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Research paper

Population pharmacokinetics and dynamics in phase II studies of the novel bioreductive alkylating cytotoxic indoloquinone EO9

JHM Schellens,[1] P Dombernowsky,[2] J Cassidy,[3] R Epelbaum,[4] L Dirix,[5] EH Cox,[1] J Wanders,[6] F Calabresi,[7] R Paridaens,[8] S Monfardini,[9] J Wolff,[10] WJ Loos,[11] J Verweij,[11] N Pavlidis[12] and A-R Hanauske[13] on behalf of the EORTC Early Clinical Studies Group

[1]The Netherlands Cancer Institute, Amsterdam The Netherlands. [2]University Hospital Copenhagen, Denmark. [3]University of Glasgow, Glasgow, UK. [4]Rambam Medical Center, Haifa, Israel. [5]University Hospital, Edegem, Belgium. [6]NDDO Oncology, Amsterdam, The Netherlands. [7]Istituto Regina Elena, Rome, Italy. [8]Universitair Ziekenhuis, Leuven, Belgie. [9]Centro di Riferimento Oncologico, Aviano, Italy. [10]5[e] Medizinische Klinik, Nurnberg, Germany. [11]Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, Rotterdam, The Netherlands. [12]University of Ionnina, Ionnina, Greece. [13]Medizinische Klinik, Klinikum rechts der Isar, Munchen, Germany.

Population pharmacokinetic–dynamic analysis was prospectively integrated in the clinical phase II programme of EO9 to determine the population pharmacokinetic profile in a larger population of patients, to estimate individual patient pharmacokinetic parameters, and to investigate relationships between drug exposure and clinical outcome. A sparse sampling method was developed, which involved three sampling times, and was implemented during course 1. A Bayesian algorithm was used to estimate individual pharmacokinetic parameters, in particular total plasma clearance (CL) of EO9 and area under the curve (AUC). In total, samples were collected of 85 (65%) of the patients. Pharmacokinetic evaluation was successful in 61 (72%) of the sampled patients. CL of EO9 showed substantial variability (median 5.08 l/min; range 2.67–6.42) and was of the same magnitude as in the phase I study where full pharmacokinetic profiles were used. No significant relationships were noticed between exposure parameters and safety, but overall limited toxicity was observed. No tumor responses were documented. Prospective implementation of large-scale population pharmacokinetic–dynamic analysis is feasible and may generate important findings, in particular when tumor responses and relevant toxicity are observed. [© 2001 Lippincott Williams & Wilkins.]

Key words: Bayesian algorithm, EO9, pharmacodynamics, pharmacokinetics, phase II, population analysis.

Correspondence to JHM Schellens, Department of Medical Oncology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.
Tel: (+31) 20 521 2569; Fax: (+31) 20 512 2572;
E-mail: jhm@nki.nl

Introduction

EO9 [3-hydroxy-5-aziridinyl-1-methyl-2-(1H-indole-4,7-indodione)-prop-b-en-a-ol] is the leading compound in a series of novel indoloquinone cytotoxic alkylating compounds structurally related to the bioreductive agent mitomycin C.[1,2] In preclinical models, EO9 exhibited an antitumor profile distinct from that of mitomycin C. EO9 demonstrated a distinct pattern of cytotoxicity *in vitro* preferentially against cell lines derived from solid tumors in the disease-oriented cell line panel of the National Cancer Institute.[2] EO9 is activated by DT-diaphorase much more efficiently than mytomycin C.[3,4] DT-diaphorase is overexpressed in experimental and clinical tumors.[5] Sensitivity for EO9 is correlated with DT-diaphorase activity in various tumor models.[6–10] *In vivo*, EO9 also demonstrated high activity against various solid tumor models.[2,6]

