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ORIGINAL ARTICLE

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A comparison of limited sampling strategies for prediction of Ecteinascidin 743 clearance when administered as a 24-h infusion

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Abstract *Purpose*: Ecteinascidin 743 (ET-743) is a novel, marine-derived anticancer agent currently under clinical development for the treatment of solid tumors. The aim of this study was to develop and validate limited sampling strategies for the prediction of ET-743 clearance in phase II studies, using two techniques: the stepwise linear regression approach and the Bayesian estimation approach. *Methods*: Data from a phase I dose-finding study were used with ET-743 administered as a 24-h infusion. Plasma concentration time data from 34 patients treated with 1200, 1500 or 1800 μg/m^2 ET-743 were randomly divided into an index data set, used for the development of the strategies, and a validation data set. With the linear regression approach, clearance (obtained by non-compartmental analysis) was correlated with the ratios of dose to the observed concentrations. For the Bayesian approach a three-compartment population pharmacokinetic model was developed; optimal time-points were selected using the D-optimality algorithm. The strategies were compared by assessment of their predictive performance of CL in the validation data set. *Results*: The linear regression method yielded a single-point sampling schedule with no significant bias and acceptable precision (–0.03% and 21%, respectively). With the Bayesian approach, a three-sample strategy was selected which resulted in less-accurate, but unbiased, predictions (bias 13%, precision 34%). *Conclusions*: Optimal sampling strategies were developed and validated for estimation of ET-743 clearance. Although the linear regression approach showed slightly better predictive performance, the Bayesian approach is preferred for the current phase II studies as it is more robust and flexible and allows the description of the full pharmacokinetic profile.

Keywords ET-743 · Pharmacokinetics · Population pharmacokinetics · Limited sampling · D-optimality

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Introduction

Ecteinascidin 743 (ET-743) is a novel anticancer agent isolated from the Caribbean tunicate *Ecteinascidia turbinata*. Preclinical studies have revealed potent activity of ET-743 against a panel of solid tumor cell lines and several human tumor xenograft models including melanoma, non-small-cell lung, ovarian, renal, prostate and breast tumors [9, 10, 11, 12, 21]. ET-743 is currently under phase II clinical development for the treatment of solid tumors.

A phase I clinical program has been conducted with ET-743 administered in several schedules and at several dose levels [15, 18, 20, 22, 24]. The pharmacokinetic profile of ET-743 administered as a 24-h infusion every 3 weeks has been described [18, 22]. In this schedule, the recommended dose for further phase II studies was 1500 μg/m^2 with neutropenia and thrombocytopenia as the dose-limiting toxicities. Hepatic toxicity was observed as well, but was reversible and not dose-limiting. ET-743 exhibits linear pharmacokinetics in the dose range tested (50–1800 μg/m^2). Considerable inter- and

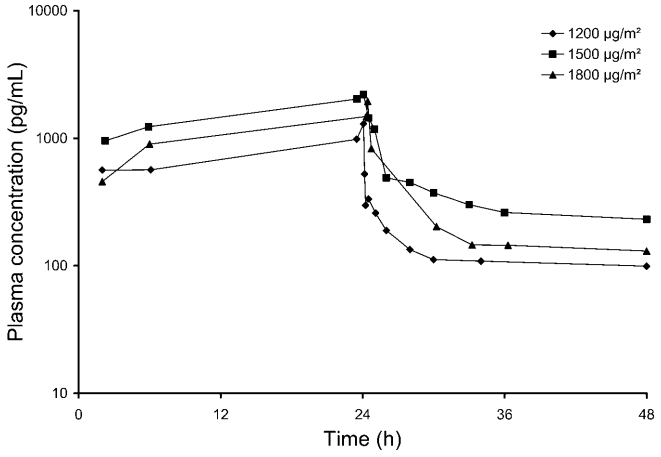

Fig. 1 Typical plasma concentration time profiles of patients treated with 1200, 1500 and 1800 µg/m^2 ET-743

intrapatient variability (45% and 28%, respectively) for the area under the plasma concentration versus time curve (AUC) was observed. ET-743 displays a long terminal half-life (at 1500 µg/m^2 the half-life was 89 h). Typical plasma concentration versus time curves of patients treated with 1200, 1500 and 1800 µg/m^2 as a 24-h infusion are depicted in Fig. 1. Combined pharmacokinetic-pharmacodynamic analyses have revealed increased hepatic and hematologic toxicity with increasing exposure to ET-743, expressed as AUC [3, 22].

