Title Page

Semi-mechanistic cell cycle type based pharmacokinetic/pharmacodynamic model of chemotherapy-induced neutropenic effects of diflomotecan under different dosing schedules

Víctor Mangas-Sanjuan, Núria Buil-Bruna,María J. Garrido, Elena Soto, Iñaki F. Trocóniz.

VM-S: Department of Engineering. Department of Pharmacy and Pharmaceutical Technology Area.University of Miguel Hernández de Elche. Carretera Alicante Valencia km 87, 03550. San Juan de Alicante, (Alicante). Spain

NB-B, MJG, IFT: Pharmacometrics and Systems Pharmacology.Department of Pharmacy and Pharmaceutical Technology.School of Pharmacy.University of Navarra.Irunlarrea 1, 31008. Pamplona, (Navarra). Spain

ES: Pharmacometrics, Pfizer, Sandwich, UK

Running Title Page

Running title: Cell cycle type PKPD based model for neutropenic effects

Corresponding author:Iñaki F. Troconiz, itroconiz@unav.es. Pharmacometrics&Systems Pharmacology.Department of Pharmacy and Pharmaceutical Technology.School of Pharmacy.University of Navarra.Irunlarrea 1, 31080. Pamplona, (Navarra). Spain

Number of text pages: 29

Number of tables:2

Number of figures: 6

Number of references: 26

Number of words in the Abstract:238

Number of words in the Introduction: 408

Number of words in the Discussion: 1019

Nonstandard abbreviations:-2LL: 2xlog(likelihood); ANC: absolute neutrophil counts; $Circ_0$ and Circ: absolute neutrophil counts at baseline, and at any time after the start of the study, respectively; FOCE: first Order Conditional Estimation method; F_{Prol}: fraction of proliferative cells that enters into the maturation chain; k_{circ}: first order rate constant representing neutrophil degradation; k_{cycle}: first order rate constant governing cell cycle dynamics within the stem cell compartment; k_{prol}:first order rate constant of proliferation; k_{TR}: first order rate constant controlling the transfer through the maturation chain; n: number of maturation compartments; MTT: mean transit/maturation time; NONMEM: Non-Linear Mixed Effect Models; pc-VPC: prediction-corrected visual predictive checks; Prol: proliferative cells; Qc_1 and Qc_2: Quiescent cells 1 and 2; SC: stem cells; TR_{1-3}: immature neutrophil levels in each of the maturation compartments; γ: parameter modulating the magnitude of the feedback mechanism.

Recommended section: Drug Discovery and Translational Medicine

Abstract

The current work integrates cell-cycle dynamics occurring in the bone marrow compartment as a key element in the structure of a semi-mechanistic pharmacokinetic/pharmacodynamic(PK/PD) model for neutropenic effects,aiming to describe with the same set of system and drug related parameters, longitudinal data of neutropenia gathered after the administration of the anticancer drug diflomotecan (9,10-difluoro-homocamptothecin) under different dosing schedules to patients (n=111) with advanced solid tumours. To achieve such objective thegeneral framework of the neutropenia models was expanded including one additional physiological process resembling cell cycle dynamics. The main assumptions of the proposed model are: (i) within the stem cell compartment proliferative and quiescent cells coexist and (ii & iii) only cells in the proliferative condition are sensitive to drug effects, and capable to follow the maturation chain.Cell cycle dynamics were characterized by the following two new parameters, F_{Prol}, and k_{cycle}, the first accounting for the fraction of proliferative cells that enter into the maturation chain, and the latter quantifying the dynamic of the transit between the different cells status. Both model parameters resulted identifiable as indicated by the results from a bootstrap analysis, and their estimates were supported by literature data. The estimates of F_{Prol} and k_{cycle} were 0.58 and 1.94 day^{-1}, respectively. The new model could describe properly the neutropenic effects of diflomotecan after very different dosing scenarios, and can be used to explore the potential impact of dosing schedule dependencies on neutropenia prediction.

Introduction

Several pharmacokinetic/pharmacodynamic (PK/PD) models have been published over the last decade describing myelosuppression responseoccurring during cancer treatment with chemotherapy agents(Minami et al., 1998; Zamboni et al., 2001; Friberg et al., 2002; Krzyzanski and Jusko, 2002; Panetta et al., 2003; Panetta et al., 2008). The most used and accepted model was developed by Friberg and co-workers(Friberg et al., 2002), hereafter the reference model, which it has demonstrated consistency among a wide variety of anti-cancer agents(Latz et al., 2006; Fetterly et al., 2008; Soto et al., 2010b), and has been used to describe neutropenic effects after drug combinations(Sandstrom et al., 2005; Soto et al., 2010a), and predict human haematological toxicity from laboratory animal data(Friberg et al., 2010).

The models mentioned above are considered semi-mechanistics and capable to discriminate between system and drug related parameters. The system related parameters include those accounting for baseline condition, cell proliferation/maturation/degradation, and rebound, whiledrug related effect parameters are represented for example by C_{50}, the drug concentration in plasma eliciting half of maximal reduction of the cell proliferation process.However, there are some examples in the literature for antitumor drugs where a change in the drug related effect parameters has been reported for anticancer drugs when the drug was given through different routes and/or different dosing schedules(Soto et al., 2011). For the case of topotecan, the drug related effect parameter was estimated 43% lower after oral administration using a different dosing schedule compared to the intravenous administration (Leger et al., 2004). Those findings suggest that there might be aspects beyond the proliferation, maturation, degradation, and rebound processesthat have also to be considered(Steimer et al., 2010).

Diflomotecan (9,10-difluoro-homocamptothecin) is a homocaptothecin and its mechanism of anti-tumoral action is related to the inhibition of topoisomerase I, a nuclear enzyme involved in the replication process. The recommended and maximal tolerated doses, as well as its pharmacokinetic and neutropenic profiles under single dosing schedules have been previously reported (Troconiz et al., 2006; Soto et al., 2011), using the reference model for neutropenia (Friberg et al., 2002).

The aim of the current work was to describe the neutropenic effects of diflomotecanadministered under different dosing schedules to patients with advanced solid tumors involved in different phase I clinical trials, using the same set of system and drugrelated parameters. To achievesuch objective, the general framework of neutropenia models had to be expanded with additional physiological process resembling cell cycle dynamics.

Materials and methods

Patient population and study design

Data from five Phase I clinical trials in advanced malignant tumours, including 111 patients were available. All participants provided written informed consent consistent with ICH-GCP (International conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use-Good Clinical Practice) and local legislation, once the nature and the intention of the investigation were fully explained. The studies were performed in accordance with the Declaration of Helsinki and were approved by the institutional review board of the ethics committee at each study site. Supplementary material table 1 lists the characteristics of the patient population.

A total of 111 patients were enrolled in five different clinical studies. Inall studies at day 1 of the first cycle of treatment, patients received diflomotecan as an intravenous infusion over 20 minutes. Additionally,(i) an intravenous infusionof 20 minutes of duration were administered on days 7 and 14 in study B (n=15 patients),(ii) five consecutive oral daily administrations were given on days 14 to 18 in studies C (n=24) and E (n=18),or (iii) four intravenous doses were administered between days 2 and 5 in study D (n=30).Patients in study A (n=24) received just the intravenous infusion over 20 minutes on day 1. Subsequent cycles in studies C-E lacked from intravenous infusion over 20 minutes on day 1 of the new cycle.

Single infusion or infusions given at days 1, 7 and 14 within a cycle were denoted as dosing schedule I, while the consecutive once daily administrations were referred as dosing schedule II. Dosing schedule II corresponds to oral solution, intravenous infusion, and oral capsules in studies C, D, and E, respectively.Cycle duration varied from the planned 28 days based on the recovery from the neutropenic toxicity.

Figure 1 summarizes the dosing scheme for all studies A-E and provides detailed information ofthe dose levels administered in cycle 1 together with the number of patients allocated to each dose group.Diflomotecan concentrations were collected during the administration days and absolute neutrophil counts (ANC) were measured in peripheral blood every 3 to 7 days.The total number of absolute neutrophil counts was 1865, and 789 (42%) were obtained during the first cycle of treatment.

Data analysis

Data were analysed using the population approach with NONMEM version 7.2(Bauer, 2011). Parameter estimation was performed based on the FOCE (first Order Conditional Estimation) method together with the INTERACTION option. Both type of data, diflomotecan concentrations and neutrophil counts, were logarithmically transformed. Inter-individual variability was described exponentially, and residual error was accounted using a combined error model on the logarithmic scale. Model building process was performed sequentially. First, the empirical Bayes estimates of the individual PK parameters were obtained from a previous published population PK model (Soto et al., 2011), which were incorporated to the dataset containing the neutrophil counts information. Given the change in the dosing paradigms from cycle 1, and the fact that data were more sparse in terms of number of patients and measurements, the model building process was performed using data only from the first treatment cycle.

Model selection was mainly based on the log-likelihood ratio test [for two nested models a decrease in 3.84 points in -2xlog(likelihood) (-2LL) for an extra added parameter was considered significant at the 5% level], and visual exploration of goodness of fit plots.

Model evaluation was performed through prediction-corrected visual predictive checks(pc-VPC)(Bergstrand et al., 2011). For each study design, one thousand simulated datasets were

generated. At specific sampling time periods, the 2.5th, 50th, and 97.5th percentiles of the simulated data were calculated. Then, the 95% prediction intervals of the 2.5th, 50th, and 97.5th percentiles were computed and displayed graphically together with the experimental data. Additionally, parameter precision was evaluated from the analysis offive hundred simulated bootstrap datasets.