In phase I studies employing different schedules of administration, the 5-min bolus i.v. administration once every week schedule was selected for phase II studies, because it resulted in the highest dose intensity.[11–13] The dose-limiting toxicity observed was reversible proteinuria, accompanied by sodium and water retention. The renal pathology resembled that of the rare drug-induced minimal change glomerulopathy.[11] Three partial responses were observed among 32 patients employing the 3-weekly schedule, two in adenocarcinoma of unknown primary site and one in bile duct cancer. The maximum tolerated dose was 27 mg/m^2 in the 3-weekly schedule and 15 mg/

m^2 in the weekly schedule, and recommended doses for phase I studies were 22 mg/m^2 in the 3-weekly and 12 mg/m^2 in the weekly schedule. The highest course dose calculated over a 3-week period could be achieved with a weekly schedule. As there was no apparent schedule-dependent difference in toxicities nor difference in activity in preclinical models between the tested schedules, this favors selection of the weekly schedule for phase II studies.[12]

Pharmacokinetics of EO9 revealed very high and variable total plasma clearance ranging from 3.2 to 24 l/min. The area under the curve (AUC) of EO9 was linarly related with dose.[11–14] In addition, the AUC was highly correlated with the proteinuria.[11] EO9 is extensively metabolized. One of the principal metabolites is EO5A, which has an open aziridine ring and much lower cytotoxicity than EO9.[7]

EO9 was selected for evaluation of its antitumor activity in a broad phase II study, in advanced breast, colorectal, pancreatic and gastric cancer. In addition, a randomized phase II study using both the weekly and 3-weekly schedule was performed in non-small cell lung cancer (NSCLC) to enable the direct comparison of toxicities versus dose intensities with the two schedules. The studies were carried out within the framework of the Early Clinical Studies Group (ECSG) of the EORTC. Currently, EO9 is being evaluated as a radiosensitizer.

Because of the the pronounced variability in clearance of EO9, and the highly significant relationship between AUC and dose-limiting toxicity in the phase I studies, a limited sampling method and population analysis were implemented in the phase II studies to enable estimation of pharmacokinetic parameters and pharmacokinetic–dynamic relationships. Implementation of such studies is increasingly seen as an essential tool for new drug development.[15–18] In the current paper results of the population analysis are presented.

Patients and methods

Patients and treatment

Data were prospectively collected from patients entered in six open, phase II trials conducted within the framework of the EORTC-ECSG. The studies were carried out in patients with breast, colorectal, gastric, pancreatic and NSCLC. EO9 was administered as a short infusion of maximally 5 min at a weekly dose of 12 mg/m^2. In the study in NSCLC, patients were randomized to receive a weekly dose of 12 mg/m^2 or a a 3-weekly dose of 18 mg/m^2. Detailed protocols and clinical results of these studies are described in the corresponding papers.[19,20] Briefly, criteria for eligibility included histologically confirmed and at least one bidimensionally measurable lesion, adequate performance score (WHO scale $\leqslant 2$) and life expectancy $\geqslant 3$ months, adequate bone marrow reserve (white blood cells $\geqslant 2000$/ml, platelets $\leqslant 75\,000$/ml), adequate renal function (serum creatinine $\leqslant 140$ mmol/l) and liver function tests (total serum bilirubin $\leqslant 26$ mmol/l, ASAT and ALAT $\leqslant 2\times$ the upper limit of normal unless related to liver metastases). In breast cancer one prior chemotherapy regimen for advanced disease was allowed, exluding mitomycin C. In all other tumor types prior chemotherapy was not allowed. Before each new drug administration a full blood count, serum creatinine and urinalysis, with special attention for proteinuria, was performed. Patients were planned to receive at least six drug administrations.

Studies were conducted in a multicenter setting within the framework of the ECSG of the EORTC. The protocols were approved by local ethics committees or institutional review boards. Prior to start patients had to give written consent.

Pharmacokinetic sampling strategy

During course 1 of treatment, three whole blood samples were collected per patient for bioanalysis and pharmacokinetics. The sparse sampling design was based on sampling times computed using preliminary population pharmacokinetic parameter estimates obtained from phase I data. Sampling times were calculated using two methods: multiple linear regression analysis and Bayesian estimation (see below). A pharmacokinetic case report form was specifically designed in order to document actual sampling times as well as time of beginning and end of infusion. EO9 and ring-opened aziridine analog EO5A were simultaneously assayed in plasma using a validated high-performance liquid chromatography (HPLC) and UV detection after solid-phase extraction.[21] Only data of EO9 were used, because EO5A is a minor and inactive metabolite.

