Dose Linearity and Proportionality

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A desirable characteristic of a drug is linear pharmacokinetic properties to facilitate dose and dose regimen adjustment in patients. "Linear pharmacokinetics" implies that any concentration–time profiles normalized for dose and time are superimposable (Ludden 1991). Thus, one of the necessary conditions for linear pharmacokinetics is dose proportionality, and its assessment is a fundamental pharmacokinetic analysis conducted during the clinical development of a new drug candidate.

Dose Proportionality

If the concentration of a drug (usually in plasma) at any given time is proportional to the dose of the drug administered, then that drug is said to be dose proportional (Smith 2004). If the dose of such a drug is doubled (or tripled or halved), so is the concentration. Mathematically, dose proportionality at a given time point implies that for any dose equal or above 0

$$C \ \alpha \ \text{dose} \tag{1}$$

or replacing the proportionality with an equality

$$C = \alpha * \text{dose} \tag{2}$$

where C is the concentration at a given time point after dosing and α is some regression constant. A relationship between dose and C in case of dose proportionality is illustrated in Fig. 1.

When the concentration is normalized for dose, Eq. 2 passes into Eq. 3 for any dose above 0, illustrating that dose-normalized concentrations being constant are conditions equivalent to dose proportionality of these concentrations.

$$C/\text{dose} = \alpha \, (\text{for dose} > 0) \tag{3}$$

Area under the curve (AUC) and maximum concentration (C_{max}) are generally used to test for dose-normality instead of comparing raw plasma drug concentrations. However, there is no reason why other dose-dependent concentration measures (e.g., trough concentrations) cannot be used. A dose-proportional compound should exhibit dose proportionality for any dose-dependent concentration measure (e.g., minimum concentration, steady-state concentration, amount excreted via kidneys in a given time period).

Dose Linearity

Dose linearity is not to be confused with linear pharmacokinetics. Dose linearity is a weaker condition, even weaker than dose proportionality. It can be described by simple linear regression of the exposure measure C against dose

$$C = \alpha_0 + \alpha * \text{dose} \tag{4}$$

where α_0 is an intercept term and α is a regression constant. If the intercept term α_0 is 0, then Eq. 4 simplifies to Eq. 2 which is the definition of dose proportionality. Dose proportionality implies no

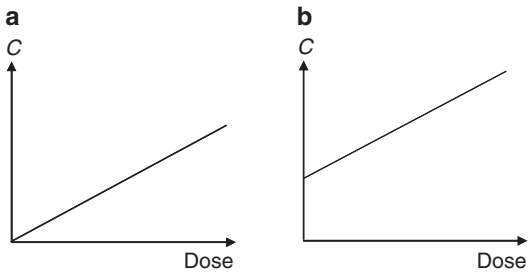

Fig. 1 Relationship between dose and concentration (C) in case of dose proportionality (**a**) and general dose linearity (**b**), both with $\alpha > 0$

drug exposure if the dose is 0. If the intercept term α_0 takes a nonzero value, the result is dose linearity, but without dose proportionality (Cawello et al. 1999). Although no dose is administered, the exposure can be larger than 0, which is typically observed for endogenous compounds. This is why a formal assessment of dose linearity and determination of the value of α is of minor practical importance of most drug candidates. The relationship between dose and C in case of dose proportionality and general dose linearity is illustrated in Fig. 1.

Mechanisms Leading to Lack of Dose Proportionality

Lack of dose proportionality (implying nonlinear pharmacokinetics) may be due to many mechanisms but is typically due to the saturation of some component in the system, such as metabolizing enzymes or transporters. Ludden (1991) classified nonlinearity into causes due to nonlinear absorption, nonlinear distribution, or nonlinear elimination.

Common causes of dose non-proportionality due to nonlinear absorption include saturation of carrier-mediated uptake, poor aqueous solubility or slow release from the formulation, and saturation of presystemic metabolism. Common mechanisms of nonlinear distribution include saturable protein binding, red blood cell binding, and tissue distribution. Lack of dose proportionality due to saturable elimination includes saturable elimination at metabolic enzymes, saturable renal elimination at transporters, and auto-induction.

Lack of dose proportionality may have implications with regard to safety and efficacy. For a drug that shows dose-dependent absorption, typically higher doses lead to less absorption and sub-proportional drug concentrations. In this case, efficacy becomes a concern. For a drug that shows saturable elimination, higher doses lead to higher than proportional concentrations and increased risk of adverse events. This becomes more of a concern when a drug has a narrow therapeutic window. Related to this is the issue of insufficient predictability of the concentration and response or toxicity from a change in dose.

Clinical Assessment of Dose Linearity/Proportionality

The assessment of dose linearity/proportionality typically starts with early exploratory single-dose clinical studies (see section "Dose Proportionality") providing PK data over a considerable dose range (Frick et al. 2006). These data support exposure–response relationships and subsequent dose selection in patients for the potential submission for new-drug approval (US FDA 2014). Dose linearity/proportionality may be assessed during drug development by more complex study designs (see section "Dose Linearity") to further support PK/PD relationships, ending with comprehensive confirmatory studies (see section "Mechanisms Leading to Lack of Dose Proportionality") to support drug labeling, dosage form modifications (EMEA CPMP 2014), or the use of several dosage strengths (EMEA CPMP 2010).

Statistical Assessment of Dose Linearity/Proportionality

Dose proportionality is a mathematically ideal concept, which physiologically will never be met in a strict sense. For instance, there is no biological setting where Eq. 2 can hold for an unlimited range of doses. In addition, even if the true

expected concentrations for a drug suggested ideal dose proportionality within a certain dose range, due to biological variability within and between subjects this could never be fully proven.

This leads to the assessment of dose proportionality being a statistical question, that is, to testing and estimation problems. Statistical analyses are intended to determine to what extent the data are compatible with the model of dose proportionality, to quantify deviations from the ideal, and to support the derivation of clinical implications.

The clinical question to be answered is whether the deviations of expected exposure from dose proportionality are of clinical relevance or not. Deviations are relevant if they are large enough to expose the patient to likely risk from concentration-dependent effects within the range of approved doses.

Descriptive Analyses

Descriptive analyses normally include the presentations of descriptive statistics for concentration-related parameters (typical statistics: number of non-missing observations, mean, standard deviation, minimum, median, maximum, geometric mean, coefficient of variation). This can be supplemented by the presentation of the corresponding descriptive statistics for dose-normalized parameters.

Graphical display could include scatterplots of PK parameters over dose for the raw or for the dose-normalized parameters. For the latter, dose proportionality is represented by a dose-independent (horizontal) level of the PK parameters (e.g., CL, V) plotted against dose. Parameters such as AUC that are expected to be dose-dependent are usually normalized to dose before plotting against the dose (e.g., Fig. 8). In addition, this presentation accounts for the fact that dose-normalization typically standardizes the variability.

Discrete Model

For any study design with a set of fixed doses, one approach to test for deviations from dose proportionality can be based on classical linear models with a fixed effect for dose for log-transformed dose-normalized parameters. If a significant dose effect is found, strict dose proportionality can be considered refuted. However, this does not necessarily imply that the deviations are of any clinical relevance.

Pairwise comparison of exposure allows estimation of the ratio of PK parameters AUC or C_{max} for two given doses. Given dose proportionality, the ratio is expected to be equal to the ratio of doses. Alternatively, ratios for dose-normalized parameters could be assessed and compared with the value of 1 which is to be expected under dose proportionality (e.g., Table 6).

Power Model

Quantifying deviations from dose proportionality for any dose demands the use of statistical models where parameters can be estimated together with measures of imprecision (usually confidence intervals) and where dose proportionality is characterized by certain values of the model parameters. Assuming log-normal distributions for exposure-related parameters like AUC and C_{max} suggests model deviations from dose proportionality that are multiplicative rather than additive. A statistically useful way to model this is known as the power model (Smith et al. 2000)

$$C = \alpha * dose^{\beta} \tag{5}$$

Ideal dose proportionality is met when $\beta = 1$: that is, when $C = \alpha$ * dose (Eq. 2).

