Eur J Cancer, Vol. 28, No. 2/3, pp. 463–469, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Phase I Trial of the Anthrapyrazole CI-941: Prospective Evaluation of a Pharmacokinetically Guided Dose-escalation

Brenda J. Foster, David R. Newell, Martin A. Graham, Lindsey A. Gumbrell, Karen E. Jenns, Stanley B. Kaye and A. Hilary Calvert

The development of new drugs in early clinical trials is currently based upon the results of preclinical antitumour and toxicity studies in animals. More recently, the use of preclinical pharmacokinetic information in mice has been proposed to also provide information that might expedite early clinical trials and more specifically phase I studies. The anthrapyrazole CI-941 was one of three chosen for phase I anticancer drug development. In addition, because of the predictability of the preclinical dose limiting toxicity and linear CI-941 pharmacokinetics in mice; a pharmacokinetically guided dose escalation scheme was attempted during the phase I trial, but had to be abandoned. 44 patients were entered who received 95 courses of treatment using a bolus injection every 21 days. The dose range was 5–55 mg/m^2. The dose limiting toxicity was leucopenia and other toxicities, which included nausea and vomiting, mucositis, diarrhoea, alopecia and skin discolouration were either mild or manageable. Pharmacokinetic studies were performed with 27 courses. There were wide interpatient variations in the dose-AUC relationship (r = 0.7496) that hampered application of the proposed pharmacokinetically guided dose escalation scheme as planned. No complete or partial responses were observed. The recommended phase II dose using this schedule is 50 mg/m^2.
Eur J Cancer, Vol. 28, No. 2/3, pp. 463–469, 1992.

INTRODUCTION

THE CURRENT conventional method of phase I cytotoxic drug development has at least three central dilemmas. Firstly, the phase I starting dose is deliberately chosen to provide an initial margin of safety [1]. Thus, the starting dose is usually substantially less than the projected optimal dose, based on toxicity and activity data from preclinical models. This results in patients being treated at subtherapeutic doses. The quick solution to patients being treated at subtherapeutic doses, i.e. rapid dose escalations, creates the second dilemma in phase I studies. Thus, escalating doses too rapidly may cause unacceptable results which include severe, irreversible toxicity or even patient deaths. The third problem involves conserving medical resources (patient, hospital and staff). Conservation of resources should be maintained while obtaining sufficient information to allow completion of the phase I endpoints, which include drug tolerance and antitumour results, and allow for planning of phase II (disease specific) trials [2].

The traditional approach to solving these phase I trial dilemmas has included a starting dose that is 1/10th of the dose that produces lethality in 10% of mice (LD_{10}) normalised for surface area and expressed in mg/m^2; provided that dose was not significantly toxic to another species [1]. Dose escalations have been based mostly on arithmetically derived schema with the steepest steps occurring initially and then tapering as the maximum tolerated dose (MTD) is approached [2]. Smaller numbers of patients are entered at the lower dose levels with more patients entered after toxicity is encountered. In addition, dose escalations can be made within the same patient, particularly if no significant toxicity has occurred at lower doses.

Collins *et al.* [3] proposed a possible alternative approach using pharmacokinetic studies and toxicity information for the bridge spanning from the preclinical results across the start of the phase I trial to the predicted end-result of the phase I trial. The hypothesis in this approach assumes that the area under the plasma concentration versus time curve, i.e. AUC or C × T, at the mouse LD_{10} is equal to the AUC at the human MTD. This assumption is based on a retrospective analysis of the results from the phase I evaluations of a number of drugs. The LD_{10} in mice and the associated pharmacokinetic results along with pharmacokinetic results at the starting dose in patients are required to evaluate this hypothesis prospectively. The pharmacokinetic results (at the mouse LD_{10} and the phase I starting dose) allows calculation of the ratio of drug exposure in the two species. The initial, and possibly subsequent, escalation(s) is based on the drug exposure ratio; provided no unacceptable toxicity is encountered in the early stages of the Phase I trial. In addition to AUC-toxicity relationships, other pharmacokinetic variables such as AUC versus dose linearity, protein binding, sexual influences on drug toxicity, species dependent metabolism and drug elimination should be investigated [4]. It was proposed that by using pharmacokinetic information to guide phase I dose escalations the number of escalation steps might be reduced by 20–50% [3]. However, major caveats to using this proposed hypothesis for pharmacokinetically guided dose

Correspondence to B.J. Foster.
B.J. Foster is at the Wayne State University School of Medicine, Division of Hematology and Oncology, P.O. Box 02188 Detroit, Michigan 48202-0188, U.S.A.; D.R. Newell, L.A. Gumbrell and A.H. Calvert are at the University of Newcastle Upon Tyne, Division of Oncology, Cancer Research Unit, Newcastle Upon Tyne; M.A. Graham and S.B. Kaye are at the University of Glasgow, Cancer Research Campaign Department of Medical Oncology, Glasgow; and K.E. Jenns is at the Imperial Cancer Research Fund Clinical Oncology Unit, Churchill Hospital, Oxford, U.K.
Revised 20 Sep. 1991; accepted 10 Oct. 1991.