Development of a limited sampling model (LSM)

The pharmacokinetic data of the phase I study with a 3-weekly schedule were used. In that study, EO9 was administered as a 5-min i.v. bolus and full pharmacokinetic profiles of EO9 were obtained by collection of 19 heparinized blood samples between 0 and 24 h after drug administration.[11] The bio-analysis of EO9

and metabolite EO5A in the previous study were carried out according to a validated HPLC assay, as outlined.

In the phase I study, the pharmacokinetic data were analyzed in a two-compartmental open model, using NONMEM (Nonlinear Mixed Effects Models, release IV; University of California San Francisco, CA). Limited sampling approaches were developed using a Bayesian estimation algorithm and data of the previous phase I study. The data of 25 patients were randomly assigned to two groups: 19 for model development and seven for model validation. A proportional error model was used for residual variabilities. Model precision and bias for prediction of AUC were determined using root mean squared error (RMSE; a measure of precision) and mean prediction error (MPE; a measure of bias), as proposed by Sheiner and Beal.[22] Results of the Bayesian algorithm for development of a LSM were compared with a model derived with multiple linear regression analysis. In this approach plasma concentration–time points, the independent variables, were correlated to the AUC of EO9, the dependent variable. Stepwise forward multiple regression was used to develop the LSM and stepwise backward elimination to check for the models.

Population analysis

In the phase II program estimates of total plasma clearance (CL) of EO9 were obtained using sparse data analysis. Only data were used if three plasma samples had been obtained, which according to the established limited sampling model were to be taken at the end of infusion, and at 5 and 10 min after the end of infusion. Population parameters were applied as prior information to estimate individual pharmacokinetic parameters from plasma concentrations measured for each patient using a Bayesian algorithm as implemented in the NONMEM computer program. A two-compartment structural model with first-order elimination was used, of which the basic parameters are CL (l/min), volume of distribution of the central compartment and intercompartmental rate constants. A proportional error model was used to model residual variability in pharmacokinetic parameters. The AUC was calculated as dose/CL.

Patient demographic and biochemical data, such as age, sex, weight, body surface area (BSA) and creatinine clearance, were used as covariables in the population model. Covariates were investigated on CL and volume of distribution at steady state (V). A two-stage approach was chosen for model development. In the first step, the different covariables were introduced separately into the pharmacokinetic model using a one-compartment model with first-order elimination from the central compartment using the NONMEM subroutine ADVAN1. The covariables were introduced on CL and V using linear relationships. Each model incorporated was tested against the model without this covariable. A covariable was considered potentially significant when the drop in the objective function was at least 3.8 points ($p < 0.05$). All potentially significant covariables were introduced in an intermediate model and subsequently the significant covariables were selected by a stepwise backward elimination process. In this analysis, a covariable was considered significant when elimination of the variable resulted in an increase of the objective function of at least 7.9 points ($p < 0.005$). Individual empirical Bayesian pharmacokinetic parameters were estimated using the POSTHOC option of NONMEM. In a second step these individual parameters were used to develop a population pharmacokinetic model. CL was expressed as the mean value of the Bayesian POSTHOC estimates.

Pharmacokinetic–dynamic analysis

Pharmacokinetic–dynamic analysis was conducted using individual estimates of CL and AUC. Also, the absolute dose given was used as a measure of exposure. The clinical endpoints considered were related to clinical efficacy and safety. Pharmacokinetic–dynamic analysis was limited to safety, because no objective responses were observed. The following safety parameters were considered: (i) neutropenia and thrombocytopenia as maximal relative decrease from baseline at first course and worst course per patient, and (ii) proteinuria CTC grade at first course and worst course per patient.

Spearman rank correlation coefficients were calculated between the exposure parameters CL, AUC and dose, and the endpoints such as neutropenia, thrombocytopenia and proteinuria. These analysis were carried out using Excel (Microsoft, release 7.0; Seattle, WA) and Stata (Stata, release 4.0; College Station, TX).

