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Comprehensive Statistical Analysis of the Pharmacokinetics, Safety and Clinical Benefit Rate of Mitotam in a Single-Center Phase I/Ib Trial in Patients with Metastatic Solid Tumors

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Supplementary Methods

Study design

The phase I study evaluated the safety of a single cycle of MitoTam across nine escalating doses (0.25, 0.5, 1.0, 1.5, 2.25, 3.0, 4.0, 5.0, and 6.0 mg/kg) and two treatment schemas. For the 0.25-3.0 mg/kg doses, one treatment cycle consisted of three MitoTam administrations (t.i.w. schema) on day 1 (D1), D3, and D5 followed by observation on D6 and D7. At the 4.0–6.0 mg/kg doses, a treatment cycle consisted of a single MitoTam administration on D1 once a week (q.w. schema) followed by two observation days (D2 and D3). Patients who completed phase I were allowed to continue in phase Ib if they met the inclusion criteria and if phase Ib was open at the time of patient screening.

Phase Ib evaluated the efficacy and safety of repeated doses of MitoTam in three different regimens. In regimen 1 (1.0 mg/kg t.i.w.), treatment comprised 4 cycles (8 weeks) of MitoTam with the option to repeat treatment for up to 16 cycles in responding patients. Regimen 2 (3.0 mg/kg q.w.) and regimen 3 (4.0 mg/kg q.w.) comprised 6 cycles (6 weeks) of therapy. The option to repeat treatment in patients with a clinical benefit was allowed in regimen 2 but not in regimen 3. The escalating doses and treatment schemes are depicted in the supplementary material (Supplemental Table 1).

MitoTam dosing was based on the patient's weight, rounded up to 0.5 kg, and the dose at which the patient was enrolled. MitoTam lyophilizate was reconstituted by dissolving 25 mg of lyophilizate in 10 mL of physiological saline (1 mL containing 2.5 mg MitoTam) and diluting to the desired infusion volume. Each patient received MitoTam as a continuous infusion (Supplemental Table 2). Premedication was not indicated. Prohibited medications are listed in the supplementary material (Supplemental Table 3).

For PK monitoring, blood samples were collected from a peripheral vein opposite to the original site of MitoTam administration or from a central route. The timing of blood sampling is listed in Supplemental Tables 4-7.

Schemes of M administration in phase I	Dosages of M (mg/kg)/No of pts
1 cycle/3 doses, D1, D3, D5 in 1 week	0,25/4, 0.5/6, 1.0/3, 1.5/3, 2.25/6, 3.0/5
1 cycle/1 dose, applied on D1	4.0/3, 5.0/6, 6.0/1
Regimen of M administration in phase Ib	Dosages of M (mg/kg)/No of pts
4 biweekly cycles*, applied on D1+D3+D5	1.0/20
6 weekly cycles*, applied on D1	3.0/9
6 weekly cycles, applied on D1	4.0/9
Note: *There was a possibility of repeating M over the 4 or 6 cycles in patients with stable disease or partial regression according to CT examination. D: day, M: MitoTam	

Supplementary Table 1: Treatment regimens and schemes of MitoTam administration.

Dose range (mg/kg)	Infusion volume (mL)	Maximal amount of MitoTam (mg)	Maximum number of vials for reconstitution
0.25-2.25	250	250	10
3.0-6.0	500	500	20
Doses >500 mg	1000	1000	40

Supplementary Table 2: Infusion sizes of MitoTam for individual dose-range levels.

