



Phase I and pharmacological study of the new topoisomerase I inhibitor GI147211, using a daily $\times 5$ intravenous administration

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Summary Topoisomerase I inhibitors are interesting anti-cancer agents with a novel mechanism of action. We performed a phase I study with intravenous GI147211, a new semisynthetic camptothecin analogue, using a daily $\times 5$ schedule administered every 3 weeks, to evaluate the side-effects and pharmacokinetics of the agent. Patients with a histologically confirmed diagnosis of a solid tumour refractory to standard forms of therapy were eligible for the study. GI147211 was given as a 30 min intravenous infusion daily for 5 consecutive days, repeated every 3 weeks. In subsequent patient cohorts the dose was escalated from 0.3 to 1.5 mg m⁻² day⁻¹. Pharmacokinetics analysis was performed on days 1 and 4 of the first course using a validated high-performance liquid chromatographic assay and non-compartmental methods. A total of 19 patients were entered into the study, one patient was not evaluable for toxicity because only one drug administration was given. Eighteen patients received a total of 67 courses through four dose levels. The dose-limiting toxicities were neutropenia and thrombocytopenia at the dose of 1.5 mg m⁻² day⁻¹. Nadirs occurred on day 15 and day 15 respectively. Other toxicities were mild and infrequent and included nausea/vomiting, headache and alopecia. The maximal tolerated dose was 1.2 mg m⁻² day⁻¹. One partial response was observed in a patient with colorectal cancer. The total plasma clearance was 999 \pm 184 ml min⁻¹ (range 640–1329). The volume of distribution was 190 \pm 46 l m⁻² and the terminal half-life was 3.7 \pm 1.2 h. The AUC increased linearly with the administered dose. A steep and significant sigmoid relationship was established between the AUC and the per cent decrease of ANC. GI147211 is a new topoisomerase I inhibitor that induced dose-limiting neutropenia and thrombocytopenia in this phase I study. The recommended dose for phase II studies with this schedule is 1.2 mg m⁻² $\times 5$ every 3 weeks.

Keywords: topoisomerase I; phase I study; GI147211

GI147211, [7-(methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)] camptothecin dihydrochloride, is a water-soluble semisynthetic analogue of camptothecin (CPT). Early clinical trials with CPT in the late 1960s showed hints of activity of this plant alkaloid in a variety of solid tumours (Gottlieb *et al.*, 1970; Muggia *et al.*, 1972; Creaven *et al.*, 1972). Its further development was stopped because of unpredictable and severe myelosuppression, gastrointestinal toxicity and haemorrhagic cystitis.

In the late 1980s two discoveries brought about a renewal of interest in CPT; firstly, topoisomerase I was identified as the single cellular target of CPT (Hsiang *et al.*, 1985; Hsiang and Liu, 1988) and, secondly, an overexpression of topoisomerase I was found in various tumour cell lines but not in normal tissues (Giovannella *et al.*, 1989; Hirabayashi *et al.*, 1992; Potmesil *et al.*, 1988). Topoisomerase I is a nuclear enzyme that resolves topological problems of the torsionally strained (supercoiled) DNA (Slichenmyer *et al.*, 1993). This is achieved by forming a covalent adduct between topoisomerase I and the DNA, termed the cleavable complex. This catalytic intermediate creates single-strand DNA breaks, allowing the DNA molecule to rotate around the intact DNA strand at the cleavage site, leading to a relaxation of the DNA molecule and in this way replication, transcription and other DNA functions can proceed. These enzyme-bridged breaks are then resealed by topoisomerase I. CPT stabilises the cleavable complexes, thereby preventing resealing of single-strand DNA breaks in the presence of the drug (Covey *et al.*, 1989; D'Arpa and Liu, 1989; Eng *et al.*, 1988). Cytotoxicity is specific to the S-phase of the cell

cycle because the double-strand breaks that occur during this phase are more difficult to repair in the presence of the drug (Horwitz and Horwitz, 1973).

Recently, several semisynthetic CPT analogues (Creemers *et al.*, 1994; Potmesil, 1994) have been developed, aiming at reduced toxicity and sustained or improved activity.

