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ORIGINAL ARTICLE

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A comparison of methods for limited-sampling strategy design using data from a phase I trial of the anthrapyrazole DuP-941

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Abstract The pharmacokinetics of a drug in individual patients can be estimated using plasma samples collected at a limited number of time points. However, different methods for a limited-sampling strategy (LSS) design exist and the optimal method has not yet been defined. Plasma concentration data were available from 27 of 74 courses in a phase I study (dose range, 5–55 mg m^{-2}) of the novel anthrapyrazole DuP-941. Three approaches to LSS development were compared. Firstly, forward stepwise regression (FSR) was used to derive equations to predict the DuP-941 area under the concentration-time curve (AUC) based on plasma concentrations measured at specified times. LSSs were developed using 14 randomly chosen data sets and were validated using the remaining 13 data sets. Secondly, "all subsets" regression (ASR) was used to develop LSSs. A jack-knife technique was also used to allow model development utilising 26 data sets and validation on the 27th data set. Thirdly, an LSS was developed using optimal sampling theory (OST), and the LSS was used in conjuction with a Bayesian algorithm. Selected sampling times for four-point LSSs were 10, 65, 185 and 485 min (FSR) and 10, 45, 200 and 480 min (OST). Ten candidate LSSs were developed using the ASR approach. ASR- and OST/Bayesian-derived four-point LSSs gave more precise ($P < 0.05$) estimates of AUC [mean absolute percentage of difference (MAD%) $\pm$ SD: ASR, $6.4 \pm 3.7\%$; OST/Bayesian, $6.8 \pm 4.6\%$] than did FSR (MAD% $= 15.1 \pm 9.9\%$). The OST/Bayesian approach is recommended because it allows estimation of all model parameters and is more flexible with regard to sample collection time and design variables.

Key words DuP-941 · Pharmacokinetics · Limited-sampling strategy

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Introduction

Understanding the relationships between the toxic and therapeutic pharmacodynamic effects of a new drug and drug exposure are fundamental to the optimisation of drug dosage, i.e. the dosage that will give the maximal likelihood of a therapeutic response whilst minimising the risk of unnecessary or unacceptable toxic side effects. This is particularly important with respect to cytotoxic drug therapy, where the therapeutic index is, for most agents, extremely narrow. It is accepted that the pharmacodynamics of many drugs are better related to pharmacokinetic parameters such as the area under the plasma concentration-time curve (AUC) or concentration at steady state than to the absolute dose [10]. If such relationships are to be defined for new anticancer drugs, it is necessary to assess the pharmacokinetics of the drug in a large number of patients.

In addition to the definition of pharmacokinetic/pharmacodynamic relationships, the accrual of pharmacokinetic data on a large number of patients will allow the development of population pharmacokinetic models, which may in turn identify physiological factors that affect the pharmacokinetics of the drug.

Standard methodology for performing pharmacokinetic studies requires the collection of 10–15 samples, many at unsociable hours, and usually requires hospital inpatient admission. Therefore, the costs of labour and a hospital admission must be added to the expense of sample analysis, making pharmacokinetic studies expensive to perform. To reduce these costs, approaches have been developed that allow the acquisition of pharmacokinetic data using a limited number of plasma samples. An approach commonly employed to estimate the AUC for the selection of sampling times is a forward stepwise regression procedure, and this has been successfully applied to a number of anti-cancer agents [6, 16, 20, 21, 23]. In this approach the time that best predicts the AUC is chosen first. The second point selected is that which provides the most additional information. However, there is no reason to suppose that the most informative pair of sampling times should include the sampling time that singly provides the most information concerning the AUC. Thus, a regression approach that considers all subsets of sampling times may be a method of limited-sampling strategy design more logical than the forward stepwise approach.

A third approach uses optimal design theory to select sampling times [3]. A Bayesian algorithm then integrates the information provided by the limited concentration data available with prior information about the population pharmacokinetic parameter values, to estimate parameter values for the individual patient. This approach has been shown to perform well for doxorubicin, allowing estimation of the total body clearance and, hence, the AUC with only two plasma samples [17]. Similar approaches have been used in the study of the anti-cancer drugs suramin [22] and hexamethylene bisacetamide (HMBA) [2] and the antibiotics ciprofloxacin [28] and ceftazidine [5].