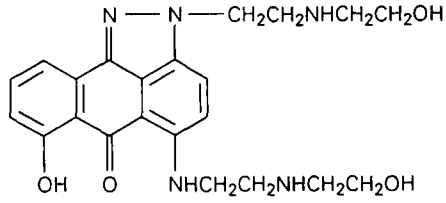

Fig. 1. CI-941.

escalations are that it may not be able to compensate for drugs with species variations in metabolism (where the drug administered is metabolised *in vivo* to one or more active species), binding, or target cell sensitivities [4]. The proposed scheme also may not compensate for highly schedule dependant toxicities unless the toxic effects are observed with one of the more easily translatable schedules, e.g. single injection, continuous infusion. However, if the toxic effects are only observed with an unusual schedule, e.g. day 1, 3 and 5 in mice; it may be difficult to translate the pharmacokinetic and toxicology results to an appropriate schedule in humans. A very practical point is that a sensitive method to measure the drug at low concentrations (likely to be encountered at the phase I starting dose) and in small samples (likely to be encountered using mice plasma samples) is required.

The synthetic DNA intercalator CI-941 [5], structure shown in Fig. 1, has a broad antitumour spectrum, similar to that of doxorubicin, in animals [6], but shows less potential for cardiotoxicity [7] based on free radical mediated mechanisms [8]. Preclinical toxicity and pharmacokinetic results with CI-941 were performed in mice as a prelude to the phase I trial [9]. The LD_{10} in mice was 20 mg/kg (60 mg/m^2) of CI-941. Pharmacokinetic studies were performed at doses of 1.5, 10, 15 and 20 mg/kg (4.5, 30, 45 and 60 mg/m^2; respectively). A linear relationship between dose and AUC was observed over the range of 1.5–15 mg/kg. However, at the 20 mg/kg dose there was a non-linear increase in AUC, i.e. the AUC at 20 mg/kg was 2.5-fold higher than the AUC at 15 mg/kg. As a result, the AUC of CI-941 in mice treated with 15 mg/kg (110 μmol/ml × min) was chosen as the target AUC value. CI-941 showed antitumour activity in preclinical models using intermittent as well as daily treatment schedules [6]. Therefore, the simplest and least costly schedule with regards to patient and staff resources, i.e. single injection every 3 weeks, was studied in this initial phase I trial. In accord with the scheme proposed by Collins *et al.* [3], pharmacokinetic studies were performed at the phase I starting dose as well as subsequent dose levels.

This study reports the clinical toxicities, antitumour results and the CI-941 dose recommended for subsequent phase II trials. In addition, the difficulties encountered during the prospective use of the dose escalation strategy proposed by Collins *et al.* [3] are presented.

PATIENTS AND METHODS

Drug

CI-941 was a generous gift from Warner Lambert Pharmaceutical Research, Usk Road, Pontypool, UK. The drug was supplied in vials as a freeze-dried solid, containing 25 mg of CI-941. The preservative-free lyophilised powder was reconstituted in 0.9% NaCl and used within 24 h.

Patient eligibility and evaluation

All patients had metastatic disease either refractory to at least one conventional treatment, or for which no standard conventional treatment exists, and performance status of better than or equal to 2 by WHO criteria [10]. Adequate haematological studies (haemoglobin ≥10.0 g/dl, leucocyte count ≥3.0 × 10^9/l), normal renal (serum urea and creatinine) and hepatic (serum liver enzymes, and bilirubin unless related to liver involvement with metastatic disease) function were required. In addition, adequate cardiac function (normal left ventricular ejection fraction [LVEF] corrected for age and sex, no evidence of recent myocardial infarct on electrocardiogram; and stable non-life threatening arrhythmias, if present) was required. A baseline physical examination, chest X-ray as well as other radiological studies to document extent of disease were required within 1 week of entering the study. Informed consent was obtained folowing the guidelines of the local Ethical Committee and the London Royal College of Physicians.

Weekly follow up with physical examination, blood or serum studies to evaluate possible bone marrow, renal and hepatic toxicity were performed. Repeat of previously positive radiological studies were performed every 6–9 weeks or sooner when indicated. LVEF was repeated when the patients went off study whenever possible. Response and toxicity were graded by standard WHO criteria [10]. The leucopenia data were analysed by a computer generated non-linear least squares regression (sigmoidal curve) using the assumption that no leukopenia occurred if no CI-941 was given.

Phase I treatment

CI-941 was reconstituted in 0.9% NaCl and given by intravenous injection over approximately 5 min. The starting dose was 5 mg/m^2 (approximately 1/10th the mouse LD_{10}, expressed as mg/m^2). It was intended to make the initial escalation level a 2-fold increase over the starting dose. Subsequent escalations would also be 2-fold until the mean CI-941 AUC in patients/target AUC determined in mice ratio was 0.4, provided no unacceptable toxicity was observed. After reaching the mean patient/target AUC ratio of 0.4, further escalations of 30–35% over the previous dose level would be made until grade 3 toxicity was observed in 2/3 of patients treated at the same dose level. Intrapatient escalations were allowed. Treatment was repeated every 21 days or when the white blood cell (WBC) count was ≥3.0 × 10^9/l. Patients received two or more courses unless obvious progressive disease was present after the first course.