Category	Details
Chronic medication	Warfarin, oral antidiabetics (metformin)
Systemic corticotherapy	Administering dexamethasone for more than five days (antiemetic prophylaxis with dexamethasone for three days was allowed)
Platelet aggregation inhibitors	Acetylsalicylic acid-type, clopidogrel, etc.
Drugs affecting CYP450	Inducers: (Administration longer than five days) carbamazepine, phenytoin, topiramate, phenobarbital, St. John's wort, efavirenz, nevirapine, pioglitazone, troglitazone (OHM), dexamethasone, modafinil Inhibitors: protease inhibitors (ritonavir, indinavir, nelfinavir, saquinavir), macrolide antibiotics (clarithromycin, telithromycin), chloramphenicol, azole antifungals (voriconazole, itraconazole, fluconazole), aprepitant, verapamil, diltiazem, erythromycin, grapefruit juice, valerian Strong CYP2D6 Inhibitors: paroxetine, fluoxetine, quinidine, cinacalcet, bupropion
Anti-tumor therapy	Including LHRH therapy in breast cancer patients
Cancer complication treatment	Anti-emetics (metoclopramide may be accepted), antidiarrheal drugs; at physician discretion
Homeopathics and natural medicines	Without prescription, vitamins except calcium
Supportive treatment and supplements	Including antioxidants (allowed only after consultation with the investigator)
Food restrictions	Grapefruit juice, alcoholic beverages (including wine and beer) throughout the study therapy
N-acetylcysteine	Reduces the effect of MitoTam (potentially used as a rescue medication)
Other drug interactions	Drugs that interact with tamoxifen could affect the effect of MitoTam; their combination with MitoTam should be consulted with a clinical pharmacologist
Abbreviations: OHM: Oral Hypoglycemic Medication; LHRH: Luteinizing Hormone-Releasing Hormone; AR: Androgen Receptor.	

Supplementary Table 3: Prohibited medications.

	Hospitalization	PK before/after; 30, 90 min, 3, 6, 12, 24, 36 h
D1	X	X
D2	X	
D3	X	X
D4	X	
D5	X	X
D6	X	
D7	X	

Supplementary Table 4: Flowchart of the blood sampling for PK analysis: Phase I, dosing 0.25-3.0 mg/kg t.i.w.

	Hospitalization	PK before/after; 30, 90 min, 3, 6, 12, 24, 36 h
D1	X	X
D2	X	
D3	X	X
D4	X	
D5	X	X
D6	X	
D7	X	
Note: Days of hospitalization and blood sampling are marked by X. D day, PK pharmacokinetic, h hour, t.i.w. three times (D1, D3, D5) in a week. Days of hospitalization and blood sampling are marked by X. D day, PK pharmacokinetic, h hour, t.i.w. three times (D1, D3, D5) in a week		

Supplementary Table 5: Flowchart of the blood sampling for PK analysis: Phase I, dosing 4.0–6.0 mg/kg qwI.

	Cycle	Hospitalization	PK before/after; 30, 60, 90 min, 3, 6, 12, 24 h
D1	1		
D3	1		
D5	1	X	X
D1	2		
D3	2		
D5	2	X	X
D1	3		
D3	3		
D5	3	X	X
D1	4		
D3	4		
D5	4	X	X
Note: Days of hospitalization and blood sampling are marked by X, D day, PK pharmacokinetic, h hour, t.i.w. three times (D1, D3, D5) in a week			

Supplementary Table 6: Flowchart of the blood sampling for PK analysis: Phase Ib, dosing 1.0 mg/kg t.i.w. biweekly.

	Cycle	Hospitalization	PK before/after; 30, 60, 90 min, 6, 12, 24 h
D1	1	X	X
D2	1	X	X
D1	2	X	X
D2	2	X	X
D1	3	X	X
D2	3	X	X
D1	4	X	X
D2	4	X	X
D1	5	X	X
D2	5	X	X
D1	6	X	X
D2	6	X	X
Note: Days of hospitalization and blood sampling are marked by X. D day, PK, pharmacokinetic, h hour, qw weekly			

Supplementary Table 7: Flowchart of the blood sampling for PK analysis: Phase Ib, dosing 3.0 and 4.0 mg/kg qw.

MitoTam analysis

MitoTam and the Internal Standard (IS) MitoTam-D15 were obtained from Smart Brain (Czech Republic). Negative fetal bovine serum was obtained from Biosera (Czech Republic), blank check serum was purchased from ACQ Science (Germany). Acetonitrile and methanol (LC-MS Chromasolv, ≥ 99.9%) were purchased from Sigma Aldrich (Germany). Formic acid (98-100%, LC-MS grade) was purchased from Fischer Chemical (Czech Republic). Deionized water was prepared using a Milli-Q water treatment system (18.2 MΩ/cm; Millipore, USA).

Working solutions of MitoTam and the IS were dissolved in acid methanol to a concentration of 1 mg (base)/mL and were stored at -20°C. Calibration samples were prepared by spiking the blank control serum with the appropriately diluted working solutions of MitoTam and IS. Three Quality Control (QC) samples for the method validation were prepared at MitoTam concentrations of 100, 6 000, and 12 000 ng/mL. The calibration and QC samples were stored at 4°C in a refrigerator.