DuP-941 (previously termed CI-941) is a novel, synthetic, anthrapyrazole anti-cancer agent [9, 24] that has been shown in experimental tumours to have a broad spectrum of activity similar to that of doxorubicin [18, 25]. However, unlike doxorubicin, DuP-941 does not readily undergo metabolic reduction to a reactive free radical [11], a factor implicated in the dose-limiting cardiotoxicity associated with doxorubicin therapy [19]. The pharmacokinetics of DuP-941 in mice are best described by a linear, open three-compartment model [12]. In the phase I study of DuP-941 given by "slow push" injection at 3-week intervals, leukopenia was dose-related and dose-limiting [8]. Subsequently, significant single-agent antitumour activity has been documented in a phase II clinical trial in women with metastatic breast cancer. There was an objective response of 63% in 30 patients treated with a dose of $50\,mg\,m^{-2}$ [27]. In human studies, DuP-941 plasma pharmacokinetics have also been found to conform to a linear, open, three-compartment model [14].

This paper compares three different approaches used to develop limited-sampling strategies for DuP-941 using pharmacokinetic data from a clinical phase I study. Comparison is made by assessing the precision and bias of individual pharmacokinetic parameter estimates derived from a limited number of samples relative to "best estimates" obtained from full data sets. The results of these comparisons can be used to guide the selection of a limited-sampling strategy for use in future pharmacokinetic, pharmacodynamic studies with DuP-941.

Patients and methods

Patients and pharmacokinetics studies

A total of 37 patients were treated in a phase I study of DuP-941 performed at the Royal Marsden Hospital, London. Plasma DuP-941 concentration data were available from 23 patients who received a total of 27 courses at doses ranging from 5 to $55\,mg\,m^{-2}$. The patients' characteristics were typical for a phase I study of an anti-cancer agent (Table I). A number of different tumour types were included, and most patients had previously been treated with anti-neoplastic agents. However, despite their having metastatic cancer, the patients' performance status was generally good. The clinical details of the phase I study have been published previously [8].

Blood samples were drawn before treatment and at 15 time points following a "slow push" intravenous injection of DuP-941. DuP-941 was provided by Parke-Davis Pharmaceuticals (Pontypool, UK). Blood samples were immediately centrifuged (1000 *g*, 10 min) to separate plasma, which was stored frozen at $-20^{\circ}C$ until analysed by reverse-phase high-performance liquid chromatography (HPLC) [13]. The limit of quantification of this assay is 10 n*M*.

Table 1 Patients' characteristics

	Clinical study	Pharmacokinetics study
Number of patients	37	23
Number of courses	74	27
Sex:		
M	13	9
F	24	14
Median age (range)	53 (27–69) years	54 (30–68) years
WHO performance status:		
0/1	14	11
2	19	11
3	4	1
Prior therapy:		
Chemotherapy	16	8
Radiotherapy	2	1
Both	15	11
Nil	4	3