One of these analogues, GI147211, demonstrated significant cytotoxicity against several xenografts of human cancers including HT-29 and SW-48 colon, PC-3 prostate, MX-1 breast, H460 lung, SKOV3 ovarian and KB epidermoid carcinomas (Emerson *et al.*, 1993, 1994, 1995). The relative effect on tumour growth was dose-schedule dependent with a greater reduction in tumour volume achieved by prolonged dosing. LD₁₀ in mice was 75 mg m⁻² (20 mg kg⁻¹) given as a single bolus injection. Animal toxicology studies by the intravenous route showed that myelosuppression was the main toxicity and was dose-limiting.

We performed a phase I and pharmacological study with intravenous GI147211 on a daily $\times 5$ regimen, repeated every 3 weeks, in patients with solid tumours.

Patients and methods

Patient selection

Patients with a histologically confirmed diagnosis of a solid tumour refractory to standard forms of therapy were eligible for this study. Other eligibility criteria included: (1) age ≥ 18 years; (2) an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 ; (3) a predicted life expectancy of at least 3 months; (4) no previous anti-cancer therapy for at least 3 weeks (6 weeks for previous nitrosoureas or mitomycin C); (5) adequate haematopoietic (WBC $\geq 3 \times 10^9$ l⁻¹, ANC $\geq 1.5 \times 10^9$ l⁻¹ and platelets 100×10^9 l⁻¹), hepatic (bilirubin within normal limits, AST, ALT and/or alkaline phosphatase $\leq 2.0 \times$ normal), and renal (serum creatinine ≤ 130 μ mol l⁻¹) functions. All patients gave written informed consent.

Treatment and dose escalation

In the published phase I studies (Rowinsky *et al.*, 1992; Verweij *et al.*, 1993) using topotecan on a daily $\times 5$ schedule, the starting dose was 1/30th of the murine LD₁₀ level because of the significant interspecies differences in toxicity. By rough estimate the murine LD₁₀ for GI147211 was equivalent to topotecan. However, the *in vitro* and *in vivo* pharmacology suggested that topotecan is 2.2-fold less potent than GI147211 (15–16). Therefore it was felt that a safe starting dose for GI147211 should be less than 1/30 of the mouse LD₁₀; 0.3 mg m⁻² day⁻¹ given as a 30 min infusion for 5 consecutive days was selected. Courses were to be repeated every 3 weeks as tolerated. Dose escalations were based on the prior dose level toxicity. For example if no toxicity was seen in the prior dose, $\leq 100\%$ dose escalation was allowed. However, if toxicity was seen, a dose escalation of 25–50%, which was determined by the worst significant toxicity, was prescribed. At least three patients were entered at each dose level. The maximum tolerated dose (MTD) was defined as one dose level below the dose that induced dose-limiting toxicities (DLTs), which were defined as at least one of the following: (1) ANC $\leq 0.5 \times 10^9$ l⁻¹ or platelets $\leq 50 \times 10^9$ l⁻¹ for more than 5 days; (2) ANC $\leq 0.5 \times 10^9$ l⁻¹ with fever requiring parenteral antibiotics, and/or non-hematological toxicity \geq CTC grade 3 in more than one-third of GI147211 naive patients (at least two of a maximum of six patients). Inpatient dose escalation was not performed.

GI147211 was supplied by Glaxo as a clear solution in vials of 2.0 ml. The vials contained a mixture of 0.5 mg of GI147211 and 100 mg of dextrose. The pH was adjusted to 3.5 with sodium hydroxide or hydrochloric acid. GI147211 was diluted in D5W. The infusion bag (GI147211 + D5W) contained 100 ml exactly.

During the first course patients were hospitalised, all other courses were given at the outpatient clinic.

Treatment assessment

Before therapy medical history was taken and complete physical examination, complete blood cell (CBC) count, serum chemistries including sodium, potassium, chloride, bicarbonate, calcium, phosphorus, creatinine, urea, uric acid, glucose, total protein, albumin, bilirubin, alkaline phosphatase, AST, ALT, were performed, as were urinalysis, coagulation parameters (APTT, PT), electrocardiogram (ECG) and chest radiograph. Weekly evaluations between the courses included history, physical examination, toxicity assessment according to the CTC criteria (National Cancer Institute, 1988) and serum chemistries. CBC and urinalysis were determined twice weekly. Tumour measurements were performed after every two courses and evaluated according to the WHO criteria for response (World Health Organization, 1979); patients were taken off protocol in case of disease progression.