For graphical and statistical analysis, the R software (http://cran.r-project.org, version 2.6.0) was used. Pc-VPC and bootstrap analysis were performed using PsN(Lindbom et al., 2005)and Xpose version 4.5.3(Jonsson and Karlsson, 1999).

Pharmacokinetic model

The population PK model for diflomotecan consisted of a three compartment model with a first order absorption and elimination processes. The population pharmacokinetics of diflomotecanwere already studied(Soto et al., 2011).

Pharmacodynamic model

Step I. The semi-mechanistic model for chemotherapy-induced myelosuppressionpreviously published by Friberg et al. (Friberg et al., 2002) was fitted to theabsolute neutrophil count versus time data obtained during schedule I. Linear, E_{MAX} and Sigmoidal E_{MAX} models were used to describe the drug effects on the first order rate constant of proliferation, k_{prol}.Then, the outcome during dosing scenario II was predicted simulating individual profiles using the individual Bayes parameter estimates obtained from the schedule I data fit. As it is illustrated in figure 2(panels B and C), the neutrophil profiles corresponding to schedule II were not well described, and values of neutrophils at nadir were in general underpredicted (not for the case of the data obtained from schedule I (figure 2A)).

Step II. A semi-mechanistic model considering the dynamics of a simplified cell cycle considering just the proliferative and quiescent sates was proposed and fitted to all cycle I data obtained from the five clinical studies. This model, represented in figure 3, assumes that: (i) within the stem cell (SC) compartment, proliferative (Prol) and quiescent(Qc) cells coexist, and cell cycle dynamics are described by first order processes governed by k_{cycle}, (ii) quiescent cells comprise two compartments (Qc_1 and Qc_2), and (iii & iv) only cells in the proliferative condition are sensitive to drug effects and capable to follow either the maturation chain, or pass to the quiescent state.

. The rest of model assumptions are equal to those presented in the original reference model(Friberg et al., 2002).

The dynamics in the SC compartment are given by the following set of equations (eq.):

$$\frac{dProl}{dt} = k_{Prol} \times Prol \times \left(\frac{Circ_0}{Circ}\right)^{\gamma} \times (1 - E_{DRUG}) + k_{cycle} \times Qc_2 - k_{TR} \times F_{Prol} \times Prol - k_{cycle} \times (1 - F_{Prol}) \times Prol \quad (eq.1)$$

$$\frac{dQc_1}{dt} = k_{cycle} \times (1 - F_{Prol}) \times Prol - k_{cycle} \times Qc_1 \quad (eq.2)$$

$$\frac{dQc_2}{dt} = k_{cycle} \times Qc_1 - k_{cycle} \times Qc_2 \ (3) \quad (eq.3)$$

Where F_{Prol} is the fraction of Prol cells that enters into the maturation chain and k_{TR} is the first order rate constant controlling the transfer through the maturation chain. In the model, k_{TR} is defined as (n+1)/MTT;being n the number of maturation compartments, and MTT, the mean transit/maturation time. $Circ_0$ and Circ represent the absolute neutrophil counts at baseline, and at any time after the start of the study, respectively. The parameter γ modulates the magnitude of the feedback mechanism.

E_{DRUG} represents drug effects which were described as a linear or non-linear (i.e., sigmoidal E_{MAX} model) function of the predicted plasma (or effect site) concentrations of diflomotecan.

The remaining compartments of the model were characterized as follows:

$$\frac{dTR_1}{dt} = k_{TR} \times F_{Prol} \times Prol - k_{TR} \times TR_1 \text{ (eq.4)}$$

$$\frac{dTR_2}{dt} = k_{TR} \times TR_1 - k_{TR} \times TR_2 \text{ (eq.5)}$$

$$\frac{dTR_3}{dt} = k_{TR} \times TR_2 - k_{TR} \times TR_3 \text{ (eq.6)}$$

$$\frac{dCirc}{dt} = k_{TR} \times TR_3 - k_{Circ} \times Circ \text{ (eq.7)}$$

Where TR_{1-3}, correspond to immature neutrophil levels in each of the maturation compartments, and k_{circ} is the first order rate constant representing neutrophil degradation. The last three differential equations are common to the reference model(Friberg et al., 2002).

Therefore in the current models, the typical system related parameters to be estimated by the model are $Circ_0$, F_{Prol}, k_{cycle}, MTT, and γ.

The initial conditions of the system are represented by the following expressions:

(i) $k_{TR} = k_{circ}$ implying that $TR_1=TR_2=TR_3=Circ_0$.

(ii) $Prol_0$, the level of proliferative cells at baseline is given by the $Circ_0/F_{Prol}$ ratio.

(iii) $Qc_1=Qc_2=(1-F_{Prol})xProl_0$, and therefore $k_{Prol}=k_{TR}$ x F_{Prol}.

During the model development process, other alternatives were also considered as assuming that diflomotecan can exert an effect also on the Qc cells, or the inclusion on an effect compartment as suggested by (Hing et al., 2008).

Results

Pharmacokinetic model

Results of the population PK model are shown in supplementalmaterial. Supplementary figure 1 shows the results of the pc-VPC indicating the population PK model provides a proper description of the drug concentration data. Population pharmacokinetics estimates are listed in supplementary material table 2. All parameters in the model were estimated with good precision based on the values of the results from the bootstrap analysis.

Modelling absolute neutrophil counts

Step I. Data obtained during dosing schedule I werefitted using the reference model for neutropenia. The E_{MAX} model provided a better fit than the linear model, however the precision of the C_{50} parameter was poor. To improve parameter precision, the E_{MAX} model was reparameterised (Schoemaker et al., 1998) where C_{50} is expressed as E_{MAX}/θ_{Slope}, where θ_{Slope} is an estimated model parameter together with E_{MAX} (the maximum attainable effect that diflomotecan can exert on k_{Prol}). Model parameters estimates are shown in table 1. Estimates showed consistency with those obtained in previous studies(Soto et al., 2011).

Figure 2A shows that nadir concentrations from schedule I were well described while those nadir levels from schedule II simulated according to parameters obtained from the analysis of the schedule I data were not adequately captured (figure 2B). This result reveals model misspecifications reflected as an underprediction of neutropenic effectsat nadir (figure 2B) and on the overall neutrophil vs time profile (figure 2C showing nine patients only).

Step II. The model described above provided a good description of the data regardless the type of dosing scenario as can be seen in figure 4A. The model predicted properlythe nadir concentrations from individuals from schedule I and schedule II. Figure 4B shows the model

performance evaluated as pc-VPC including all individuals from the five clinical studies, where both the general tendency and the dispersion of the neutrophil data were well described.In this case, E_{DRUG} was best characterised by a linear model of the form SLP x C_P, where the SLP parameter drives the relationship between the parameter k_{Prol} and the plasma concentrations of diflomotecan (C_P). Better fit was obtained with two Qc compartments with respect a single Qc compartment. Increasing the number of Qc compartments did not improve data description.Table 2 lists the population parameter estimates of the selected model incorporating cell cycle dynamics. All parameters were estimated with good precision, as indicated by the bootstrap analysis where none of the 2.5-97.5th percentiles included the null value, and the parameter values estimated are within the 2.5-97.5th percentiles from the bootstrap analysis. Figure 4C shows that for the nine selected patients included in previous Figure 2C, the current model provides a good description of the individual profiles in both dosing scenarios. The three panels show slight trends suggesting some degree of model misspecification. Models incorporating an effect compartment or considering quiescent cells also sensitive to drug effects did not provide a better description of the data.

Figure 5 summarizes the results of figures 2C and 4C with focus on the degree of neutropenia at nadir after the administration of schedule II. For half of the selected patients the reference model predicted grade III neutropenia while raw data indicated grade IV. The new model provided a 100% match between raw and predicted degree of neutropenia at nadir.Both, the reference and the current models show very similar estimates of the parameters $Circ_0$, and MTT (see tables 1 and 2). The F_{Prol}parameter was estimated as 0.58 indicating that, at any time, approximately more than a half of the proliferative cells follow the maturation chain, and slightly less than a half transit to a quiescent state. The turnover process within the stem cell compartment is 2.5 fold faster than the maturation process, as indicating by the difference between the median cell cycle time calculated as ($3/k_{cycle}$) and MTT (1.6 vs 3.8 days).

Drug effects were best characterized with a linear model in the case of the new proposed model. The slope parameter showed large differences between the two models (1.5 vs 9.5 mL/ng) which might explain at least partly the underprediction of neutropenic effects after administration of schedule II, when using the empirical Bayes individual parameters obtained from the analysis of the schedule I data.

The estimate of the inter-individual variability in the slope parameter was 108%. There is the possibility that individual pharmacokinetic datawere not sufficiently informative and so pharmacokinetic variability has been assigned to pharmacodynamics. To test that possibility a simultaneous fit was attempted, but we could not achieve convergence, and all additional models were terminated without providing parameter estimates. On the other hand in a previous analysis (Troconiz et al., 2006) performed with only part of the data included in this study (study A) an estimate of inter-individual variability of 61% was already obtained. It is expected that pooling data from additional individuals with advanced cancer from other studies, the magnitude of inter-individual variability increases..

As a final modelling exercise, the reference model was also fit to all data (schedules I & II), and a significant decrease in -2LL was found in favor of the proposed model (Δ_{-2LL} =5.2; $p<0.05$). However, the main difference in model performance as shown in figures 2C and 4C is at the level of the nadir, which has a profound impact on adverse effect characterization but in our case less impact on -2LL.