ET-743 administered as a 24-h infusion is currently undergoing further clinical development in phase II studies in this schedule to further determine its antitumor activity in different tumor types, including advanced soft tissue sarcoma, melanoma, and breast and renal carcinoma. Promising clinical activity of ET-743 in multiple tumor types has been reported [4, 7, 8, 25]. During the phase II studies the concentration-time profile of ET-743 will be assessed in a large number of patients which will enable further exploration of the population pharmacokinetics and pharmacokinetic-pharmacodynamic relationships.

Standard procedures for the performance of pharmacokinetic studies usually involve extensive sampling, which is very impractical, particularly when studies are performed in multiple centers. By applying optimal sampling strategies however, the number of samples taken can be reduced without a reduction in the accuracy and precision of the estimates of the pharmacokinetic parameters.

Several different techniques are used for the development of optimal sampling schedules. An approach that has been commonly used in oncology is a multivariate linear regression procedure [13, 17, 23]. Plasma concentrations at certain time-points are correlated with the pharmacokinetic parameters of interest, usually AUC or clearance (CL), and one or more time-points that most accurately predict the pharmacokinetic parameter are included in the limited sampling strategy. A second approach uses the D-optimality theory for the selection of optimal time-points, based on previously established population pharmacokinetic parameters [5]. Bayesian estimates of individual pharmacokinetic parameters can be generated based on the prior population pharmacokinetic parameters and the individual plasma concentration-time data available [1, 19].

In this study, these two techniques were applied to the development of limited sampling strategies to assess the pharmacokinetic parameter CL when ET-743 was administered as a 24-h infusion. This parameter was selected as it determines the individual's exposure to the drug (expressed as AUC) after administration of a dose. Exposure to ET-743 has been shown to be correlated with toxicity during treatment [4, 15, 18]. The validation of the developed sampling schedules was performed on a distinct data set, by comparing the predicted pharmacokinetic parameters with reference values, obtained on the basis of full pharmacokinetic profiles. The different strategies were compared by assessment of their predictive performance.

Methods

Patients and pharmacokinetic studies

The development and validation of the optimal sampling strategies was performed using pharmacokinetic data from a phase I dose-finding study with ET-743 administered as a 24-h infusion every 3 weeks. In total 52 patients were treated at nine dose levels ranging from 50 to 1800 µg/m^2. At the recommended phase II dose of 1500 µg/m^2, a total of 25 patients was recruited to better characterize the safety profile of ET-743, before commencing an extensive phase II program. During the first course of treatment of all patients, serial blood samples were collected at 15 time-points: preinfusion, at 2, 6 and 23.5 h during the infusion, and at 5, 10, 15 min and 0.5, 1, 2, 4, 6, 9, 12 and 24 h after the end of administration. As ET-743 displays a long terminal half-life, the sampling schedule was extended at the highest dose levels in order to obtain the complete terminal part of the curve. Blood samples were then collected weekly, up to 504 h after the end of administration. The samples were analyzed using a sensitive analytical method which combines miniaturized liquid chromatography with two mass analyzers [14]. This method yields a linear detection range of 10–2500 pg/ml.

In the phase II program with ET-743, patients are treated with 1500 µg/m^2 ET-743 administered as a 24-h intravenous infusion. For the development of limited sampling strategies for this schedule, only the phase I data from the first course of patients treated at the dose levels 1200, 1500 and 1800 µg/m^2 were used. Patients were randomly divided into an index data set, which was used for the development of the limited sampling strategies, and a second data set, used for the validation of the created schedules.