After collection, blood samples were allowed to clot for 30 min at room temperature. Serum was separated by centrifugation (1500 × g, 5 min, 4°C) and stored at -20°C until analysis. A 100-µL sample of biological sample was added to an Eppendorf tube, followed by the addition of 10 µL of the IS solution (MitoTAM-D15, 25 000 ng/mL in methanol) and methanol (1 mL). The mixture was mixed for 1 min. and centrifuged at 13 400 RPM (1 min). The organic supernatant (200 µL) was transferred to a glass insert with deionized water (200 µL).

MitoTam was quantified using a Nexera X2 liquid chromatography system (Shimadzu, Japan) coupled to a ABSciex QTrap 5500 mass spectrometer interfaced with an electrospray ion source (AB Sciex, USA). Separation was achieved with a chromatographic column (Luna Omega, 3 µm PS C18 100 A, 50 × 2.1 mm; Phenomenex, USA) connected to a precolumn. The mobile phases consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) were used at a flow rate of 0.5 mL/min and temperature of 40°C. The following gradient was used: 0-0.5 min 20% B, 1.5 min 50% B, 2.0-3.0 min 95% B, 4.5 min 20% B.

Quantitation was done in the multiple reaction monitoring (MRM) mode to monitor the protonated precursor → product ion transition

of m/z 744.239 → 345.100 for MitoTam and 759.4021 → 304.100 for MitoTam-D15. The capillary voltage was set to 2000 kV, the temperature of source was 500°C. The data were processed and evaluated using Analyst 1.7.3 software (AB Sciex).

The performance of the method was evaluated according to the recommendations of the Scientific Working Group for Forensic Toxicology. The test range of the assay was 10-15 000 ng/mL. The intra-assay coefficient of variation was <15% (Supplementary Tables 8 and 9).

Subject ID	Sex (M/F)	Age	C diagnosis (MKN)	Patients transited to phase Ib (Y-N)
P1-0.25	F	71	34	N
P2-0.25	F	62	50	N
P3-0.25	M	61	18	N
P4-0.25	M	45	74	N
P1-0.5	F	63	49	N
P2-0.5	F	62	17	N
P3-0.5	M	39	34	Y
P4-0.5	F	48	25	Y
P5-0.5	M	45	75	Y
P6-0.5	F	47	20	N
P1-1.0	F	51	80	N
P2-1.0	M	43	34	N
P3-1.0	M	48	80	Y
P1-1.5	M	54	18	N
P2-1.5	M	58	18	N
P3-1.5	F	67	20	N
P1-2.25	M	63	64	Y
P2-2.25	F	71	24	N
P3-2.25	F	58	20	Y
P4-2.25	F	46	23	Y
P5-2.25	F	67	34	Y
P6-2.25	M	68	25	Y
P1-3.0	M	59	20	Y
P2-3.0	F	70	74	N
P3-3.0	F	66	34	N
P4-3.0	F	50	63	Y
P5-3.0	M	62	64	Y
P1-4.0	F	54	20	N
P2-4.0	F	70	56	Y
P3-4.0	M	72	64	Y
P2-5.0	M	68	18	Y
P1-5.0	M	73	64	Y
P3-5.0	F	66	48	N
P4-5.0	F	63	64	N
P5-5.0	M	62	64	N
P6-5.0	M	67	64	Y
P1-6.0	M	68	20	Y

Note: The subject ID consists of the patient's serial number, phase and dose level, for example P1-1.00 is patient No. 1, phase 1, MitoTam dose level 1.0 mg/kg. M: Male; F: Female; Y: Yes; N: No; MKN-code for malignant neoplasms according to the international classification of diseases and related health problems

Supplementary Table 8: Overview of patients enrolled in phase I.