Deviations from dose proportionality ($C = \alpha$ * dose) are modeled as factors depending on β, which can be seen in

$$C = \alpha^{*} dose^{\beta} = \alpha^{*} dose^{*} dose^{\beta-1} \tag{6}$$

For an r-fold change of a given dose an $r^{\beta-1}$-fold change of the exposure can be expected if the drug demonstrates dose proportionality.

$$C/\text{dose} = \alpha^{*}\text{dose}^{\beta-1} \quad (7)$$

Values of β above 1 represent higher than proportional drug exposure (super-proportional), whereas values of β below 1 represent lower exposure (sub-proportional). Restated, the factor $r^{\beta-1}$ can be considered as the dose-normalized ratio between exposure following the r-fold change of a dose versus the initial dose. Different scenarios are given as examples in Table 1 below.

For instance, for a drug with a value of 1.25 for β, in case of doubling the dose (r = 2) the exposure would be more than doubled: it would be 19% higher than expected under dose proportionality. Figure 2 shows the deviation from dose-proportional exposure for different values of β.

It will be of clinical importance to assess whether the range for "r" is large enough to cover clinically relevant dose ranges.

One important beneficial statistical feature of the power model is that log transformation leads to a very simple linear model with a normal distribution for log(C):

$$\log(C) = \log(\alpha) + \beta^{*}\log(\text{dose}) + \text{Error} \quad (\text{for dose} > 0) \quad (8)$$

Estimates of β together with its confidence limits can be derived using standard statistical methods for linear models (Gough et al. 1995; Smith et al. 2000). Based on that, it follows that for a given dose ratio a confidence interval for the "deviation factor" $r^{\beta-1}$ can be calculated. This leads to the analysis being linked to questions of bioequivalence. If, for instance, a classical range of 0.8–1.25 can be considered for a drug as an acceptable range for bioequivalence, the deviation from dose proportionality may be considered as irrelevant as long as the confidence interval for $r^{\beta-1}$ does not violate this interval of 0.8–1.25. A 90% confidence interval is commonly used (US FDA 2001).

Table 1 Factor for deviation from dose proportional exposure for different values of β and r

	β				
Dose factor r	0.5	0.75	1	1.25	1.5
0.1	3.16	1.78	1.00	0.56	0.32
0.5	1.41	1.19	1.00	0.84	0.71
1.0	1.00	1.00	1.00	1.00	1.00
2.0	0.71	0.84	1.00	1.19	1.41
10	0.32	0.56	1.00	1.78	3.16

Example 1: Exploratory Assessment of Dose Linearity/Proportionality – Single Dose Study Design

Purpose and Rationale

The primary objective of first-in-human studies is generally to assess the safety and tolerability of a drug in healthy volunteers. Pharmacokinetic and pharmacodynamic data are often collected in these dose-escalation studies, so a secondary objective is thus the evaluation of pharmacokinetic parameters to describe the dose effect on drug absorption (t_{max}, t_{lag}), elimination ($t_{1/2z}$), and exposure (C_{max}, AUC) to assess the drug linearity/proportionality. The selection of the starting dose in these initial human trials is generally based on preclinical data from the most sensitive species in toxicology studies. The tested dose range should cover the potential therapeutic dose and should allow the determination of a maximum tolerated dose in humans and the safety margin.

The design of an exploratory assessment of dose linearity/proportionality during conduct of a first-in-human study for candidate drug "X001" is presented below. The description is limited to pharmacokinetic data although safety/tolerability and pharmacodynamic data were also obtained.

Primary objectives were to assess the clinical and laboratory safety/tolerability of X001, and secondary objectives were to assess the pharmacokinetics (PK) and pharmacodynamics (PD) following ascending single oral doses of X001 under fasted conditions. It was a single center, double-blind, placebo-controlled, randomized, escalating single oral dose study.

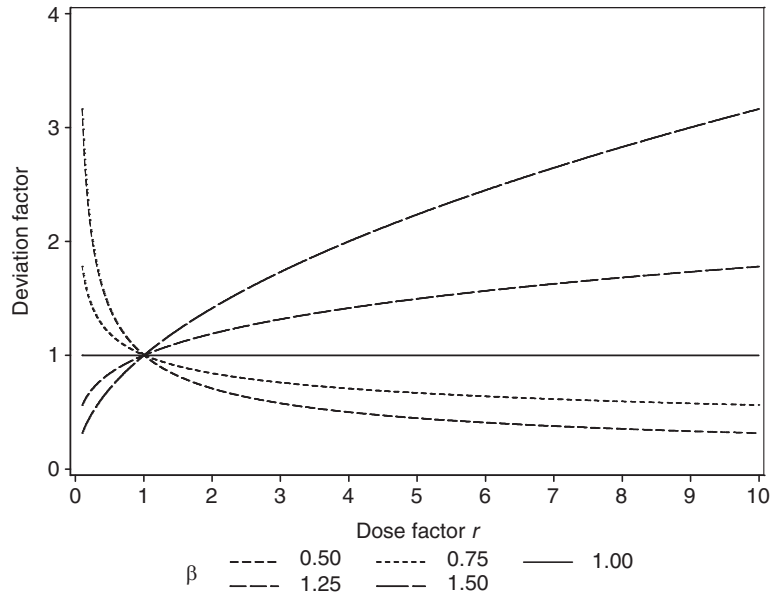

Fig. 2 Factor for deviation from dose proportional exposure for different values of beta (β)

Study Design

Healthy young male subjects aged between 18 and 45 years with a body weight between 50 and 90 kg and a body mass index between 18 and 28 kg/m2 were recruited. These subjects were randomized to receive X001 oral odoses of 1, 2, 5, 10, 20, 40, 80, 160, 300 or 500 mg, or placebo. The placebo-controlled study design was chosen for purposes of safety and PD assessment, and was not mandatory for the PK objective. Six subjects in each dose step were randomized to X001 and two subjects to a matching placebo. The capsules were administered with 240 mL of non-carbonated water after an overnight fast and with a 4 h post-administration fasting period.

Eighty subjects were recruited, plus eight additional subjects for optional dose levels.

Starting with the lowest dose, each of the subsequent doses was administered only if the preceding dose was safe and well tolerated. The decision to proceed to the next higher dose (n + 1) was based on the full range of safety parameters of the last dose (n) and pharmacokinetic data of the previous dose (n − 1). Subjects entered the study unit the evening before the study of drug administration and were assessed for their baseline characteristics on the morning of the day of drug administration. Subjects remained in the study unit for 48 h after the dose was administered. Standard safety/tolerability criteria (e.g., adverse events, biochemistry, hematology, urinalysis, ECG, and vital signs) and additional pharmacodynamic parameters (e.g., postprandial blood glucose) were assessed in this study but are out of the scope of this chapter.

Blood samples to determine X001 were collected before dosing, and at 0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 24 h, 36 h, and 48 h post-dose. X001 concentrations in plasma were assayed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with a validated lower limit of quantification (LLOQ) for X001 of 1 ng/mL.

The following pharmacokinetic parameters were calculated using standard non-compartmental techniques: maximum concentration (C_{max}), time to maximum concentration (t_{max}), area under the concentration–time curve from time of drug administration to last quantifiable concentration time point (AUC_{last}), area under the concentration–time curve from time of drug administration extrapolated to infinity (AUC), terminal elimination half-life ($t_{1/2z}$), and time to the first quantifiable concentration

(t_{lag}). Plasma concentrations and pharmacokinetic parameters were listed by standard descriptive statistics (N, mean, SD, SE, min, median, max, CV%, and geometric mean).