Pharmacokinetic analyses

All solvents and reagents were either analytical-reagent grade or high performance liquid chromatography (HPLC) grade and obtained from standard suppliers. Plasma samples were obtained from heparinised blood taken prior to treatment and 5, 10, 15, 20, 45, 60, 120, 180, 240, 300, 480, 720, 1080 and 1440 min following treatment. Urine was collected for the first 24 h following treatment when possible. Patient samples were analysed using the method described previously by Graham *et al.* [11]. In brief, aliquots (1–2 ml) of plasma or urine were passed through 200 mg/3 ml C_2 Bond Elut columns (Jones Chromatography Ltd). Drug standards were prepared at 50 and 500 ng/ml in pooled human plasma or urine from healthy volunteers and similarly passed through the columns. The columns were solvated prior to use with approximately 3 ml methanol followed by 10 ml deionised water. Plasma or urine contaminants were eluted in 10 ml deionised water prior to eluting the analyte in a

mixture of 2 ml methanol/hydrochloric acid 10.2 mol/l (19:1 v/v). The patient samples or standards were evaporated to dryness under a stream of nitrogen at 45°C, reconstituted in 200 μl HPLC mobile phase, vortexed and centrifuged at 1000 *g* for 5 min prior to analysis. The reconstituted fractions were analysed by HPLC using a 15 cm × 0.46 cm Spherisorb C_6 analytical column (5 μmol/l particle size) (Phase Separations, Queensferry, UK.) fitted with a CO:PELL ODS (C_{18}) precolumn (Whatman). The analyte was eluted isocratically at a flow rate of 1.5 ml/min at ambient temperature. The mobile phase consisted of acetonitrile/methanol/0.25 mol/l ammonium formate (adjusted to pH 3.0 with 98% formic acid) (1:1:8 v/v). CI-941 present in the HPLC effluent was detected by ultraviolet absorbance at 492 and 385 nm. The standards (plasma and urine) were analysed before and after each set of samples and quantitation achieved by external standardisation. The minimum detection limit of the assay was 1 ng/ml for a 2 ml sample. The standard curve was linear over the concentration range detected in patients plasma and urine samples. Intra- and interassay coefficients of variation for plasma and urine were ≤10%.

The pharmacokinetic data were analysed by a computer generated non-linear least squares regression analysis with a weighting of $1/(y + \hat{y})^2$ [12, 13]. The best estimates were obtained by fitting a triexponential model to the data points using the equation

$$C = Ae^{-\alpha t} + Be^{-\beta t} + Ze^{-\gamma t}$$

where C is the plasma CI-941 concentration at time t. A, B, and Z are the concentration constants for the 1st, 2nd, and 3rd phases, respectively and α, β and γ are the 1st order rate constants for the three respective phases [14]. The area under the plasma CI-941 concentration versus time curve (AUC) was determined using the equation

$$\text{AUC} = A/\alpha + B/\beta + Z/\gamma$$

and plasma clearance

$$\text{Cl} = \text{Dose/AUC}.$$

The 1st, 2nd and 3rd phase half lives ($t_{1/2}$) were calculated from the 1st order rate constants, i.e.

$$t_{1/2} = 0.693/\text{1st order rate constant}.$$

RESULTS

Patients' characteristics

44 patients (31 females, 13 males) entered the study. Details of patient characteristics are shown in Table 1. The numbers of patients treated per dose level and courses administered with pharmacokinetics are shown in Table 2. Twelve dose levels (5–55 mg/m²) were studied. 6 or more new patients were treated at three of the top 4 dose levels. 27 of the 95 courses administered were subject to pharmacokinetic studies. Although the intent was to use the mean AUC at each dose level to determine subsequent dose levels to reach the target AUC (110 μmol/l × min), this approach was abandoned because of interpatient AUC variability (see pharmacokinetics).

Table 1. CI-941 phase I trial patient characteristics

No. of patients entered	44
Patients lost to follow-up or early deaths	3
Females	31
Males	13
Median age (range 23–69)	54
Performance status (WHO)	
0–1	20
2	20
3	4
Diagnosis	
Ovarian carcinoma	8
Breast carcinoma	11
Other	25
Prior therapy	
None	7
Doxorubicin and/or mitozantrone	6
Other chemotherapy	35
Radiotherapy	19

Table 2. CI-941 dose levels, No. of patients treated and courses administered with pharmacokinetics

Dose (mg/m²)	No. new*	Total	No. of courses pharmacokinetics	Total
5	3	3	3	3
7.5	—	1	1	1
10	1	3	2	4
15	3	3	2	3
20	3	6	3	7
25	3	4	3	9
30	4	6	2	7
35	5	5	3	10
40	6	7	4	20
45	6	6	2	7
50	7	7	1	17
55	3	3	1	7

*Patients previously untreated with CI-941

CI-941 toxicities

Leukopenia. The phase I starting dose was 5 mg/m² (near 1/10th the mouse LD_{10}). Escalations of 5 mg/m² were used to 55 mg/m². The dose limiting toxicity (DLT) was leukopenia and is shown in Table 3. WHO grade 3 leukopenia was initially observed at 35 mg/m². Leukopenia ≥ grade 3 was observed in 2 of 3 patients (4 of 7 courses) at 55 mg/m². The leukopenia generally occurred during the second week following therapy and reversed by day 21. There was no marked evidence of cumulative leukopenia as shown in Fig. 2a and b at doses of 40, 50 and 55 mg/m² in patients who received 3–6 courses. 2 patients had dose reductions (35 decreased to 30 mg/m², 45 decreased to 40 mg/m²) and 1 patient had treatment delay (45 mg/m²) due to leukopenia. 2 patients (35 mg/m² and 55 mg/m²) developed infections during drug induced WHO grade 4 leukopenia. Both of these patients had metastatic colon carcinoma and infection entry sites at the skin from metastatic disease. Although the sepsis in one patient (35 mg/m²) was successfully treated, the other patient (55 mg/m²) died 11 days after treatment during the sepsis. The other early death that occurred on study was in a patient with metastatic lung carcinoma treated at 20 mg/m²,

