ipsen Pharma S.A., Barcelona, Spain.

Plasma concentrations of diflomotecan, BN80942 and P-20, were determined by an on line SPE-LC-MS/MS method using the stable isotope labelled analogues as internal standards. Briefly, the analytical method consisted of direct injection of the filtered plasma sample in the on-line SPE-LC-MS/MS system, with the mass spectrometer in multiple reaction monitoring (MRM) mode. The validated calibration range was 0.05–50 ng/mL for diflomotecan, BN80942 and P-20. The limit of quantitation for all compounds was 0.050 ng/mL (50 pg/mL). Acceptable inter-assay precision and accuracy ranges were obtained (5.3 to 11.3% and −3.47% to 3.03 for precision and accuracy, respectively).

The maximum drug concentration (C_{max}), terminal half-life ($t_{1/2}$), area under the plasma concentration time curve (AUC), apparent volume of drug distribution at steady state (V_{ss}) and total drug clearance (Cl) were determined for diflomotecan and the C_{max}, $t_{1/2}$ and AUC were determined for its two metabolites, BN80942 and P-20. All PK variables for diflomotecan and its two main metabolites were presented by descriptive summary statistics including number of patients, means, standard deviations and coefficients of variation.

Erythromycin breath test

An EBT was performed prior to the administration of diflomotecan in both cycles 1 and 2. The activity of the microsomal enzyme Cytochrome P450 3A4 (CYP3A4) can sometimes explain inter-patient variability in clearance of a particular drug. We evaluated this using an EBT. A small amount of radioactive erythromycin labelled with 3 μCi of ^{14}C in the *N*-methyl position was injected intravenously over approximately 1 min. A breath sample was collected after 20 min by having the patient exhale 3–6 times into a balloon provided by Metabolic Solutions, Nashua, NH. The quantity of exhaled radioactive carbon dioxide, which correlates with CYP3A4 activity, was measured by Metabolic Solutions, Nashua, NH.

Results

Thirteen patients were recruited into the study at three centres in the UK between June 2004 and February 2005. Patient characteristics at baseline are summarised in Table 2. Tumour types included oesophageal cancer (n = 3), gastric cancer (2), colon cancer (2), and miscellaneous (6). All 13 patients had received 1–3 lines of prior chemotherapy, and 4 patients had also received prior radiotherapy. Two patients had also received a non-cytotoxic agent within other Phase 1 clinical trials.

Table 2 Patient characteristics

Characteristic	Patients (n = 13)
Age	
Mean	55
Range	38–74
Male	9
Female	4
Number of previous chemotherapy treatments	
1	4
2	5
3	4

Administration of diflomotecan

Patients were treated with a starting dose of either 2 mg of diflomotecan (8 patients) or 4 mg of diflomotecan (5 patients) and they received a total of 22 cycles of treatment (median = 1; range 1–4). Three of the eight patients treated with a starting dose of 2 mg diflomotecan were withdrawn after one treatment cycle, and two of these patients were replaced so that a total of 6 patients were evaluable in this dose cohort. Four patients in this dose cohort received a second cycle of diflomotecan with dose escalation to 4 mg (2 patients), 3 mg (1 patient) and with no dose escalation in 1 patient. One further patient received a total of 4 cycles of diflomotecan with escalation of the dose to 4 mg for the second, and subsequent, treatment cycles. The total administered doses of diflomotecan ranged from 2 to 14 mg in this dose cohort. Three of the 5 patients treated with a starting dose of 4 mg diflomotecan were withdrawn after 1 cycle, and the remaining 2 patients received a total of 2 cycles of diflomotecan without any intra-patient dose escalation.

Toxicity

All 13 patients were evaluable for toxicity. Treatment toxicities are listed in Table 3. All DLTs were characterised by Grade 4 neutropenia, but no associated febrile neutropenia or infection was reported. DLTs were observed in 1 patient enrolled at the 2 mg starting dose level (grade 4 neutropenia which lasted for 8 days) and in 2 of 5 patients enrolled at the starting dose of 4 mg diflomotecan (grade 4 neutropenia which lasted for 11 days; grade 4 neutropenia which resulted in withdrawal from the study). Consequently, no further patients were recruited into the 4 mg diflomotecan dose cohort, and no further dose escalation was performed. Cumulative grade 3/4 haematologic toxicity (worst grade per patient—all cycles) included grade 3/4 neutropenia (7 patients), grade 3/4 thrombocytopenia (2) and grade 3/4 anaemia (1). Cumulative grade 3/4 non-haematologic drug-related toxicity (worst grade per patient—all cycles) included one case each of anaphylactic reaction, diarrhoea, peripheral neuropathy, oral candidiasis, fatigue, cholangitis and pleural effusion. Most of these adverse events were considered by the investigator to be possibly related to the trial treatment. One patient died during study treatment. A 44-year-old man with metastatic oesophageal cancer developed a pulmonary embolism on day 20 of cycle 2 of diflomotecan, and died 2 days later. Pulmonary embolism was confirmed as the cause of death on autopsy, and this was considered unrelated to the study drug.

