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ORIGINAL ARTICLE

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Population pharmacokinetics of the novel anticancer agent KRN7000

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Abstract *Purpose:* KRN7000 is a novel anticancer agent, acting through stimulation of the immune system. The first clinical trial with this agent, which included pharmacokinetic studies, has recently been completed. The aim of the study presented here was to develop a population pharmacokinetic model for KRN7000. *Methods:* Plasma concentration-time data were gathered from 24 patients enrolled in a phase I trial in which KRN7000 was administered as a weekly slow injection at doses ranging from 50 to 4800 μg/m^2. These data were used to build a pharmacokinetic model using the nonlinear mixed-effect modeling (NONMEM) program. The model was validated by performance of 200 bootstraps. *Results:* A three-compartment model with interindividual variability on the central and two peripheral volumes of distribution (V1, V2 and V3) and on clearance (CL) adequately described the data. The final estimates were: V1 2.34 l, V2 2.61 l, V3 2.13 l, and CL 0.130 l/h. Of 24 covariates tested, including both demographic and pathophysiological factors, none showed a significant relationship with the pharmacokinetic parameters obtained. The bootstrap analysis provided parameter estimates within approximately 15% of the original estimates, indicating stability of the model. *Conclusion:* The pharmacokinetic behavior of KRN7000 in the clinical trial could be described by a three-compartment model. Hence, KRN7000 demonstrates linear pharmacokinetics over the investigated dose range. The pharmacokinetics of KRN7000 are not influenced by patient demographic or pathophysiological characteristics.

Keywords KRN7000 · Population pharmacokinetics · NONMEM · Model building · Model validation

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Introduction

KRN7000, (2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyl)-2-(*N*-hexacosanoylamino)-1,3,4-octadecanetriol, (Fig. 1) is a novel antineoplastic agent extracted from the marine sponge *Agelas mauritianus*. It has demonstrated strong antitumor effects in both in vitro and in vivo studies. The highest efficacy has been observed in murine liver and lung metastatic models, with very little toxicity to the animals [9, 10, 11]. The mechanism of action of this agent is believed to involve stimulation of the host's immune response, primarily through activation of natural killer (NK) T cells [5, 13]. NK T cells are a distinct type of lymphocyte, characterized by the expression of a specific T-cell receptor consisting of a Vα24-chain paired with a Vβ11-chain [3, 6]. Physiologically, NK T cells are probably involved in the regulation of several autoimmune diseases and the control of malignancies [2, 8].

KRN7000 is currently undergoing clinical evaluation, and the first phase I trial has recently been completed.

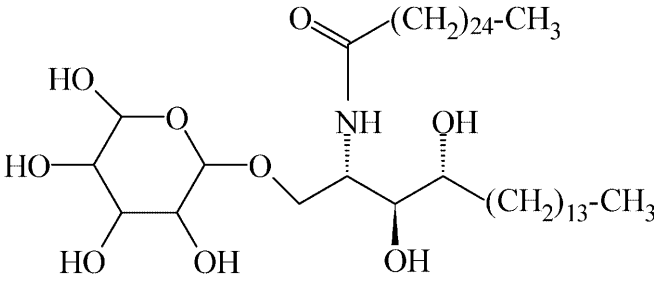

Fig. 1 Structural formula of KRN7000

The aim of this trial, in which KRN7000 was administered as a weekly slow injection, was to determine a suitable dose for further clinical testing based either on observed biological activity or on toxicity. A secondary objective was to study the pharmacokinetics of KRN7000. For this last goal, pharmacokinetic studies were performed in all patients included. For future clinical development, it would be useful to assess pharmacokinetics over a range of doses as well as possible interfering covariates at an early stage. Here, we present a population pharmacokinetic model built using the nonlinear mixed-effect modeling (NONMEM) program [1].