Table 3. CI-941 toxicities: leukopenia (95 courses evaluated)

Dose mg/m²	WHO grade 0	1	2	3	4
5	1	2	—	—	—
7.5	1	—	—	—	—
10	—	3	1	—	—
15	2	1	—	—	—
20	2	4	1	—	—
25	—	4	5	—	—
30	1	1	5	—	—
35	—	2	5	2	1
40	5	4	9	2	—
45	1	2	2	2	—
50	—	2	7	8	—
55	1	1	1	3	1

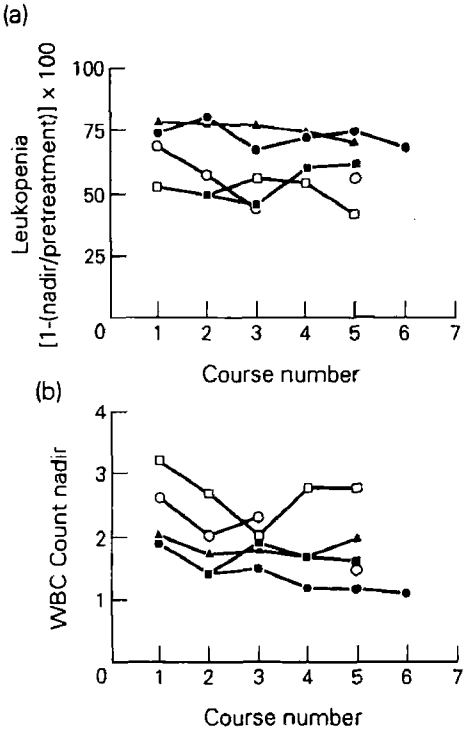

Fig. 2. Leukopenia vs. CI-941 treatment course in patients treated with (■ · · —, □ — · —) 40 mg/m²; (● — —, ○ ——) 50 mg/m²; (▲ · · ·) 55 mg/m². Values on each line are from one patient. (a) Leukopenia expressed as a percentage of the WBC decrease. (b) Leukopenia expressed as absolute WBC count nadir.

who died 3 days after treatment of a pulmonary embolus (confirmed by autopsy). His death was considered to be disease rather than drug related.

Other toxicities. The occurrence of nausea and vomiting is shown in Table 4. Although the majority of treatment courses were associated with some degree of nausea with or without vomiting, the severity did not clearly increase with dose. Other CI-941 toxicities are summarised in Table 5. Mucositis, diarrhoea and skin toxicities occurred in 19, 4 and 4%, respectively, of the courses evaluated. Although some form of alopecia was

Table 4. CI-941 toxicities : nausea and vomiting (73 courses evaluated)

Dose mg/m²	WHO grade 0	1	2	3	4
5–15	9	1	—	—	—
20	2	—	3	—	—
25	2	4	—	3	—
30	3	1	2	1	—
35	2	3	2	2	—
40	3	6	6	1	—
45	1	2	1	—	—
50	1	3	3	3	—
55	—	1	1	2	

Table 5. CI-941 toxicities: other (courses evaluated)

Gastrointestinal	
Mucositis (73)	
Grade 1*	5
Grade 2	9
Diarrhoea (75)	
Grade 2	1
Grade 3	2
Alopecia (74)	
Grade 1	12
Grade 2	16
Skin (73)	
Vein discoloration	2
Grade 2	1
Cardiac (74)	None

*WHO grade

reported in 38% of the evaluated courses, the maximum was reversible WHO grade 2. Often these patients had a history of prior alopecia. Of particular notice was that no evidence of cardiac toxicity (measured by pretreatment and off-study LVEF) or renal toxicity (measured by serum urea and creatinine) was detected. Although the majority of patients received a low cumulative dose, LVEF measured at the time they went off CI-941 treatment remained normal after cumulative doses of 205–300 mg/m² in 5 patients.

Recommended phase II dose

No complete or partial responses were observed. The MTD was 55 mg/m² and the recommended phase II dose using this schedule is 50 mg/m².