Pharmacokinetics

For pharmacokinetic analysis whole blood samples (7 ml) in heparinised tubes were collected from an indwelling i.v. canula, placed in the arm contralateral to that receiving the drug, before infusion and at 15, 25, 45 min and 1, 1.5, 2, 4, 6, 8, 10, 12 h after the initiation of the infusion on days 1 and 4 of the first course. Urine was collected within a 2 hour interval before the dosing and over the intervals; 0–4, 4–8, 8–12 and 12–24 h. Both blood and urine samples were analysed for the lactone form using a validated chromatographic assay, according to a method published by Stafford and St. Claire (1995). The AUC was calculated using the trapezoidal method with extrapolation of the curve to infinity on day 1 and extrapolated to 24 h on day 4. The terminal half-life was calculated as $\ln 2/\lambda$ where λ is the elimination rate constant, the total plasma clearance (Cl) as dose/AUC and the apparent volume of distribution at steady state (V_{dss}) as

$$\frac{\text{Dose} \times \text{AUMC}}{\text{AUC}^2} - \frac{\text{Dose}(0.5)}{2\text{AUC}}$$

Sigma Plot for Windows (release 2.0, Jandel scientific) and PCNONLIN (release 4.0, SCI software) were used for pharmacological data analysis. The sigmoid E_{max} model (Hill equation) was used to explore relationships between pharmacokinetic parameters and per cent decrease ANC and per cent decrease thrombocytes. The Gauss–Newton algorithm was used without weighing factor. The concentration of the drug in the infusion bags was also quantitated by high-performance liquid chromatography (HPLC).

Results

A total of 19 patients entered the study. Patient characteristics are given in Table I. All patients were eligible but one patient with NSCLC was considered not evaluable for toxicity and response as the patient was taken off study after the first drug administration, because of development of broncho-oesophageal fistula. In total 18 patients were evaluable for toxicity and response. The total number of evaluable courses was 67. The median number of courses per patient was four (range 4–10). Dose levels studied were 0.3, 0.6, 1.2 and 1.5 mg m⁻² day⁻¹.

Hematological toxicity

Neutropenia and thrombocytopenia were the dose-limiting toxicities of GI147211 on this schedule (Table II). No myelotoxicity was observed at the first two dose levels. At the third dose level (1.2 mg m⁻² day⁻¹), grade 3–4 neutropenia and thrombocytopenia were seen in 7/15 and 6/15 of the courses respectively. The median ANC nadir at this dose level was 1.29×10^9 l⁻¹ (range 0.08–5.6), for platelets it was 74×10^9 l⁻¹ (range 33–379). The median duration of severe myelosuppression, expressed as the number of days between the first occurrence of grade 3–4 toxicity and recovery to \leq grade 2 toxicity, was 10 days for neutropenia and 7 days for thrombocytopenia (Table III). In three of six patients first receiving 1.2 mg m⁻² day⁻¹, the dose was subsequently reduced to 0.9 mg m⁻² day⁻¹; in two of the patients because of slow recovery from myelosuppression (with retreatment being permitted on day 28 and day 35 respectively); and in one patient because of febrile neutropenia with sepsis during the first course. At 1.5 mg m⁻² day⁻¹ the dose-limiting toxicity was reached (Figure 1). Grade 3–4 neutropenia was noted in 13/15 courses (86%). The median ANC nadir was 0.09×10^9 l⁻¹

Table I Patient characteristics

Number of patients	19
Sex (male/female)	10/9
Median age (range)	59 (34–74)
Median performance score (ECOG)	
0	11
1	8
2	0
Prior therapy	
Chemotherapy	6
Radiotherapy	1
Both	7
None	5
Tumour types	
NSCLC	2
Colorectal cancer	9
Sarcoma	3
Unknown primary	1
Pancreatic cancer	1
Breast cancer	1
Mesothelioma	1
Oropharyngeal cancer	1

Table II Haematological toxicity

Dose (mg m ⁻² day)	Number of patients	Number of evaluable courses	Leucocytes				Neutrophils CTC grades				Platelets						
			1	2	3	4	1	2	3	4	1	2	3	4			
0.3	3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.6	3	18	8	0	0	0	4	1	0	0	1	0	0	0	0	0	0
0.9	3	9	3	1	1	0	0	4	1	0	4	2	1	0	0	0	0
1.2	6	15	1	1	4	2	2	2	1	6	0	1	6	0	0	0	0
1.5	6	15	0	9	3	3	2	0	2	11	2	6	4	3	0	0	0