Subject ID	Sex (M/F)	Age	Diagnosis (MKN)	Histogenetic origin of the tumor	Response to MitoTam (R vs. NR)	Number of applied cycles
P1b-1.0	M	63	64	ME	R	16
P2b-1.0	M	73	23	EN	NR	4
P3b-1.0	F	69	50	EC	NR	4
P4b-1.0	M	67	25	EN	NR*	1
P5b-1.0	M	40	34	EN	R	4
P6b-1.0	M	71	64	ME	R	12
P7b-1.0	F	58	20	EN	NR	4
P8b-1.0	M	48	80	UNK	NR	4
P9b-1.0	F	46	23	EN	R	8
P10b-1.0	M	69	7	EN	R	4
P12b-1.0	F	49	25	EN	NR*	3
P13b-1.0	F	64	50	EN	NR	4
P14b-1.0	M	45	75	EC	R	12
P11b-1.0	F	68	34	EC	NR	4
P15b-1.0	M	68	25	EN	NR*	4
P16b-1.0	M	59	20	EN	NR	4
P17b-1.0	F	55	18	EN	NR	4
P18b-1.0	F	50	53	ME	NR	4
P19b-1.0	M	57	60	ME	NR	4
P20b-1.0	M	62	64	ME	NR	4
P1b-3.0	F	70	56	ME	NR	6
P2b-3.0	F	53	56	ME	R	12
P3b-3.0	M	72	64	ME	R	12
P4b-3.0	M	46	20	EN	NR*	4
P5b-3.0	M	68	18	EN	R	6
P6b-3.0	M	73	64	ME	R	6
P8b-3.0	F	66	52	EN	R	6
P7b-3.0	F	59	18	ME	R	5
P9b-3.0	M	67	64	ME	R	10
P1b-4.0	F	67	50	EC	NR	4
P2b-4.0	M	68	20	EN	NR	4
P3b-4.0	M	55	20	EN	R	6
P4b-4.0	M	54	18	EN	NR	4
P5b-4.0	M	57	20	EN	NR	6
P6b-4.0	F	63	25	EN	NR	4
P7b-4.0	M	64	20	EN	NR*	1
P8b-4.0	F	57	25	EN	NR	6
P9b-4.0	M	69	19	EN	NR*	4

Note: The subject ID consists of the patient's serial number, phase and dose level, for example P1b-1.00 is patient No. 1, phase 1b, MitoTam dose level 1.0 mg/kg. One series of therapy comprised 4 cycles of MitoTam in regimen 1 and 6 cycles in regimens 2 and 3.

R: Responder, NR: Non-Responder (in patients marked * a control CT scan was not done and progressive disease was recorded according to the patients' clinical status), M: Male; F: Female; MKN: Code for malignant neoplasms according to the international classification of diseases and related health problems; EC: Ectoderm; ED: Endoderm; ME: Mesoderm; C80: Tumor of Unknown (UNK) origin, histogenetic origin cannot be determined.

Supplementary Table 9: Overview of patients enrolled in phase Ib.

Appendix

Detailed statistical analysis of pharmacokinetics

A statistical analysis of three pharmacokinetic parameters-elimination half-time in serum, area under the curve to the last quantifiable concentration, and maximal observed concentration (in serum)-is performed and the corresponding detailed results are provided here.

Elimination half-time in serum

A longitudinal data analysis of the elimination half-time in serum is performed using the random effects mixed models (i.e., generalized linear mixed models – GLMM) for repeated observations over subjects (patients). From a mathematical point of view, the following stochastic model for the elimination half-time tel is considered

$$\log E\left[t_{ij}^{el}\right]=\frac{v_{ij}}{\alpha}=\beta_0+\beta_1 1\left\{Regime_{ij}=I1\right\}+\beta_2 1\left\{Regime_{ij}=Ib1\right\} \quad \text{.....(1)}$$
$$+\beta_3 1\left\{Regime_{ij}-Ib3\right\}+\beta_4 1\left\{Regime_{ij}-Ib4\right\}+b_{0i}+b_{1i}Dose_{ij};$$
$$t_{ij}^{el}\sim Gamma(v_{ij},\alpha)$$
$$f(y_{ij}\mid v_{ij},\alpha)=\frac{\alpha^{v_{ij}}}{\Gamma(v_{ij})}y_{ij}^{v_{ij}-1}\exp\left\{-\alpha y_{ij}\right\}; \quad \text{.....(2)}$$
$$\left[b_{0i},b_{1i}\right]^{\mathrm{T}}\sim N_2(0,\begin{bmatrix}\frac{\sigma_{11}^2}{\rho\sigma_{11}\sigma_{22}} & \frac{\rho\sigma_{11}\sigma_{22}}{\sigma_{22}^2}\end{bmatrix}) \quad \text{.....(3)}$$
$$\left[b_{0i},b_{1i}\right]^{\mathrm{T}}\sim N_2(0,\begin{bmatrix}\frac{\sigma_{11}^2}{\rho\sigma_{11}\sigma_{22}} & \frac{\rho\sigma_{11}\sigma_{22}}{\sigma_{22}^2}\end{bmatrix}) \quad \text{.....(4)}$$