Development of limited sampling strategies

With both techniques, limited sampling strategies were developed to assess the pharmacokinetic parameter CL. This parameter was selected as it determines the exposure to ET-743 (expressed as AUC) according to the following equation: CL = dose/AUC.

Linear regression approach

For all patients of the index and validation data sets, the CL was calculated applying non-compartmental pharmacokinetic analysis

using the WinNonlin program (Standard Edition, version 3.0, 1999). CL was determined by dividing dose by the AUC. The AUC was determined using the log-linear trapezoidal rule with extrapolation to infinity. The index data set was used to develop the limited sampling strategies. Values for CL were correlated with dose divided by concentration (dose/concentration). A forward multiple regression analysis was performed to select the time-points that yielded significant correlations between CL and dose/concentration. A stepwise backward elimination procedure was then applied as a check for the strategies. Statistical analyses were performed with SPSS (Statistical Product and Service Solutions, version 6.1 for Windows, 1994). All tests for significance were two-tailed and the level of significance (P) was set at 0.05.

The predictive performance of the developed schedules was assessed using the validation data set. Values for CL were calculated on the basis of the limited sampling scheme and the corresponding measured concentrations and were compared with the CLs determined using the full pharmacokinetic curve.

Bayesian estimation approach

Development of a population pharmacokinetic model

A population pharmacokinetic model was developed for ET-743 using the index data set. Data analysis was performed using the NONMEM program (double precision, version V, level 1.1) operating under Windows 98 [2]. A three-compartment structural model with elimination from the central compartment (PREDPP package, ADVAN 11, TRANS 1), was fitted to the data of the index set. The basic pharmacokinetic parameters were CL (l/h), volume of distribution of the central compartment V (l) and intercompartmental rate constants K12, K21, K13 and K31 (h^{-1}). The elimination rate constant K was defined as CL/V. Interindividual variability for the pharmacokinetic parameters was modeled using a proportional error model, assuming constant coefficients of variation. Residual variability was described with a combined additive and proportional error model.

Selection of optimal time-points

The selection of optimal time-points was performed using the D-optimality algorithm [5], as implemented in the software package ADAPT II (release 4, 1997) [6]. The D-optimality theory minimizes the total overall variance of the parameter estimates. The number of time-points in the design at least equals the number of parameters to be estimated. In this study, several optimal sampling schemes were designed, based on the sampling times generated by D-optimality. The sampling schemes needed to be practical in the clinic in view of the phase II studies and therefore the strategies were designed to provide sampling times within different time windows: 24–48 h, 24–96 h and 24–168 h after the start of infusion. A sample just before the end of infusion (t = 23.5 h) was included in all schedules. In addition, the limited sampling schedule that was previously incorporated in the early phase II protocol was tested for its predictive performance (samples at 6, 23.5, 27, 48 and 96 h after the start of administration).

The plasma concentration-time data of the validation data set were used to validate the optimal sampling strategies. Individual Bayesian estimates of CL were calculated using the values for the population pharmacokinetic parameters for both fixed and random effects of the index data set and the plasma concentrations at the optimal time-points generated with D-optimality using the POSTHOC option in NONMEM.

Determination of pharmacokinetic reference parameters

The development of the population pharmacokinetic model indicated that the pharmacokinetic profile of ET-743 could be best described by a three-compartment model. For the validation of the optimal sampling strategies, reference individual values of CL for all patients of the validation data set were generated. These individual estimates of CL were obtained using the complete plasma concentration-time data and individual modeling of a three-compartment model using NONMEM. For several patients, the individual data were not sufficient for the description of the second distribution phase of the curve. In these cases, a two-compartment model was applied. For the two-compartment model, the estimated pharmacokinetic parameters were V (l), CL (l/h) and the intercompartmental rate constants K12 and K21 (h^{-1}). Residual variability was modeled using a combined additive and proportional error model.