Figure 6 explores how the model behaves. In figure 6Athe typical profiles in each compartment for the reference and current proposed models after a 20 minutes intravenous infusion of 0.5 mg/m^2 at day 1 (schedule I), followed by a five consecutive 20 min intravenous infusions of 0.1 mg/m^2 given once daily, starting at day 14 are shown. After a single drug infusion both models behaves very similar. However, after consecutive

administrations and due to the turnover dynamics in the stem cell compartment, the absolute depletion of the proliferative cells is increased in the current model, eliciting higher neutropenic effects. The schedule dependency seen for diflomotecan is caused possibly by the fast decline of its drug concentrations. After a short intravenous infusion, proliferative cells are affected, but by the time the quiescent cells are converted into proliferative cells, most of the drug has been eliminated from the body. In figure 6B we explored the differences in the overall neutrophil profiles between the two models corresponding to different scenarios where the total plasma clearance (CL) of the drug was reduced (half-life was augmented). The prediction discrepancy between the two models diminished as the CL is reduced. Figure 6C shows the typical plasma drug concentration profiles for each of the PK scenarios highlighting the median cell cycle time of 1.58 days.

Discussion

In the current work a model describing the neutropenic effects of diflomotecan administered under different dosing schedules involving either a short (20 minutes) intravenous infusion, or a five daily intravenous or oral administrations within a cycle of chemotherapy has been developed.

The proposed model was built from the physiological platform of the reference model of neutropenia(Friberg et al., 2002). In addition to the proliferation, maturation, rebound, and degradation processes, cell cycle dynamics occurring within the stem cell compartment were incorporated. Such extra physiological element in the model was represented by two new parameters, F_{Prol}, and k_{cycle}, the first accounting for the fraction of the proliferative cells that enter into the maturation chain, and the latter quantifying the dynamic of the transit between the different cells status. Both model parameters resulted identifiable, as indicated by the results from the bootstrap analysis. It should be noted that parameter identifiability of those two parameters could only be obtained when data of the two types of schedules were analyzed together, otherwise the current model collapsed to the reference model (i.e., $F_{Prol} = 1$) with a smaller estimate of the slope parameter associated to the schedule I data.An attempt was made to fit all available data (all treatment cycles) using the proposed model; however it was not possible get precise and reliable estimates for the F_{prol} and k_{cycle}, due the sparse nature of the data (samples were recorded at times far from nadir, usually at the end of each treatment cycle), and the fact that the number of subjects providing data after the first cycle of treatment decreased at least by a half. This result stresses the need of rich data to apply the model proposed in the current work, which is generally the case of phase I studies at least during the very first cycles of treatment.

The F_{Prol} and k_{cycle} estimates obtained in this work are in accordance with values obtained from literature data (Reddy et al., 1997; Quesenberry et al., 2015). Proliferative cells are able

to replicate into new stem cells, which might remain into the same cell type or differentiate into a new one along the maturation chain compartments. These mechanisms are controlled by different biochemical and cell cycle checkpoint signals(Pietras et al., 2011), which might increase or reduce cell differentiation and/or stem cell growth. The cell growth rate estimated in the current model ($k_{Prol} = k_{TR}$ x $F_{Prol} = 0.6\ d^{-1}$) is in accordance with the cell growth rate values of $0.56\ d^{-1}$ published elsewhere (Clairambault, 2012). On the contrary, higher cell growth rate values were estimated using the reference model ($0.83\ d^{-1}$). This discrepancy might be explained because, at any time, two pools of stem cells are present in the quiescent compartments and, because of the cell cycle turnover process, lower cell growth rates are needed to satisfy circulating neutrophils demand. The cell cycle duration in the current model ($3/k_{cycle}$) was 37.9 h, indicating physiological resemblance with the published cell cycle duration window for hematopoietic stem cells (Reddy et al., 1997; Pietras et al., 2011).

As discussed above cell cycle dynamics could only be detected and modelled when data from the two schedules were fitted simultaneously. Then, for a particular anticancer drug under investigation or clinical use, and in the case of neutrophil data available just under the same or similar schedules, the question of how to anticipate relevant dosing schedule dependency limiting the prediction of neutropenic effects after different dosing scenarios remains open.

We showed in figures 6B-C that the greater the remaining area under the plasma drug concentration vs time curve (AUC) outside the time corresponding to the estimated cell cycle duration, the lower the differences between the neutrophil profiles predicted by the two models (suggesting a lack of impact of predicting neutropenia when ignoring cell cycle dynamics). However, we have not explored formally the relationship between for example, the percentage of total AUC predicted over the duration of the cell cycle and the degree of discrepancy between the two models; in addition, other factors like drug potency might play a role.

We propose the following workflow to explore the potential impact of dosing schedule dependencies on neutropenia prediction. Taking advantage that the system related parameters of the reference model have shown repeatedly consistency across drugs, and that F_{Prol} and $k_{cycle}$are supported by literature, neutrophil data obtained after administration of one schedule were analysed under (i) the reference model estimating the full set of parameters, and (ii) the current model fixing F_{Prol} and k_{cycle} to the estimates obtained in the current evaluation, and the rest of parameters to be estimated. Then simulations are performed under different scenarios to evaluate discrepancies between the outcome (i.e., percentage of patients experiencing grade 4 neutropenia) of the two models.

When that approach was performed on the data from schedule I in the current work, we obtained that after the administration of 0.2 mg/m^2 on day 1 and five consecutive oral doses of 0.35 mg on days 14-18, the median simulated percentage of patients showing grade 4 receiving diflomotecan was 77% and 94% using the parameters obtained from thereference and current developed model, respectively.

To summarize the results from the current investigation, a model describing the neutropenic effects after administration of diflomotecan following two different dosing administration schemes was developed, incorporating cell cycle dynamics in addition to the proliferation, maturation, degradation, and rebound processes described in previous published semi-mechanistic models. The new model accounted for the schedule dependent parameters obtained when the reference model was applied. Cell cycle dynamics were characterized by new parameters, F_{Prol}, and k_{cycle}, the first accounting for the fraction of proliferative cells following the maturation chain, and the latter quantifying the dynamic of the transit between the different cells status. Both model parameters resulted identifiable, and their estimates were supported by literature data.

Authorship contribution

Performed data analysis: Mangas-Sanjuan V, Buil-Bruna N, Garrido MJ, Soto E, Troconiz IF

Wrote or contributed to the writing of the manuscript: Mangas-Sanjuan V, Buil-Bruna N, Garrido MJ, Soto E, Troconiz IF.

References

Bauer R (2011) *NONMEM users guide: introduction to NONMEM 7.2.0.* . ICON Development Solutions, Ellicott City.

Bergstrand M, Hooker AC, Wallin JE and Karlsson MO (2011) Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. *The AAPS journal***13**:143-151.

Clairambault JO, F. (2012) Physiologically structured cell population dynamic models with applications to combined drug delivery optimisation in oncology, in *Mathematical modelling of cancer growth and treatment* (Springer ed).

Fetterly GJ, Grasela TH, Sherman JW, Dul JL, Grahn A, Lecomte D, Fiedler-Kelly J, Damjanov N, Fishman M, Kane MP, Rubin EH and Tan AR (2008) Pharmacokinetic/pharmacodynamic modeling and simulation of neutropenia during phase I development of liposome-entrapped paclitaxel. *Clin Cancer Res***14**:5856-5863.

Friberg LE, Henningsson A, Maas H, Nguyen L and Karlsson MO (2002) Model of chemotherapy-induced myelosuppression with parameter consistency across drugs. *J Clin Oncol***20**:4713-4721.

Friberg LE, Sandstrom M and Karlsson MO (2010) Scaling the time-course of myelosuppression from rats to patients with a semi-physiological model. *Invest New Drugs***28**:744-753.

Hing J, Perez-Ruixo JJ, Stuyckens K, Soto-Matos A, Lopez-Lazaro L and Zannikos P (2008) Mechanism-based pharmacokinetic/pharmacodynamic meta-analysis of trabectedin (ET-743, Yondelis) induced neutropenia. *Clin Pharmacol Ther***83**:130-143.

Jonsson EN and Karlsson MO (1999) Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. *Computer methods and programs in biomedicine***58**:51-64.

Krzyzanski W and Jusko WJ (2002) Multiple-pool cell lifespan model of hematologic effects of anticancer agents. *J Pharmacokinet Pharmacodyn***29**:311-337.

Latz JE, Karlsson MO, Rusthoven JJ, Ghosh A and Johnson RD (2006) A semimechanistic-physiologic population pharmacokinetic/pharmacodynamic model for neutropenia following pemetrexed therapy. *Cancer Chemother Pharmacol***57**:412-426.

Leger F, Loos WJ, Bugat R, Mathijssen RH, Goffinet M, Verweij J, Sparreboom A and Chatelut E (2004) Mechanism-based models for topotecan-induced neutropenia. *Clin Pharmacol Ther***76**:567-578.

Lindbom L, Pihlgren P and Jonsson EN (2005) PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. *Computer methods and programs in biomedicine***79**:241-257.

Minami H, Sasaki Y, Saijo N, Ohtsu T, Fujii H, Igarashi T and Itoh K (1998) Indirect-response model for the time course of leukopenia with anticancer drugs. *Clin Pharmacol Ther***64**:511-521.

Panetta JC, Kirstein MN, Gajjar AJ, Nair G, Fouladi M and Stewart CF (2003) A mechanistic mathematical model of temozolomide myelosuppression in children with high-grade gliomas. *Math Biosci***186**:29-41.

Panetta JC, Schaiquevich P, Santana VM and Stewart CF (2008) Using pharmacokinetic and pharmacodynamic modeling and simulation to evaluate importance of schedule in topotecan therapy for pediatric neuroblastoma. *Clin Cancer Res***14**:318-325.