Table III Neutropenia and thrombocytopenia at the two highest dose levels

	Median day of occurrence of grade 3-4 thrombocytopenia	Day nadir	Median number of days from grade 3-4 to recovery ≥ grade 2 thrombocytopenia	Median day of occurrence of grade 3-4 neutropenia	Day nadir	Median number of days from grade 3-4 to recovery ≥ grade 2 neutropenia
1.2 mg m ⁻² day ⁻¹	15 (15-15) ^a	15 (10-19) ^a	7 (2-8) ^a	15 (10-19) ^b	22 (13-22) ^b	10 (1-10) ^b
1.5 mg m ⁻² day ⁻¹	15 (10-15) ^b	15 (13-15) ^b	8 (5-12) ^b	10 (5-25) ^c	15 (13-22) ^c	11 (4-25) ^c

^an=6. ^bn=7. ^cn=13.

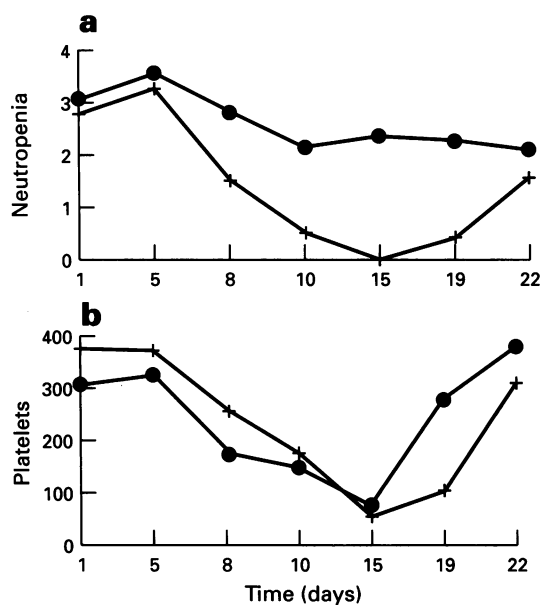


Figure 1 Comparison of the median ANC (a) and median platelet (b) counts of all given courses at doses of 1.2 (●) and 1.5 (+) mg m⁻² day⁻¹.

(range 0.01-1.7). The median number of days to recover from grade 3-4 neutropenia was 11 days (range 4-25). Three infectious complications were observed, two of them were life-threatening septic complications caused by gram-negative bacteria. In addition, thrombocytopenia grade 3-4 was seen in 7/15 courses (46%). At this dose of 1.5 mg m⁻² day⁻¹ the median platelet nadir was 62 × 10⁹ l⁻¹ (range 10-116). The recovery of grade 3-4 thrombocytopenia occurred in 8 days (range 5-12). Thrombocytopenia was complicated by gastrointestinal bleeding in two patients. Anaemia occurred frequently but was never severe. Regularly, subsequent courses had to be postponed, owing to delayed recovery of myelosuppression. At the dose of 1.2 mg m⁻² day⁻¹ a treatment delay of 1 week was required in four courses and a delay of 2 weeks was required in two courses. At 1.5 mg m⁻² day⁻¹ a treatment delay of 1 week was necessary in three courses and a delay of 2 weeks in two courses.

Cumulative myelotoxicity was not observed. The pattern of myelosuppression during the first course predicted the pattern in all subsequent courses (Figure 2).

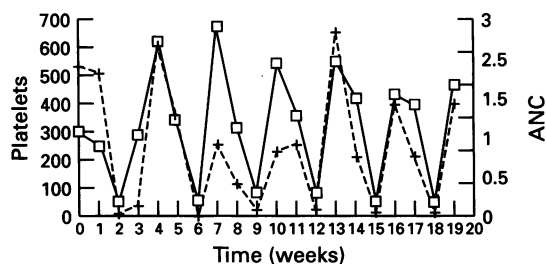


Figure 2 Graph showing course of ANC (-□-) and platelets (+) during subsequent cycles with GI47211 of patient 16 at the dose of 1.5 mg m⁻² day⁻¹.