Comparison of the strategies

Validation of the limited sampling strategies was conducted on a data set distinct from the index data set. The predictive performance of the various strategies was evaluated using the mean relative prediction error (%MPE) and the corresponding 95% confidence interval (CI) as a measure of bias and the root mean squared relative prediction error (%RMSE) and the corresponding 95% CI as a measure of precision [16, 23]. The %MPE and MSE (mean squared error) and the corresponding standard errors are given by:

$$\%\mathrm{MPE} = \frac{\sum_{i=1}^{N}(\mathrm{pei})}{N} \times 100\% \quad \%\mathrm{SE} = \sqrt{\frac{\sum_{i=1}^{N}(\mathrm{pei}-\mathrm{MPE})^2}{N\times(N-1)}} \times 100\%$$

$$\mathrm{MSE} = \frac{\sum_{i=1}^{N}(\mathrm{pe}_i^2)}{N} \quad \mathrm{SE\ MSE} = \sqrt{\frac{\sum_{i=1}^{N}(\mathrm{pe}_i^2-\mathrm{MSE})^2}{N\times(N-1)}}$$

in which N is the number of pairs of true with predicted values and pe_i is the relative prediction error for each pair [$\ln(CL_{predicted})$–$\ln(CL_{true\ value})$]. The %RMSE is calculated as the square root of the MSE multiplied by 100% and the corresponding 95% CI is defined as the square root of the endpoints of the 95% CI of the MSE multiplied by 100%.

In general, optimal sampling schedules should be highly precise and not significantly biased.

Results

Patients and pharmacokinetic studies

At the three highest dose levels of 1200, 1500 and 1800 μg/m^2, 34 patients were treated in multiple courses. The data from the first course were used for the development of the optimal sampling strategies. The data set consisted of 34 patients, of whom 5 received 1200 μg/m^2, 25 received 1500 μg/m^2 and 4 received 1800 μg/m^2. Both the index and the validation data set consisted of 17 patients.

Limited sampling strategies

Linear regression approach

ET-743 plasma concentrations at each of the time-points in the index data set were correlated with CL, calculated using a non-compartmental analysis. Significant correlation coefficients (i.e. $P < 0.05$) ranged from 0.58 at t = 30 h and t = 48 h, to 0.78 at t = 26 h.

A stepwise forward multivariate linear regression analysis was performed for CL with dose/concentration at all time-points as explanatory variables. With this procedure, the concentrations at t = 26 h were included in the sampling schedule as they showed the best correlation with CL. This schedule produced a statistically non-significant bias of the prediction of CL of –0.03% and an acceptable precision of 21% (Table 1). Incorporation of any other time point in the schedule was not statistically significant in linear regression (95% level). In Fig. 2, the predicted values of CL using this schedule are plotted against the reference values.

The time-point with the second best correlation coefficient with CL was t = 33 h ($r = 0.73$). A sampling strategy based on this time-point, however, showed a large, statistically significant bias (–71%) and also a markedly worse precision (77%). A third strategy included the plasma concentrations at t = 6 h ($r = 0.70$). This strategy also yielded biased (35%) predictions of CL and was less precise (%RMSE 65%) than the first schedule. Linear regression indicated that inclusion of other time points in both schedules was not statistically significant at the 95% level.

Bayesian estimation approach

Population pharmacokinetic model

The data of the index set could be adequately described by a three-compartment model. The results of the fit are summarized in Table 2. Pharmacokinetic modeling of the validation data set yielded comparable estimates of all parameters (Table 2). In both the index and validation data set interindividual variability could be quantified for V, CL and K31. Addition of interindividual variability for other parameters did not improve the fit. For K31, interindividual variability was large in both the index and validation set (410% and 400%, respectively). Residual variability consisted of an additive error of 28 pg/ml for the index set and 19 pg/ml for the validation set; the proportional error was 35% and 41%, respectively.

Determination of pharmacokinetic reference parameters

Individual reference pharmacokinetic parameters of the validation data set were calculated using individual fitted curves with NONMEM. A three-compartment model could be fitted to eight curves; the other nine curves were fitted to a two-compartment model.

