

OBJECTIVE

Describing the *in vitro* and *in vivo* anticancer effect of etoposide by a PK/PD model. Comparison of the *in vitro* and *in vivo* PK/PD parameters.

MATERIALS AND METHODS

In the *in vivo* tumor growth inhibition experiments, W256 tumor-bearing Wistar rats received etoposide 5 or 10 mg/kg i.v. *bolus* every day for 8 and 4 days, respectively. Tumor volume was monitored daily during and after drug treatment. The protocol of the *in vivo* studies was approved by UFRGS Ethics Committee on Animal Use (#22302). *In vitro* antitumor activity was determined against Walker-256 (W256) carcinosarcoma cells exposed to different constant concentrations and to dynamic concentration-time profiles that mimic the concentrations observed *in vivo*. Data were analyzed starting from the Del Bene [1] and Simeoni [2] TGI models (<http://repository.ddmore.eu>). An $E_{\max}$ function was introduced both *in vitro* and *in vivo* in the PD model to take into account the dependence of k_2 (drug potency) parameter on the concentration (Figure 1). A four-compartment popPK model was previously developed to describe the drug disposition in plasma and tumor in a separate group of animals. PK/PD analysis was conducted using Monolix v.4.3.3, on average data for *in vitro* study and by mean of a nonlinear mixed-effect model for *in vivo* data.

$$\begin{aligned} \frac{dZ_1(t)}{dt} &= \frac{\lambda_0 \cdot Z_1(t)}{\left[1 + \left(\frac{\lambda_0}{\lambda_1} \cdot w_1(t)\right)^{\frac{1}{\eta}}\right]^{\eta}} - k_{2,variable} \cdot C(t) \cdot Z_1(t) \\ \frac{dZ_2(t)}{dt} &= k_{2,variable} \cdot C(t) \cdot Z_1(t) - k_1 \cdot Z_2(t) \\ \frac{dZ_3(t)}{dt} &= k_1 \cdot Z_2(t) - k_1 \cdot Z_3(t) \quad Z_1(0) = w_0 \\ \frac{dZ_4(t)}{dt} &= k_1 \cdot Z_3(t) - k_1 \cdot Z_4(t) \quad Z_{2,3,4}(0) = 0 \\ w(t) &= Z_1(t) + Z_2(t) + Z_3(t) + Z_4(t) \end{aligned}$$

$$k_{2,variable} = k_{2,max} \left(1 - \frac{C(t)}{IC_{50} + C(t)}\right)$$

Figure 1. Overall system of differential equations of the PK/PD model used to fit the *in vitro* and *in vivo* data.

RESULTS

The nonlinear mixed-effect model was capable to describe the sources of between-subject variability in the *in vivo* tumor growth and fitting the data using either free tumor or total plasma concentrations. In the *in vitro* experiments, the model estimated on data from constant exposure well predicted the antitumor activity for the dynamic concentration-time profile experiments. The comparison between *in vivo* and *in vitro* PK/PD parameters showed an increased etoposide anticancer activity *in vivo* than *in vitro*.

