Characterising Gemcitabine effects administered as single agent or combined with carboplatin in mice pancreatic and ovarian cancer xenografts. A semi-mechanistic pharmacokinetic/pharmacodynamics tumour growth-response model

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Gemcitabine Preclinical PKPD tumour growth-response model

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Nonstandard abbreviations: -2LL: 2xlog (likelihood); FOCE: first Order Conditional Estimation method; IIV, inter-individual variability; NONMEM: Non-Linear Mixed Effect Models; pc-VPC: prediction- corrected visual predictive checks.

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Abstract

In this work, a semi-mechanistic tumour growth response model for gemcitabine in pancreatic (administered as single agent) and ovarian (given as single agent and in combination with carboplatin) cancer in mice was developed. Tumour profiles were obtained from nude mice, previously inoculated with KP4, ASPC1, MIA PACA2, PANC1 (pancreas), A2780 or SKOV3xluc (ovarian) cell lines, and then treated with different dosing schedules of gemcitabine and/or carboplatin. Data were fitted using the population approach with NONMEM 7.2. In addition to cell proliferation, the tumour progression model for both types of cancer incorporates a carrying capacity representing metabolite pool for DNA synthesis required to tumour growth. Analysis of data from the treated groups revealed that gemcitabine exerted its tumour effects by promoting apoptosis as well as decreasing the carrying capacity compartment. Pharmacodynamic parameters were cell-specific and overall had similar range values between cancer types. In pancreas, a linear model was used to describe both gemcitabine effects with parameter values between $3.26 \times 10^{-2}$ and $4.2 \times 10^{-1}$ L.mg$^{-1}$ day$^{-1}$. In ovarian cancer, the apoptotic effect was driven by an $E_{\text{MAX}}$ model with an efficacy/potency ratio of 5.25-8.65 L.mg$^{-1}$.days$^{-1}$. The contribution of carboplatin to tumour effects was lower than the response exerted by gemcitabine and was incorporated in the model as an inhibition of the carrying capacity. The model developed was consistent in its structure across different tumour cell lines and two tumour types where gemcitabine is approved. Simulation-based evaluation diagnostics showed that the model performed well in all experimental design scenarios including dose, schedule, and tumour type.
Introduction

Nowadays, drug development constitutes a great deal in terms of the cost-efficacy relationship, especially in the oncology area where the attrition rate is very high; in fact, a recent review indicates that up to 95% of the compounds tested in early clinical phases do not reach marketing authorisation (Moreno and Pearson, 2013). One approach to reduce cancer attrition rates is to look for strategies to better predict clinical trial outcome from early preclinical information (Zhang et al., 2006). In this context, preclinical (semi) mechanistic pharmacokinetic/pharmacodynamics (PKPD) analysis could be considered as a critical phase to understand the effect of a drug and its correlation to the clinic, taking into account that despite several successful applications (Claret et al., 2013; Stein et al., 2012; Wang et al., 2009), modelling response data in oncology presents more limitations than in other therapeutic areas (Mould et al., 2015). Recently, it has been shown that models developed in the in vivo preclinical setting can drive the model structure in the clinical scenario where data are in general sparse and conditioned by high drop-out rates (Ouerdani et al., 2015). In addition, other works have shown that it is possible to scale-up relevant model-derived descriptors and model parameters from animal to human either for efficacy or hematotoxicity (Friberg et al., 2010; Rocchetti et al., 2007).

This work is focused on studying the pharmacodynamics of the anticancer drug Gemcitabine in xenograft mice models. Gemcitabine, a cytotoxic/cytostatic antimetabolite, is a pro-drug of the active form (dFdCTP) which inhibits cell growth and induces apoptosis through incorporation into replicated DNA (Hui and Reitz, 1997; Storniolo et al., 1997; Teicher, 2007). It is approved in the treatment of locally advanced or metastatic pancreatic cancer as single agent and in the treatment of non-small-cell lung, breast and ovarian cancer in combination (Hui and Reitz, 1997).

Information regarding Gemcitabine dose-response relationship and the factors contributing to its magnitude of response is still limited. To the best of our knowledge there is a lack of publicly available reports dealing with the PKPD characteristics of gemcitabine apart from the recent in vivo pre-clinical analysis in breast cancer xenografts (Yuan et al., 2015), and
the mechanistic models developed with in vitro data (Hamed et al., 2013; Miao et al., 2016; Zhu et al., 2015). Regarding clinical PKPD models, the effect of gemcitabine administered in combination in non-small cell lung, breast and ovarian cancer patients have been described linking model predicted changes in tumour size with overall survival (Tate C et al., 2013; Tham et al., 2008; Zecchin et al., 2016).