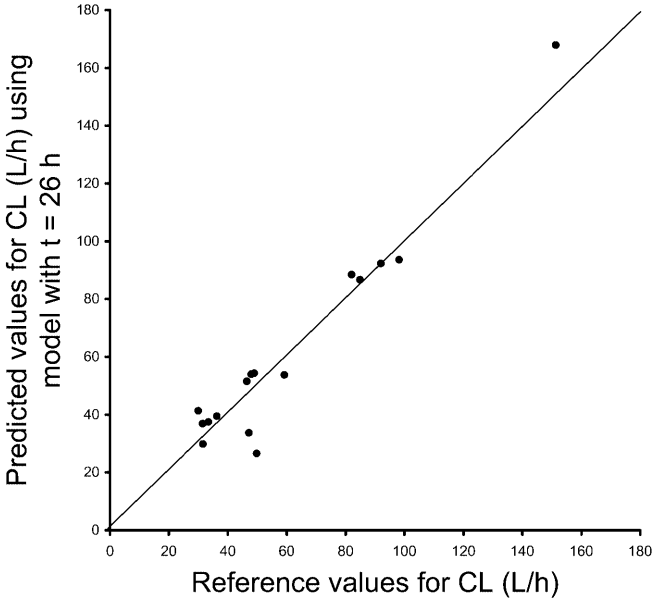

Fig. 2 Predicted values for CL using the limited sampling strategy with t = 26 h versus reference values for CL. The solid line represents the line of identity

Table 2 Population pharmacokinetic parameters of ET-743

	Index data set		Validation data set	
	Estimate	Relative standard error	Estimate	Relative standard error
Parameter				
V (l)	52	0.18	49	0.5
CL (l/h)	75	0.20	68	0.13
K12 (h^{-1})	0.62	0.29	0.32	0.34
K21 (h^{-1})	0.26	0.37	0.19	0.54
K13 (h^{-1})	1.1	0.37	0.78	0.25
K31 (h^{-1})	0.0051	0.32	0.0056	0.38
Interindividual variability (%)				
V	24	1.59	53	1.77
CL	73	0.73	57	1.31
K31	410	1.51	400	1.31
Residual variability				
Proportional error (%)	36	0.20	41	0.30
Additive error (pg/ml)	28	0.17	19	0.15

Table 1 Limited sampling strategies and corresponding predictive performance for prediction of ET-743 CL (*dose* total dose in micrograms, *conc* plasma concentration in picograms per milliliter, *CI* confidence interval)

Sampling times after start of infusion (h)	Strategy	Percent bias (95% CI)	Percent precision (95% CI)
6	$CL = 23.9 \times dose/conc_{t=6}$	35 (4.3 to 67)	65 (0 to 97)
26	$CL = 7.15 \times dose/conc_{t=26}$	–0.032 (–12 to 12)	21 (0 to 32)
33	$CL = 2.23 \times dose/conc_{t=33}$	–71 (–88 to –54)	77 (60 to 91)

Optimal sampling strategies

With the D-optimality algorithm, two time-points were selected for each given time window, i.e. t = 25.7 h after the start of infusion and a time-point as late as possible within the time window. Therefore, time-points at t = 48 h and t = 96 h were added to the schedules. Furthermore, from several patients additional weekly samples were collected from 168 h up to 504 h (3 weeks) after the first treatment course with ET-743. These weekly samples were also incorporated in the schedules. Several combinations of all sampling times were evaluated. The optimal sampling schedules involved eight variations as summarized in Table 3. The sampling scheme previously proposed in the early phase II protocol included a sample at 27 h after the start of infusion. As no sample was taken at that time in the phase I study, two different strategies were tested, including either t = 26 h or t = 28 h after the start of administration (schedules 7 and 8). A Bayesian estimation of CL was also obtained with the full validation data set (schedule 9). In Table 4 the predictive performance of each sampling schedule is summarized, and Fig. 3 depicts the percentage bias including the 95% confidence interval for each schedule. Bayesian estimates of CL using the full data set resulted in unbiased, although not very precise, predictions (%RMSE 46%). All but three optimal sampling strategies yielded statistically significantly biased estimates of ET-743 CL. The schedule with only one sample taken at t = 23.5 h (schedule 1) yielded the least-precise estimates of CL.