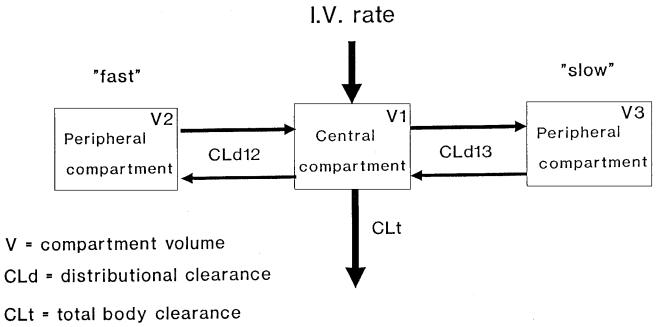

Fig. 1 Open, linear 3-compartment model fitted to DuP-941 pharmacokinetic data

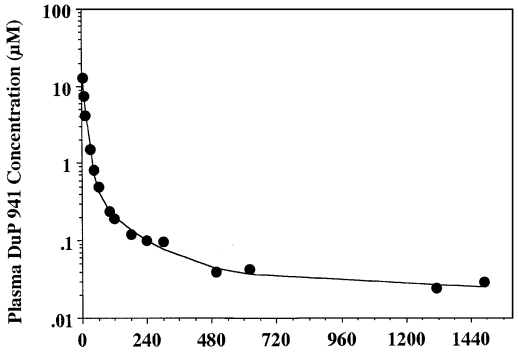

Fig. 2 Plasma concentration profile obtained in a patient treated at 55 mg m^{-2}, with model fit

Pharmacokinetic modeling of individual data sets

The "slow push" intravenous injection was modeled as a 5-min infusion, and plasma concentration data were available for the following nominal times: $t = 10, 15, 20, 25, 35, 50, 65, 125, 185, 245, 305, 485, 725, 1085$ and 1445 min, where $t = 0$ was the start of the injection. An open, linear three-compartment model with drug elimination from the central compartment (Fig. 1) was fitted to the plasma concentration data (Fig. 2). Estimation of individual pharmacokinetic parameters was performed using the maximum-likelihood algorithm included in the modeling program ADAPT II [4], run on a Hewlett Packard HP9000/835 mini-computer operating under UNIX. The maximum-likelihood algorithm estimates both pharmacokinetic [three volumes (V1, V2 and V3), two distributional clearances (CLd12 and CLd13) and total body clearance (CLt); see Fig. 1] and error-variance parameters. The error-variance model assumed that the standard deviation (SD) of the observation varied linearly with the plasma concentration (C) according to the following equation:

$$SD = \theta_1 C + \theta_2,$$

where θ_1 and θ_2 are constants.

Development of a population pharmacokinetics model

Taking the mean and variance of the maximum-likelihood-derived pharmacokinetic parameter estimates as the corresponding parameter distribution in the population represents a standard two-stage analysis. The results of a standard two-stage analysis were used to define the prior probability distribution of the population pharmacokinetic parameters for an iterative two-stage analysis [26] of the patients' data sets. The iterative two-stage analysis uses a Bayesian algorithm (also from ADAPT II) to refine individual estimates and the population model recursively until means and covariances are stable. The population model was considered to be stable when both pharmacokinetic parameter estimates and the associated elements of the covariance matrix varied by less than 1% in successive iterations. This analysis was performed using PopIT, a computer program developed at the University of Maryland Cancer Center [7]. PopIT interfaces with ADAPT II and allows multiple iterations to be performed in an automated fashion. The mean of individual patient error-variance estimates of θ_1 and θ_2 were used in all subsequent analyses to describe the population error variance.

Using PopIT and ADAPT II, individual and population parameter estimates were obtained from three data sets: (1) all 27 data sets, (2) a randomly selected subset of 14 patients used for limited-sampling strategy development, and (3) the remaining 13 data sets, which were used as a validation subset. The individual parameter estimates from analysis 1 (all 27 data sets) were subsequently taken as "best estimates" of the parameter values.

Limited-sampling strategy development

Forward stepwise regression approach

Individual patients' total body clearance (CLt) estimates from the final iteration of an iterative two-stage analysis of the 14 "development" data sets were used to calculate the AUC value ($AUC = Dose/CLt$) for DuP-941. As there is no evidence of nonlinearity of the pharmacokinetics of DuP-941 in humans AUC values and the observed plasma concentrations (C_{time}) were scaled to the smallest dose (5 mg m^{-2}) by multiplying by 5 and then dividing by the dose. A forward stepwise linear-regression analysis (The Statistician, Quant Systems, Charleston, S.C., USA) of AUC estimates (dependent variable) against C_{time} (independent variables) was then performed. This analysis produced an equation of the form $AUC = A_1 \times C_1 + A_2 \times C_2 .. A_n \times C_n + B \times (dose)$, where A_x and B are coefficients and there are n samples. The equations derived for 1, 2, 3, 4 and 6 timed plasma samples were applied to plasma DuP-941 concentration data from the 13 "validation" data sets. These limited-sampling strategy-derived AUC estimates were then compared with AUC estimates derived from the "best estimates" of total body clearance (see above). The bias of the limited-sampling strategy-derived estimates was assessed by calculating the mean percentage of difference (MD%) from the "best estimates", where percentage difference = [(derived estimate - best estimate)/best estimate] $\times 100\%$, and precision was assessed by calculating the mean absolute percentage of difference (MAD%).