Results

Study design and implementation

During the phase I study 25 patients were fully evaluable for pharmacokinetics. They were randomly assigned to a training dataset of 19 patients and validation set of six patients. Concentration–time data of the training set are given in Figure 1. Population analysis revealed that a two-compartment model described the data better than a one-compartment model ($p < 0.01$). The population median value of CL

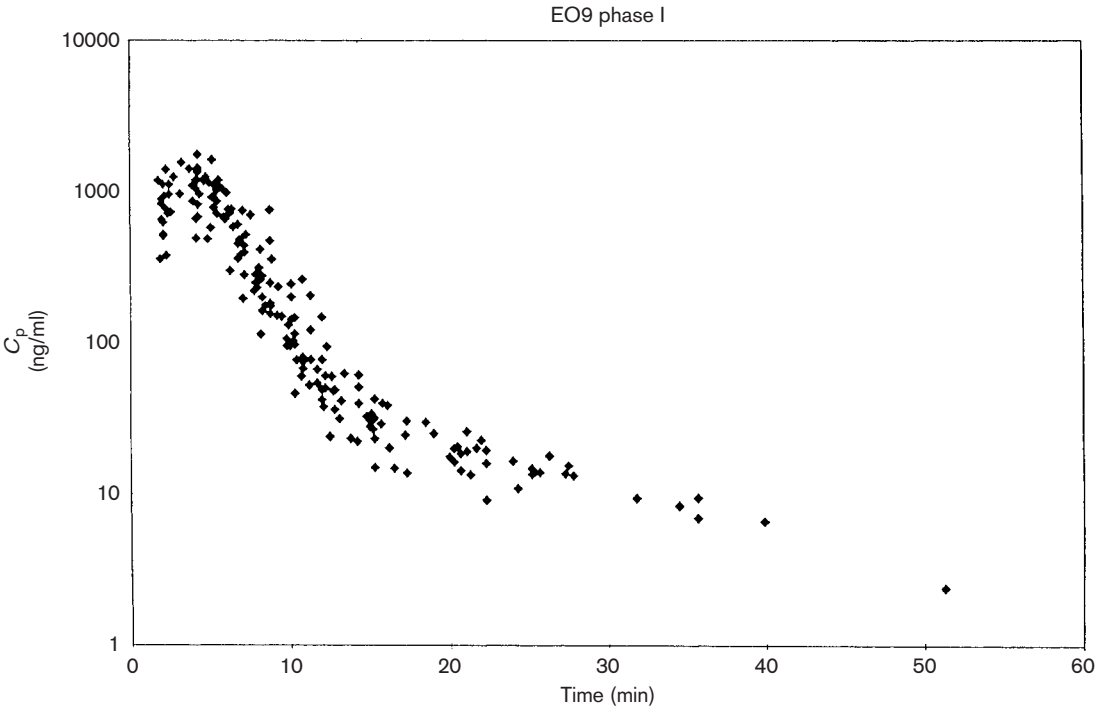

Figure 1. Plasma concentration–time data (C_p) of 19 patients of a single-center phase I study used to develop a preliminary population and LSM.

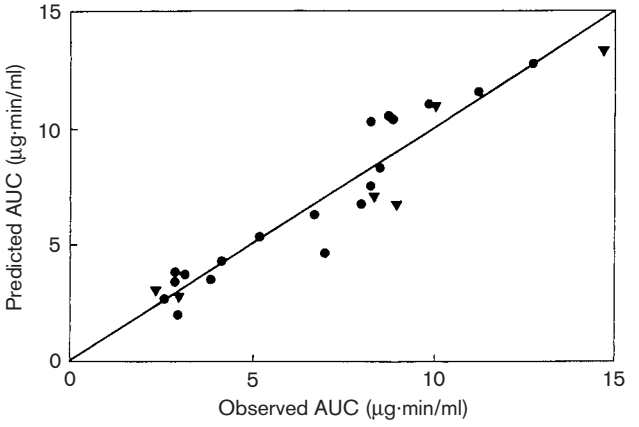

Figure 2. Relationship between predicted and observed AUC of EO9 in the phase I study in the training (●) and validation set (▼). R^2=0.88.

was 5.37 l/min (range 3.73–7.14 l/min). V was 18.6 l. A number of three concentration time points was necessary to obtain sufficiently accurate and precise estimates of individual CL values, using a Bayesian approach. The optimal time points were 5, 10 and 15 min after the start of the 5-min infusion. The MPE% was −12.1% (SE 3.7%) and RMSE 31.8% (SE 3.0%). The Pearson R^2 value was 0.88. In the validation set the MPE was 15.6% (SE 4.5%) and RMSE% 32.7 (SE 5.3%). The R^2 value was also 0.88. See Figure 2.