A three-point sampling scheme including a sample at t = 48 h and at t = 96 h resulted in unbiased predictions of CL (schedule 4). When the weekly samples were incorporated in the model, both bias and precision slightly improved (schedule 5). The addition of a sample during the infusion (schedule 6) did not improve the predictive performance. The incorporation of samples shortly after the end of infusion (schedules 7 and 8) resulted in significantly biased estimates of CL. Schedule 5, which included a sample just before the end of infusion, and at 48 h, 96 h and 1 week after the start of infusion, showed no significant bias (12%) and displayed the best precision. In Fig. 4, the predicted values for CL using schedule 5 are plotted against the reference values of CL.

Discussion

The aim of this study was to develop and validate limited sampling strategies for the prediction of ET-743 plasma clearance for the pharmacological support of phase II studies with the drug administered as a 24-h infusion. These strategies were developed using full plasma concentration versus time curves of a phase I study performed at this schedule.

ET-743 is dosed in the microgram range, resulting in nanomolar plasma concentrations. Although a very sensitive bioanalytical assay has been developed for ET-743 [14], the terminal phase of the curve could not be completely determined at the lower dose levels (50–900 μg/m^2). Therefore, the limited sampling strategies were designed using data from the three highest dose levels, 1200, 1500 and 1800 μg/m^2. Moreover, 1500 μg/m^2 is the recommended dose for moderate- or good-risk patients in the phase II program, and 1200 μg/m^2 is the suggested dose reduction step.

Bayesian estimation of individual pharmacokinetic parameters requires a priori established population pharmacokinetic parameters of the study population. A population pharmacokinetic model was designed for ET-743 on 17 full curves of the index data set. The pharmacokinetics of ET-743 were adequately described using a three-compartment model with elimination from the central compartment. Interpatient variability could be quantified for V, CL and K31; the data did not allow

Table 4 Predictive performance of Bayesian estimation of ET-743 clearance with the different sampling strategies

Strategy	Number of samples	Percent bias (95% CI)	Percent precision (95% CI)
1	1	35 (16 to 53)	49 (34 to 61)
2	2	20 (3.2 to 38)	38 (21 to 50)
3	3	25 (11 to 40)	37 (24 to 46)
4	3	13 (–3.4 to 30)	34 (19 to 44)
5	4	12 (–4.5 to 28)	33 (17 to 43)
6	5	14 (–1.6 to 30)	33 (0 to 48)
7	5	20 (4.9 to 36)	36 (0 to 51)
8	5	18 (2.2 to 33)	34 (0 to 50)
9	Full data set	6.6 (–17 to 31)	46 (16 to 63)

CI confidence interval

Table 3 Optimal sampling strategies

Strategy	Number of samples	Sampling times after start of infusion						
1	1		23.5 h					
2	2		23.5 h				96 h	
3	3		23.5 h	26 h		48 h		
4	3		23.5 h			48 h	96 h	
5	4		23.5 h			48 h	96 h	Weekly
6	5	6 h	23.5 h			48 h	96 h	Weekly
7	5	6 h	23.5 h	26 h		48 h	96 h	
8	5	6 h	23.5 h		28 h	48 h	96 h	
9	Full data set							