Pharmacokinetics

Plasma pharmacokinetics were studied at all dose levels (27 of 95 courses). The decline in CI-941 concentration in plasma was triexponential with mean (S.D.) $t_{1/2}\ \alpha$ = 8(2) min (range 5–13), $t_{1/2}\ \beta$ = 65(27) min (range 25–135) and $t_{1/2}\ \gamma$ = 20(8) h (range 6–35). The AUC values of the 3 patients treated at the initial dose level (5 mg/m²) were 17, 23 and 54 μmol/l × min. Although the AUC in one of these patients was approximately twice the AUC in the others and half the target AUC, none exhibited toxicity. This variability in AUC values [31(20)] prevented the use of AUC's at the starting dose to calculate

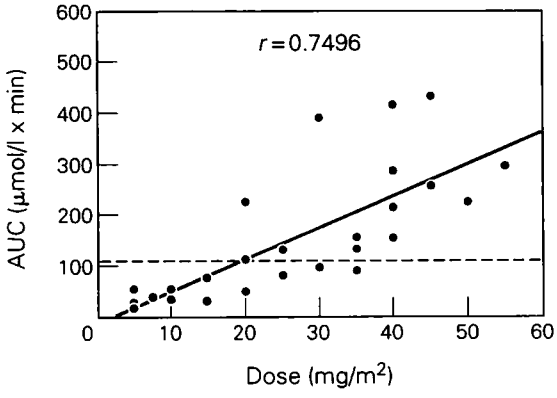

Fig. 3. CI-941 plasma AUC vs. dose in patients who had pharmacokinetic studies. The solid line (—) is the linear regression of all points. The dashed line (– –) is the target AUC of 110 μmol/l × min.

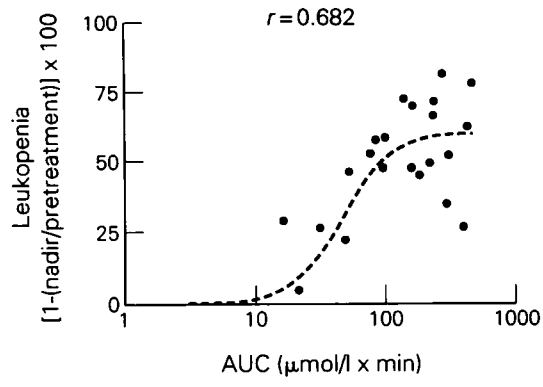

Fig. 5. First course leukopenia (expressed as a percentage of the WBC decrease) vs. CI-941 plasma AUC in patients who had pharmacokinetic studies. The line is the nonlinear least squares regression (sigmoid curve) of all points.

the interspecies drug exposure ratio necessary to allow dose escalations based on AUC values. Pharmacokinetic studies performed at subsequent dose levels confirmed the initial observations of interpatient AUC variability and lack of any clear correlation between AUC and toxicity severity. The AUC levels at the 12 dose levels are shown in Fig. 3 and the linear regression coefficient (r) was 0.7496 ($P < 0.001$).

As stated above, the DLT was leukopenia. The leukopenia associated with the first course of CI-941 treatment in patients who had pharmacokinetic studies is shown in Fig. 4 ($r = 0.745$, $P < 0.001$). By comparison, the leukopenia associated with the first course AUC of CI-941 is shown in Fig. 5 (same group of patients as shown in Fig. 4). In the latter case (Fig. 5) the r value was 0.682 ($P < 0.001$). Therefore, the leucopenia correlated no better with CI-941 AUC than with dose expressed as mg/m^2.

The wide variations in AUC values are a reflection of the wide variation in CI-941 clearance in patients which was not readily explained by renal, hepatic or cardiac function; or by sex, age, malignant disease type or performance status. One of the initial dose level AUC values was at least half-way to the target AUC of 110 μmol/l × min. Thus, in view of the nonlinear pharmacokinetics observed in mice treated at higher doses and the steep dose–toxicity curve observed in mice [9], cautious increments of 5 mg/m^2 were used. The one exception was the patient with a AUC of 54 μmol/l × min without toxicity at the starting level. The dose escalation in this instance was 2.5 mg/m^2 over the previous level.

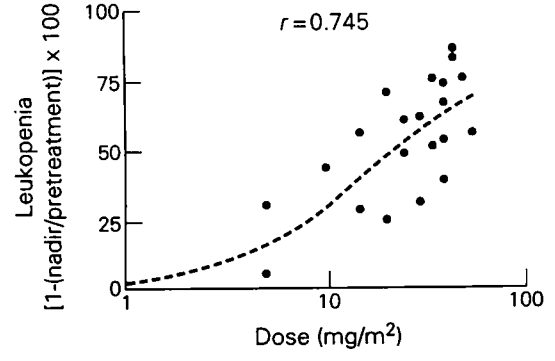

Fig. 4. First course leukopenia (expressed as a percentage of the WBC decrease) in patients who had pharmacokinetic studies. The line is the nonlinear least squares regression (sigmoid curve) of all points.

CI-941 renal excretion was <10%. No evidence of significant CI-941 metabolism was observed as assessed by the lack of significant components other than CI-941 in the patient samples when analysed by HPLC.

DISCUSSION

There have always been efforts to improve methods of drug development in phase I trials. In 1966 Freireich *et al.* published results that established interspecies relationships between dose (normalised for surface area and expressed as mg/m^2) and toxicity [15]. This provided a rationale, that was substantiated with data for numerous drugs, on the use of preclinical studies in mice to provide toxicity predictions for phase I trials. The results of Freireich *et al.* were based on a retrospective analysis. However, subsequent independent retrospective analyses on the use of preclinical toxicity data, as well as clinical experience, has confirmed that a safe phase I starting dose is provided by mouse toxicity results for the majority of cytotoxic drugs [1, 16–18]. Based on retrospective analysis of a variety of drugs, Collins *et al.* proposed dose escalations based on pharmacokinetic data generated at the mouse LD_{10} and the starting dose of the Phase I trial [3]. Implicit in the use of the dose escalation scheme proposed by Collins *et al.* is the concept that the DLT will be the same in mice and patients or that the dose limiting normal tissues will have the same quantitative drug exposure-toxicity relationship. Furthermore, the availability of a sensitive assay method to measure the drug both at low concentrations (as expected at the phase I trial starting dose) and in limited volume samples (such as mouse plasma samples), is a requirement.