Table 3 Cumulative haematologic and non haematologic toxicity recorded as the worst toxicity (NCI-CTC version 3.0) per patient (all cycles)

	Cumulative toxicity	
	Grade 3	Grade 4
Haemoglobin	1	–
Absolute neutrophil count	3	4
Platelet count	1	1
Diarrhoea	1	–
Peripheral neuropathy	1	–
Oral candidiasis	1	–
Fatigue	1	–
Cholangitis	1	–
Pleural effusion	1	–
Anaphylaxis	–	1

Efficacy

Seven of the 13 patients had a repeat CT scan and were evaluable for objective response by the RECIST criteria. The best recorded response per patient included one minor response, 3 patients with stable disease, and 3 patients with progressive disease at the first disease assessment. The minor response was a 28% reduction in the sum of the longest diameters of all target lesions in a patient with oesophageal cancer with lymph node and liver metastases. In addition, the remaining 6 patients were withdrawn after only one course of treatment and were not evaluable for response. These patients were withdrawn from the study due to DLT (3 patients), symptomatic disease progression not confirmed by objective response measurement (1), anaphylaxis (1), and tumour-associated cholangitis (1).

Pharmacokinetic analyses

Blood samples for PK analyses were available from the first treatment cycle for all 8 patients who were treated with a starting dose of 2 mg diflomotecan, and from the second treatment cycle for the 3 patients who went on to receive 4 mg diflomotecan and the 1 patient who went on to receive 3 mg diflomotecan. Blood samples for PK analyses were available for 3 of the 5 patients who were treated with a starting dose of 4 mg diflomotecan, including 2 patients from the first treatment cycle and 1 additional patient from the second treatment cycle. The 24-h PK concentration from the first treatment cycle from this final patient was unusually high, and so data from the first treatment cycle was not included in the PK analyses. EBT results were also available from the same patients as for PK analyses with the exception of one patient who was treated with 2 mg diflomotecan in the first treatment cycle, and with 4 mg in the second treatment cycle.

The results of the PK analyses for diflomotecan and two of its metabolites (BN80942 and P-20), and of the EBT, are summarised in Table 4. The mean values for the PK parameters were calculated from 8 patients treated at 2 mg diflomotecan (all first treatment cycle), 1 patient treated at 3 mg diflomotecan (second treatment cycle), and 6 patients treated at 4 mg diflomotecan (2 in the first treatment cycle, and 4 in the second treatment cycle).

The concentration of diflomotecan declined rapidly after discontinuation of the i.v. drug infusion. Concentrations of the metabolites, BN80942 and P-20, gradually increased after starting the diflomotecan infusion and maximum levels were achieved approximately 1–2 h after the end of the drug infusion. Subsequently, the plasma concentration–time curves of the metabolites and parent drug declined in parallel, although with higher plasma levels for BN80942.

The mean values for the EBT were calculated from 7 patients treated at 2 mg diflomotecan (all first treatment cycle), 1 patient treated at 3 mg diflomotecan (second treatment cycle), and 5 patients treated at 4 mg diflomotecan (2 in the first treatment cycle, and 3 in the second treatment cycle). The correlation between EBT (% of dose/h) and diflomotecan clearance (L/h) is shown in Fig. 1.

There appeared to be a less than dose-proportional increase in exposure parameters of diflomotecan and its main metabolites at increasing doses. The mean plasma clearance of diflomotecan, associated with a high degree of inter-patient variability (from 50 to 64%), was estimated to be between 24.5 and 32.8 L/h for cycle 1 and 2, respectively. The apparent drug volume of distribution at steady state ($V_{ss} \sim 100$ L) was higher than the physiological water volume (43 L), and the half-life associated to the terminal phase was relatively short (approximately 5 h). In comparison with historical data from previous studies at comparable dose levels, there appeared to be no large difference in the PK parameters of diflomotecan and BN80942. The exposure of the metabolite P-20 occurred at a similar magnitude to the parent drug.