Selection of only two concentration time points resulted in unacceptable low precision ($R^2 < 0.7$) and more bias.

In the multiple linear regression analysis a deviation of 20% from the chosen time points and the infusion duration was accepted. The same number and combination of time points, combined with the infusion duration, resulted in the best estimate of CL. The results of precision and bias were of the same order as with the Bayesian approach. The Bayesian approach was selected for application during the phase II studies, because of superior flexibility

Patient characteristics at baseline of the phase II study

Patient characteristics are summarised in Table 1. Data are given of 61 patients evaluable for pharmacokinetics. In the phase II study in total 131 patients were registered, of whom 128 were fully eligible. Samples were collected from 85 patients (65%). Samples of 24 patients were not adequate, because tubes were broken during transport (n=4) or during processing (n=1), only two blood samples were taken (n=7), due to too late sampling times resulting in plasma

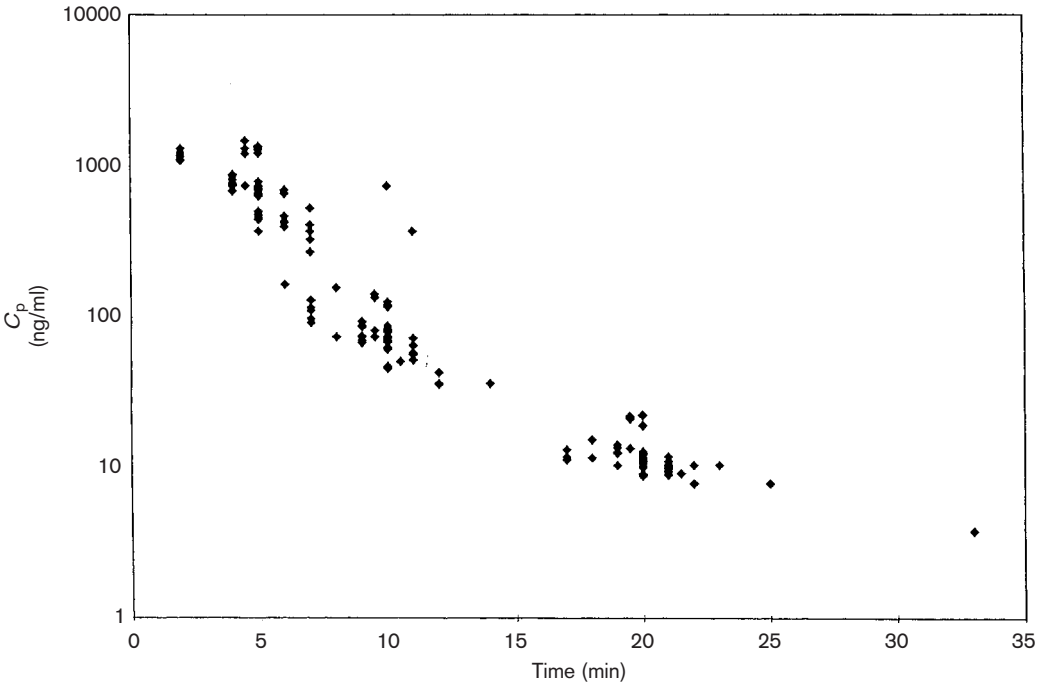

Figure 3. Population pharmacokinetics of EO9 in phase II studies: plasma concentration–time data (C_p) of 61 patients, five studies, 19 centers.