Materials and methods

Patients and treatment

A phase I clinical trial was designed with KRN7000 administered intravenously as a weekly slow injection (3–6 min). Patients with advanced solid malignancies were entered into the study. Inclusion criteria were: age >18 years, WHO status <3, minimal life expectancy of 3 months, last chemotherapy given at least 4 weeks previously, last immunotherapy given at least 3 months previously, and normal bone marrow, renal and liver function. An interpatient dose escalation scheme was used starting at 50 $\mu g/m^2$, which is one-tenth of the nontoxic dose in rats. Subsequent dose levels were 150, 300, 600, 1200, 2400 and 4800 $\mu g/m^2$. Three patients were to be included per dose level, and three extra in the case of dose-limiting toxicity. The study was approved by the Committees of Medical Ethics of the cooperating hospitals, and written informed consent was obtained from all patients.

Pharmacokinetic sampling and bioanalysis

Whole-blood samples were collected during the first week of treatment at the following times: immediately prior to injection, and then 15, 30 and 60 min and 2, 4, 6, 10, 24, 48, 96 and 168 h afterwards. During the third course, pharmacokinetics were also assessed using a reduced number of samples: before administration, and 15 min and 1, 4, 10, 24, 96 and 168 h later. Blood samples were centrifuged and the resulting plasma was stored at –20°C until analysis. All samples were analyzed using a validated high-performance liquid chromatography electrospray ionization tandem mass spectrometry assay (LC/ESI/MS/MS). In brief, KRN7000 and internal standard were extracted from serum using a liquid-liquid extraction with ethyl acetate. The organic layer was evaporated to dryness and the remainder was reconstituted in methanol. Next, the sample was injected onto a C8 column and analyzed by an LC/ESI/MS/MS detection method. ^{13}C-Labeled KRN7000 was used as internal standard. The lower limit of quantitation of this method was 12.5 ng/ml. Chromatographic data were processed by Mac Quan analysis software (SCIEX, Thornhill, Ontario, Canada).

Pharmacokinetic data evaluation

Pharmacostatistical population analysis was performed with the NONMEM program (double precision, version V, level 1.1) [1]. The first-order conditional estimation (FOCE) method was used.

Structural pharmacokinetic model

Two- and three-compartment structural kinetic models with first-order elimination from the central compartment were used to model the plasma-time profiles of KRN7000. The pharmacokinetics were parameterized in terms of volume of distribution of the central compartment (V1), clearance from the central compartment (CL), volume of distribution of the first peripheral compartment (V2), intercompartmental clearance for the first peripheral compartment (Q2), volume of distribution of the second peripheral compartment (V3) and intercompartmental clearance for the second peripheral compartment (Q3) (NONMEM PREDPP subroutines ADVAN3- and ADVAN11-TRANS4).

Interpatient variability was estimated using an exponential error model. For instance, variability in CL was estimated from the equation:

$$CL_i = CL_{pop} * \exp(\eta_i)$$

where CL_i represents the CL of the ith individual, CL_{pop} is the population value and η is the interindividual random effect with mean 0 and variance ω^2. The difference between the jth measured concentration in the ith patient (C_{obsij}) and its respective prediction (C_{predij}) was modeled with a combined additive-proportional error model:

$$C_{obsij} = C_{predij}(1+\varepsilon_2)+\varepsilon_1$$

where ϵ is an independent random variable with mean 0 and standard deviation σ.

Refinement of the statistical submodel

Since plasma concentration versus time profiles were determined after two dosages (course 1 and course 3), interoccasion ("between course") variability could be estimated. The following expression was used for Cl:

$$CL_{ij} = CL_{pop} * \exp(\eta_i + \kappa_{ij})$$

where CL_{ij} represents the CL of the ith individual at occasion j, CL_{pop} is the population value and η and κ are the interindividual and interoccasion random effects with mean 0 and variances ω^2 and π^2, respectively. The correctness of the interindividual variability model was further checked by evaluation of the covariances between the parameters in the model.