All-subsets regression approach

Using the "best estimates" of the AUC for each of the 27 patients, an all-subsets regression of the AUC against the concentrations at the given sampling times was carried out using the computer program BMDP 9R [1]. As with the forward stepwise approach, all AUC and concentration values were scaled to the smallest dose. The ten "best" subsets of four sample times (where "best" means the highest R^2 value) were determined. For each of these subsets and for each of the 27 patients, a jack-knife prediction of the AUC was made. A jack-knife prediction [15] is made when the regression equation to predict the AUC was derived using the 4 concentrations from 26 of the patients, and this equation is used to predict the AUC of the 27th patient. Thus, for each subset of sample times a slightly different regression equation is used to predict the AUC of each patient. The bias and precision of the limited-sampling strategy-derived estimates

from the ten best sampling strategies were assessed as described for the forward stepwise selection approach.

Optimal-sampling theory approach

The *sample* module of ADAPT II provides a sampling strategy using optimal sampling theory, based on a single set of parameters [4]. Limited-sampling strategies were produced for each individual from the 14 "development" data sets based on the parameter values determined for that patient in the iterative 2-stage analysis. The specified error variance used the same values of θ_1 and θ_2 that were utilised in the iterative two-stage analysis, and D-optimality criteria were specified. To allow the determination of limited-sampling strategies with fewer points than the number of model parameters, the population mean and interpatient variance estimates were specified as "observed", in addition to individual plasma concentrations. From the 14 individual strategies produced, a final representative 4-point strategy was selected. In general, the selected strategy approximated the mean and/or median of each time point (t_{1-4}) from each individual strategy.

The remaining 13 data sets were used to validate the limited-sampling strategies. Plasma concentrations at each of the selected times were fitted using a Bayesian algorithm (ADAPT II) with priors developed from the iterative 2-stage analysis of the 14 "development" data sets. The bias and precision of individual patient AUC estimates derived using this sampling strategy were assessed by calculating the mean percentage of difference and mean absolute percentage of difference as described for the regression approaches.

Results

Population pharmacokinetics models

In Table 2 are shown the population pharmacokinetic models developed using the 3-compartment model (Fig. 1) for the following data sets: (1) all 27 data sets, (2) the 14 data sets in the development subset, and (3) the 13 data sets in the validation subset. The random separation of subjects resulted in a significant difference in estimated CLt between the population pharmacokinetic models for the "development" and "validation" subsets: 275 ± 122 ml min^{-1} m^{-2} for the development subset and 382 ± 140 ml min^{-1} m^{-2} for the validation subset.

Limited-sampling strategies

Forward stepwise regression approach

From the 14 "development" data sets, this approach identified 185 min as the most informative sampling time, and this single plasma concentration correlated well with the AUC ($R^2 = 0.930$). The succeeding most informative sample times (and cumulative R^2) were: 10 (0.990), 485 (0.991), 65 (0.991), 245 (0.994) and 15 min (0.995). From this analysis, the equation derived to estimate the AUC using four sample times was:

$$\mathrm{AUC} = 14.3 \times \mathrm{C}_{10} - 14.8 \times \mathrm{C}_{65} + 1643 \times \mathrm{C}_{185} + 49.0 \times \mathrm{C}_{485\mathrm{min}} + 1.17 \times (\mathrm{dose}),$$

where *dose* is expressed in milligrams per square meter of body surface area. Equations with a similar form were derived for one-, two-, three- and six-point sampling strategies. The precision (MAD%) of the two-, three-, four- and six-point strategies are shown in Table 3; however, for all strategies this value was greater than 10% (12.2–15.1%). When a single plasma sample (185 min) was used to estimate the AUC the precision worsened considerably (MAD%, 30.7 ± 21.7%). All the forward stepwise linear-regression-derived limited-sampling strategies gave biased (systematically high) estimates of individual AUC values.