for i (respectively k) over all patients and j over all repeated observations per patient. Let us recall that . There are five levels of the nominal categorical variable Regime differentiating with respect to the phase and the cohort (i.e., dose), where the P haseI3 level corresponds to a baseline (represented by the intercept in our model):

- P haseI3 (Cohorts 0.25-3 mg/kg) . . . administration on Days 1, 3 and 5;
- P haseI1 (Cohorts 4, 5, and 6 mg/kg) . . . single administration;
- P haseIb1 (Cohort 1 mg/kg) . . . administration on Days 1, 3 and 5 in biweekly regimen;
- P haseIb3 (Cohort 3 mg/kg) . . . administration on Day 1 in weekly regimen;
- P haseIb4 (Cohort 4 mg/kg) . . . administration on Day 1 in weekly regimen.

The estimates of the unknown parameters, which are statistically significant, from the model (1)-(4) are listed in Table 1 for the fixed effects and in Table 2 for the random effects. The overall effect of the administration regime is highly significant (p-value 4.3 × 10-10) with respect to the Wald χ2-test.

From the interpretation point of view, the expectation of the elimination half-time in serum in the regime PhaseI1 is exp {(β_1)^ } = exp{0.8528352} ≈ 2.346-times higher than in the regime PhaseI3 (represented by the intercept).

Moreover, there are non-significant effects of the cycle (p-value 0.0901), day of administration (0.1600), dose (0.1746), sex (0.6372), and the age of the patient (0.7203) on the elimination half-time in serum.

Effect	Parameter	Estimate	Std. error	t-value	P-value
Intercept	β0	2.9278449	0.1043857	28.048340	<0.0001
PhaseI1	β1	0.8528352	0.2503024	3.407219	0.0011
PhaseIb1	β2	0.6491770	0.1391598	4.664975	<0.0001
PhaseIb3	β3	0.0405436	0.1548373	0.261846	0.7942
PhaseIb4	β4	−0.2789037	0.1761020	−1.583763	0.1180

Table 1: Estimated fixed effects from linear mixed model (1)-(4).

Parameter	1⁄α	σ_11	σ_22	ρ	ϱ
Estimate	0.7126153	5.100542 × 10−4	1.432424 × 10−7	0.000	0.2669009

Table 2: Estimated random effects from linear mixed model (1)-(4).

Area under the curve to the last quantifiable concentration

Analogously, another longitudinal data analysis of the area under the curve (AUC) from the time of dosing to the time of the last quantifiable concentration is performed using the random effects mixed models for repeated observations over patients as in the previous section. From a mathematical point of view, the following stochastic model for the area under the curve from the time of dosing to the time of the last quantifiable concentration AUC is considered

$$\log E\left[AUC_{ij}\right]=\frac{v_{ij}}{\alpha}=\beta_0+\beta_1 Age_{ij}+\beta_2 1\left\{Sex_{ij}=M\right\}+\beta_3\log(\mathrm{D}ose_{ij}) \quad \text{.....(5)}$$
$$\log E\left[AUC_{ij}\right]=\frac{v_{ij}}{\alpha}=\beta_0+\beta_1 Age_{ij}+\beta_2 1\left\{Sex_{ij}=M\right\}+\beta_3\log(\mathrm{D}ose_{ij})$$

$$f\left(y_{ij}\mid v_{ij},\alpha\right)=\frac{\alpha^{v_{ij}}}{\Gamma(v_{ij})}y_{ij}^{v_{ij}-1}\exp\left\{-\alpha y_{ij}\right\}; \quad \text{.....(6)}$$
$$b_i\sim N\left(0,\ \sigma^2\ \right); \quad \text{.....(7)}$$

for i (respectively k) over all patients and j over all repeated observations per patient. The estimates of the unknown parameters, which are statistically significant, from the model (5)-(8) are listed in Table 3 for the fixed effects and in Table 4 for the random effects. The overall effect of the day of administration is significant (p-value 0.011) with respect to the Wald χ2-test.