IN VITRO

(A) Static concentrations

(B) Dynamic concentrations

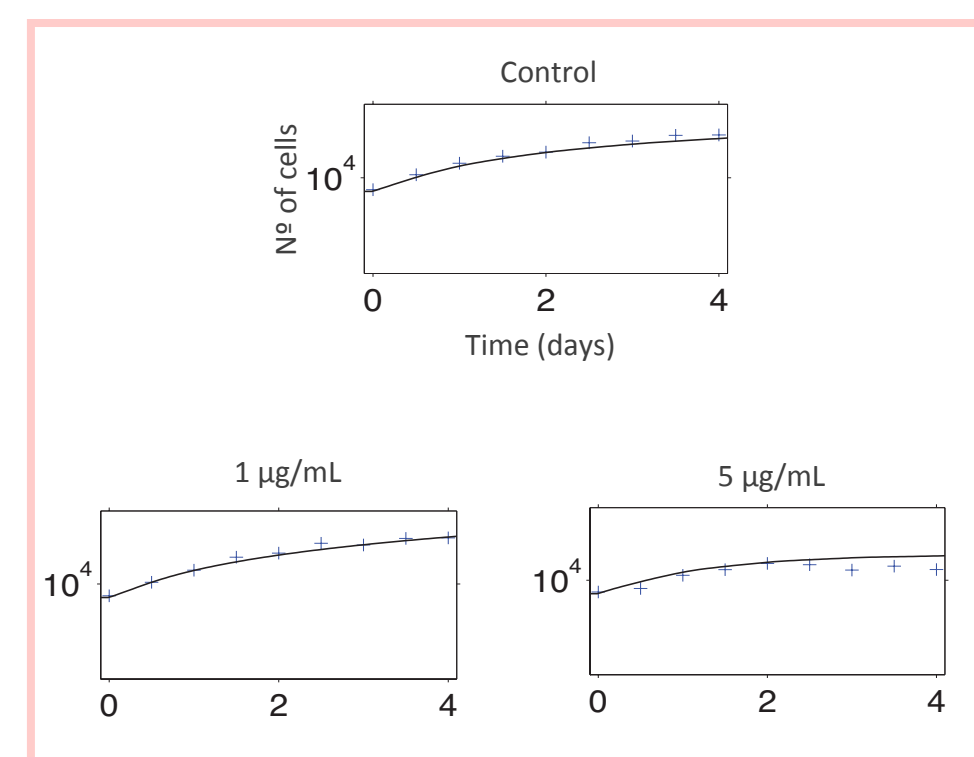
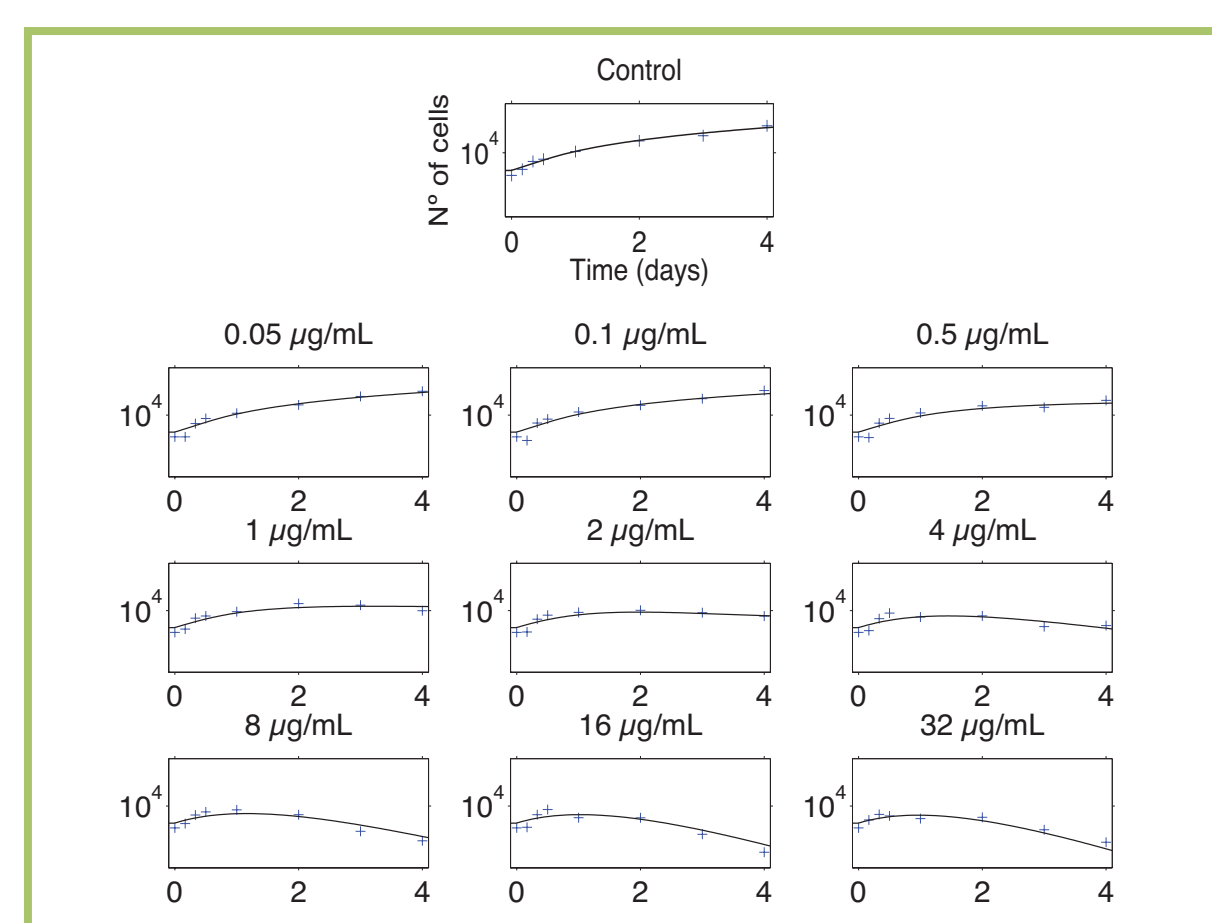