Dose Proportionality

Dose proportionality of C_{max} and AUC was evaluated using the log-transformed power model with dose as the fixed effect:

$$\begin{aligned}\text{Log}\,(\text{PK parameter}) = {} & \text{Log}\,(\alpha) \\ & + \beta^{*}\text{Log}\,(\text{dose}) \\ & + \text{Error}\end{aligned} \tag{9}$$

This model was tested for goodness-of-fit using the plot of residuals. Since there was no evidence of goodness-of-fit (residuals randomly distributed around the origin), estimates for β with 90% confidence intervals (CI) were obtained by ordinary least squares regression. Estimates and 90% CI for PK parameter associated with an r-fold (r = 2 and r = highest dose/lowest dose) increase in dose were obtained by exponentiating r to the powers of the β estimate ($\widehat{\beta}$) and confidence limits:

$$r^{\widehat{\beta} \pm t_{0.95},\, df\, SE\left(\widehat{\beta}\right)} \tag{10}$$

Dose Effect

The effect of dose upon $t_{1/2z}$ was assessed using a linear fixed effects model on log-transformed values:

$$\text{Log}\left(t_{1/2z}\right) = \text{dose} + \text{Error} \tag{11}$$

Point estimate and 90% CI for the geometric means of $t_{1/2z}$ were pooled across dose levels and separately for each dose level.

Results

Mean X001 plasma concentration time profiles following a single oral dose of 1 mg, 2 mg, 5 mg, 10 mg, 20 mg, 40 mg, 80 mg, 160 mg, 300 mg, and 500 mg X001 are presented in Fig. 3. X001 was rapidly absorbed, showing peak plasma concentrations approximately 1 h post-dose, irrespective of dose.

A summary of the descriptive statistics of main X001 PK parameters is given in Table 2. The relationship of individual AUC values versus dose, with linear regression and the 95% confidence range is illustrated in Fig. 4. The results of dose proportionality analysis are summarized in Table 3. X001 exposure increased with increasing doses. 90% CI of β-estimates for C_{max} and AUC were 0.97–1.03 and 1.00–1.06, respectively, thus including the unity and demonstrating dose proportionality. The mean terminal elimination half-life ($t_{1/2z}$) increased from 1 h to 2 h with increases in dose. Statistical analysis revealed that the dose had a significant effect ($p < 0.001$) on the terminal elimination half-life ($t_{1/2z}$).

Typically, this kind of early first-in-human study covers a very broad dose range, often leading to dose disproportionality over the entire dose range. At very low doses, PK parameters such as elimination half-life are often not reliable due to the limits of analytical quantification. At high doses, saturation of absorption or elimination processes or limitations in drug release from the dosage form due to insolubility may decrease the exposure and prevent a dose proportional increase. In these cases a pivotal investigation of dose linearity/proportionality becomes necessary. It typically includes at least three different doses covering the anticipated therapeutic dose range proposed for regulatory approval. More details are provided in section "Mechanisms Leading to Lack of Dose Proportionality."

Discussion

As shown in Fig. 3 for the two lower doses of 1 mg and 2 mg, X001 could only be detected up to 2 h and 4 h, respectively, resulting in very limited data points in the elimination phase of the drug. Therefore, the quantification limit of the analytical method might have compromised the reliability of the PK parameters obtained for these doses (1 mg

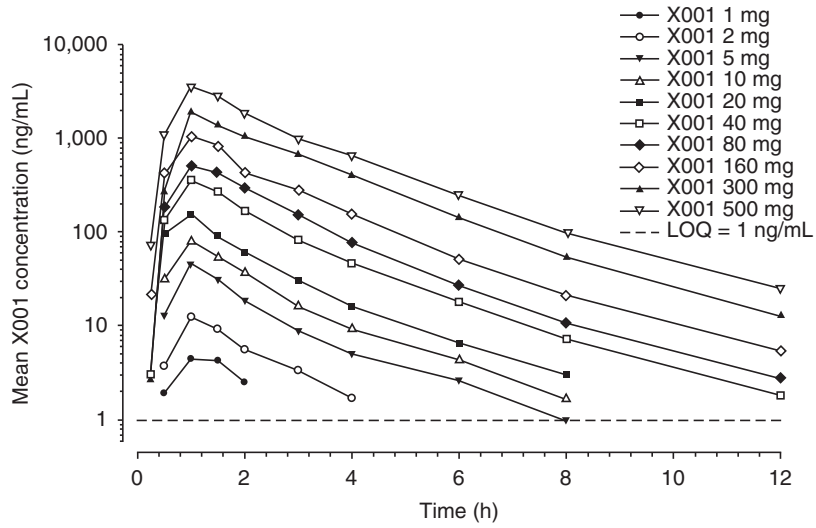

Fig. 3 Mean X001 plasma concentrations (semi-logarithmic scale)

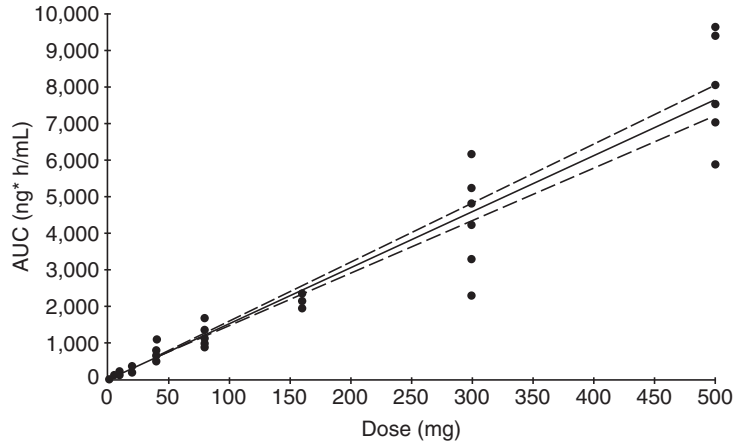

Fig. 4 Relationship of individual AUC values for X001 versus dose with linear regression (bold line) and the 95% confidence range (dashed line)

and 2 mg). For example, the low value of approximately 1 h for the calculated $t_{1/2z}$ for the 1 mg and 2 mg doses (Table 2) differs from the value of approximately 2 h for the doses from 5 mg to 500 mg. If the low doses are of further interest (e.g., as pharmacologically active dose), the lower limit of quantification of the analytical method could be improved in order to guarantee a reasonable PK assessment.

Evaluation of dose-proportionality is part of the first study in humans which is always a single-dose study but could also be applied to early multiple-dose studies. This kind of explorative data related to dose linearity/proportionality can be used to predict the exposure for further planned dose steps, including doses outside the dose range investigated so far. If a notable nonlinear effect is seen by this exploratory evaluation, a more elaborate study will need to be performed as described in section "Mechanisms Leading to Lack of Dose Proportionality." In some cases dose linearity/proportionality assessment using the described explorative method is not possible due to high interindividual variability in PK characteristics. In these cases an intra-individual crossover design is preferred as described in section "Dose Linearity."

Table 2 Key PK parameters and descriptive statistics of X001 by dose

	AUC (ng*h/mL)				C_{max} (ng/mL)				$t_{1/2z}$ (h)			
Dose (mg)	Mean	CV%	SD	Geometric mean	Mean	CV%	SD	Geometric mean	Mean	CV%	SD	Geometric mean
1	10.5	20	2.1	10.4	6.99	36	2.5	6.61	0.968	63	0.609	0.852
2	25.7	37	9.4	24.4	13.1	5	0.7	13.1	1.36	27	0.36	1.31
5	84.4	35	29.1	80.5	50.5	42	21.0	47.3	1.93	18	0.36	1.90
10	160	18	29	158	93.0	28	26.4	90.2	1.62	18	0.29	1.60
20	283	33	94	269	164	40	66	152	1.58	4	0.07	1.58
40	714	33	237	683	390	30	117	375	1.79	19	0.33	1.77
80	1,170	26	301	1,140	554	21	117	543	1.76	14	0.25	1.74
160	2,150	7	155	2,140	1,210	28	334	1,170	1.78	12	0.22	1.77
300	4,350	32	1,370	4,150	2,140	16	346	2,110	2.07	31	0.65	2.00
500	7,910	18	1,440	7,800	3,880	14	540	3,850	2.04	28	0.57	1.98

Table 3 Estimates with 90% CI for r-fold increase in dose

Parameter	Dose ratio	Ratio	
		Estimate	90% CI
C_{max} (ng/mL)	r = 2	2.00	1.96–2.05
	r = 500	506	414–619
	β-Estimate	1.00	0.97–1.03
AUC (ng∗h/mL)	r = 2	2.04	1.99–2.08
	r = 500	592	487–720
	β-Estimate	1.03	1.00–1.06

r = 500 = highest/lowest dose

Example 2: Exploratory Assessment of Dose Linearity/Proportionality: Crossover Study Design

Purpose and Rationale

Drug candidate "X002" had already been carefully explored before this study for pharmacodynamics, pharmacokinetics, and safety in a large number of subjects. However, no formal evaluation of the dose–exposure–response relationship had yet been conducted.