Non-haematological toxicity

Overall, non-haematological toxicities were relatively mild. Nausea and vomiting were not dose-related and occurred in 30 (44%) and 15 (22%) of the given courses respectively and were never worse than grade 1-2. These symptoms were present only during the periods of drug administration and could easily be circumvented by the prophylactic use of standard anti-emetics. Alopecia was dose related, alopecia grade 1 was observed in three patients at the highest two dose levels. Mild headache was not dose-dependent and occurred in eight courses (12%). Prophylactic use of analgesics such as paracetamol during the days of drug administration prevented this symptom. No other toxicities were seen. There was no diarrhoea, mucositis, liver or renal toxicity.

Pharmacokinetics and kinetic-dynamic relationships

Complete plasma sampling was obtained from all 18 patients on days 1 and 4 during the first course.

The pharmacokinetic data are summarised in Tables IV, V and VI. The AUC was linearly related to the dose (Figure 3). Total plasma clearance, determined on day 1, was 1014 ± 177.0 ml min⁻¹ m⁻² (mean ± s.d.), V_{dss} was 193 ± 45.9 l m⁻² (mean ± s.d.), t_{1/2} was 3.54 ± 0.99 h (mean ± s.d.) and mean residence time (MRT) was 3.53 ± 0.93 h (mean ± s.d.).

Significant sigmoid relationships were observed between the AUC on day 4 of treatment and the per cent decrease ANC (Figure 4). No significant influence of pretreatment on these relationships was observed (Figure 5).

The fraction of the drug excreted unchanged in the urine (fe) on day 1 was 0.14 ± 0.07 and the renal clearance was 140 ± 74 ml min⁻¹ m⁻² (Table IV).

Responses One partial response was seen in a patient with metastatic colorectal cancer. His tumour had previously been shown to be resistant to 5-FU/folinic acid. The remission achieved by GI147221 lasted 6 weeks. A minor response was observed after two courses in a patient with liver metastasis of a leiomyosarcoma of the stomach, previously progressive after two courses of doxorubicin/ifosfamide. The patient refused further treatment because the second course was

complicated by a non-drug-related upper gastrointestinal bleeding. Stable disease was seen in ten patients.

Discussion

The characterisation of the inhibition of topoisomerase I as the mechanism of action of CPT has resulted in the

Table IV Pharmacokinetic data after i.v. administration of GI147211 on day 1 of the first course

Patient number	Dose (mg m ⁻²)	Day	AUC _{inf} (ng ml ⁻¹ h)	C _{max} (ng ml ⁻¹)	t _{max} (h)	t _{1/2} (h)	MRT (h)	Clearance (ml min ⁻¹ m ⁻²)	V _{dss} (l m ⁻²)
1	0.3	1	4.93	3.63	0.42	2.96	2.89	1014	161
2	0.3	1	4.04	4.27	0.42	1.99	1.66	1238	104
3	0.3	1	4.77	3.73	0.42	2.63	3.33	1048	194
		Mean	4.58	3.88	0.42	2.52	2.63	1100	153
4	0.6	1	10.10	9.16	0.42	3.35	4.01	990	223
5	0.6	1	15.63	9.41	0.42	2.81	3.74	640	134
6	0.6	1	14.00	11.46	0.42	2.80	3.52	714	140
		Mean	13.25	10.01	0.42	2.98	3.76	781	166
7	1.2	1	17.57	17.33	0.42	3.62	3.01	1138	189
8	1.2	1	24.95	11.97	0.42	4.04	5.18	802	237
9	1.2	1	16.70	15.99	0.42	2.54	2.94	1198	193
10	1.2	1	15.05	12.83	0.42	2.40	3.04	1329	223
11	1.2	1	19.86	14.11	0.25	5.10	3.98	1007	225
12	1.2	1	19.42	17.65	0.42	2.97	2.66	1030	149
		Mean	18.93	14.98	0.39	3.45	3.47	1084	203
13	1.5	1	24.66	15.08	0.25	4.98	5.11	1014	296
14	1.5	1	—	27.48	0.42	—	—	—	—
15	1.5	1	24.39	19.21	0.42	4.62	3.57	1025	204
16	1.5	1	23.32	19.53	0.25	4.37	3.32	1072	197
17	1.5	1	22.64	18.91	0.25	4.61	3.65	1104	225
18	1.5	1	28.37	15.90	0.75	4.42	3.78	881	186
		Mean	24.68	19.35	0.39	4.60	3.89	1019	222

AUC_{inf}, AUC after extrapolation to infinity; C_{max}, maximal plasma concentration; t_{max}, time to maximal plasma concentration; t_{1/2}, elimination half-life; clearance, total plasma clearance; V_{dss}, apparent volume of distribution at steady state; MRT, mean residence time.