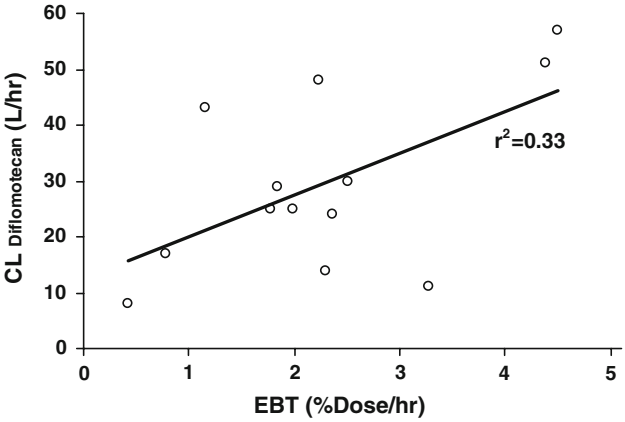

Fig. 1 Individual diflomotecan clearance (L/h) plotted as a function of EBT (% of dose/h)

The dose-limiting toxicities of homocamptothecin derivates are predominantly haematologic. The most sensitive marker of haematologic toxicity, grade of neutropenia, was analysed with the AUC as a parameter of drug exposure (Fig. 2), and there was a trend to increased toxicity with increased drug exposure. The mean drug exposure for patients with grade 0 neutropenia was twofold lower than that of patients with grade > 3 neutropenia (97 and 202 ng h/mL, respectively).

Discussion

In previous studies, diflomotecan has been largely well tolerated, and the toxicities observed have been haematologic or gastro-intestinal in nature [13, 15, 18]. When administered in a previous study using the schedule used in our study (20-min intravenous infusion every 21 days), there

Table 4 Summary of EBT and PK analysis

Dose (mg)	Descriptive statistics	EBT (%/h)	Diflomotecan					BN80942			P-20		
			$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC (ng h/mL)	Cl (L/h)	V_{ss} (L)	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC (ng h/mL)	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC (ng h/mL)
2	*n*	7	8	8	8	8	8	8	8	8	8	8	8
	Mean	2.16	4.32	31.04	114.50	24.50	93.88	5.85	20.41	229.00	4.42	13.18	98.63
	SD	1.42	1.65	11.73	72.23	15.70	26.17	0.98	9.48	166.50	1.43	10.47	53.81
	CV%	66	38	38	63	64	28	17	46	73	32	79	55
3		1.99	4.56	37.7	120	25	113	5.76	23.8	205	4.32	11.9	83
4	*n*	5	6	6	6	6	6	6	6	6	6	6	6
	Mean	2.49	3.26	45.25	156.83	32.83	112.50	5.49	22.28	242.67	4.08	23.67	148.83
	SD	1.17	1.06	13.93	88.21	16.41	37.76	0.88	10.18	162.88	0.30	10.23	11.23
	CV%	47	33	31	56	50	34	16	46	67	7	43	8

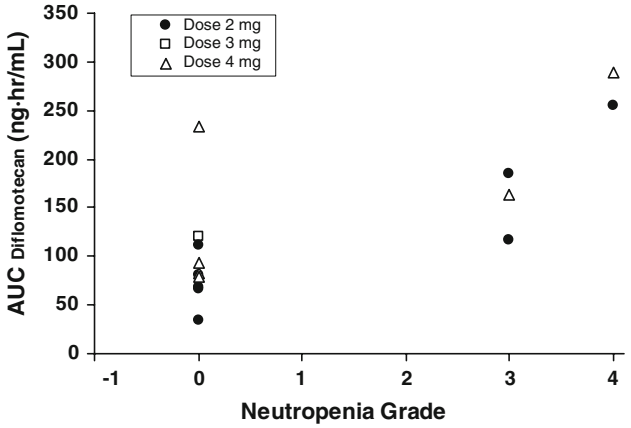

Fig. 2 Individual diflomotecan exposure ($AUC_{Diflomotecan}$) plotted as a function of neutropenia grade

was no haematologic DLT at either 2 or 4 mg/m^2, but grade 4 neutropenia and thrombocytopenia were observed at 6 mg/m^2 [18]. In contrast, dose-limiting haematologic toxicity (protracted grade 4 neutropenia) was seen at lower doses in our study, with 2 mg diflomotecan being the maximum tolerated dose. Furthermore, the patients in our study had received 1–3 lines of prior chemotherapy, and so were not likely to have had more myelotoxic previous treatments than the standard phase 1 patient population that were treated in the previous study.