The primary objective of this study was to investigate the dose–exposure–response relationship of X002 after single subcutaneous injections of 0.075 units/kg body weight (U/kg), 0.15 U/kg, and 0.3 U/kg. The secondary objective was to assess the safety and tolerance of X002. The description is limited to pharmacokinetic data, although safety, tolerability, and pharmacodynamic data were also obtained.

The number of doses was restricted to three to allow for intra-subject comparisons in a crossover design.

Study Design

This was a single-center, single-blind, randomized, single dose, three-way crossover study comparing three single doses of X002 (0.075 U/kg, 0.15 U/kg, and 0.3 U/kg) injected subcutaneously. The design was a full crossover including all six possible treatment sequences.

All subjects were male patients between 18 and 55 years, a body mass index between 18 kg/m^2 and 30 kg/m^2, and within the context of the underlying disease had normal findings in the following assessments: medical history, physical examination, laboratory values, electrocardiogram (ECG), blood pressure, pulse rate, and core body temperature, unless the investigator considered any abnormality to be clinically irrelevant and not interfering with the safety of the subject and the scientific integrity of the study.

Subjects were randomized to one of the six sequences of three dose levels of X002. The study consisted of five trial periods – trial period 0 (screening visit), trial periods 1, 2, 3 (X002 treatment visits), and trial period 4 (follow-up visit). X002 was injected subcutaneously into the predefined body region on three different study days.

Serum concentrations were measured at time point 0 (prior to dosing) and after 10 min, 20 min, 30 min, 40 min, 50 min, 60 min, 70 min, 80 min, and 90 min as well as after 2 h, 2.5 h, 3 h, 4 h, 5 h, 6 h, 8 h, and 10 h after the injection of study medication. If the end of the period for pharmacodynamic assessment occurred earlier than the maximum of 10 h post-study medication injection, no further samples were taken. Serum concentrations of X002 were analyzed using a radioimmunoassay. The lower limit of quantification (LLOQ) was 5.0 μU/mL.

Area under the concentration–time curve for the time between 0 h and 2 h after dosing ($AUC_{(0–2h)}$, μU∗min/mL) was considered the primary PK parameter and was derived using standard non-compartmental methods as were $AUC_{(0–end)}$ (μU∗min/mL), and mean residence time (MRT, h).

Maximum concentration (C_{max}, μU/mL) and time to C_{max} (t_{max}, h) were derived using a compartmental method. No adjustments of the alpha levels were made for multiple analyses.

Serum concentrations and pharmacokinetic parameters were listed individually and summarized per dose by standard descriptive statistics (number of non-missing observations, geometric mean, mean, standard deviation, standard error of

the mean, minimum, median, 25%- and 75%-quantiles, maximum, coefficient of variation (%)). Individual profiles per subject and median profiles were plotted by dose. Box and whisker plots of primary pharmacokinetic variables were generated per dose.

No formal sample size calculation was performed for this study. The sample size of the total 18 subjects, 3 subjects per sequence, was considered a standard approach for evaluation of dose–exposure–response relationship.

Assessment of the Dose–Exposure Relationship

AUC-values ($AUC_{(0–2\ h)}$ and $AUC_{(0–end)}$) and C_{max} were plotted over the dose per kg body weight (U/kg) as well as over the total dose (U), for each subject. Corresponding plots were generated for the exposure parameters normalized to a dose of 0.15 U/kg or to 10 U, respectively.

Geometric means for AUC-values and C_{max} were plotted over dose with a regression line forced through the origin point. Geometric means for AUC-values and C_{max} normalized to a dose of 0.15 U/kg were also plotted over dose together with a regression line.

AUC values (through 2 h and through the end of sampling), C_{max}, and MRT were natural log-transformed and compared among the three dose levels using a linear ANOVA model with adjustment for dose, period, sequence, and subject within sequence (discrete model). 95% confidence intervals for pairwise dose differences were calculated and retransformed to derive the respective confidence limits for mean ratios of the pairwise treatment comparisons, that is, for 0.15 U/kg versus 0.075 U/kg, and for 0.3 U/kg versus 0.15 U/kg. Dose proportionality within the commonly accepted bioequivalence criteria (0.80–1.25) is confirmed for a doubling of the dose, when the confidence interval for a treatment ratio is within 1.60–2.50. T_{max} was analyzed by nonparametric analysis for pairwise comparisons with 95% nonparametric confidence intervals for the respective median difference in dose. A power model was applied to assess dose proportionality. This analysis was based on the actual doses of X002 administered.

Results

All 18 randomized subjects were treated, completed the study, and were evaluable for the PK component of the study. Median X002 serum concentration time profiles following the single subcutaneous doses of 0.075 U/kg, 0.15 U/kg, and 0.3 U/kg are presented in Fig. 5. X002 was rapidly absorbed, showing peak serum concentrations approximately 1 h post-dose.

All subjects showed a monotonically increasing dose–exposure relationship in $AUC_{(0\text{-}2\ h)}$, $AUC_{(0\text{-end})}$, and C_{max} for X002. T_{max} generally increased slightly with dose. Descriptive statistics for key PK parameters of X002 are given in Table 4. Boxplots for $AUC_{(0–2\ h)}$ of X002 are given in Fig. 6, showing the monotonic relationship and expected increased variability with higher doses.

Geometric means for $AUC_{(0–2\ h)}$ of X002 are shown in Fig. 7, suggesting dose proportionality.

Geometric means for $AUC_{(0–2\ h)}$ of X002 normalized on a dose of 0.15 U/kg are given in Fig. 8. The point estimates for treatment ratios together with 95% confidence intervals are presented in Table 5. All 95% confidence intervals are fully contained within the range of 1.60–2.50, and as a consequence the 90% confidence intervals are within that range as well. Thus, exposure can be assumed to behave according to dose proportionality for a doubling of doses within the dose range investigated.

Due to the variation in body weight, the administered, weight-based doses varied between 4 U and 31 U, a 7.75-fold range. Individual plots of $AUC_{(0–2\ h)}$ over the total dose (U) are given in Fig. 9. The figure also represents the finding of dose monotony in this exposure parameter for each subject.

Individual plots of $AUC_{(0–2\ h)}$ dose normalized for 10 U, over the total dose (U) are given in Fig. 10.