Pietras EM, Warr MR and Passegue E (2011) Cell cycle regulation in hematopoietic stem cells. *J Cell Biol***195**:709-720.

Quesenberry PJ, Goldberg LR and Dooner MS (2015) Concise reviews: a stem cell apostasy: a tale of four h words. *Stem Cells***33**:15-20.

Reddy GP, Tiarks CY, Pang L, Wuu J, Hsieh CC and Quesenberry PJ (1997) Cell cycle analysis and synchronization of pluripotent hematopoietic progenitor stem cells. *Blood***90**:2293-2299.

Sandstrom M, Lindman H, Nygren P, Lidbrink E, Bergh J and Karlsson MO (2005) Model describing the relationship between pharmacokinetics and hematologic toxicity of the epirubicin-docetaxel regimen in breast cancer patients. *J Clin Oncol***23**:413-421.

Schoemaker RC, van Gerven JM and Cohen AF (1998) Estimating potency for the Emax-model without attaining maximal effects. *J Pharmacokinet Biopharm***26**:581-593.

Soto E, Staab A, Freiwald M, Munzert G, Fritsch H, Doge C and Troconiz IF (2010a) Prediction of neutropenia-related effects of a new combination therapy with the anticancer drugs BI 2536 (a Plk1 inhibitor) and pemetrexed. *Clin Pharmacol Ther***88**:660-667.

Soto E, Staab A, Tillmann C, Trommeshauser D, Fritsch H, Munzert G and Troconiz IF (2010b) Semi-mechanistic population pharmacokinetic/pharmacodynamic model for neutropenia following therapy with the Plk-1 inhibitor BI 2536 and its application in clinical development. *Cancer Chemother Pharmacol***66**:785-795.

Soto E, Keizer RJ, Troconiz IF, Huitema AD, Beijnen JH, Schellens JH, Wanders J, Cendros JM, Obach R, Peraire C, Friberg LE and Karlsson MO (2011) Predictive ability of a semi-mechanistic model for neutropenia in the development of novel anti-cancer agents: two case studies. *Invest New Drugs***29**:984-995.

Steimer JL, Dahl SG, De Alwis DP, Gundert-Remy U, Karlsson MO, Martinkova J, Aarons L, Ahr HJ, Clairambault J, Freyer G, Friberg LE, Kern SE, Kopp-Schneider A, Ludwig WD, De Nicolao G, Rocchetti M and Troconiz IF (2010) Modelling the genesis and treatment of cancer: the potential role of physiologically based pharmacodynamics. *Eur J Cancer***46**:21-32.

Troconiz IF, Garrido MJ, Segura C, Cendros JM, Principe P, Peraire C and Obach R (2006) Phase I dose-finding study and a pharmacokinetic/pharmacodynamic analysis of the neutropenic response of intravenous diflomotecan in patients with advanced malignant tumours. *Cancer Chemother Pharmacol***57**:727-735.

Zamboni WC, D'Argenio DZ, Stewart CF, MacVittie T, Delauter BJ, Farese AM, Potter DM, Kubat NM, Tubergen D and Egorin MJ (2001) Pharmacodynamic model of topotecan-induced time course of neutropenia. *Clin Cancer Res***7**:2301-2308.

FOOTNOTES

VM-S, received a predoctoral grant from Ministry of Education and Science of Spain and Miguel Hernandez University [FPU AP2010-2372]. NB-B, was supported by a predoctoral fellowship from Asociación de Amigos de la Universidad de Navarra. This work was supported by the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115156, resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. The DDMoRe project is also supported by financial contribution from Academic and SME partners. This work does not necessarily represent the view of all DDMoRe partners.

Part of the content of this work was presented at Population Approach Group of Europe Meeting, held in Alicante 2014.

Reprint request: Dr Iñaki F Troconiz, Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra. C/ Irunlarrea 1, 31008 Pamplona, Navarra, Spain Phone: +34948 425600 (ext 806507) Fax: +34948425649 E-mail: itroconiz@unav.es

Figure Legends

Figure 1. Schematic representation of the administration schemes proposed in each study. Schedule I is represented as blue arrows. Schedule II is represented by arrows in red (oral solution), green (intravenous infusions), and yellow (oral capsules administered). In parenthesis, number of patients in each dose level.

Figure 2. Model performance of the reference model for neutropenia. **A.**Observed vs Predicted nadir concentration corresponding to schedule I: studies A, B, C and E.**B.**Observed vs Predicted nadir concentration from studies C, D and E with schedule II. Blue dotted line represents the identity line and red solid line shows the result of linear regression. **C.** Individual predicted profiles obtained from the reference model considering only schedule I data (solid lines in blue). Simulated profiles corresponding to the schedule II based on the individual predicted Bayes parameters estimates obtained from schedule I (solid lines in red) using the reference model. Dots represent the individual observations.

Figure 3. Semi-mechanistic cell cycle based PKPD model of chemotherapy-induced neutropenic effects. Terms are defined in the text.

Figure 4. Current neutropenia model performance. **A.**Observed vs Predicted nadir concentration from all studies. Blue dots represent schedule I from studies A, B, C and E and red dots represent schedule II from studies C, D and E. Blue dotted line represents the identity line and red solid line shows the linear regression obtained from the correlation. **B.** pc-VPC from all studies in the first cycle. Lines represent the 2.5^{th}, 50^{th} and 97.5^{th} experimental percentiles and grey area is the 95% confidence interval of 2.5^{th}, 50^{th} and 97.5^{th} percentile of the simulated data. **C.** Individual predicted profiles obtained from the current model. Solid lines in blue and red correspond to schedule I and II, respectively. Black dots represent the individual observations.

Figure 5. Each column of symbols represents the predicted absolute neutrophils counts at nadir obtained from the reference (blue) and current proposed (orange) models, and individual observed values (black crosses) for each of the selected nine patients, whose neutrophil vs time profiles were shown in figures 2C and 4C. Dotted horizontal lines highlight the limits for each grade of neutropenia.

Figure 6A. Typical simulated profiles in each of the compartmentsrepresenting the reference (blue) and current model proposed (orange) models. Simulations were performed assuming the administration of a single 20 infusion of 0.5 mg/m^2 at day 1 and a daily single 20 min intravenous infusion of 0.1 mg/m^2 during five days starting at day 14. **B.**Simulated typical model predicted ANC profiles. Simulations were performed considering the administration of a daily single intravenous 20 min infusion of 0.05 mg/m^2 during five days and when clearance was reduced by 0, 25, 50 and 75% with respect the population clearance value estimated in the final PK model(supplementary material table 2).**C.** Simulated typical model predicted diflomotecan concentration profiles when clearance was reduced by 0, 25, 50 and 75% with respect the population clearance value estimated in the final PK model. Simulations were performed considering the administration of a single intravenous 20 min infusion of 0.05 mg/m^2. Vertical line represents the mean cell cycle time estimated from the current model (1.54 days).

TABLES

Table 1.Population pharmacodynamic parameter estimates corresponding to the reference model for neutropenia (Friberg et al., 2002)

			Bootstrap (n=500)	
Parameters	**Estimate**	**Shrinkage (%)**	**Median**	**2.5-97.5th Percentiles**
$Circ_0$ (x10^9/L)	4.70		4.65	3.9-5.4
MTT(d)	4.81		4.75	3.9-6.0
θ_{Slope} (mL/ng)	1.49		1.51	0.6-3.2
E_{MAX}	4.03		3.96	2.1-11.2
γ	0.14		0.14	0.08-0.2
IIV_{Circ0} (%)	52	7.3	47	15-63
IIV_{MTT} (%)	26	15.9	25	10-34
IIV_{SLP} (%)	88	20.3	83	16-115
$IIV_{Residual\ Error}$ (%)	19	34.6	18	0.6-44
Residual error [log (x10^9/L)]	0.4	13.7	0.4	0.3-0.5

CV, coefficient of variation; IIV, inter-individual variability expressed as CV(%); n, number of bootstrap datasets; Model parameters are defined in the text.

Table 2. Population pharmacodynamics parameter estimates obtained from the final model incorporating cell-cycle dynamics.

			Bootstrap (n=500)	
Parameters	**Estimate**	**Shrinkage (%)**	**Median**	**2.5-97.5th Percentiles**
$Circ_0$ ($x10^9$/L)	4.6		4.8	4-5.4
MTT(d)	3.8		4.1	3.8-4.8
SLP (mL/ng)	9.5		9.4	8.2-10.2
k_{cycle} (d^{-1})	1.9		1.9	1.5-2.1
F_{prol}	0.58		0.55	0.5-0.6
γ	0.37		0.37	0.3-0.4
IIV_{Circ0} (%)	33	19.6	29	15-35
IIV_{MTT} (%)	12	30.1	12	10-22
IIV_{SLP} (%)	108	23.1	109	78-116
IIV_{Fprol} (%)	32	21.4	30	16-33
$IIV_{Residual\ Error}$ (%)	28	48.9	32	27-44
Additive [log ($x10^9$/L)]	0.05	18.4	0.05	0.01-0.09
Proportional (%)	66		63	51-73

CV, coefficient of variation; IIV, inter-individual variability expressed as CV(%); n, number of bootstrap datasets; Model parameters are defined in the text.