In the previous phase 1 trial using the schedule used in our study [18], administration of diflomotecan as a 20-min intravenous infusion gave mean AUC values at 4 and 6 mg/m^2 of 363 ± 164 and 1,004 ± 163 ng h/mL, respectively, and mean C_{max} values of 119 ± 31 and 175 ± 33 ng/mL, respectively [18]. At the highest doses used in our study (4 mg) the mean AUC levels (157 ± 88 ng h/mL) and the mean C_{max} values (45 ± 14 ng/mL) were significantly lower than those described in the previous study [19], yet these lower doses and exposures resulted in greater myelosuppression and haematologic toxicity. However, dose-limiting toxicities were found in the two patients with the highest drug exposure (255 and 289 ng h/mL after 2 and 4 mg drug administration, respectively), whose drug AUC values fall within the range of drug exposure at which dose-limiting toxicity was observed in the previous study. As a consequence of the high degree of inter-patient variability associated with diflomotecan plasma clearance (between 50 and 64%), drug exposure after administration of 2 mg in some patients is higher than that after administration of 4 mg in other patients. Consequently, dose-limiting toxicity was observed in two patients treated at different dose levels but with similar drug exposure, suggesting that drug-induced toxicity is related to actual drug exposure rather than the administered dose. This high degree of inter-patient variability has also been observed with other camptothecin derivatives (e.g. 63% for the irinotecan metabolite, SN-38) [18]. A less than dose-proportional increase in exposure of diflomotecan and its metabolites, BN80942 and P-20, was observed at increasing administered doses. However, conclusions about linearity of diflomotecan and its metabolites should be interpreted with caution due to the low number of patients included in each dose group and the narrow dose range tested (2–4 mg). Similarly, as the number of patients that were recruited to this study was smaller than initially planned due to the observed toxicities, the sample size is insufficient to robustly determine whether other variables such as mild impairment of organ function could have influenced these AUC observations in our study. The AUC of the metabolite P-20 was similar in magnitude to that noted for the parent drug. Again, the inter-individual variability in drug clearance remained high in our study, as noted in previous studies with diflomotecan [13, 18].

The activity of the microsomal enzyme cytochrome P450 3A4 (CYP3A4) varies substantially among individuals and this variability can sometimes explain inter-patient variability in clearance of a specific drug. In this study, CYP3A4 activity was evaluated using the EBT. Several in vivo probes of CYP3A4 have been proposed and their relative merits widely discussed [19]. At the time that this study was initiated, the EBT was arguably the most widely accepted method [20] and significantly correlated with drug metabolism in cancer patients even in the presence of confounding factors such as metastatic liver disease, altered protein binding, or co-medication [21]. A similar degree of inter-patient variability for the EBT results as that of drug exposure was observed with the first cycle of diflomotecan administration. There was a trend to an association between drug clearance and CYP3A4 activity as measured by the EBT. However, it is likely that the variability of CYP3A4 activity alone does not explain the inter-patient variability of diflomotecan exposure in the limited patient numbers treated within this study. Since this study was initiated there has emerged some evidence, from analysis of the patient samples from the study of oral administration of diflomotecan, linking variant ABCG2 alleles to altered drug exposure of diflomotecan and suggesting that inter-individual variability in substrate drug effects might be influenced, in part, by ABCG2 genotype [22].

Some anti-tumour activity has been described with diflomotecan in previous studies [13, 18] including one partial response in a patient with breast cancer at a dose of 6 mg/m^2 by 20 min intravenous infusion at 3 weekly intervals, reduction in tumour markers in 3 patients (one with breast cancer and two with colon cancer) and also disease stablization was observed after two cycles of diflomotecan in 4 of 12 [13] and 5 of 20 evaluable patients with refractory disease [14]. One minor response was observed in this

study, with a 28% reduction in the sum of the largest diameters of all target lesions in a patient with oesophageal cancer, and a further 3 patients had stable disease at their first disease re-assessment after 2 cycles of dilomotecan.

In conclusion, there is a significant inter-patient variability in drug exposure after administration of diflomotecan as a 20-min intravenous infusion on a 3-weekly schedule which is similar to the variability seen with other camptothecin derivatives. In contrast, alternative dosing schedules of diflomotecan administered orally using a daily-times-five schedule have been evaluated in phase I clinical trials showing a more favourable PK/PD and safety profile. Given this data and the preliminary indications of anti-tumour activity, further evaluation of diflomotecan in Phase II clinical trials using the oral dosing schedule should be performed.

Acknowledgments The authors are grateful to all the staff who contributed to the care of the patients included in this study, and to Fiona Conway for assistance in preparing this article.

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