A variety of anthrapyrazoles have been synthesised in the search for a DNA binding agent with an antitumour spectrum similar or superior to doxorubicin without cardiotoxic potential [19]. CI-941 is one such candidate [6, 20] and was chosen for clinical development. CI-941 was selected as a candidate for prospective evaluation of pharmacokinetically guided dose escalation for the following reasons: (a) a sensitive method for the measurement of CI-941 had been developed [11]; (b) preclinical results indicated similar protein binding in mice and human plasma [9]; (c) pharmacokinetic studies analysed by HPLC in mice indicated a lack of significant *in vivo* metabolism [9] and *in vitro* studies with anthrapyrazoles indicated that the parent drug was the active species [5]; (d) linear pharmacokinetics were observed in mice over a 10-fold dose range that included the equivalent of the phase 1 starting dose (expressed as mg/m^2) and

allowed the selection of a target AUC [9]; and (e) the DLT in mice was leukopenia which was predicted to be similarly observed in patients based on the predictability of bone marrow toxicity [21, 22] as well as its prevalence associated with the use of cytotoxic drugs with similar structure characteristics as the anthrapyrazoles, i.e. anthracyclines and anthraquinones [23, 24].

In practice, the DLT in patients was leucopenia (as in mice) and the MTD was 55 mg/m^2 (LD_{10} in mice was 60 mg/m^2). However, due to the interpatient AUC variability as shown in Fig. 3, it was not possible to use the mean AUC at any dose level to guide escalations at subsequent levels. The AUC range was very broad and often (21 of 26) included AUC values that were half or more of the target AUC value. Furthermore, the correlation between the DLT (leukopenia) and AUC was no better than the correlation between the phase I dose level (mg/m^2) and leukopenia, $r = 0.682$ ($P < 0.001$) and 0.745 ($P < 0.001$), respectively (figures 5 and 4). Obviously, a very important key is to identify the mechanism(s) responsible for the variations in plasma clearance, such as was done with carboplatin [25] because this would allow accurate AUC predictions. Another study of CI-941 using a weekly ×3 schedule every 6 weeks also observed interpatient variations of the AUC and the DLT was leukopenia with few other side effects [26]. To date, the aetiology of the variations in CI-941 plasma clearance that resulted in AUC variations has not been defined.

Additional studies (preclinical and phase I) are needed to assess further whether the problem of inter patient AUC variability encountered was peculiar to CI-941 and whether dose escalation schemes can be based on AUC values. One approach would be to determine the likelihood of AUC variation in preclinical studies prior to phase I trials. For example, in addition to studies in mice, pharmacokinetic and toxicity studies might be assessed in larger animals, e.g. rats and/or dogs. Other species are already often used to check the safety of the recommended phase I starting dose [1]. The addition of pharmacokinetics to toxicity studies in larger animal species would not only allow inter-species comparisons, but also further intra-species and intra-animal comparisons at equivalent and varying dose levels. However, the addition of more preclinical studies might delay the start of phase I trials, escalate the cost of preclinical drug development, and suggest toxicities that are later shown to be species specific and not of clinical importance. To illustrate the latter issue, nephrotoxicity has been reported in rats treated with doxorubicin [27], but has not surfaced as being clinically relevant [28]. A similar renal lesion has recently been reported in rats treated with CI-941 [29]. Although CI-941 associated nephrotoxicity is of interest, particularly since preclinical studies demonstrated a high CI-941 concentration in mice kidneys [9], it was not observed in the phase I trial of CI-941. In spite of the possible drawbacks (delayed clinical development, higher preclinical costs, false positive toxicity predictions); the additional pharmacokinetic studies with toxicity evaluation would still provide useful additional information for deciding which drugs might be favourable candidates for pharmacokinetically guided dose escalation during phase I studies.

Other investigators have also prospectively evaluated the dose escalation scheme proposed by Collins *et al.* [3]. Two were results from phase I trials by separate institutions of another anthrapyrazole, oxantrazole or piroxantrone [30, 31]. In both studies, too few samples from patients treated at the starting and first escalation doses had detectable plasma levels of drug to allow for accurate calculation of AUC. However, in one study AUC determinations at the third and fourth dose level (despite interpatient variations) allowed a 2-fold escalation to the fifth level [30]. It was estimated by the authors that this 2-fold escalation allowed 6–9 fewer patients to be treated in this phase I study. The other study found that the estimated AUC at the third dose level was equal to or greater than the target AUC and further escalations were made using a modified Fibonacci scheme [31]. Thus, one oxantrazole study used the proposed pharmacokinetically guided dose escalation scheme and one did not.