The goal of this study is to develop a semi-mechanistic tumour growth model to describe the effects of gemcitabine in both pancreas (administered as a single agent) and ovarian (given in combination with carboplatin) mouse xenograft models, including different tumour cell lines for both types of tumour. Our aim was to select a common model structure for all experimental conditions (cell lines, tumour type, single vs combination therapy, and dosing schedule) providing a robust reference for future translational purposes. This work appears timely given recent articles where the concentration-response of gemcitabine in combination with other anti-cancer drugs has been evaluated at the in vitro (Hamed et al., 2013; Miao et al., 2016; Zhu et al., 2015), and in vivo pre-clinical (Yuan et al., 2015) and clinical levels (Tate C et al., 2013; Tham et al., 2008; Zecchin et al., 2016).
Material and Methods

Experimental Data and Studies Design

Longitudinal tumour growth profiles were obtained from 14 xenograft studies where gemcitabine, or the combination of gemcitabine and carboplatin was administered i.p. to athymic or CD1 nu/nu male and female nude mice, weighing a mean of 25 g (18-34 g), previously inoculated subcutaneously into the flank of mice with $10^6$ or $10^7$ tumour cells from human derived pancreatic (KP4, ASPC1, MIA PACA2, or PANC1) or ovarian (A2780, or SKOV3x) tumour cell lines. No survival experiments and analysis were performed in the current evaluation.

Figure 1 provides an overall view of the experimental designs used to perform the current evaluation showing key descriptors. Briefly, in all the studies, mice were randomly allocated either to a control group receiving saline or to different groups receiving active treatment. The number of mice in each group for each study varied between 5 and 15 (with a median of 10 mice per group). No design optimization was performed with regard to the number of animals per group.

Gemcitabine and carboplatin were given at different doses varying from 15 to 200 mg/kg and 25 to 100 mg/kg, respectively, and under different dosing schedules (see figure 1). Tumour measurements [volume ($\text{mm}^3$)] were obtained around five or ten days after tumour cell inoculation and were regularly collected over the course of the study. The dimensions of the tumour were measured using a caliper, reporting measures of two longest diameters and transformed into tumour volume following equation 1 (Pierrillas et al., 2016; Simeoni et al., 2004):

$$\text{Tumour volume (mm}^3) = \text{length (mm)} \times \text{width}^2 (\text{mm}^2)/2$$  \hspace{1cm} (1)

All the protocols followed during the performance of the animal studies were approved by the Eli Lilly and Company Institutional Animal Care and Use Committee.

Data analysis

Tumour volume versus time data profiles were described based on the population approach using NONMEM version 7.2 (Bauer, 2011) and the First Order Conditional Estimation
(FOCE) method together with the INTERACTION option. Data were logarithmically
transformed for the analysis. Inter-animal variability in the model parameters was modelled
exponentially as follows: $P_{m,i} = P_{m,\text{pop}} \times e^{\eta_{n,i}}$, where $P_{m,i}$ and $P_{m,\text{pop}}$ represent the individual and
typical values of the m parameter in the model, respectively, and $\eta_{n,i}$ which represents the
discrepancy between $P_{m,\text{pop}}$ and $P_{m,i}$, is a random variable assumed to be symmetrically
distributed around 0 with variance equal to $\omega_m^2$. Residual error in the response variable was
described with an additive error model on the logarithmic domain of the transformed data:

$$T_{ij} = f(D_i, t_j, \phi_i) + \epsilon_{ij}.$$ $T_{ij}$ is the log of the tumor volume observed for $i^{th}$ animal at the $j^{th}$ time after
the start of the experiment, $f$, reflects the structure of the population PKPD model, $D_i$
represents the dosing scheme given to the $i^{th}$ animal, the set of individual PKPD parameters
are represented by $\phi_i$, and $\epsilon_{ij}$ represents the discrepancy between the observed and
predicted $T$ [given by $f(D_i, t_j, \phi_i)$]. The set of the differences between observations and
predictions constitutes a random variable symmetrically distributed around 0 with variance
$\sigma^2$.

**Model building**

Data corresponding to each of the different tumour cell lines (either pancreas or ovarian)
were analysed separately. Within each cell line, tumour measurements obtained from
different studies were pooled and analysed together. A more appropriate procedure would
have been to pool all data together for a joint modelling exercise of all cells lines, and use
the type of cell line as a categorical covariate. However when we tried that approach, we
faced the complexity of estimating a great number of parameters, and we did not manage to
get convergence and parameter estimates in the analyses.