Figure 2. Observed data (cross) and fitted growth curves (line) for both untreated and treated W256 cancer cells exposed to different concentrations of etoposide. (A) static concentrations and (B) dynamic concentrations predicted with parameter estimates based on data from static experiments.

Table 1. Parameter estimate of the *in vitro* PK/PD model using data from static and dynamic experiments.

Parameter	Fixed effect (RSE %)
λ_0 (day ⁻¹)	0.966 (14)
λ_1 (cells.day ⁻¹)	7.24x10 ³ (10)
w_0 (cells)	4.37x10 ³ (4)
k_1 (day ⁻¹)	1.76 (39)
$k_{2,max}$ (mL.µg ⁻¹ .day ⁻¹)	0.90 (20)
IC_{50} (µg.mL)	2.38 (46)
Proportional error	0.19 (8)
AIC	1389.52

Parameter	Fixed effect (RSE %)
λ_0 (day ⁻¹)	0.799 (21)
λ_1 (cells.day ⁻¹)	9.53x10 ³ (8)
w_0 (cells)	6.89x10 ³ (8)
k_1 (day ⁻¹)	1.76 (fixed)*
$k_{2,max}$ (mL.µg ⁻¹ .day ⁻¹)	0.9 (fixed)*
IC_{50} (µg.mL)	2.38 (fixed)*
Proportional error	0.155 (14)
AIC	513.68