Table 1. Patient characteristics at baseline of the phase II study and EO9 exposure

No. of patients evaluable for pharmacokinetics	61
Age (median and range; years)	57 (33–83)
Sex	
male	33
female	28
WHO performance score (median and range)	1 (0–2)
EO9 treatment and exposure	
initial dose (mg/m^2)	
12 (no. patients)	51
22 (no. patients)	10
absolute dose given (mg)	
12 mg/m^2 dose level (median, range)	22 (16–25)
22 mg/m^2 dose level (median, range)	40 (37–50)
infusion duration (min) (median and range)	4.7 (2–11)
CL (l/min) (median and range)	5.08 (2.67–6.42)
AUC (mg·min/ml) (median and range)	4.76 (3.20–8.34)

concentrations below the lower limit of quantitation (LLQ) of the assay (LLQ=5 ng/ml),[11] or sampling error resulting in a weighed residual of the predicted concentration which deviated more than 10 SEM.[1] In total data of 61 patients (72% of the sampled patients) could be used for pharmacokinetic evaluation (Figure 3). The continuous line in Figure 4 is a fit of patient data obtained using Bayesian estimation.

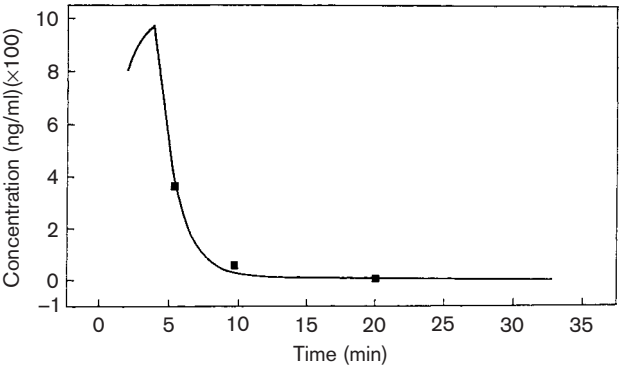

Figure 4. Phase II population pharmacokinetics: fit of data of an individual patient (subject 71) using Bayesian estimation.

Individual pharmacokinetic parameter estimates

Individual estimates of pharmacokinetic parameters and exposure parameters are given in Table 1. The

continuous line in Figure 4 is a fit of a patient data using Bayesian estimation. In this relatively large population of patients, the population median value of CL was 5.08 l/min, which is very close to the value of 5.37 l/min previously estimated in the phase I study using 19 full plasma concentration–time profiles. Covariables age, sex, BSA and creatinine clearance did not significantly reduce the objective function. Weight was significantly correlated, but resulted in a minor and clinically unimportant reduction of the objective function (decrease of 7 points, $p < 0.05$). A significant correlation was observed between the dose given and AUC of EO9 (Pearson $R = 0.77$).

Pharmacokinetics–pharmacodynamics

Efficacy No objective responses have been observed in the patients entered in the phase II program. Hence, relationships with pharmacokinetics could not be evaluated.

Safety. Overall most side effects of the therapy were mild and only incidentally greater than CTC grade 2. They consisted of mild nausea and vomiting, neutropenia, anemia, and fatigue. Neutropenia CTC grade 1 was observed in two out of 61 patients evaluable for pharmacokinetics (3%), grade 2 in one patient (1.6%) and grade 3 in one patient (1.6%). There was no significant relationship or correlation between any exposure parameter and neutropenia, which was scored as relative decrease from baseline value as well as according to CTC grade. Thrombocytopenia was almost absent. The most prominent side effect was proteinuria, the dose-limiting toxicity in the phase I study. In all 128 eligible patients, proteinuria grade 1 was observed in 65 patients (51%), grade 2 in 23 (18%), grade 3 in two (1.6%) and grade 4 in one (0.8%). In the 61 patients evaluable for pharmacokinetics, one grade 3 and no grade 4 proteinuria was observed. Grade 1 proteinuria developed in 29 patients (48%) and grade 2 in 10 patients (16%).

Only categorical CTC-graded proteinuria data were available. The relationship between AUC of EO9 and CTC grade proteinuria is given in Figure 5. No significant relationship was observed between any exposure parameter and proteinuria.