All-subsets regression approach

The bias (MD%) and precision (MAD%) of the estimated AUC values are shown in Table 4. All candidate sampling strategies are unbiased and there is little difference between subset 1 and subset 10 (selected using the R^2 value) in terms of precision. It should be noted that none of the 10 selected subsets includes the 185-min sample favoured by the forward stepwise linear-

Table 2 Iterative 2-stage-derived population models for all 27 patients and the development and validation subsets

Parameter (units)	All patients ($n = 27$) mean (CV)	Development ($n = 14$) mean (CV)	Validation ($n = 13$) mean (CV)
V1 (l/m^2)	4.89 (52%)	4.55 (45%)	3.80 (53%)
V2 (l/m^2)	3.62 (42%)	3.64 (44%)	3.62 (39%)
V3 (l/m^2)	62.4 (50%)	69.5 (48%)	57.1 (50%)
CLd12 (ml min^{-1} m^{-2})	68.8 (48%)	65.3 (41%)	77.7 (52%)
CLd13 (ml min^{-1} m^{-2})	67.0 (48%)	64.2 (50%)	77.3 (52%)
CLt (ml min^{-1} m^{-2})	330 (50%)	275 (45%)*	382 (37%)*

*$0.01 < P \leq 0.05$ (unpaired t-test) for the difference between 275 ± 122 and 382 ± 140

Table 3 Bias and precision of equations derived using forward stepwise regression and used to estimate the DuP-941 AUC in each of the patients in the validation subset

Number of samples	Samples times (min)	MD% (± SD, $n = 13$)	MAD% (± SD, $n = 13$)
1	185	27.6 ± 26.8%*	30.7 ± 21.7%
2	10,185	14.2 ± 10.6%*	14.8 ± 9.6%
3	10, 185, 485	14.3 ± 10.7%*	15.0 ± 9.6%
4	10, 65, 185, 485	14.6 ± 10.8%*	15.1 ± 9.9%
6	10, 15, 65, 185, 245, 485	11.9 ± 12.6%*	12.2 ± 12.4%

*$P < 0.05$, biased (*t*-test)

Table 4 Results of limited-sampling strategy development using the all-subsets approach

Four-sample strategies		
Times (min)	Bias MD% (± SD)	Precision MAD% (± SD)
15, 65, 245, 1085	0.7 ± 7.5%	6.4 ± 3.7%
10, 15, 65, 725	1.3 ± 8.5%	6.2 ± 5.9%
10, 15, 245, 1085	0.8 ± 7.3%	5.9 ± 4.3%
15, 25, 245, 1085	0.8 ± 8.0%	6.6 ± 4.4%
15, 20, 65, 725	0.8 ± 7.5%	6.4 ± 3.7%
15, 50, 65, 1085	1.6 ± 8.2%	6.6 ± 4.8%
15, 35, 245, 1085	0.9 ± 8.6%	6.7 ± 5.3%
10, 15, 245, 725	0.3 ± 7.7%	6.2 ± 4.3%
15, 25, 65, 725	0.9 ± 7.4%	6.3 ± 3.2%
15, 20, 245, 1085	0.7 ± 8.4%	6.8 ± 4.8%

regression approach. The equation derived from subset 1 is:

$$\mathrm{AUC} = 13.8 \times C_{15} + 41.6 \times C_{65} + 413 \times C_{245} + 560 \times C_{1085} + 0.43 \times (\mathrm{dose}).$$

An alternative equation, which gave the best precision (lowest MAD%), was:

$$\mathrm{AUC} = 2.25 \times C_{10} + 11.6 \times C_{15} + 768 \times C_{245} + 297 \times C_{1085} + 0.26 \times (\mathrm{dose}),$$

although the difference in precision was not statistically significant (*t*-test).