*Drug related parameters were fixed from static experiments

IN VIVO

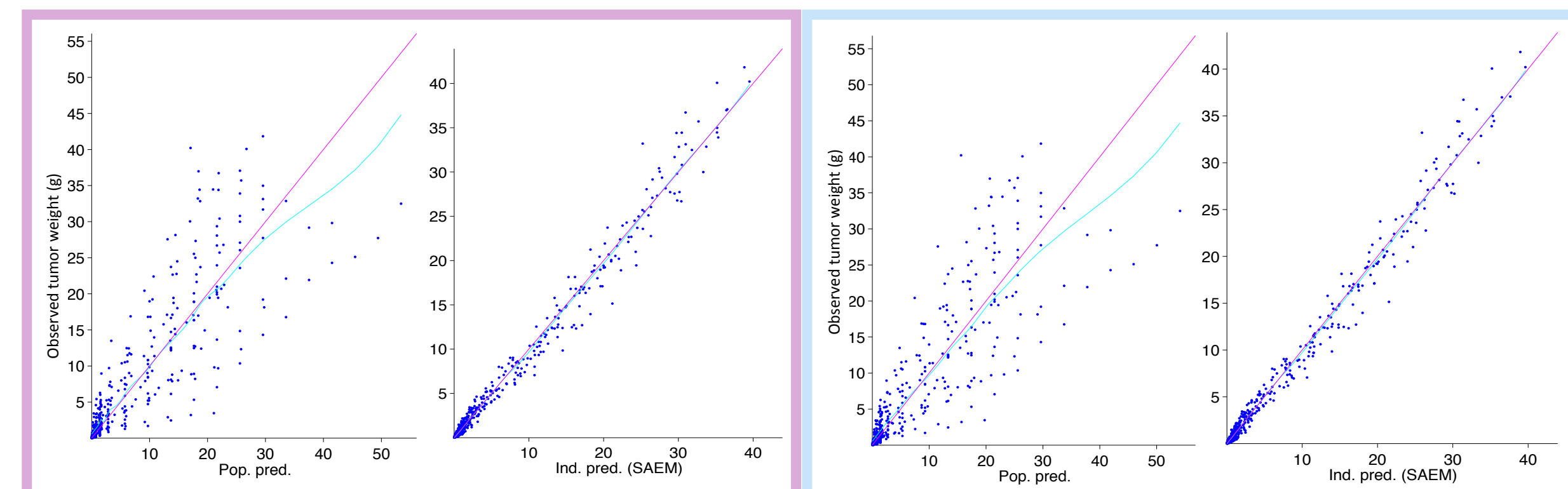


Figure 3. Basic goodness-of-fit plots with observed and model-predicted tumor size using **total plasma** (left) and **free tumor** (right) concentrations. Blue dots represent the observations and pink and blue lines indicate the linear regression fit and the identity line, respectively.

Table 2. Parameter estimate of the *in vivo* PK/PD model.

Parameter	Fixed effect (RSE %)		Random effect (RSE %)	
	Plasma ^a	Tumor ^b	Plasma ^a	Tumor ^b
λ_0 (day ⁻¹)	0.704 (2)	0.718 (2)	0.08 (19)	0.08 (19)
λ_1 (g.day ⁻¹)	3.97 (11)	3.94 (11)	0.61 (15)	0.59 (14)
w_0 (g)	0.042 (12)	0.039 (11)	0.54 (14)	0.54 (15)
k_1 (day ⁻¹)	1.64 (3)	1.61 (3)	-	-
$k_{2,max}$ (mL.µg ⁻¹ .day ⁻¹)	11.9 (1)	26.6 (1)	-	-
IC_{50} (µg.mL)	1.04 (1)	1.03 (1)	-	-
Residual error (Proportional)	0.40 (3)	0.404 (3)		
AIC	1154.59	1233.50		