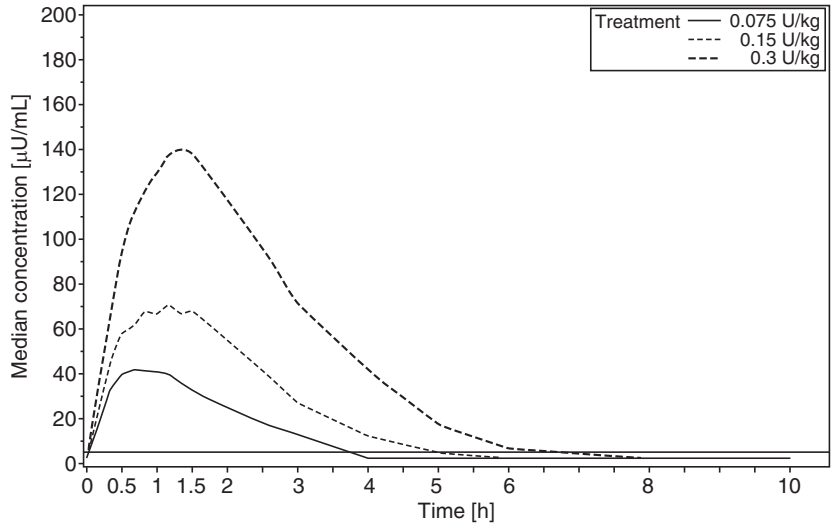

Fig. 5 Median X002 concentration (µU/mL) profiles over time after dosing

Table 4 Key PK parameters of X002 and descriptive statistics by dose

	Geometric mean (arithmetic mean ± SD)		
Variable (Unit)	0.075 U/kg ($N = 18$)	0.15 U/kg ($N = 18$)	0.3 U/kg ($N = 18$)
$AUC_{(0–2\ h)}$ (µU∗min/mL)	3,792 (3,855 ± 677)	6,676 (6,832 ± 1,461)	12,992 (13,237 ± 2,559)
$AUC_{(0–end)}$ (µU∗min/mL)	5,341 (5,372 ± 589)	11,196 (11,284 ± 1,456)	24,891 (25,076 ± 3,209)
C_{max} (µU/mL)	42 (43 ± 9)	72 (73 ± 16)	140 (142 ± 25)
MRT (min)	115 (122 ± 50)	121 (125 ± 34)	134 (136 ± 28)
T_{max} (min)[a]	47 (34−99)	57 (44−93)	72 (50−112)

[a]Median (minimum–maximum) reported

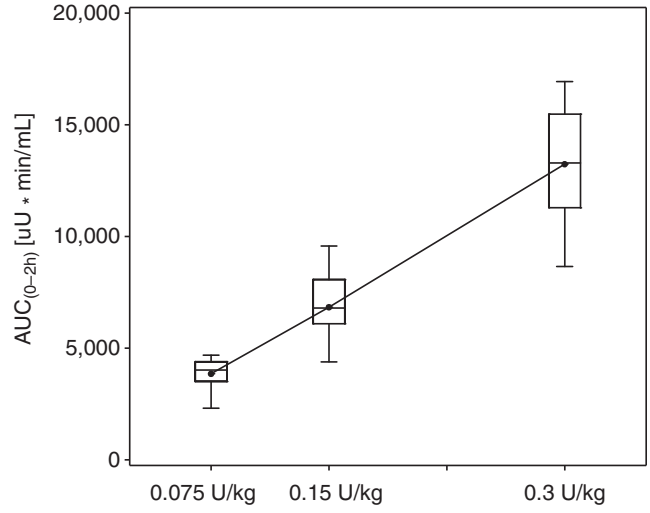

Fig. 6 Boxplots of $AUC_{(0–2\ h)}$ (µU∗min/mL) for X002 per dose

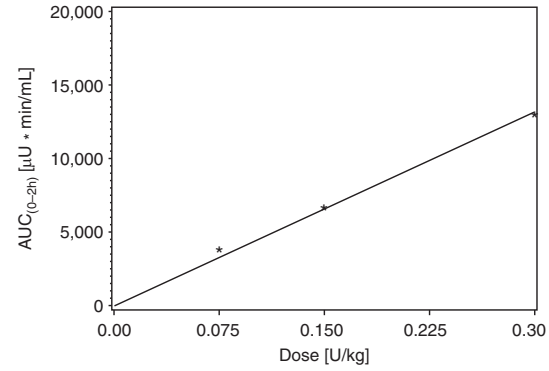

Fig. 7 Plots of geometric mean $AUC_{(0–2\ h)}$ (µU∗min/mL) for X002 per dose

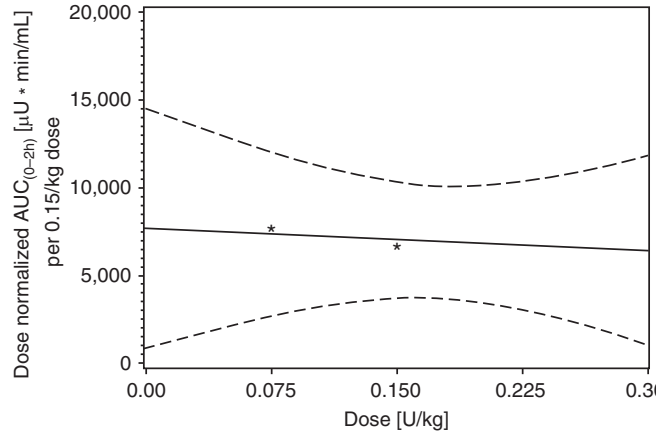

Fig. 8 Plots of geometric mean $AUC_{(0–2\ h)}$ (µU∗min/mL) for X002 – dose normalized on 0.15 U/kg

Table 5 Pairwise comparisons of key PK parameters for X002

	Point estimate (95% confidence interval)	
Variable	Ratio of 0.15–0.075 U/kg dose	Ratio of 0.3–0.15 U/kg dose
$AUC_{(0–2\ h)}$	1.8 (1.6–1.9)	1.9 (1.8–2.1)
$AUC_{(0–end)}$	2.1 (2.0–2.2)	2.2 (2.1–2.3)
C_{max}	1.7 (1.6–1.9)	2.0 (1.8–2.1)

Each deviation from a horizontal line indicates a deviation from dose proportional results for an individual. However, deviations are generally small.

The results of the analysis of the power model for the dose-proportionality of increasing total doses of X002 are presented in Table 6. The 90% confidence intervals for the dose normalized ratios for a value of r = 2 are fully contained within the classical bioequivalence range, confirming the dose proportional behavior of exposure for a doubling of dose. A factor of 7.75 between the highest and the lowest total dose was measured instead of the expected factor of 4. According to the results from the power model, dose proportionality could not be shown for the full, 7.75-fold dose range. However, it was determined by extrapolation that up to a 3.7-fold (r) for $AUC_{(0–2\ h)}$, up to 5.0 r for $AUC_{(0–end)}$, and up to 3.0 r for C_{max} satisfied criteria for dose-normalized dose-proportionality. These dose ranges, for which dose proportionality can be assumed, were considered to cover clinical needs and expected range of actual dose adjustments of the drug.

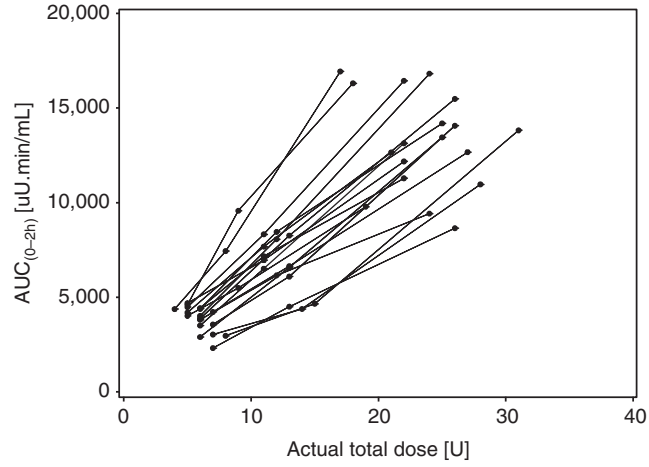

Fig. 9 Individual $AUC_{(0–2\ h)}$ (μU∗min/mL) over actual total dose (U) – per subject

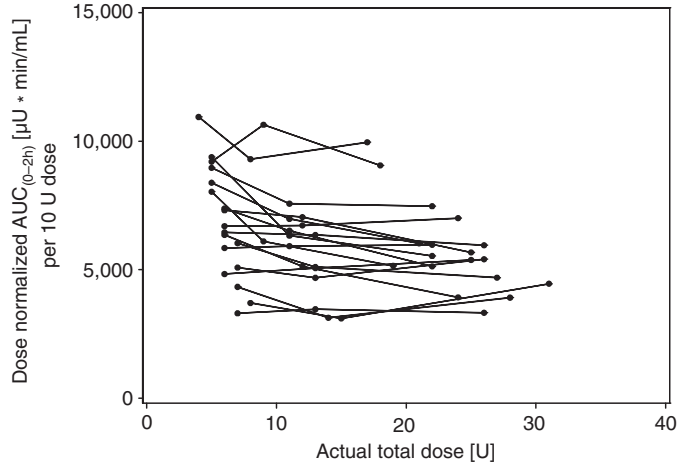

Fig. 10 Individual $AUC_{(0–2\ h)}$ (μU∗min/mL) over actual total dose (U) – per subject and dose normalized on 10 U

Discussion

The study was designed as an explorative study, because a strictly confirmatory study was not deemed necessary at this stage of the development of X002. In another setting this might be required. For this case, a single primary analysis used to decide about dose proportionality will have to be specified upfront.