Covariate model

Individual empirical Bayesian estimates of parameter values were obtained from the structural pharmacokinetic model with no covariates included using the POSTHOC option in NONMEM [7]. For each subject, individual pharmacokinetic parameters were calculated taking both individual observations and population parameters into account. The individual estimates were plotted against covariate values for visual inspection using Xpose (version 3.0) [4], as implemented in the statistical program S-plus (version 2000 Professional Release I, Mathsoft, Cambridge, Mass.). All covariates were tested for inclusion in the population pharmacokinetic model by stepwise addition into the basic model. Continuous variables were centered to their median values. For instance the relationship between central volume of distribution (V1) and weight (WT) was described as follows:

$$V1_{pop} = \theta 1 + \theta 2 * (WT - 70)$$

where $V1_{pop}$ represents the population value, $\theta 1$ the value of a (median) 70-kg patient, and $\theta 2$ is the increment of V1 per kilogram weight difference. Dichotomous variables were modeled as follows:

$$V1_{pop} = \theta 1 * (\theta 2 \text{^} \text{LIVM})^2$$

where $V1_{pop}$ represents the population value, $\theta 1$ the population value in the absence of liver metastases (LIVM = 0) and $\theta 2$ represents the change of V1 when liver metastasis are present (LIVM = 1). The presence of liver metastases was unknown in some of the patients included. Therefore, two covariates, LIVM1 and LIVM2, were tested. In the case of LIVM2, patients with an unknown status were considered positive, whereas the opposite was true for LIVM1. Unknown pleural effusion status was handled in a similar manner.

The objective function was used to evaluate the increase in goodness of fit. A decrease of at least 3.8 identified a covariate as being significant ($P < 0.05$). This criterion is based on the objective function having an approximate chi-squared distribution with one degree of freedom. During backward elimination (with all the significant covariates in a multivariate intermediate model) a stricter criterion was used ($P < 0.001$): an increase in objective function of more than 10.8 was required for retention of a covariate in the final model.

The final model was validated by performing 200 bootstrap analyses, each time leaving out 15% of the data set [7]. The mean parameter estimates obtained from these bootstraps were compared with those obtained in the final model of the original data set.

Results

A total of 24 patients were entered into the study. The last three received KRN7000 as a 30-min infusion, whereas in all other patients KRN7000 was given as a slow injection. At the first dose level (50 μg/m^2) six patients were entered whereas at all other dose levels (150, 300, 600, 1200, 2400 and 4800 μg/m^2) three patients were treated. Patient characteristics, and the abbreviations used for the variables in the covariate analysis (see below) are given in Table 1. If baseline hematology or blood chemistry was not obtained on the last day prior to treatment, the closest previous measurement was used for the analysis. Pharmacokinetic samples from courses 1 and 3 were available for all patients included. In total, 483 plasma samples were obtained. Mean observed plasma profiles per dose level are depicted in Fig. 2. Clinical data obtained in this phase I trial are reported separately (Giaccone et al., submitted for publication).

Population pharmacokinetic analysis

Attempts to describe the data on the basis of a two-compartment model resulted in the parameter estimates presented in Table 2 (model 1). In this model, interindividual variability for V1, CL and V2 was estimated. The addition of a second peripheral compartment (parameters V3 and Q3) reduced the objective function by 90 points (model 2). Furthermore, no trend was present in the plot of WRES versus time (data not shown). Hence, the two-compartment model was rejected. Introduction of interindividual variability on V3 improved the fit of the three-compartment model: the objective function decreased by 28.6 points (model 3). Estimation of interindividual variability for Q2 and Q3 did not improve the

Table 1 Patient characteristics at inclusion ($n = 24$)

	Number of patients	Median	Range
Demographics			
Age (years)		53	31–69
Weight (kg)		70.5	53.6–105
Height (cm)		171	154–183
Body surface area (m^2)		1.8	1.5–2.3
Sex (M/F)	14/10		
Tumor involvement (Y/N)			
Liver metastasis	5/8 (11 unknown)		
Renal tumor	2/22		
Ascites	0/20 (4 unknown)		
Pleural effusions	1/23 (1 unknown)		
Baseline hematology			
Hemoglobin (mmol/l)		7.8	4.9–9.2
WBC (10^9/l)		6.4	3.8–13.3
Neutrophils (10^9/l)		4.6	1.8–7.4
Lymphocytes (10^9/l)		1.3	0.4–2.5
Platelets (10^9/l)		250	126–510
Baseline blood chemistry			
Serum creatinine (μmol/l)		86	57–132
Creatinine clearance (ml/min)[a]		83	57–164
Alkaline phosphatase (U/l)		84	45–514
Bilirubin (total) (μmol/l)		6	3–32
Aspartate amino transferase (U/l)		16	7–67
Alanine amino transferase (U/l)		14	5–73
Gamma glutamyl transferase (U/l)		40	12–288
Lactate dehydrogenase (U/l)		247	149–711
Total protein (g/l)		74	58–83
Albumin (g/l)		36	24–46

[a]Calculated using the Cockroft-Gault formula

fit (the objective function decreased by 0.1 and 1.8 points, respectively). This should not be interpreted as an absence of variability, but only that the data did not contain enough information to estimate these parameters.