Figure 1

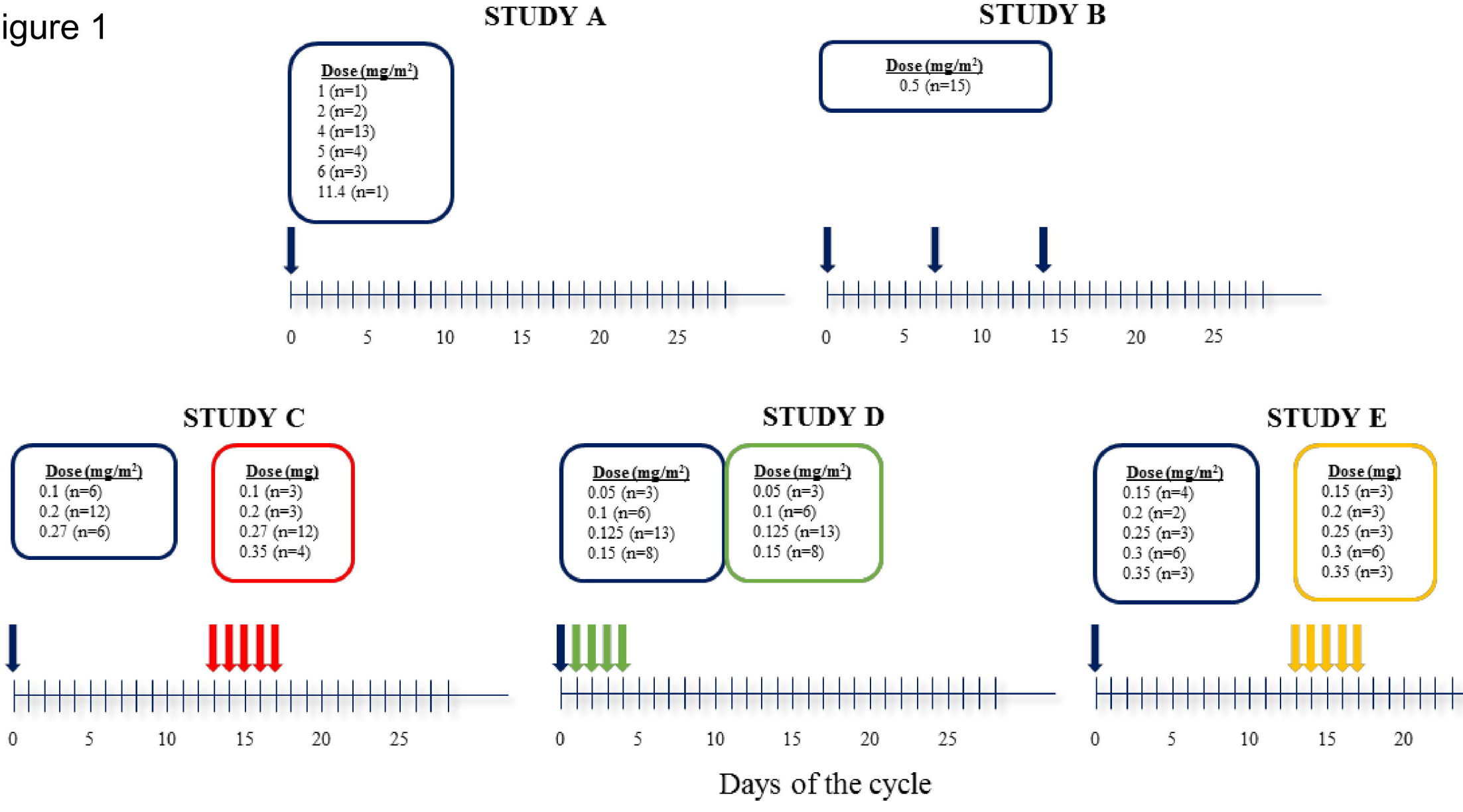
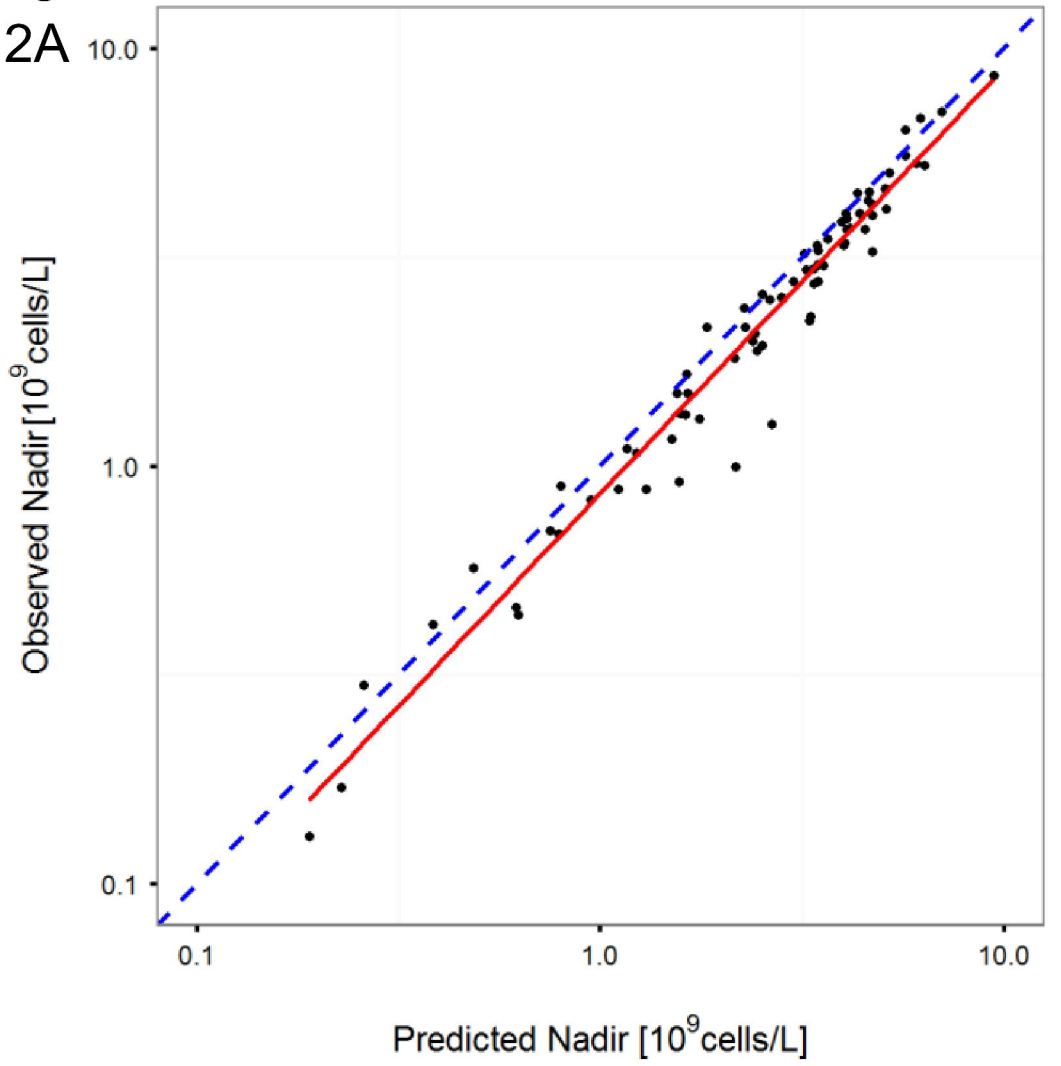
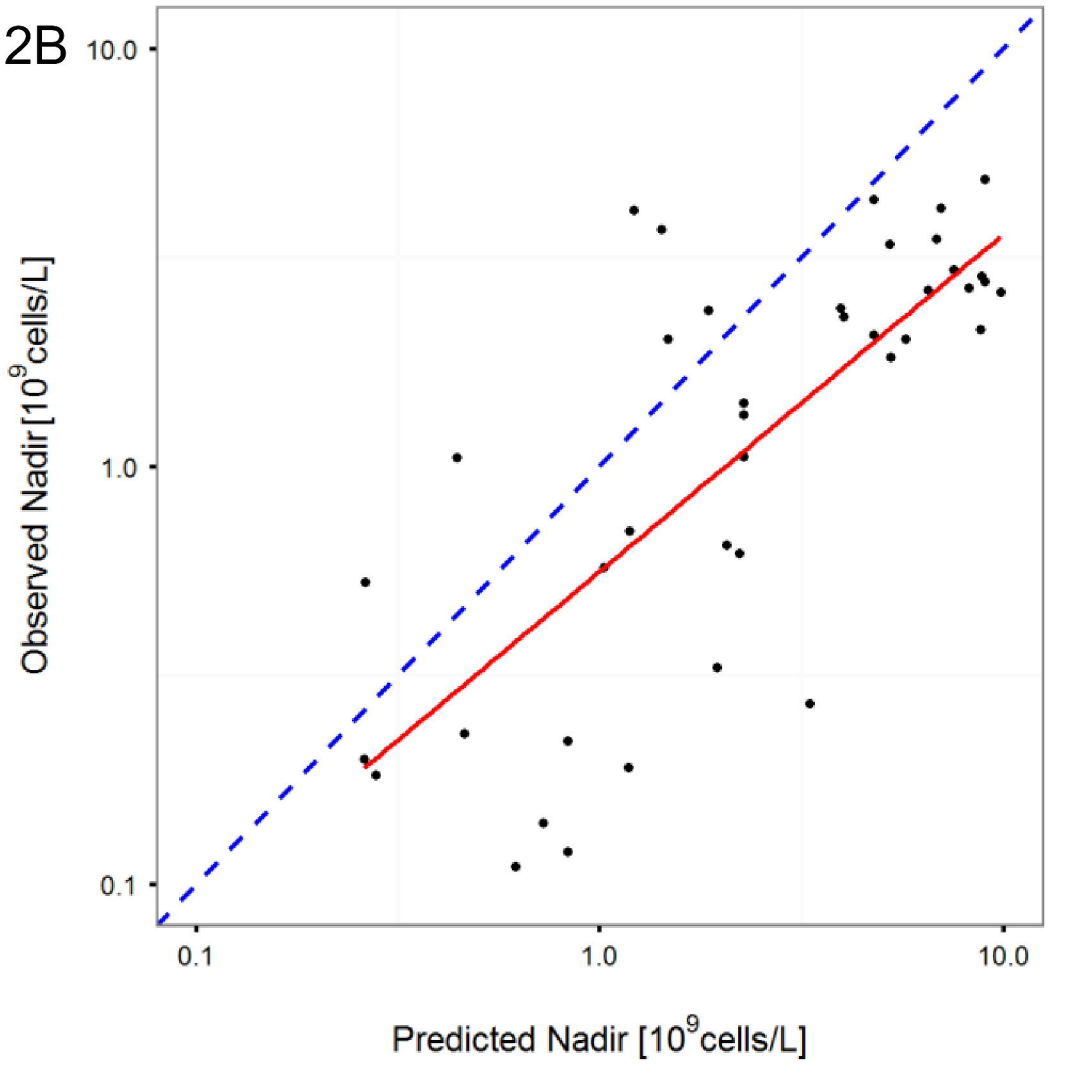