Based on the crossover design, the study design allowed to assess dose–exposure relationship and to investigate dose proportionality based on intra-subject comparisons, which is not possible in any setting with parallel groups. In addition, due to the dosing per kg of body weight and the variability of body weight between subjects, a broader range of doses could be observed.

Due to the crossover design with complete blocks, the number of doses investigated had to be limited to a small number. For situations where a broader dose range has to be investigated, crossover designs with incomplete blocks could be set up.

Different statistical approaches were used and delivered consistent findings. The study allowed to draw conclusions for a clinical relevant range of doses.

Table 6 Estimates with 90% CI for r-fold increase in dose

Parameter	Dose ratio	Ratio		Dose-normalized ratio	
		Estimate	90% CI	Estimate	90% CI
$AUC_{0-2\ h}$	r = 2	1.8440	1.7784, 1.9119	0.9220	0.8892, 0.9559
	r = 7.75	6.0964	5.4781, 6.7838	0.7866	0.7069, 0.8753
	$r^* = 3.7324$	3.1985	2.9859, 3.4260	0.8570	0.8000, 0.9179
	β-Estimate	0.8828	0.8306, 0.9350		
AUC_{0-end}	r = 2	2.1576	2.1145, 2.2016	1.0788	1.0572, 1.1008
	r = 7.75	9.6960	9.1353, 10.2917	1.2511	1.1788, 1.3280
	$r^* = 5.0073$	5.9723	5.6989, 6.2591	1.1927	1.1381, 1.2500
	β-Estimate	1.1094	1.0803, 1.1385		
C_{max}	r = 2	1.8164	1.7425, 1.8936	0.9082	0.8713, 0.9468
	r = 7.75	5.8315	5.1580, 6.5940	0.7524	0.6655, 0.8508
	$r^* = 3.0718$	2.6284	2.4574, 2.8115	0.8557	0.8000, 0.9153
	β-Estimate	0.8611	0.8012, 0.9211		

(r^*) = highest dose ratio compatible with dose proportionality by the equivalence approach

Example 3: Confirmatory Assessment of Dose Linearity/Proportionality – Single and Repeated Dose Crossover Design

Purpose and Rationale

The aim of a confirmatory dose proportionality study is to assess the PK of a drug at doses bracketing the anticipated therapeutic dose (i.e., usually at one dose below therapeutic dose and one dose above), using the most appropriate design (i.e., crossover, within-subject comparison) and an adequate number of subjects, in order to assess what are the variations in exposure when the dose needs to be adjusted, for example, in special populations or in case of concomitant medications.

The design of the confirmatory dose linearity/proportionality study during advanced clinical development for candidate drug "X003" is presented below. The study description is limited to the primary objective of pharmacokinetic data, although safety/tolerability data were also obtained as secondary objective.

In the case of candidate drug X003 the exploratory assessment of dose linearity/proportionality from the first-in-human study did not allow accurate characterization of the deviation from proportionality of X003 pharmacokinetics because of the parallel group design, low number of subjects, and relatively high variability of X003 PK. For a twofold increase in dose, there was a threefold increase in exposure with large 95% confidence interval of 1.9–4.1. In addition, no multiple dose PK data were available at doses below the anticipated therapeutic dose of 400 mg administered twice daily (BID).

Study Design

This was a single center, randomized, non-placebo-controlled, open-label, single and repeated BID oral dose, three treatment, three period, crossover study with a washout of 14 days between periods.

Healthy young white men aged between 18 and 35 years with a body weight between 50 kg and 90 kg and a body mass index between 18 kg/m^2 and 28 kg/m^2 were included. Eighteen subjects were accrued in order to have at least 12 subjects complete the study (US FDA 2001). Subjects were randomized and treated with 200 mg,

400 mg, and 800 mg of X003. The tablets were administered with 200 mL of non-carbonated water on Day 1 at 8:00 a.m. after the end of a standardized breakfast (single dose), on Days 2–13 at 8:00 a.m. and 8:00 p.m., and on Day 14 at 8:00 a.m. after the end of a standardized meal. Tablets containing 100 mg and 400 mg X003 were used to provide 200 mg (2 × 100 mg), 400 mg (1 × 400 mg), and 800 mg (2 × 400 mg) doses.

Subjects were hospitalized the evening before first drug administration (Day 1) to Day 2 (morning) and from Day 13 (evening) to Day 15 (morning) of each period. The subjects were discharged and then visited the study unit every morning and every evening from Days 2 to 13 for blood-sampling and/or study drug administration.

After the last dose was administered on the morning of Day 14, subjects returned for blood sampling on the evening of Day 15, and on the mornings of Days 16 through 18. The duration of study participation for each subject was in total 12–15 weeks: 3–21 days for subject selection, 14 days for period 1, 14 days for washout period 1, 14 days for period 2, 14 days for washout period 2, 14 days for period 3, and 10–12 days for follow-up period.

Blood samples to determine X003 were collected before dosing and at 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 24 h, 36 h, 48 h, 72 h, and 96 h after dosing on Days 1 and 14, and before morning dosing for trough determinations on Days 6, 7, 8, 9, 10, and 12. X003 concentrations in plasma were determined using a validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) method with a limit of quantification (LOQ) for X003 of 0.5 ng/mL. Standard safety/tolerability criteria (e.g., adverse events, biochemistry, hematology, urinalysis, ECG, and vital signs) were assessed in this study but are not the subject of this chapter.

Primary and secondary objectives were to assess the deviation from dose proportionality and safety/tolerability, respectively, of X003 after 200 mg, 400 mg, and 800 mg single and twice daily repeated oral doses of X003 for 10 days. The following pharmacokinetic parameters were assessed using non-compartmental analysis:

Day 1:
Maximum concentration (C_{max}), time to maximum concentration (t_{max}), area under the concentration time curve from time of drug administration to time 12 h ($AUC_{(0–12\ h)}$) and to last quantifiable concentration time point (AUC_{last}), area under the concentration–time curve from time of drug administration extrapolated to infinity (AUC), terminal elimination half-life ($t_{1/2z}$).
Day 14:
C_{max}, T_{max}, $AUC_{(0–12\ h)}$, $t_{1/2z}$.
Days 6, 7, 8, 9, 10, and 12: trough concentration (C_{trough}).

Plasma concentrations and pharmacokinetic parameters were listed by standard descriptive statistics (N, mean, SD, SE, min, median, max, CV%, geometric mean) for each dose level on Days 1 and 14. X003 C_{max} (Days 1 and 14), AUC (Day 1), $AUC_{(0–12\ h)}$ (Day 14), and $t_{1/2z}$ were log-transformed, and t_{max} was rank-transformed.