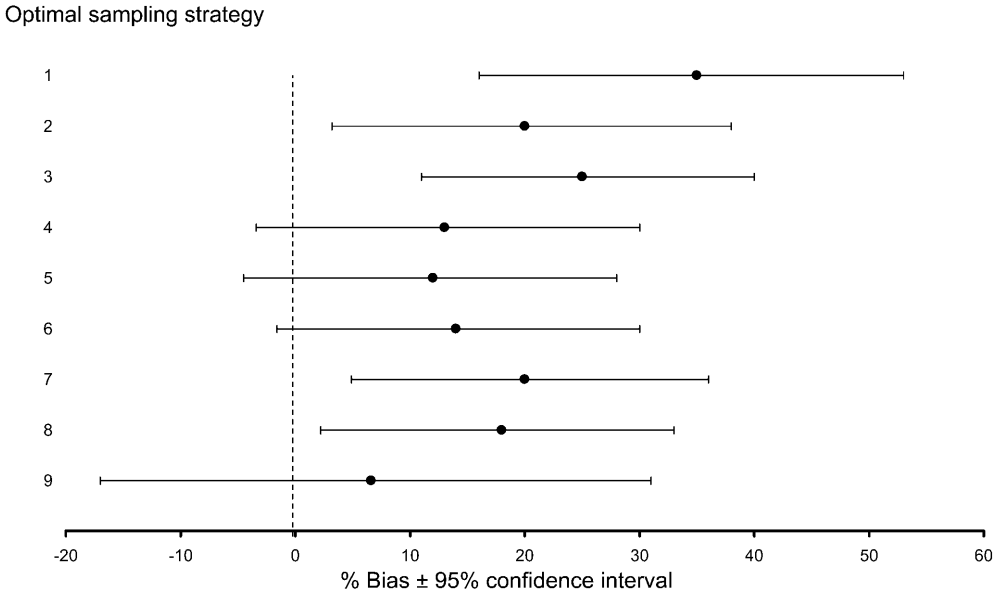

Fig. 3 Graphical representation of % mean prediction error (bias) with 95% confidence interval for each optimal sampling strategy

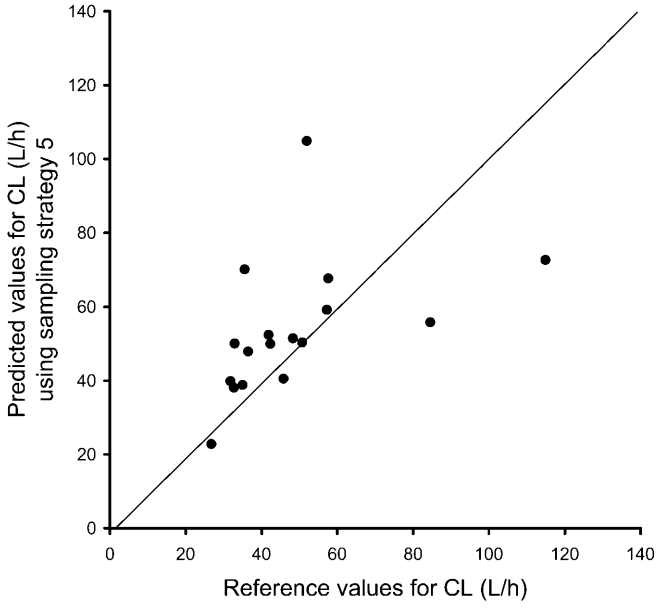

Fig. 4 Predicted values for CL using optimal sampling strategy 5 versus reference values for CL. The solid line represents the line of identity

quantification of interpatient variability in other parameters. Modeling of the validation data set yielded essentially equal estimates for all parameters, indicating that the developed population model was sufficiently robust. In both data sets, values for the rate constant K31 were small (0.0051 and 0.0056 h^{-1} for the index and validation set, respectively), which corresponds to the long terminal half-life of ET-743 (20–40 h) reported previously [22]. Furthermore, the variability between patients was large for this parameter.

The residual variability was modeled with a combined additive and proportional error model, in which the proportional component was substantial for both data sets (36% and 41% for the index and validation data set, respectively). This high unexplained residual variability was mainly caused by irregularities during the 24-h continuous infusion. As can be seen in Table 2 and Fig. 1, ET-743 showed a relatively rapid distribution to the peripheral compartments (K12, K13), shortly after the end of infusion, followed by a long terminal elimination phase. Therefore, small alterations in the infusion rate would markedly influence the concentration-time profile of ET-743, which would subsequently affect the analyses performed with these data.