STUDY A
Dose (mg/m²)
1 (n=1)
2 (n=2)
4 (n=13)
5 (n=4)
6 (n=3)
11.4 (n=1)
0 5 10 15 20 25
STUDY B
Dose (mg/m²)
0.5 (n=15)
0 5 10 15 20 25
STUDY C
Dose (mg/m²)
0.1 (n=6)
0.2 (n=12)
0.27 (n=6)
Dose (mg)
0.1 (n=3)
0.2 (n=3)
0.27 (n=12)
0.35 (n=4)
0 5 10 15 20 25
STUDY D
Dose (mg/m²)
0.05 (n=3)
0.1 (n=6)
0.125 (n=13)
0.15 (n=8)
Dose (mg/m²)
0.05 (n=3)
0.1 (n=6)
0.125 (n=13)
0.15 (n=8)
0 5 10 15 20 25
STUDY E
Dose (mg/m²)
0.15 (n=4)
0.2 (n=2)
0.25 (n=3)
0.3 (n=6)
0.35 (n=3)
Dose (mg)
0.15 (n=3)
0.2 (n=3)
0.25 (n=3)
0.3 (n=6)
0.35 (n=3)
0 5 10 15 20
Days of the cycle

Figure 2

2A



2B



2C

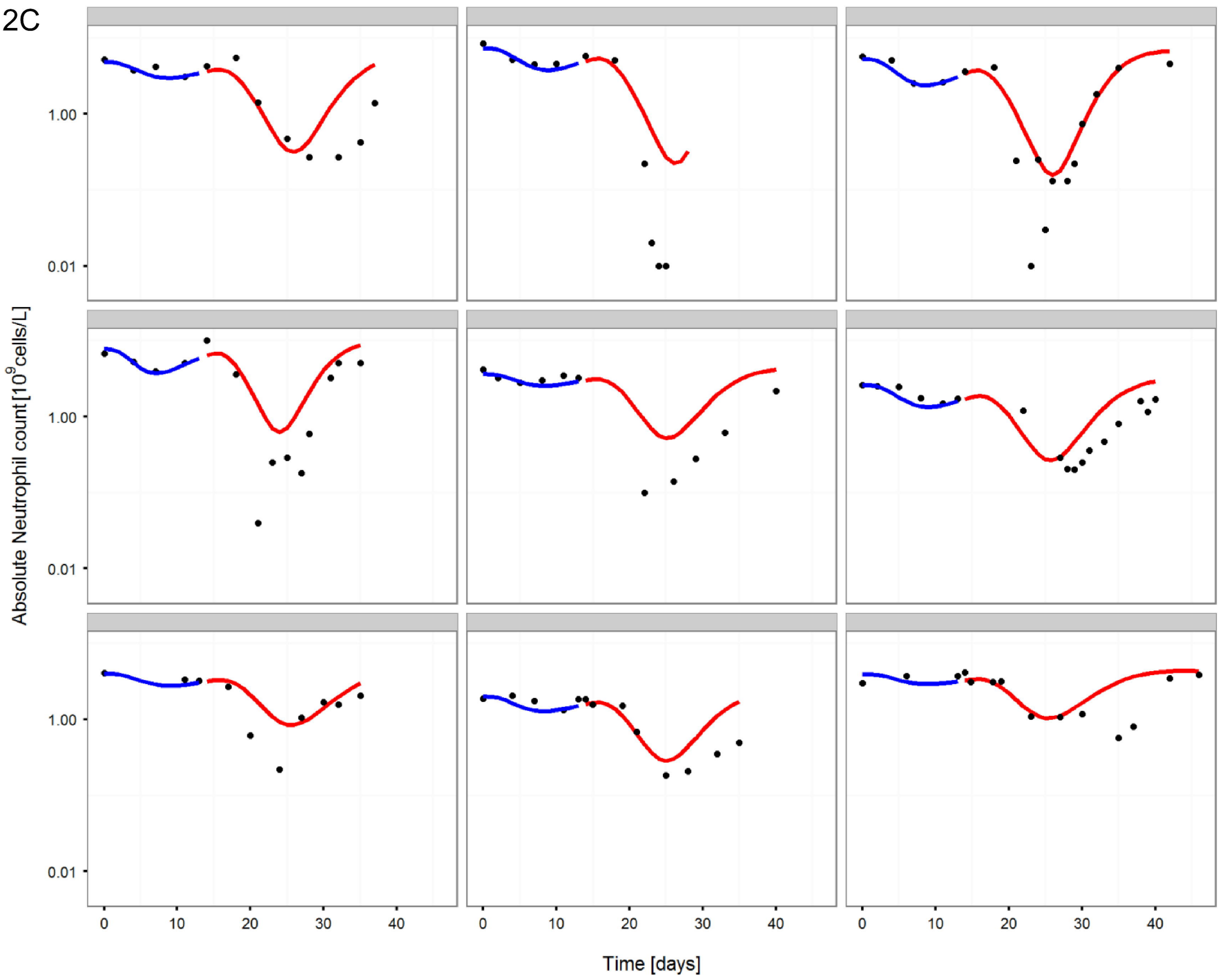


Figure 3

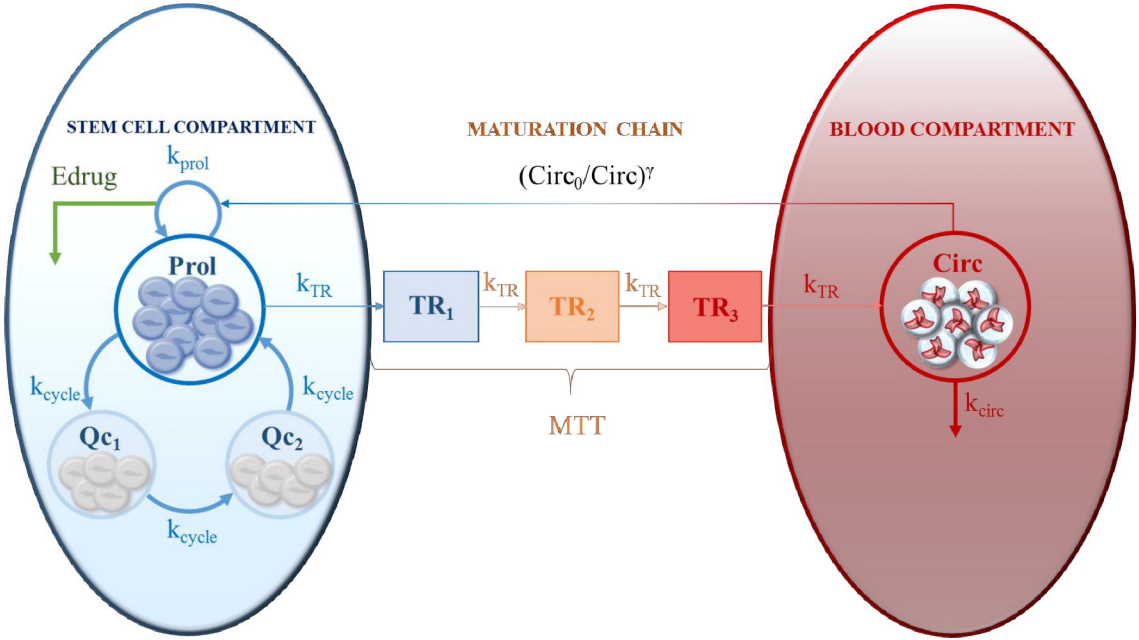
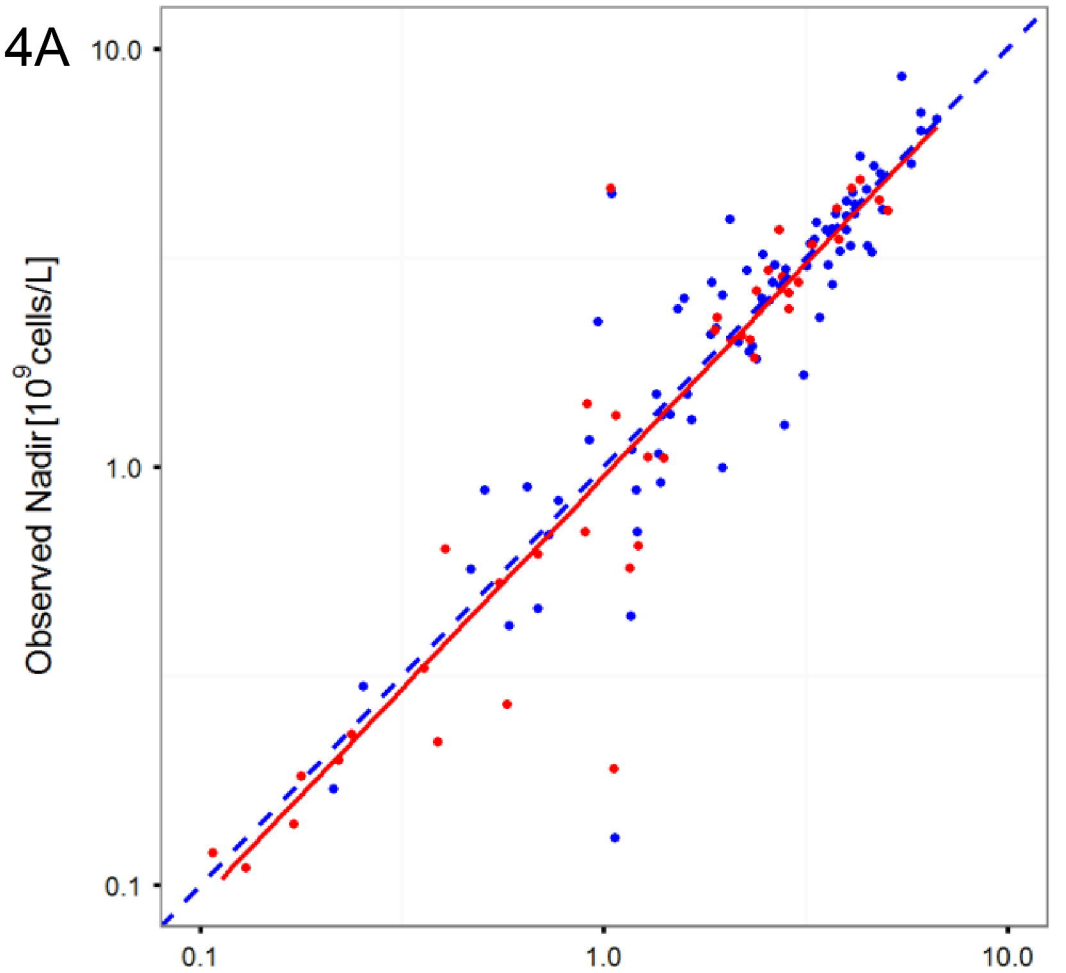
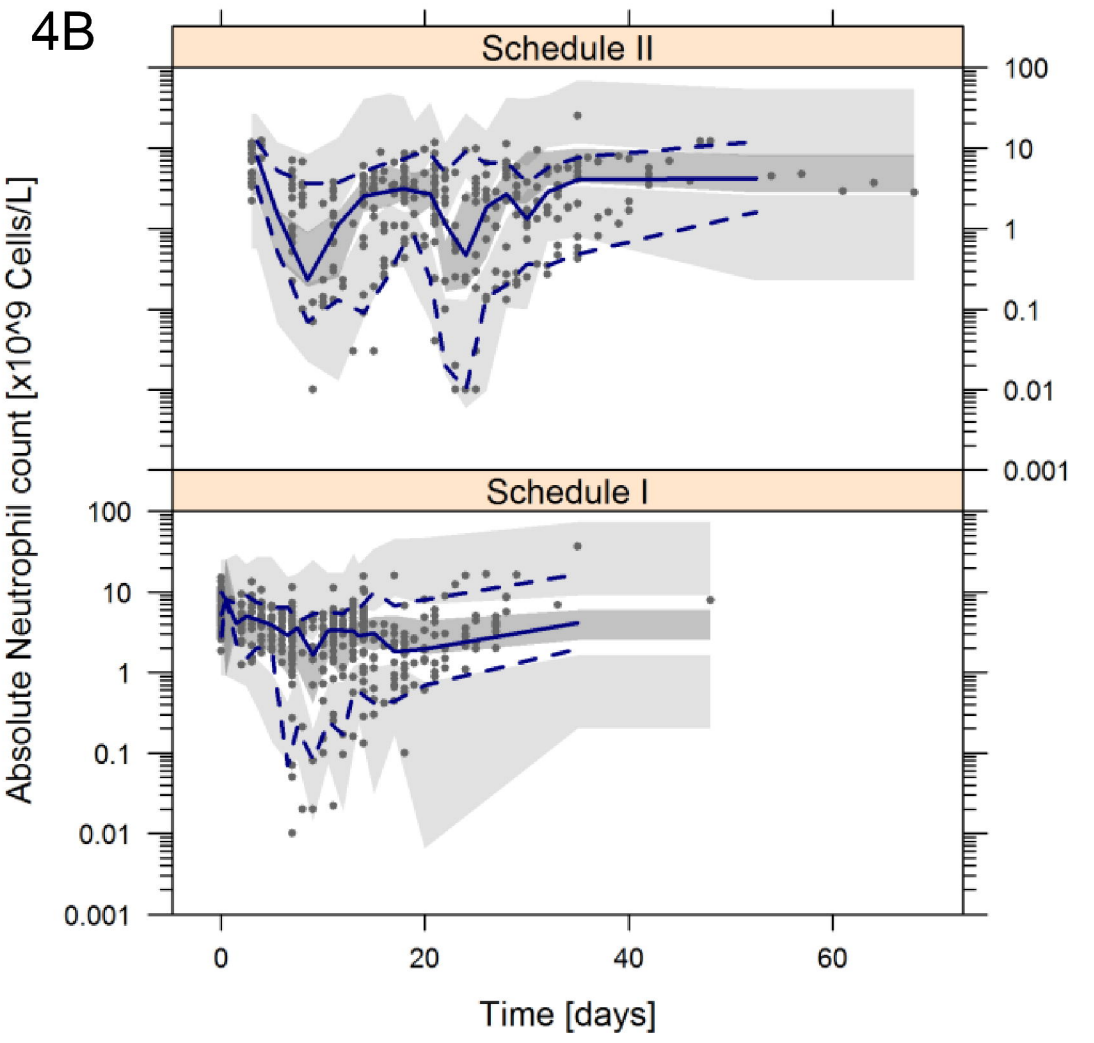

STEM CELL COMPARTMENT
MATURATION CHAIN
BLOOD COMPARTMENT
Edrug
k_{prol}
Prol
k_{TR}
TR_1
k_{TR}
TR_2
k_{TR}
TR_3
k_{TR}
$(Circ_0/Circ)^{\gamma}$
Circ
k_{circ}
k_{cycle}
k_{cycle}
Qc_1
Qc_2
k_{cycle}
MTT