Table 6. Comparative CI-941 phase I dose escalations

Dose levels (mg/m^2)	
Modified Fibonacci	Actual
5	5
10	7.5
16.5	10
25	15
35	20
45	25
60	30
(50)	35
(55)	40
	45
	50
	55

(Probable levels)

Other results from a phase I trial of a new anthracycline, iodo-doxorubicin, reported marked pharmacokinetic and metabolism differences in mice and humans [32]. These differences prevented use of pharmacokinetically guided dose escalations in the initial stages of the trial. Yet, through very careful pharmacokinetic and toxicity studies during these earlier stages of the trial, the reasons for the species differences were identified. This allowed the determination of the critical interspecies drug exposure ratio; which in turn allowed a 2-fold escalation between dose levels 6 and 7. Thus, a pharmacokinetically guided dose escalation was possible during the latter portion of this phase I trial. This iodo-doxorubicin study demonstrated one of the main caveats to the use or pharmacokinetically guided dose escalations in phase I trials based on mice results previously expressed [4], i.e. species specific metabolism. Clearly, more prospective evaluations of the escalation scheme as proposed by Collins *et al.* are necessary before recommendations concerning its utility may be suggested.

The CI-941 clinical evaluation showed that the pharmacokinetically guided dose escalation scheme as proposed by Collins *et al.* [3] could not be successfully applied. This lack of success occurred even though problems of metabolism, protein binding, end organ toxicity and drug measurement were not present. Although the proposed pharmacokinetically guided dose escalation scheme was not used in the Phase I trial of CI-941, the pharmacokinetics were helpful in the decision to make cautious fixed escalations of 5 mg/m^2. If the more traditional modified Fibonacci dose escalation scheme had been used, the MTD would have been reached in six steps instead of 11 as shown in Table 6. However, the dose of 60 mg/m^2 would have exceeded the MTD. Probably the intervening levels of 50 and 55 mg/m^2 would have still been studied had the modified Fibonacci scheme been used, resulting in a possible total of nine levels. Thus, overall the attempted use of a pharmacokinetically guided dose

escalation strategy probably did not overly compromise the phase I evaluation of CI-941.

In summary, the phase I study of CI-941 was straightforward and phase I endpoints were successfully accomplished. The recommended phase II dose of CI-941 with this schedule is 50 mg/m^2. The myelosuppression was predictable and readily reversible. No significant other toxicities were seen. In particular, it is encouraging that no evidence of cardiotoxicity was elicited despite the fact that several patients received multiple courses. Therefore, it seems that CI-941 has fulfilled its preclinical promise of being an extremely well-tolerated DNA binding agent. Early phase II studies in breast cancer have already been published in abstract form and suggest a high response rate of about 60% [33]. Although no complete or partial responses were observed in the phase I study reported herein, these preliminary phase II results were obtained in a different population of patients, i.e. those not previously treated with anthracycline-like drugs. If these preliminary phase II results are maintained, CI-941 would clearly be a good candidate both for the adjuvant therapy of breast cancer and for use at high doses in combination with haematopoietic growth factors. In addition, phase II studies should be performed in other histologies sensitive to doxorubicin and mitozantrone, e.g. small cell lung cancer.