Optimal-sampling theory approach

The chosen four-point limited-sampling strategy was 10, 45, 200 and 480 min ($t = 0$ at the start of the "slow push"). Plasma DuP-941 concentration data collected at times closest to the selected strategy (10, 50, 185 and 485 min) were fitted using the Bayesian program (ADAPT II) with prior population estimates derived from the IT2S analysis of the 14 "development" data sets. Parameter estimates and AUC (calculated using $AUC = Dose/CLt$) were compared with "best estimates" (Table 5). AUC estimation using the four-point strategy gave a low MAD% (6.8 ± 4.6%), although the estimates remained biased (MD% ± SD, 4.6 ± 6.9%).

Table 5 Bias and precision of estimates of parameter values derived using an optimal-sampling theory-derived limited-sampling strategy (4-point) an in conjunction with a Bayesian algorithm

Parameter	Bias MD% (± SD)	Precision MAD% (± SD)
AUC	4.6 ± 6.9%*	6.8 ± 4.6%

*$P < 0.05$, biased

Discussion

This paper describes the development of limited-sampling strategies for a novel anticancer agent, DuP-941. These strategies were developed using pharmacokinetic data from a phase I study of DuP-941. During a phase I study, it is common for patients to be closely monitored as inpatients, and this allows the collection of full pharmacokinetic profiles from ten or more blood samples, but in a relatively small number of patients. However, in phase II studies, drugs are commonly given to outpatients, and admission for intensive blood sampling would be both inconvenient for patients and prohibitively expensive. Therefore, a strategy using a limited number of samples that allows the accurate estimation of the pharmacokinetics of a drug in individual patients is very valuable, particularly if admission and sampling at "unsociable" hours are avoided. In addition, the cost of sample acquisition and assay are also significantly decreased by the reduction in the number of samples. The differences between the population pharmacokinetics models developed for the development and validation subsets demonstrates the need for the development of population models based on adequate numbers of patients. The development of appropriate limited-sampling strategies will facilitate this.

It is imperative in the development of novel antineoplastic drugs that limited-sampling strategies are available for use during phase II testing of a new agent. Commonly, phase III–IV studies involve the use of combination regimens, and the opportunity to study the pharmacokinetics and pharmacodynamic/pharmacokinetic relationships of the new drug in the absence of potential drug-drug interactions is lost. This

highlights the importance of demonstrating that such a strategy can be developed in a phase I dose-ranging study.

Forward stepwise linear regression has been used to develop limited-sampling strategies for a number of anti-cancer drugs [6, 16, 20, 21, 23]. However this approach generally allows only estimation of a single pharmacokinetic parameter (usually AUC), requires consistent timing of venepuncture and infusion duration, and cannot accommodate patients with missing specimens. In addition, in the development of a limited-sampling strategy for subsequent patients, the stepwise regression approach can consider only time points drawn in the original study. In the current study, a four-point limited-sampling strategy derived by stepwise regression gave only moderately precise estimates of the DuP-941 AUC in the validation subset (MAD% $\pm$ SD, 15.1 $\pm$ 9.9%) and tended to over-estimate the DuP-941 AUC (MD% $\pm$ SD, 14.6 $\pm$ 10.8%) as compared with available "best estimates" of the AUC. However, the stepwise regression approach is straightforward to apply and does not require the development of a population model, which in turn involves complicated statistical analyses and a significant computing expertise/resource.

An all-subsets regression approach was also evaluated. Although this requires more computer time than does forward stepwise analysis, it is more appropriate and remains within the capability of a modest personal computer. In this analysis a jack-knife approach was also used to allow strategies to be developed utilising 26 data sets for validation using the 27th data set. The "best" subset (MAD%, 6.4 $\pm$ 3.7%) produced more precise ($P \leq 0.05$) estimates as compared with the forward stepwise regression-derived strategy. Indeed, the results of the ten best subsets in the all-subset regression approach were very similar (MAD%, 5.9–6.8%), and this would allow the selection of a "clinically appropriate" strategy rather than being tied to the single strategy selected using the stepwise approach. The optimal-sampling theory-derived strategy, when used in conjunction with the Bayesian algorithm (single iteration), also gave more precise estimates of AUC (MAD% $\pm$ SD, 6.8 $\pm$ 4.6%) than did the stepwise regression-derived equations ($P < 0.005$) . There was no evidence of a statistically significant difference between the precision of the optimal sampling approach and the all-subsets approach.