Figure 4

4A



4B



4C

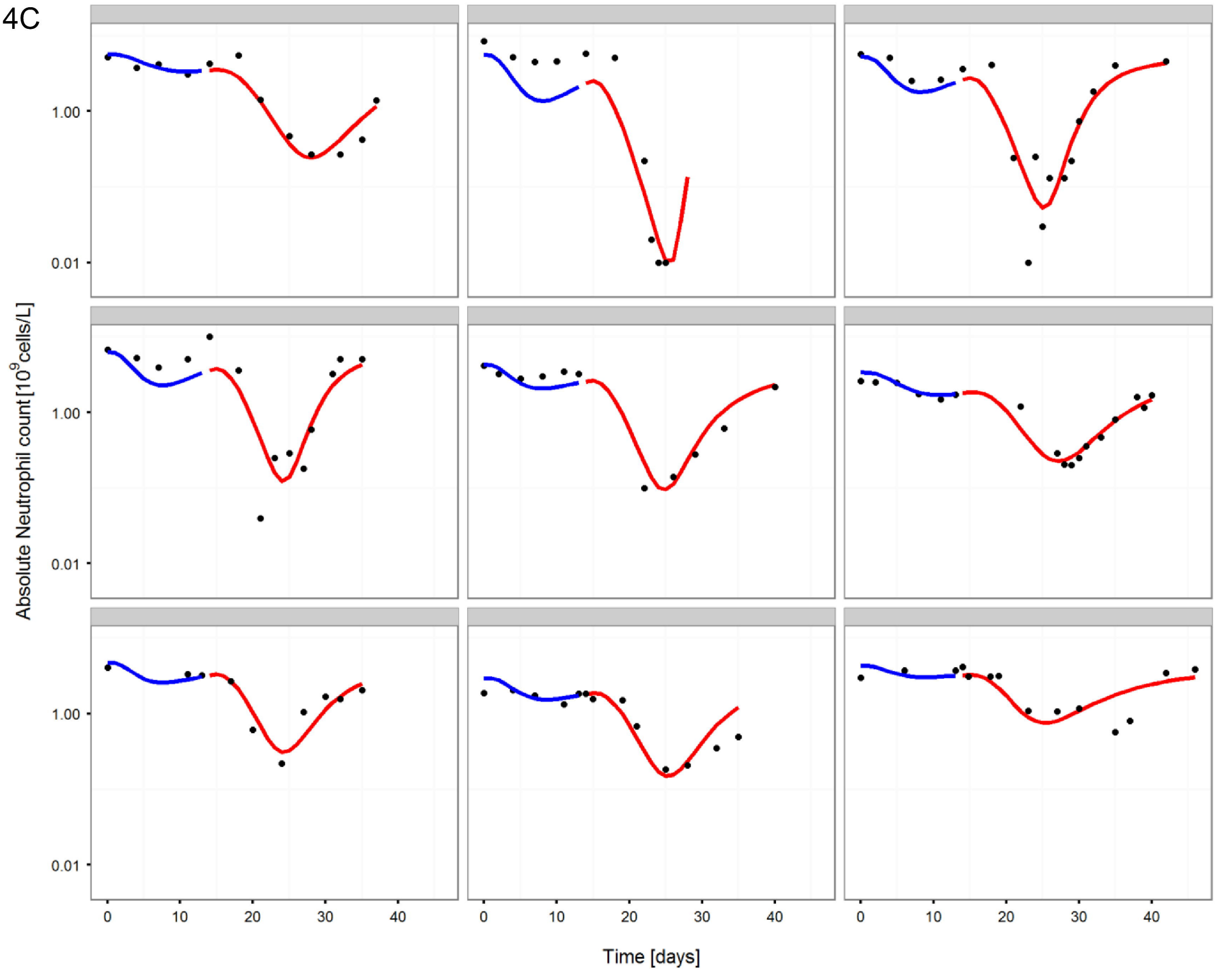


Figure 5

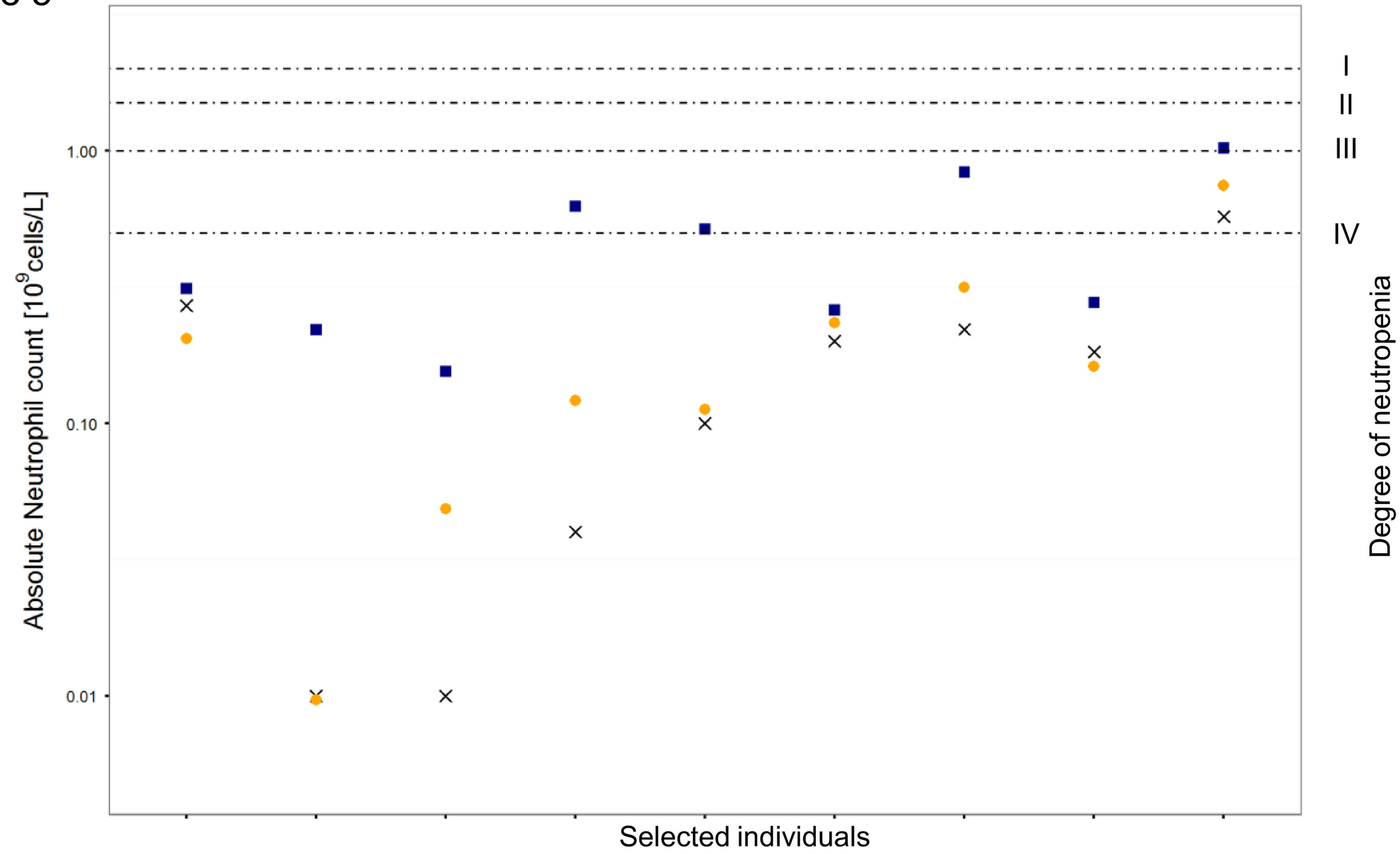
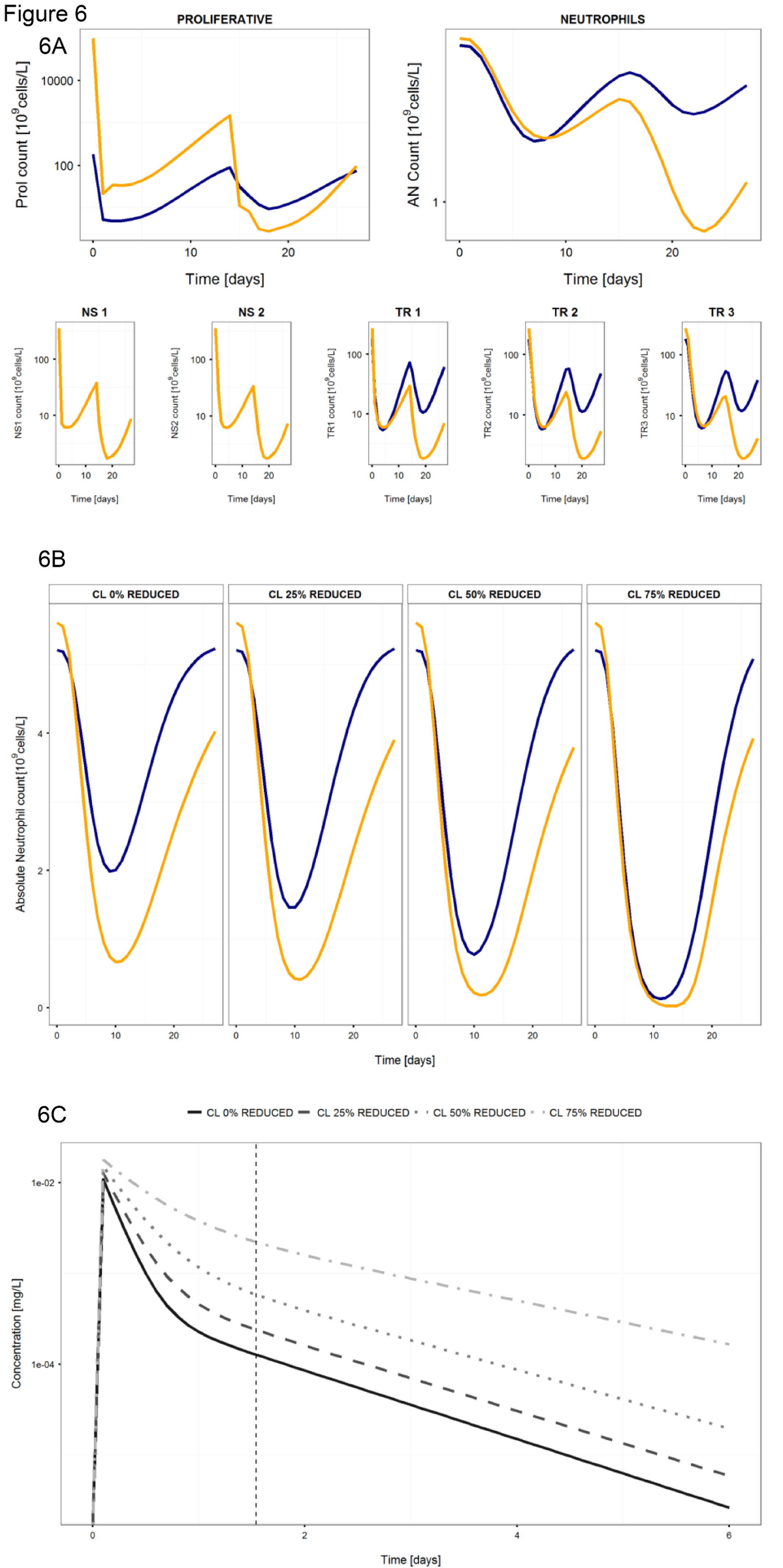

Absolute Neutrophil count [10^9cells/L]
1.00
0.10
0.01
Selected individuals
I
II
III
IV
Degree of neutropenia

Figure 6


6A
PROLIFERATIVE
Prol count [10^9 cells/L]
10000
100
0
10
20
Time [days]
NEUTROPHILS
AN Count [10^9 cells/L]
1
0
10
20
Time [days]
NS 1
NS2 count [10^9 cells/L]
TR1 count [10^9 cells/L]
TR2 count [10^9 cells/L]
TR3 count [10^9 cells/L]
100
10
Time [days]
6B
CL 0% REDUCED
CL 25% REDUCED
CL 50% REDUCED
CL 75% REDUCED
Absolute Neutrophil count [10^9 cells/L]
4
2
0
Time [days]
6C
CL 0% REDUCED
CL 25% REDUCED
CL 50% REDUCED
CL 75% REDUCED
Concentration [mg/L]
1e-02
1e-04
0
2
4
6
Time [days]