^a total plasma concentrations from the PK model

^b free tumor concentrations from the PK model

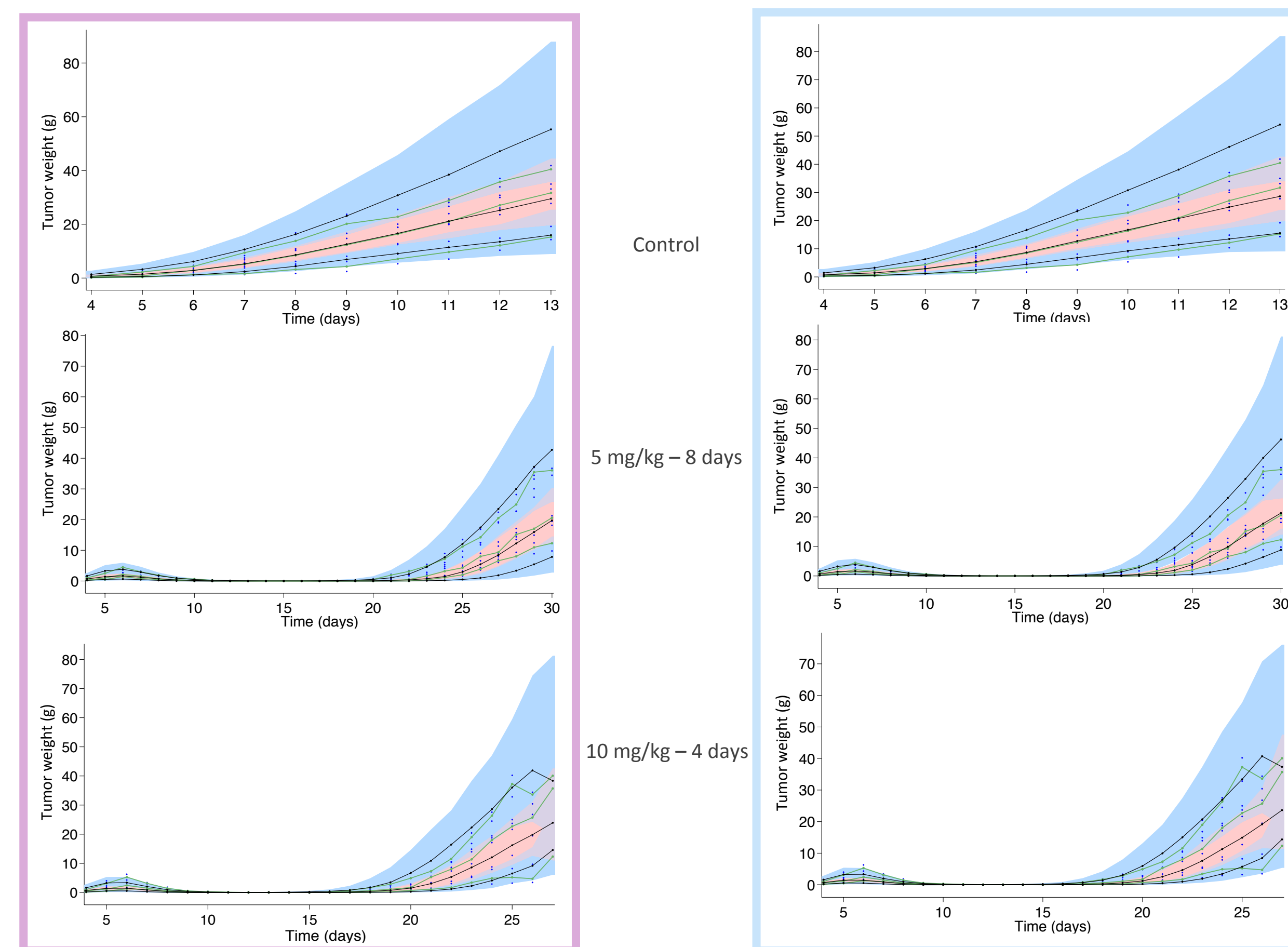


Figure 4. Visual predictive check (VPC) plot of the final PK/PD model stratified by group using **total plasma** (left) and **free tumor** (right) concentrations based on 500 simulated replicates of the original data. The green and black lines show the 10th, 50th and 90th percentiles of observed and predicted data, respectively; the areas represent the confidence intervals for prediction intervals.

CONCLUSION

The same TGI model can successfully predict the etoposide effect after *in vivo* and *in vitro* exposure. The *in vivo* data were well fitted independently of the concentrations used in the model free tumor or total plasma. In the *in vitro* approach, the antitumor effect following dynamic concentrations can be simulated using the parameters estimates from constant exposures, without the need of time-consuming experiments.

REFERENCES

- [1] Del Bene F, Germani M, De Nicolao G, Magni P, Re CE, Ballinari D, Rocchetti M. A model-based approach to the *in vitro* evaluation of anticancer activity. *Cancer Chemother Pharmacol* (2009) 63 (5):827– 36.
- [2] Simeoni M, Magni P, Cammia C, De Nicolao G, Croci V, Pesenti E, Germani M, Poggesi I and Rocchetti M. Predictive pharmacokinetic-pharmacodynamic modeling of tumor growth kinetics in xenograft models after administrations of anticancer agents. *Cancer Res* (2004) 64: 1094-1101.

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