Next, interoccasion variability was estimated for V1 and CL (model 4). In comparison with model 3, the objective function was reduced by 93.7 points. Estimation of interoccasion variability of V2 and V3 did not further improve the goodness of fit: the reduction in the objective function was 6.1 and 0 points, respectively. The degree of correlation between ηV1, ηCL, ηV2, ηV3, κV1 and κCL were evaluated and quantified. Correlations between ηV1, ηCL, ηV2 and ηV3 were observed (Fig. 3), being high between ηV1 and ηV2 as well as between ηV1 and ηV3 ($r > 0.99$). As a result, these last correlations were fixed at a value of 1. Consequently, the interindividual random effects of V2 (ηV2) and V3 (ηV3) were expressed as:

$$\eta V2 = \theta_{V1-V2} * \eta V1$$

$$\eta V3 = \theta_{V1-V3} * \eta V1$$

This resulted in model 5, the covariance model (Table 2). In this model, the correlation between the central volume of distribution (V1) and clearance (CL) was estimated at 0.943.

Finally, all covariates were added separately to the NONMEM model. The following covariates were found to be significant ($P < 0.05$): for V1, sex (SEX), the presence of a renal tumor (RETM) and the presence of liver metastases (LIVM2). For clearance, only SEX was significant, and for V2 SEX and RETM. No covariates had a significant influence on V3.

The significant covariates were combined in an intermediate multivariate model. One covariate significantly increased the objective function value when deleted from the intermediate model: RETM vs V1. The corresponding increase was 13.1 points. The resulting equation for V1 was:

$$V1 = 2.13 * (0.111 \text{\^{}} RETM)$$

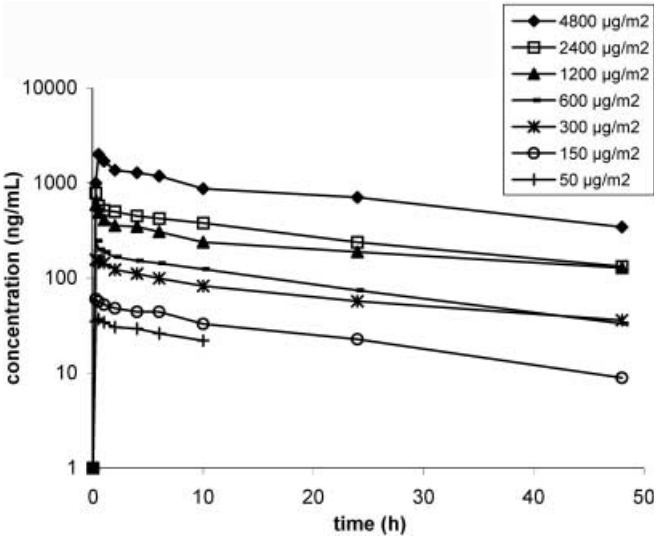

Fig. 2 Mean observed plasma concentrations after the first KRN7000 administration per dose level

Table 2 Estimates of population pharmacokinetic parameters for KRN7000 of the two-compartment model (model 1), the three-compartment model (model 2), the three-compartment model with interindividual variability on V1, CL, V2 and V3 (model 3), the three-compartment model with interoccasion variability (model 4), the covariance and final model (model 5) and the covariate model (model 6). Numbers in parentheses are the coefficient of variation (CV) estimation expressed as percentages