C_{max} and AUC at Day 1, and C_{max} and $AUC_{(0–12\ h)}$ at Day 14 were analyzed with a "random intercepts and random slopes" mixed model in SAS PROC MIXED separately for Days 1 and 14. The parameters were assumed to follow a multiplicative power model, which is equivalent to the log-transformed power model, and has the form:

$$\begin{aligned}\mathrm{Log}(\text{parameter}) = {} & [\mathrm{Log}(\alpha)\mathrm{i} - \mathrm{Log}(\alpha)] \\ & + [\beta_i - \beta] \\ & \times \mathrm{Log}(\text{dose}) \\ & + \text{period} + \text{Error}\end{aligned} \tag{12}$$

In the model, Log(α) and β were the estimates of intercept and slope, respectively, estimated by generalized least squares (GLS) with restricted maximum likelihood (REML) estimates of random effects.

Goodness-of-fit was assessed by visual inspection of residuals plots. If there was no evidence of lack-of-fit (residuals randomly distributed around the origin), estimates for β with 90% confidence intervals were computed within the mixed model framework. Estimates with 90% CI for PK parameter increases associated with an r-fold (r = 2 and r = 4 = highest dose/lowest dose) increase in dose were obtained by exponentiating r to the powers of the $(\widehat{\beta})$ and confidence limits (Eq. 10) and also, subsequently, converting these to a dose-normalized scale by dividing by r. Three cases were considered: r = 2 (doubling of dose), r = 4 (high/low dose), and the maximum dose ratio "r" compatible with dose proportionality by an equivalence approach (i.e., 90% confidence limits for the dose normalized increase in the PK parameter is within 0.80–1.25).

Dose Effect

Differences of $t_{1/2z}$ and T_{max} between doses were tested for significance with p-values from the linear fixed effects model with fixed terms for sequence, period, day, dose, and the dose-by-day interaction, and a random term for subjects within sequence.

If the dose-by-day interaction was not significant ($p \geq 0.05$), the interaction term was dropped from the model and the model was refit. The p-values for the dose and day effects were reported in the context of the reduced model.

If the dose-by-day interaction was significant ($p < 0.05$), the p-value for the dose effect was computed in a mixed effects model. Days 1 and 14 were fit separately with fixed terms for sequence, period and dose, and a random term for subjects within sequence. The p-value for the day effect was computed in a mixed effect model, fit separately for each dose with fixed terms for sequence and day and a random term for subjects within sequence. Each model described above was fit by GLS with REML estimates of random effects, using SAS PROC MIXED.

Accumulation Effects

To assess accumulation effects from Days 1 to 14, the PK parameters were analyzed with a mixed effects model with fixed terms for sequence, period, day, dose and the dose-by-day interaction, and a random term for subjects within sequence. The model was fit again by GLS with REML estimates of random effects using SAS PROC MIXED.

If the dose-by-day interaction was significant ($p < 0.05$), accumulation was assessed for each dose group separately within the mixed model framework. Otherwise, the term was dropped from the model and accumulation effects were assessed for all dose groups. For C_{max} and $AUC_{(0–12\ h)}$, the difference in means between Days 14 and 1, with 95% CI, was computed within the mixed model framework and converted to an accumulation ratio of adjusted geometric means by the antilog transformation.

Steady State

The occurrence of steady state for X003 was assessed separately at each dose level by fitting the trough values with a nonlinear mixed effects model using the SAS NLMIXED procedure. The BID trough values corresponding to days 6, 7, 8, 9, 10, and 12 were utilized for the steady-state assessment. Model goodness-of-fit was evaluated by graphical inspection of the fitted curves and the within-subject residuals, and by graphical inspection of histograms of the estimated subject-specific random parameters (e.g., evidence of outliers, or subpopulations by gender, age, metabolizer status). For each subject and each dose level, the day at which 90% of the estimated subject-specific steady-state trough concentration is reached was predicted from the model. The overall time-to-steady state was determined as the 50th percentile (for average steady state) and the 90th percentile (for individual steady state) of these individual predicted values at each dose level. The 95% CIs for the 50th and 90th percentiles were calculated by nonparametric methods.

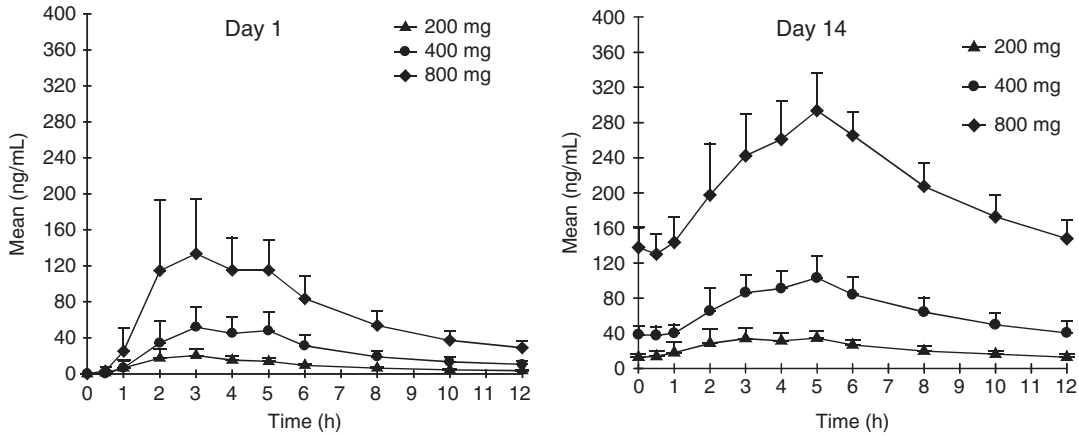

Fig. 11 Mean (SD) X003 plasma concentrations on Day 1 after a single and on Day 14 after repeated BID oral administration of X003 (linear scale)

Table 7 Mean (CV%) X003 pharmacokinetic parameters observed after a single and repeated oral administration of X003 BID

PK parameters	Day	200 mg $N = 17$	400 mg $N = 16$	800 mg $N = 17$
C_{max} (ng/mL)	1	23.1 (38)	67.2 (36)	162 (40)
	14	40.3 (30)	111 (17)	298 (13)
t_{max} (h)[a]	1	3 (2; 3)	3 (2; 5)	3 (2; 6)
	14	5 (2; 5)	5 (3; 6)	5 (2; 6)
$AUC_{0-12\ h}$(ng*h/mL)	1	111 (24)	310 (28)	846 (27)
	14	276 (23)	798 (19)	2,510 (12)
$t_{1/2z}$ (h)	1	9.81 (33)	17.6 (56)	19.6 (33)
	14	26.9 (32)	30.0 (29)	31.2 (32)
AUC (ng*h/mL)	1	160 (27)	474 (33)	1,310 (26)

[a]Median value (min; max)

Results

Plasma Concentrations and Pharmacokinetic Parameters

Mean (SD) X003 plasma concentration versus time curves observed after single (Day 1) and repeated BID (Day 14) oral administrations of X003 are presented in Fig. 11. X003 plasma concentrations were higher following a 10-day repeated BID oral administration (Day 14) compared to the single dose at Day 1 and reached peak levels approximately 5 h post-dose. Twelve hours post-dose plasma concentrations were not below LOQ.

A summary of X003 main pharmacokinetic parameters observed on Day 1 after a single oral administration and on Day 14 after a 10-day repeated BID oral administration of X003 is presented in Table 7.

Steady State

Mean (SD) X003 C_{trough} observed from Days 6 to 14 during repeated BID X003 administrations are graphically summarized in Fig. 12. Individual steady state, as expressed by 90th percentile, was reached after 3–5 treatment days. Average steady state, as expressed by 50th percentile, was reached after 3–4 treatment days, regardless of the dose.