However, there are additional advantages associated with the use of the Bayesian approach, which should be considered in selecting the approach to use. The first is where the times when samples are actually obtained differ considerably from the time specified for them to be obtained. The Bayesian approach utilises the *actual* time sampled, in contrast to the regression approaches, which must assume that the sample was taken at the *specified* time. The Bayesian approach also provides information on model parameters other than the AUC (e.g. the volume of distribution) and, therefore, simulations can be performed to study the whole plasma-concentration time course in an individual patient. This may allow investigation of other pharmacokinetic/pharmacodynamic relationships, such as the duration above a threshold concentration, in addition to relationships utilising AUC as the sole pharmacokinetic parameter.

The results of the iterative two-stage analyses of the two subsets ("development" and "validation") highlight the dangers of basing "population" analyses on small data sets. The mean parameter estimates derived from the "validation" sets varied from the "development" estimates by up to 39%. Most important was the significant ($P \leqslant 0.05$) difference in total body clearance (CLt), which was estimated to be 39% higher in the "validation" subset. Because CLt is inversely related to AUC, the differences in CLt found between the two groups may well explain the systematic over-estimation (MD% $\pm$ SD, 14.6 $\pm$ 10.8%) of AUC using the stepwise regression-derived four-sample equation. However, the optimal sampling theory/Bayesian approach, also utilising four time points, was capable of compensating almost entirely for the CLt inequalities between the subsets (AUC MD% $\pm$ SD, 4.6 $\pm$ 6.9%). The imbalance between the "development" and "validation" subsets was also negated by use of the jack-knife approach in combination with the all-subsets analysis.

The degree of precision (MAD%) achieved in this study (6.8% with a four-point optimal-sampling theory-derived strategy, 5.9–6.8% with the all-subset-derived strategies) compares favourably with the values reported for other applications of a stepwise regression approach: amonafide, 16% using a two-point strategy [21], cyclophosphamide, 9% using a three-point strategy [6] ; and oral etoposide, 8% using a two-point strategy [16]. A three-point sampling strategy (10, 185 and 485 min) was also developed for DuP-941 using the OST/Bayesian method described above. However, this gave imprecise (MAD%, 20.4 $\pm$ 14.5%) estimates of the DuP-941 AUC and, hence, is not recommended for future studies.

In conclusion, we describe the development of limited-sampling strategies for estimating the pharmacokinetics of the novel anthrapyrazole DuP-941, and we recommend that these sampling strategies be used in any future development of this drug. Because the strategies were developed in a dose-ranging phase I study, the assumption was made that there was a linear relationship between dose and AUC. However, only 1 data set was obtained at the recommended phase II dose (50 mg m^{-2}) and plasma samples were collected for only 24 h. Therefore, we would recommend obtaining full data sets including later time points from the first ten patients treated in future studies for further validation of these sampling strategies. It is hoped that the introduction of a limited-sampling strategy at this early stage in the clinical development of new drugs will

facilitate the investigation of relationships between their pharmacodynamics and pharmacokinetics and, in turn, allow more effective clinical use of new drugs.

We recommend the use of the optimal-sampling theory-derived strategy (10, 45, 200 and 480 min in combination with the Bayesian algorithm, as this would avoid sampling at "unsociable" hours and avoid the need for an overnight hospital admission and inconvenience to the patient. The Bayesian algorithm also allows for incorrect sample timing (provided that the *actual* time is known), can be used with different administration schedules and allows approximation of the full pharmacokinetics model, useful if simulation of plasma concentration profiles may yield further information. If multiple linear regression-derived limited-sampling strategies are to be used to estimate the AUC alone, we caution against the use of the forward stepwise approach and suggest that the more statistically appropriate all-subsets analysis be used.

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