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
θ_{V1} (l)	3.18 (21%)	1.79 (9%)	1.59 (8%)	2.43 (13%)	2.34 (29%)	2.13 (23%)
$\theta_{V1\text{-}RETM}$ (l)	–	–	–	–	–	0.111 (38%)
θ_{CL} (l/h)	0.130 (14%)	0.129 (15%)	0.134 (11%)	0.122 (10%)	0.130 (11%)	0.126 (11%)
θ_{V2} (l)	3.86 (46%)	3.50 (22%)	3.37 (16%)	2.43 (19%)	2.61 (26%)	2.96 (17%)
θ_{Q2} (l/h)	2.40 (78%)	7.97 (12%)	8.90 (6%)	6.65 (9%)	6.86 (28%)	7.46 (15%)
θ_{V3} (l)	–	1.57 (23%)	2.06 (19%)	2.01 (21%)	2.13 (16%)	2.12 (17%)
θ_{Q3} (l/h)	–	0.077 (28%)	0.140 (31%)	0.138 (34%)	0.145 (23%)	0.151 (24%)
Interindividual variability						
ω_{V1} (%)	40 (56%)	49 (71%)	55 (37%)	38 (67%)	29 (78%)	20 (83%)
ω_{CL} (%)	51 (65%)	60 (48%)	58 (46%)	61 (43%)	53 (42%)	55 (44%)
ω_{V2} (%)	101 (57%)	69 (35%)	65 (50%)	73 (40%)	–	–
ω_{V3} (%)	–	–	73 (42%)	67 (53%)	–	–
$\theta_{V1\text{-}V2}$ (%)	–	–	–	–	2.16 (40%)	2.39 (54%)
$\theta_{V1\text{-}V3}$ (%)	–	–	–	–	1.99 (32%)	3.21 (39%)
$corr_{V1\text{-}CL}$	–	–	–	–	0.943	0.940
Interoccasion variability						
π_{V1} (%)	–	–	–	35 (40%)	35 (71%)	42 (54%)
π_{CL} (%)	–	–	–	10 (40%)	10 (42%)	10 (39%)
Residual variability						
σ_1 (%)	15.5 (21%)	13.0 (25%)	12.5 (12%)	8.02 (12%)	8.68 (12%)	8.78 (11%)
σ_2 (µg/l)	4.58 (26%)	4.47 (27%)	3.78 (18%)	3.55 (23%)	3.54 (22%)	3.29 (23%)
Objective function	2816.3	2726.4	2697.8	2604.1	2540.2	2527.1

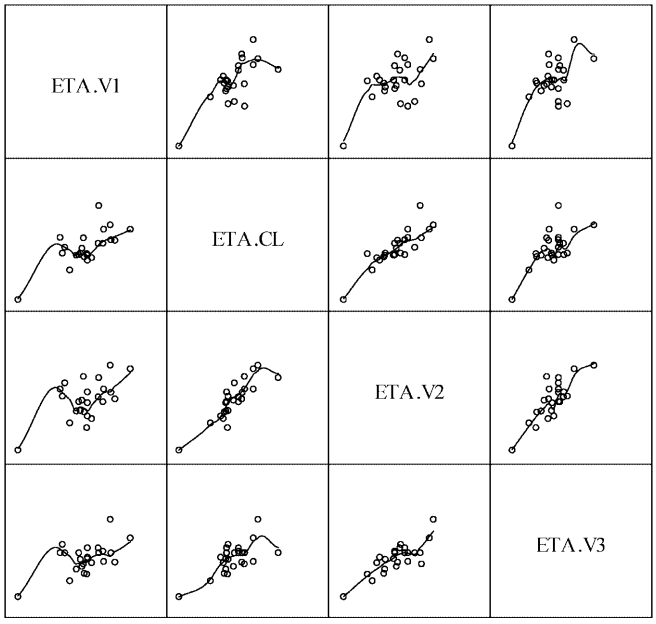

Fig. 3 Matrix plot of the interindividual random effects of V1 (ETA1), CL (ETA2), V2 (ETA3) and V3 (ETA4)

with RETM = 0 (no) or 1 (yes), which resulted in model 6 (Table 2). Physiological interpretation of the equation for V1 indicated that the central volume of distribution for patients with a renal tumor was 0.24 l. The volume of the central compartment most likely corresponds to the plasma, of which the volume is, depending on weight, approximately 2.8 l. As a result, it was concluded that the covariate model was an artifact. Model 5 (without any covariates) was therefore assigned as the final model. A visual indication of goodness of fit of this final model can be seen in Fig. 4.