Table V Pharmacokinetic data after i.v. administration of GI147211 on day 4 of the first course

Patient number	Dose (mg m ⁻²)	Day	AUC _{inf} (ng ml ⁻¹ h)	C _{max} (ng ml ⁻¹)	t _{max} (h)	t _{1/2} (h)	MRT (h)	Clearance (ml min ⁻¹ m ⁻²)	V _{dss} (l m ⁻²)
1	0.3	4	6.19	4.73	0.25	4.82	4.62	808	207
2	0.3	4	6.79	4.30	0.25	2.34	4.73	736	199
3	0.3	4	7.46	5.11	0.42	3.81	5.30	670	199
		Mean	6.81	4.71	0.31	3.65	4.88	738	202
4	0.6	4	11.21	7.60	0.42	3.42	4.89	892	246
5	0.6	4	15.10	8.13	0.42	3.78	5.38	662	203
6	0.6	4	15.94	9.29	0.25	4.15	6.35	6.27	224
		Mean	14.08	8.34	0.36	3.78	5.54	727	224
7	1.2	4	20.59	18.66	0.25	3.02	3.06	971	163
8	1.2	4	37.83	20.03	0.42	4.07	5.62	529	167
9	1.2	4	21.10	16.21	0.42	2.70	3.47	948	182
10	1.2	4	14.84	9.62	0.42	2.20	3.96	1348	299
11	1.2	4	26.62	12.74	0.42	6.99	7.06	802	307
12	1.2	4	16.96	11.63	0.42	4.99	4.44	1179	291
		Mean	22.99	14.82	0.39	3.99	4.60	963	235
13	1.5	4	26.92	20.02	0.42	4.95	5.06	929	261
14	1.5	4	29.51	23.81	0.42	3.44	2.56	847	117
15	1.5	4	30.85	25.41	0.25	4.35	3.04	810	144
16	1.5	4	34.51	32.19	0.42	5.62	4.47	724	178
17	1.5	4	24.85	21.18	0.25	5.82	5.75	1006	319
18	1.5	4	52.70	28.25	0.42	4.43	4.74	474	126
		Mean	33.22	25.14	0.36	4.77	4.27	798	191

AUC_{inf}, AUC after extrapolation to infinity; C_{max}, maximal plasma concentration; t_{max}, time to maximal plasma concentration; t_{1/2}, elimination half-life; clearance, total plasma clearance; V_{dss}, apparent volume of distribution at steady state; MRT, mean residence time.

Table VI Pharmacokinetic data after i.v. administration of GI147211 on days 1 and 4 of the first course

Patient number	Day	<i>f_e</i>	<i>Cl_r</i> (ml min ⁻¹)	<i>Cl_r</i>	Day	<i>f_e</i>	<i>Cl_r</i> (ml min ⁻¹)	<i>Cl_r</i> (ml min ⁻¹ m ⁻²)
1	1	0.114	232	116	4	0.109	175	88
2	1	0.081	184	101	4	0.095	128	70
3	1	0.212	333	222	4	0.428	430	287
4	1	0.115	231	115	4	—	—	—
5	1	0.156	161	101	4	0.181	192	120
6	1	0.149	194	107	4	0.197	225	123
7	1	0.106	201	122	4	0.122	196	119
8	1	0.101	151	84	4	0.219	212	118
9	1	0.088	203	107	4	0.133	241	127
10	1	0.085	226	113	4	0.128	344	172
11	1	0.116	208	119	4	0.239	314	179
12	1	0.347	663	358	4	0.210	459	248
13	1	—	—	—	4	—	—	—
14	1	0.191	—	—	4	—	—	—
15	1	0.135	280	150	4	—	—	—
16	1	—	—	—	4	0.177	218	128
17	1	—	—	—	4	0.090	182	91
18	1	—	—	—	4	0.125	101	59
	Mean	0.143	251	140		0.0175	244	138
	s.d.	0.070	133	74		0.087	106	64
	CV (%)	49	53	53		50	43	47

f_e, fraction of the drug excreted unchanged; *Cl_r*, renal clearance.

development of several semisynthetic CPT analogues, of which some are under extensive clinical investigation. This is the first report of a clinical phase I study with GI147211.