The D-optimality algorithm can be used for the selection of optimal time-points for limited sampling strategies [5]. Within a given time window, optimal time-points are calculated which would yield best estimates of the parameters of interest. Several strategies, which were based on the optimal time-points selected with D-optimality, were tested for their predictive performance. It appeared that inclusion of time-points 48 and 96 h after the start of infusion was essential to yield unbiased estimates of CL. Addition of samples obtained weekly after treatment (schedule 5) resulted in the best predictive performance, although the predictions remained rather imprecise (RMSE 33%). In view of the execution of large scale phase II studies it is, however, not feasible to obtain samples on a weekly basis. The addition of samples from shortly after the end of infusion, as were included in the early phase II protocol, did not improve the predictions in the present analyses (schedules 7 and 8).

The Bayesian estimates for CL based on the full validation data set yielded unbiased but imprecise predictions. This unexpected increase in %RMSE could mainly be ascribed to the addition of samples to the strategies obtained during the infusion and the rapid distribution phase of ET-743. This was also seen when

samples obtained during and shortly after the end of infusion were added to the sampling strategies (schedules 6, 7 and 8). In all, with the Bayesian approach, the three-point model including samples at 23.5, 48 and 96 h after the start of administration (schedule 4) is preferred as it yielded unbiased estimates of CL and will be most practical in view of the phase II studies.

With the linear regression approach, correlations between plasma concentrations at certain time-points and CL were investigated. A sampling strategy based on concentrations at 26 h after the start of the infusion yielded unbiased and acceptably precise predictions of CL. Incorporation of any other time point in the schedule was not statistically significant in linear regression analysis. Two additional schedules, based on other time-points with comparable correlation coefficients, were also tested for their predictive performance. These strategies, however, produced both biased and imprecise predictions of CL (Table 1). This was rather unexpected as the correlation coefficients of all three time-points were comparable. We can explain these differences in predictive performance between the strategies by the fluctuations in infusion rate which dramatically influenced the concentration profile. Furthermore, none of the time-points tested showed a very good correlation with CL (range 0.58–0.78). Moreover, for several time-points close to $t = 26$ h (e.g. 24.5 and 28 h), the correlation with CL was not statistically significant (i.e. $P > 0.05$). These findings imply that small deviations from the selected sampling time of 26 h after the start of infusion, would yield biased and imprecise predictions of CL. These counterintuitive results illustrate the drawbacks of this approach.

Nevertheless, the stepwise regression approach has been used in the development of limited sampling strategies for a number of anticancer agents [18, 22, 23]. It is a straightforward method, does not require the development of a population pharmacokinetic model [19] and enables simple calculation of the CL and thus of the AUC. Another limitation of this technique, however, is that the strategies can only be applied in identical dosing regimens; duration of infusion and sampling times should be very precise. Furthermore, limited sampling strategies developed using this approach can only be used for the estimation of a single pharmacokinetic parameter. In contrast, Bayesian strategies are more general as they allow description of the full pharmacokinetic profile. In addition, Bayesian estimation does not require exact sampling and allows more flexibility in dosing regimens. In view of these advantages, the Bayesian approach is preferred, although in this study the linear regression approach yielded a strategy with a slightly better predictive performance.

In conclusion, we have developed and validated limited sampling strategies for the prediction of the clearance of ET-743. The linear regression approach yielded a somewhat more accurate model than the Bayesian estimation method. However, based on the objectives of the ongoing phase II studies, the three-point model including samples at 23.5, 48 and 96 h developed with Bayesian estimation is preferred, as it is more flexible and consistent and allows estimation of other model parameters for each individual. The practical time-points of the optimal sampling strategy facilitate pharmacokinetic monitoring during the phase II program with ET-743 in this schedule. As the pharmacokinetic profile of ET-743 shows considerable variability, additional samples will be taken during the phase II studies whenever feasible. Detailed knowledge of the pharmacokinetics of ET-743 is essential for further characterization of the relationships between the exposure to the drug and the toxicity and response profile. It may enable the definition of a safe and effective target value for AUC, from which an individually adapted dose can be derived. Limited sampling strategies may then be applied for pharmacokinetically guided dosing.

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