1. Grieshaber CK, Marsoni S. Relation of preclinical toxicology to findings in early clinical trials. *Cancer Treat Rep* 1986, **70**, 65–72.
2. Calvert AH, Balmanno K. Anticancer drugs: phase I trials. *Cancer Topics* 1987, **6**, 51–52.
3. Collins JM, Zaharko DS, Dedrick RL, Chabner RA. Potential roles for preclinical pharmacology in phase I clinical trials. *Cancer Treat Rep* 1986, **70**, 73–80.
4. E.O.R.T.C. Pharmacokinetics and Metabolism Group. Commentary and proposed guidelines on "pharmacokinetically-guided dose escalation in phase I clinical trials." *Eur J Clin Oncol* 1987, **23**, 1083–1087.
5. Fry DW, Boritzki TJ, Besserer JA, Jackson RC. *In vitro* DNA strand scission and inhibition of nucleic acid synthesis in L1210 leukemic cells by a new class of DNA complexes, the anthra[1, 9-cd] pyrazole-6-(2H)-ones (anthrapyrazoles). *Biochem Pharmacol* 1985, **34**, 3499–3508.
6. Leopold WR, Nelson JM, Plowman J, Jackson RC. Anthrapyrazoles, a new class of intercalating agents with high level, broad spectrum activity against murine tumors. *Cancer Res* 1985, **45**, 5532–5539.
7. Fagan MA, Hacker MP, Newman RA. Cardiotoxic potential of substituted anthra(1,9-cd)pyrazole-6-(2H) ones (anthrapyrazoles) as assessed by the fetal mouse heart organ culture. *Proc Am Assoc Cancer Res* 1984, **25**, 302.
8. Graham MA, Newell DR, Butler J, Hoey B, Patterson LH. The effect of the anthrapyrazole antitumour agent CI-941 on rat liver microsome and cytochrome P450 reductase mediated free radical processes; inhibition of doxorubicin activation *in vitro*. *Biochem Pharmacol* 1987, **36**, 3345–3351.
9. Graham MA, Newell DR, Foster BJ, Calvert AH. The pharmacokinetics and toxicity of the anthrapyrazole anticancer drug CI-941 in the mouse: a guide for rational dose escalation in patients. *Cancer Chemother Pharmacol* 1989, **23**, 8–14.
10. WHO Handbook for Reporting Results of Cancer Treatment: World Health Organization. *WHO Offset Publication* Geneva 1979, **48**.
11. Graham MA, Newell DR, Calvert AH. Determination of the anthrapyrazole anticancer drug CI-941 in plasma and urine by solid phase extraction and high performance liquid chromatography. *J Chromatogr Biomed Appl* 1989, **491**, 253–261.
12. Jennrich RI, Sampson PF. Application of stepwise regression to non-linear least squares estimation. *Technometrics* 1968, **10**, 63–72.
13. Ottaway JH. Normalization in the fitting of data by iterative methods. *Biochem J* 1973, **134**, 729–736.
14. Wagner JG. *Fundamentals of Clinical Pharmacokinetics*. Illinois, Drug Intelligence Publications Inc., 1975.
15. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemotherapy Rep* 1966, **50**, 219–244.
16. Goldsmith MA, Slavik M, Carter SK. Quantitative prediction of drug toxicity in humans from toxicology in small and large animals. *Cancer Res* 1975, **35**, 1354–1364.
17. Guarino AM, Rozencweig M, Kline I, *et al.* Adequacies and inadequacies in assessing murine toxicity data with antineoplastic agents. *Cancer Res* 1979, **39**, 2204–2210.
18. Rozencweig M, Von Hoff DD, Staquet MJ, *et al.* Animal toxicology for early clinical trials with anticancer agents. *Cancer Clin Trials* 1981, **4**, 21–28.
19. Showalter HDW, Johnson JL, Hoftiezer JM, *et al.* Anthrapyrazole anticancer agents. Synthesis and structure—activity relationships against murine leukemias. *J Med Chem* 1987, **30**, 121–131.
20. Werbel LM, Elslager EF, Fry DW, Jackson RC, Leopole WR, Showalter HDH. 5-aminoanthrapyrazoles (CI-937, CI-941, CI-942): a novel class of DNA binders with broad-spectrum anticancer activity. In: Harrap KR, Connors TA, eds. *New Avenues in Developmental Cancer Chemotherapy*. London, Academic Press, 1987, 355–365.
21. Owens AH. Predicting anticancer drug effects in man from laboratory animal studies. *J Chronic Dis* 1963, **15**, 223–228.
22. Schein PS, Davis RO, Carter SK, Newman J, Schein DR, Rall DP. The evaluation of anticancer drugs in man. *Clin Pharm Ther* 1970, **14**, 3–40.
23. Young RC, Ozols RF, Myers CE. The anthracycline antineoplastic drugs. *N Engl J Med* 1981, **305**, 139–153.
24. Shenkenberg TD, Von Hoff DD. Mitoxantrone: a new anticancer drug with significant clinical activity. *Annals Int Med* 1986, **105**, 67–81.
25. Calvert AH, Newell DR, Gumbrell LA, *et al.* Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *J Clin Oncol* 1989, **7**, 1748–1756.
26. Allan SG, Cummings J, Evans S, *et al.* Phase I study of the anthrapyrazole biantrazole: clinical results and pharmacology. *Cancer Chemother Pharmacol* 1991, **28**, 55–58.
27. Van Hoesel QGCM, Steevenberg PA, Dormans JAMA, de Jong WH, Wildt DJ, Vos JG. Time course study on doxorubicin-induced nephropathy and cardiomyopathy in male and female LOU/M/WSL rats: lack of evidence for a causal relationship. *J Natl Cancer Inst* 1986, **76**, 299–307.
28. Schilsky RL. Renal and metabolic toxicities of cancer treatment. In: Perry MC; Yarbro JW, eds. *Toxicity of Chemotherapy*. New York, Grune and Stratton, 1984, 317–342.
29. Camplings D, Robbins MEC. Marked nephrotoxicity associated with the anthrapyrazole anticancer drug CI-941. 4th International Nephrotoxicity Symposium, Surrey U.K. 1989.
30. Ames MM, Loprinzi CL, Collins JM, *et al.* Phase I clinical and pharmacological evaluation of pirozantrone hydrochloride (oxantrazole). *Cancer Res* 1990, **50**, 3905–3909.
31. Hantel A, Donehower RL, Rowinsky EK, *et al.* Phase I study and pharmacokinetics of piroxantrone (NSC 349174), a new anthrapyrazole. *Cancer Res* 1990, **50**, 3284–3288.
32. Gianni L, Vigano L, Surbone A, *et al.* Pharmacology and clinical toxicity of 4'-iodo-4'-deoxydoxorubicin: an example of successful application of pharmacokinetics to dose escalation in phase I trials. *J Natl Cancer Inst* 1990, **82**, 469–477.
33. Mansi JL, Smith IE, Button D, Judson I, Calvert AH. Phase II study of anthrapyrazole CI-941. A highly active drug against advanced breast cancer. *Proc ASCO* 1990, **9**, 84.

Acknowledgements—This work was supported by grants from the Cancer Research Campaign and Medical Research Council. The phase I study was performed under the auspices of the Cancer Research Campaign Phase I Committee. During the time this work was performed, Dr Foster was receiving an E.O.R.T.C./N.C.I. Fellowship Award. CI-941 was a generous gift from Warner Lambert Pharmaceutical Research, Pontypool, U.K.