To validate the proposed pharmacokinetic model, 200 bootstraps were generated by resampling the data set with replacement. The mean parameter values are summarized in Table 3. The parameter estimates of the final model were all within approximately 15% of the mean bootstrap.

Discussion

The plasma-concentration time data from 24 patients in a phase I trial with KRN7000 could well be described by a three-compartment pharmacokinetic model developed using the NONMEM software for population pharmacokinetic analysis. Thus, KRN7000 demonstrates linear pharmacokinetics over the investigated dose range. The final model gave mean estimates for the three volumes of distribution (central and peripheral) of 2.34, 2.61, and 2.13 l, respectively. Therefore, the steady-state volume of distribution is approximately 7.2 l. The clearance of KRN7000 from the central compartment had a mean value of 0.130 l/h. The accuracy of this final model was high, for the residual variability was small. In the observed concentration range of 12.5 to 2250 ng/ml, the residual variability was 4.6 to 199 ng/ml.

KRN7000 demonstrated considerable interindividual variability, for example 53% for CL (Table 2). The interoccasion or intrapatient variability, on the other hand, was low. Since there was also little accumulation of KRN7000 in this weekly schedule (demonstrated by predose levels of zero or almost zero in all patients), the pharmacokinetic parameters (AUC, Cl, V etc) derived from sampling during course 1, will generally be good

Fig. 4a–c Diagnostic plots of the final model (model 5). Presented are: **a** plot of population predictions (*PRED*) versus observed concentrations (*DV*; *solid line* represents x = y); **b** plot of individual predictions (*IPRED*) versus observed concentrations (*DV*; *solid line* represents x = y); **c** plot of the weighted residuals associated with the population predictions (*WRES*) versus time; the line represents a smooth