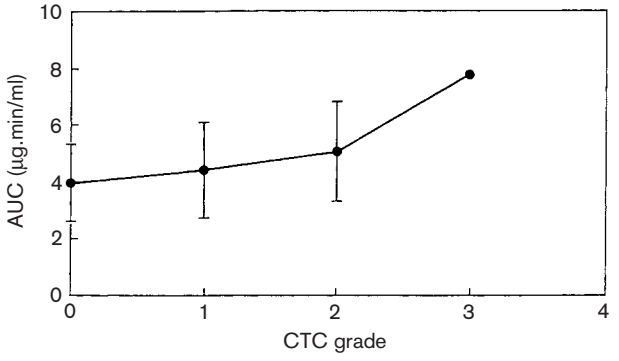

Figure 5. Relationship between proteinuria and AUC of EO9. The proteinuria was graded according to CTC.

Discussion

Large-scale pharmacologic evaluation was fully and prospectively integrated in the early clinical studies of EO9, in order to determine its interpatient pharmacokinetic variability and relationships with safety parameters and response if applicable. Results of the phase I study revealed pronounced interpatient variability in exposure and relatively low correlation between dose administered and AUC as main exposure parameter.[11,13,23] In the phase I study, proteinuria was the dose-limiting toxicity.[11,14] In that study continuous data of proteinuria were available.

Prior to implementation of pharmacologic evaluation in the multicenter phase II study, a limited sampling model was developed. For this aim two approaches were followed: a Bayesian estimation and multiple linear regression analysis. The advantage of the former is its superior flexibility and often superior performance. The advantage of the regression analysis is its simplicity. Preliminary results of the phase I study revealed that the pharmacokinetic profile of EO9 is best described by a two-compartment open model, i.e. four parameters have to be estimated. In the D-optimality theory this often results in a sampling schedule of four time-points per patient. However, Bayesian estimation as well as multiple regression analysis revealed that with three concentration–time points and the known infusion duration an almost unbiased estimate could be obtained of CL (and therefore AUC) the major parameter of interest. Also, the precision of the estimate of CL was considered sufficiently adequate for large scale application of the three-point limited sampling model.

In total, 85 patients were sampled (66% of the eligible patients), which indicates that a limited sampling procedure can be carried out successfully within the framework of the ECSG. Pharmacokinetic evaluation was successful in 61 (72%) of the sampled patients, somewhat lower than can be achieved in large multicenter studies.[18] This is at least partly related to the difficult pharmacokinetic characteristics, in particular the very short terminal half-life of

EO9, which necessitates that the last blood sample is collected no later than around the planned time. The number of obtained pharmacokinetic data is considered adequate to describe interpatient variability in pharmacokinetics and major predictors of CL. Also, it should be sufficient to describe major relationships with safety parameters, if they exist. However, much larger populations of patients are needed for the population pharmacokinetic analysis to enable validation of the model in small patient subgroups with altered pharmacokinetics, as has been shown for docetaxel.[18] The estimate of CL in the phase II population was very close to the preliminary population estimate in the phase I study using 19 full pharmacokinetic curves, which may be an indication that the estimate is not significantly biased. Weight had a significant, but minor influence on the variability in CL. BSA had no significant influence, indicating that dose normalization using BSA is not necessary, which is often the case for anticancer agents.[24]

Pharmacokinetic–response relationships could not be determined, because unfortunately the applied schedule was not active. No objective responses were noted. No significant relationships were observed between exposure parameters, such as dose, AUC, CL and safety parameters. The applied schedule induced only limited bone marrow suppression, which is in line with the data observed in the phase I studies. The major toxicity parameter was proteinuria, the dose-limiting toxicity in the two previous phase I studies.[11,14] In one of the phase I studies a highly significant sigmoidal relationship was observed between the proteinuria expressed as a continuous parameter in g/l.[11] The likelihood of development of severe proteinuria increased sharply at AUC levels above 8.5 (mg·min/ml). In the present study no such relationship was observed. Firstly, categorization of data by CTC grading resulted in substantial loss of information. Secondly, the highest observed AUC was 8.34, which is not yet associated with a high risk of development of proteinuria. The latter observation confirms that relatively low AUC levels are not associated with pronounced proteinuria.

This study confirms that large-scale prospective implementation of population pharmacokinetic–dynamic analysis is feasible. The limited sampling model was based on three sampling time-points and the infusion duration, to describe a two-compartmental model for EO9. It resulted in sufficiently precise and accurate estimates of CL. This approach may turn out very useful to describe pharmacokinetic–response and –safety relationships for drugs which induce responses and toxicity.

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