From the interpretation point of view, the AUC from the time of dosing to the time of the last quantifiable concentration becomes exp{β ^_3 × log 2)} = 2 × exp{2.28882} ≈ 19.727-times higher when the dose increases twice. Finally, to assess the overall goodness-of-fit, the value of R2=0.9971927 being close to one indicates suitability of the considered statistical model.

Effect	Parameter	Estimate	Std. error	t-value	P-value
Intercept	β0	-52.06563	1.0002517	-52.05252	<0.0001
Age	β1	1.36042	0.0165671	82.11554	<0.0001
Sex = M	β2	20.44602	0.2957409	69.13491	<0.0001
log(Dose)	β3	2.28882	0.2850714	8.02895	<0.0001
Day = 3	β4	0.61131	0.5668304	1.07846	0.2821
Day = 5	β5	-0.82876	0.4125645	-2.00880	0.0459
Cycle	β6	1.61294	0.0476118	33.87678	<0.0001

Table 3: Estimated fixed effects from linear mixed model (5)-(8).

Maximal observed concentration (in Serum)

Similarly, additional longitudinal data analysis of the maximal observed concentration (in serum) is performed using the random effects mixed models for repeated observations over patients as in the two previous sections. From a mathematical point of view, the following stochastic model for the maximal observed concentration (in serum) Cmax is considered

$$\log E\left[C_{ij}^{\max}\right]=\frac{v_{ij}}{\alpha}=\beta_0+\beta_1 Dose_{ij}+\beta_2 1\left\{Day_{ij}=3\right\}+\beta_3 1\left\{Day_{ij}=5\right\} \quad \text{.....(9)}$$

$$+\beta_4 1\left\{Regime_{ij}=I3\right\}+\beta_5 1\left\{Regime_{ij}=Ib1\right\}$$

$$+\beta_6 1\left\{Regime_{ij}=Ib3\right\}+\beta_7 1\left\{Regime_{ij}=Ib4\right\}+b_i;$$

$$C_{ij}^{\max}\sim Gamma\left(v_{ij},\alpha\right)$$

$$f\left(y_{ij}\mid v_{ij},\alpha\right)=\frac{\alpha^{v_{ij}}}{\Gamma(v_{ij})}y_{ij}^{v_{ij}-1}\exp\left\{-\alpha y_{ij}\right\}; \quad \text{.....(10)}$$

$$b_i\sim N\left(0,\ \sigma^2\ \right); \quad \text{.....(11)}$$

for i (respectively k) over all patients and j over all repeated observations per patient. The estimates of the unknown parameters, which are statistically significant, from the model (9)-(12) are listed in Table 5 for the fixed effects and in Table 6 for the random effects. The overall effects of the administration regime as well as the day of administration are significant (p-values 2.6 × 10-5 and 0.032, respectively) with respect to the Wald χ2-test. From the interpretation point of view, the expectation of the maximal observed concentration becomes exp {(β_1)ˆ}=exp{0.777707} ≈ 2.176-times higher when the dose increases by 1mg. Moreover, there are non-significant effects of the cycle (p-value 0.1931), sex (0.7744), and the age of the patient (0.1827) on the maximal observed concentration (in serum).

Effect	Parameter	Estimate	Std. error	t-value	P-value
Intercept	β0	- 4.925039	0.7249989	6.793167	<0.0001
Dose	β1	0.777707	0.1394035	5.578822	<0.0001
Day = 3	β2	0.328833	0.1765595	1.862450	0.0639
Day = 5	β3	0.570170	0.2243428	2.541510	0.0118
Phase I3	β4	1.040164	0.5595685	1.858867	0.0675
Phase Ib1	β5	1.924003	0.6363471	3.023512	0.0036
Phase Ib3	β6	1.417902	0.4117993	3.443188	0.0010
Phase Ib4	B7	0.683376	0.3643173	1.875772	0.0651

Table 5: Estimated fixed effects from linear mixed model (9)-(12).

Summary

The pharmacokinetic analysis provides affirmative significant effect of the dose concentration on the area under the curve to the last quantifiable concentration as well as on the maximal observed concentration in serum. Although, there is no statistical evidence for the dose concentration impact on the elimination half-time in serum.