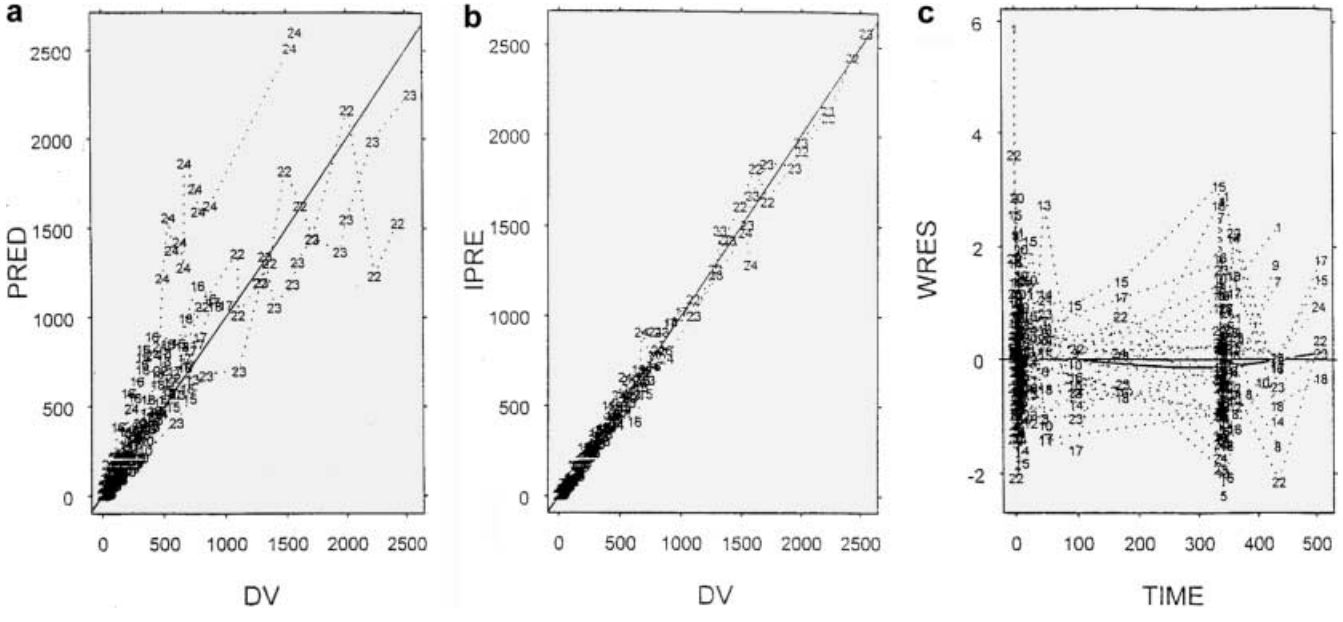

Table 3 Results of the bootstrap analysis

	Mean estimate (CV%)	Difference between final model and bootstrap estimate (%)
θ_{V1} (l)	2.33 (32%)	0.4
θ_{CL} (l/h)	0.132 (10%)	1.5
θ_{V2} (l)	2.68 (27%)	2.7
θ_{Q2} (l/h)	6.37 (29%)	7.1
θ_{V3} (l)	2.15 (15%)	1.0
θ_{Q3} (l/h)	0.136 (24%)	6.2
Interindividual variability		
ω_{V1} (%)	35 (55%)	21
ω_{CL} (%)	51 (22%)	3.8
ω_{V2} (%)	–	–
ω_{V3} (%)	–	–
$\theta_{V1\text{-}V2}$ (%)	2.49 (73%)	15.3
$\theta_{V1\text{-}V3}$ (%)	2.32 (79%)	16.6
$corr_{V1\text{-}CL}$	0.936	1.3
Interoccasion variability		
π_{V1} (%)	38 (44%)	8.6
π_{CL} (%)	10 (24%)	0
Residual variability		
σ_1 (%)	8.50 (12%)	2.1
σ_2 (μg/L)	3.41 (27%)	3.7

predictors of the pharmacokinetics in later courses. In the covariate analysis, 24 factors were tested, but only the presence of a kidney tumor appeared to influence the pharmacokinetic behavior of KRN7000. However, in the phase I trial used to develop the population pharmacokinetic model, only two patients with a renal tumor were included. Therefore, further investigation of this phenomenon is warranted before definite conclusions can be drawn. Nevertheless, there is probably little need for dosage adjustments in specific patients.

The covariance between the parameters (CL, V1, V2 and V3) was high in this model. This is often the result of a correlation of each of these to a single covariate, such as weight. We tested this by plotting post-hoc values of V1, CL, V2 and V3 of model 4 versus weight and body surface area (BSA). In model 4, covariance between the parameters was not estimated. No relationships were observed, which was further confirmed by separate inclusion of the covariates in the pharmacokinetic model: neither weight nor BSA improved the goodness of fit. Inclusion of covariance (model 5), however, did improve the model. Thus, the variation in weight or BSA in the studied patients could not explain the observed significant correlations between interindividual variabilities of V1, CL, V2 and V3.

The population pharmacokinetic model of KRN7000 presented here was based on a relatively small number of patients, so a thorough validation was desirable. As yet, no other clinical trials with this drug have been performed, so the model could not be evaluated against a second data set. When the possibility of performing such an external validation is precluded, a good alternative is to carry out an internal validation, as we did by bootstrap analysis [12]. We performed 200 bootstraps, each time leaving out 15% of the data set. The resulting estimates of the parameter values were all close to the parameter values of the original model, indicating stability of the model.

KRN7000 is at an early stage of clinical development. As yet, little is known about metabolism and routes of excretion in humans. However, preclinical data are in line with our findings, e.g. dose-proportional pharmacokinetics in mice and rats. Furthermore, metabolism in animals appears to be limited, for KRN7000 is retrieved in rat serum and tissues mainly in the unchanged form (Kirin Brewery Company, data on file). This aspect will need further investigation in humans. Moreover, the pharmacokinetic behavior in other dosing schedules should be assessed, as well as possible relationships between pharmacokinetics and pharmacodynamics, such as changes in NK cell count, or observed toxicities of this agent. Based on our data a limited sampling model could be developed, enabling the calculation of pharmacokinetic parameters with just a few samples per patient.

In conclusion, we developed and validated a three-compartment pharmacokinetic model for KRN7000, which accurately described the data of 24 patients enrolled in a phase I trial. This means that KRN7000 demonstrates linear pharmacokinetics over the investigated dose range. No clinically relevant relationship with any of the covariates tested was found. This model could be used in future clinical trials to estimate the individual pharmacokinetic parameters using the Bayesian approach with limited individual data.

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