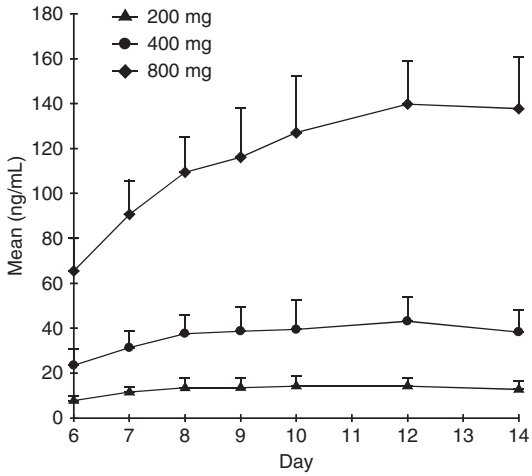

Fig. 12 Mean (SD) X003 trough concentrations from Days 6 to 14 during repeated BID oral administration of X003

Accumulation

In the accumulation assessment, the dose-by-dose interaction was not significant for C_{max} and $AUC_{(0–12\ h)}$, allowing the assessment of a single accumulation ratio across doses for each PK parameter of X003. After a 10-day repeated BID oral administration, an accumulation ratio of 1.84 (95% CI, 1.65–2.04) in C_{max} and 2.72 (2.52–2.94) in $AUC_{(0–12\ h)}$ was observed, regardless of the administered dose (Table 8).

Dose Proportionality

Results of the dose proportionality assessment, at Days 1 and 14, are summarized in Table 9.

C_{max} and t_{max}: After a 10-day repeated BID oral doses of X003, doses ranging from 200 mg to 800 mg X003 C_{max} values were reached 5 h after drug intake, and no significant dose, day, or dose-by-day interaction effects were observed on T_{max}. As measured by ratio estimate and associated 90% CI, X003 C_{max} increased more than expected by dose proportionality: a twofold increase in dose led to a 2.62 (2.39–2.88) and 2.77 (2.62–293) increase in X003 C_{max} on Days 1 and 14, respectively.

$AUC_{(0–12\ h)}$: Individual and mean (SD) values of X003 $AUC_{(0–12\ h)}$ on Day 14 are presented in Fig. 13. As measured by ratio estimate and associated 90% CI, X003 AUC increased more than expected by dose proportionality (Table 9): a twofold increase in dose led to a 2.86 (2.67–3.06) and 3.06 (2.92–3.20) increase in X003 AUC on Days 1 and 14, respectively.

Table 8 Accumulation ratio (R_{ac}) with 95% CI for X003 C_{max} and $AUC_{0–12\ h}$

PK parameters	R_{ac} estimate	95% CI
C_{max} (ng/mL)	1.84	1.65; 2.04
$AUC_{0–12\ h}$ (ng*h/mL)	2.72	2.52; 2.94

$t_{1/2z}$: The p-value for the dose-by-day interaction was significant in the analysis of $\log(t_{1/2z})$ for X003 ($p > 0.001$). There was a dose effect at Days 1 and 14, and a Day effect for X003 at 200 mg, 400 mg, and 800 mg. From 200 mg to 800 mg, the mean $t_{1/2z}$ increased significantly from 9.8 h to 19.6 h at Day 1 and from 26.9 h to 31.2 h at Day 14 (Table 7).

Discussion

The described evaluation provides confirmatory data on the lack of dose linearity/proportionality for X003, which allows dose adjustment recommendations in the submission package of a drug. This type of study also supports bracketing approaches in bioequivalence studies in which different formulations and dose strengths are to be tested. In several cases it was accepted by authorities that bioequivalence for only the lowest and highest dose strength had to be demonstrated (US FDA 2014). In a three-period crossover design, the investigator should recruit approximately 50% additional subjects in order to have enough subjects completing all three periods and to guarantee appropriate PK evaluation. Overall, the crossover design is preferable for the assessment of dose linearity/proportionality because it minimizes the variability in PK parameters.

In case steady-state conditions are known to be achieved within a specified dosing regimen, it might be sufficient to evaluate dose proportionality at steady state or alternatively single dose conditions only. The investigator should also consider evaluating dose proportionality for major, active metabolites.

Table 9 Ratio estimates and associated 90% CIs for dose proportionality assessed for a twofold increase in dose

PK parameters	Day	Estimate	95% CI
C_{max} (ng/mL)	1	2.62	2.39; 2.88
C_{max} (ng/mL)	14	2.77	2.62; 2.93
AUC (ng/mL)	1	2.86	2.67; 3.06
$AUC_{0–12\ h}$ (ng*h/mL)	14	3.06	2.92; 3.20

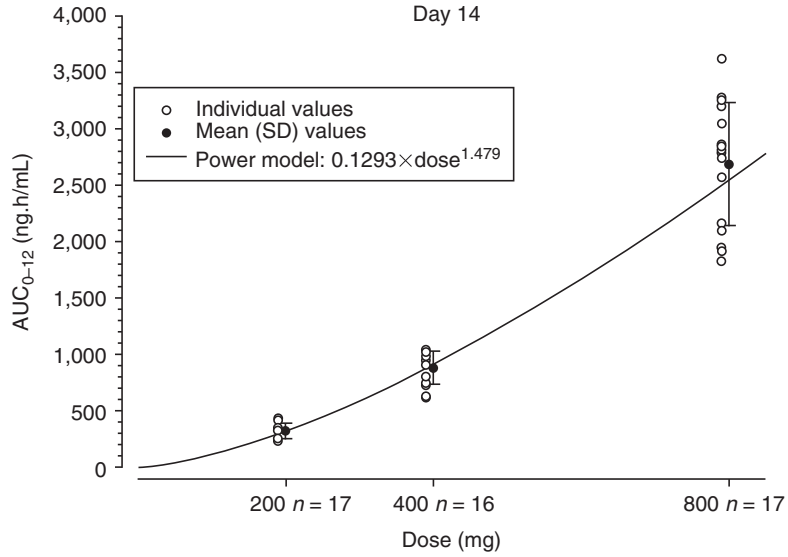

Fig. 13 Individual and mean (SD) X003 $AUC_{(0–12\ h)}$ values observed after repeated BID oral administration of X003

References and Further Reading

Cawello W, Brett M, Weimann H-J, Zimmerman H, Pabst G, Sierakowski B, Gieschke R, Baumann A (1999) Parameters for compartment-free pharmacokinetics: standardisation of study design, data analysis, and reporting. Shaker Verlag, Aachen

EMEA CPMP (2010) Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr)

EMEA CPMP (2014) Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms (EMA/CPMP/EWP/280/96 Corr1)

Frick A, Marshallsay C, Steinstraesser WR (2006) Clinical studies – typical designs. In: Vogel HG, Maas J, Mayer D, Hock F (eds) Drug discovery and evaluation: safety and pharmacokinetic assays. Springer, Berlin/Heidelberg

Gough K, Hutchison M, Keene O, Byrom B, Ellis S, Lacey L, McKellar J (1995) Assessment of dose proportionality: report from the Statisticians in the Pharmaceutical Industry/Pharmacokinetics UK Joint Working Party. Ther Innov Regul Sci 29(3):1039–1048

Hummel J, McKendrick S, Brindley C, French R (2009) Exploratory assessment of dose proportionality: review of current approaches and proposal for a practical criterion. Pharm Stat 8:38–49. https://doi.org/10.1002/pst.326

Ludden TM (1991) Nonlinear pharmacokinetics: clinical implications. Clin Pharmacokinet 20:429–446

Sethuraman VS, Leonov S, Squassante L, Mitchell TR, Hale MD (2007) Sample size calculation for the Power Model for dose proportionality studies. Pharm Stat 6:35–41. https://doi.org/10.1002/pst.241

Smith BP (2004) Assessment of dose proportionality. In: Bonate P (ed) Pharmacokinetics in drug development: vol 1, clinical study design and analysis. AAPS Press, Arlington

Smith BP, Vandenhende FR, DeSante KA, Farid NA, Welch PA, Callaghan JT, Forgue ST (2000) Confidence interval criteria for assessment of dose proportionality. Pharm Res 17:1278–1283

US FDA (2001) Guidance for industry: statistical approaches to establishing bioequivalence. Office of Training and Communications, Division of Communications Management, Maryland

US FDA (2014) Draft guideline for industry: bioavailability and bioequivalence studies submitted in NDAs or INDs – general considerations, Mar 2014