The dose-limiting toxicity of GI147211 administered as a 30 min i.v infusion for 5 consecutive days in patients with solid tumours was neutropenia in conjunction with thrombocytopenia. The dose of 1.2 mg m⁻² day⁻¹ was considered the maximally tolerated dose. At this dose the onset of neutropenia grade 3–4 occurred between days 10 and 19, with a median ANC nadir of 1.29 × 10⁹ l⁻¹ (range 0.08–5.6). The median day of the platelet nadir and the first day of grade 3–4 toxicity was day 15. The median number of days to recovery was 7 (range 2–8). The recovery of neutropenia was even more prolonged, it lasted 10 days (range 1–10). Owing to this prolonged myelosuppression subsequent courses had to be postponed in 6/15 courses at this dose level of 1.2 mg m⁻² day⁻¹. At the dose of 1.5 mg m⁻² day⁻¹ the dose-limiting toxicity was reached. At this dose grade 3–4 neutropenia already was noticed as early as day 10 (range 5–25) and lasted for 11 days (4–25) with a median ANC nadir of 0.09 × 10⁹ l⁻¹ (range 0.01–1.7). This deep and

prolonged recovery from neutropenia was complicated by three septic episodes, and resulted in treatment delay in 5 of the 15 courses given at this dose level. Although the depth, the time of occurrence and recovery from thrombocytopenia at this dose level was equal to the dose of 1.2 mg m⁻² day⁻¹, at this dose the thrombocytopenia was complicated in two courses by gastrointestinal bleeding.

As only one patient was heavily pretreated (ten courses of anthracyclin-containing chemotherapy) at the higher dose levels, these treatment delays were not related to prior myelosuppressive therapies. There were no indications of cumulative myelosuppression, the pattern of myelosuppression in subsequent courses was equal (see Figure 2). The use of haematopoietic growth factors might be helpful in preventing infections, but will be of limited value in further dose escalation of GI147211, since dose-limiting thrombocytopenia occurred in conjunction with neutropenia.

The pharmacokinetic analysis reveals moderate interpatient variability. The pharmacokinetic data, obtained on days 1 and 4 of course one, demonstrate limited inpatient variability. There was a linear relationship between the AUC

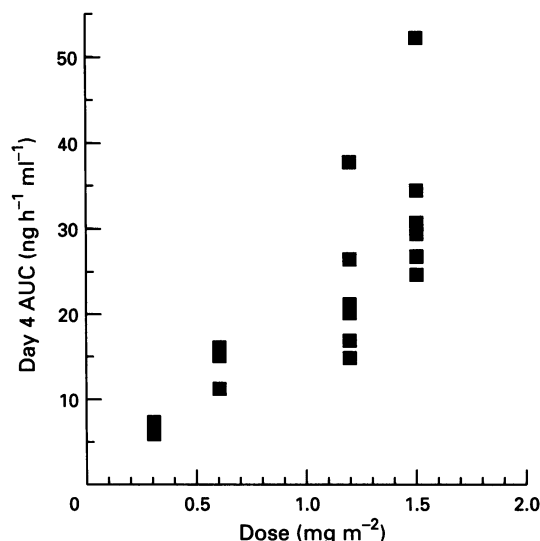


Figure 3 Relationship between the dose m⁻² and the area under the curve (AUC) of GI147211 determined on day 4 of course 1.

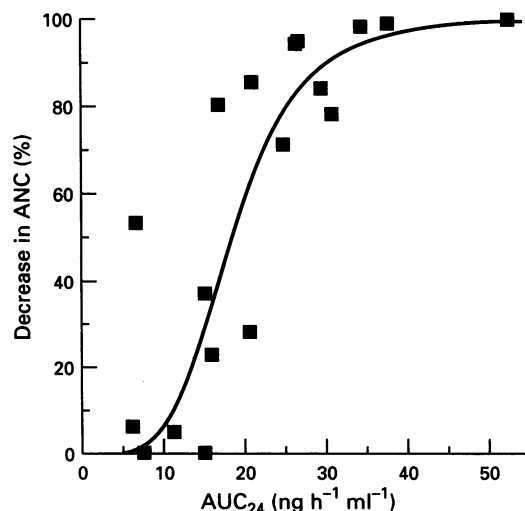


Figure 4 Relationship between the AUC of GI147211 determined on day 4 of the first course and the per cent decrease in ANC. The sigmoid *E_{max}* model was applied.

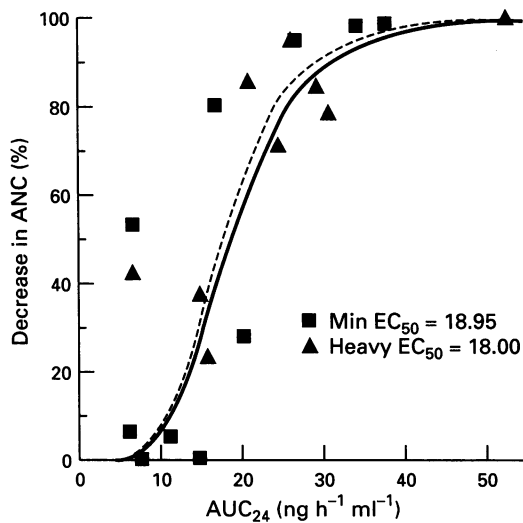


Figure 5 Relationship between the AUC of GI147211 determined on day 4 of the first course and the per cent decrease in ANC, in minimally and heavily pretreated patients. EC_{50} value in minimally pretreated patients, 18.95 (■). EC_{50} value in heavily pretreated patients, 18.00 (▲).

and the administered dose. A steep sigmoid relationship was observed between the AUC on day 4 and per cent decrease ANC, indicating that the AUC is predictive for the myelosuppression.

In this study one short-lasting partial response was noted in a patient with metastatic colorectal cancer. This fits with the observations of activity of GI147211 in preclinical models against colorectal cancer. In addition, it is of interest that we observed a minor response in a patient with leiomyosarcoma. In a recently reported phase II study in metastatic soft tissue sarcoma the topoisomerase I inhibitor topotecan only showed responses in patients with leiomyosarcoma (Eisenhauer *et al.*, 1994).

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In phase I studies with a daily $\times 5$ schedule of topotecan the dose-limiting toxicity was also myelosuppression, predominantly severe neutropenia of brief duration not necessitating treatment delays (Rowinsky *et al.*, 1992; Verweij *et al.*, 1993). Thrombocytopenia mainly occurred in prolonged continuous regimens and the myelosuppression was not cumulative (Hochster *et al.*, 1994). As the daily $\times 5$ schedule appeared to be most active in early phase I studies many different phase II studies were initiated with this scheme. Although a randomised comparison is obviously lacking, GI147211 seems to induce more prolonged myelosuppression than topotecan. Presumably related to this, in contrast to topotecan, GI147211 administration necessitated relatively frequent delays of retreatment. These human data therefore confirm preclinical studies in bone marrow cultures where GI147211 was found to be more myelotoxic than topotecan. In preclinical studies this increased myelotoxicity of GI147211 seems to coincide with more anti-tumour activity (Emerson *et al.*, 1995).

The other topoisomerase I inhibitor in a well-advanced stage of clinical development, Irinotecan (CPT-11) induces neutropenia in addition to diarrhoea. Diarrhoea was not observed at all for GI147211. Unlike GI147211, which is the active compound, CPT-11 is a prodrug. CPT-11 has to be converted to the active metabolite SN-38. It has been hypothesised that the conversion in the intestinal mucosa might be responsible for the diarrhoea. The fact that such a conversion is not required for GI147211 may result in relatively low intestinal mucosal drug levels as compared with SN-38, and thereby less mucosal damage.

Preclinical data have indicated that topoisomerase I inhibitors, like topoisomerase II inhibitors, demonstrate more efficacy with prolonged continuous exposure (Burriss *et al.*, 1992; Giovanella *et al.*, 1989; Houghton *et al.*, 1993). Therefore, future development of GI147211 will be focused on prolonged infusions, and the apparent bioavailability of oral administration will be determined.

The recommended dose for phase II studies with a daily $\times 5$ intravenous schedule is $1.2 \text{ mg m}^{-2} \text{ day}^{-1}$ repeated every 3 weeks. Phase II studies in various tumour types have recently been initiated.

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