Model development was performed sequentially. First, the disease progression model (i.e.,
tumour dynamics in the absence of treatment) was characterised, and then the model for
drug effects was established (see below). At each modelling step all model parameters
were re-estimated.
Pharmacokinetics

Blood and tumour samples to determine drug concentrations were not considered in the current evaluation. In addition, experimental data to characterise pharmacokinetics of gemcitabine and carboplatin were not available. Gemcitabine pharmacokinetic parameters in mice were obtained from an internal Eli Lilly pharmacokinetic model developed with human data (Zhang et al., 2006) using bodyweight to scale down the pharmacokinetic parameters from human to mice. The pharmacokinetic model in mice is composed of three compartments with the following parameter values: CL=2.5 L/h/kg, Q_2=4.9 L/h/kg, Q_3=0.767 L/h/kg, V_1=0.281 L/kg, V_2=0.411 L/kg and V_3=5.35 L/kg, where CL and Q_n are the total and inter-compartment clearance, respectively, and V_n is the distribution volume in each compartment. In the case of carboplatin, a one compartment model was used and the estimates of the pharmacokinetic parameters were obtained from literature (Wang et al., 2004a; Wang et al., 2004b). During the analysis, the mean pharmacokinetic population parameters were used to simulate typical drug concentration vs time profiles in plasma corresponding to each drug and dosing schedule, which were used to describe the drug effects of tumour dynamics. It was assumed that (i) drug absorption after i.p administration was fast and complete (and treated as an intravenous bolus injection), and independent from the dose level and time, and (ii) there was not pharmacokinetic interaction between gemcitabine and carboplatin (Wang et al., 2004a; Wang et al., 2004b).

In the following, the selected model is described in detail. However, during the model building exercise several other models and variants were also explored, as indicated in the results section.

Disease progression model

Different disease progression models were explored and are represented in Table 1 (Claret et al., 2009; Frances et al., 2011; Simeoni et al., 2004; Wang et al., 2009). The model providing the best description of the data regardless of the type of cancer cell lines was the model published by Hahnfeldt (Hahnfeldt, 1999), in which nutrient supply represents a key
element of the model structure. The model is described mathematically by the following two ordinary differential equations:

\[
\frac{dT}{dt} = -\lambda_1 \times T \times \log(T/K) \quad (2)
\]

\[
\frac{dK}{dt} = -\lambda_2 \times K + B \times T - D \times K \times T^2 \quad (3)
\]

Where T and K represent the tumour volume and the carrying capacity, the latter representing nutrient supply. \(\lambda_1\) and \(\lambda_2\) are the first order rate constants of tumour proliferation and carrying capacity degradation. The parameters B and D account for the stimulatory and negative feedback mechanisms of tumour volume on the dynamics of the carrying capacity.

**Drug effect model**

**Gemcitabine**

The selected model describing gemcitabine response consists of two different effects: (i) \(E_{\text{Gem1}}\), promoting apoptosis within the tumour, and (ii) \(E_{\text{Gem2}}\), decreasing the source of nutrients.

\(E_{\text{Gem1}}\) was found to be delayed compared to the predicted typical time course of gemcitabine in plasma (\(C_{p_{\text{Gem}}}\)). Delayed response was characterised by a chain of three transit compartments as described by the next set of differential equations, where \(TR_{1-3}\) are the predicted concentration/active signal in each of the transit compartments. \(K_{TR}\) is the first order rate constant of transfer between transit compartments.

\[
\frac{dTR_1}{dt} = K_{TR} \times (C_{p_{\text{Gem}}} - TR_1) \quad (4)
\]

\[
\frac{dTR_2}{dt} = K_{TR} \times (TR_1 - TR_2) \quad (5)
\]

\[
\frac{dTR_3}{dt} = K_{TR} \times (TR_2 - TR_3) \quad (6)
\]

\(E_{\text{Gem1}}\) has the form of \(f(TR_3)\), where \(f()\) represents linear or non-linear expressions of \(TR_3\), and was incorporated into the tumour model as:

\[
\frac{dT}{dt} = -\lambda_1 \times T \times \log(T/K) - E_{\text{Gem1}} \times T \quad (7)
\]

The second mechanism of action, represented by \(E_{\text{Gem2}}\), could be linked directly to the predicted \(C_{p_{\text{Gem}}}\) using a model of the form \(g(C_{p_{\text{Gem}}})\), where \(g()\) represents a linear or non-linear expressions of \(C_{p_{\text{Gem}}}\). \(E_{\text{Gem2}}\) was incorporated into the tumour model as:
\[
\frac{dK}{dt} = -\lambda_2 \times K + B \times T - D \times K \times T^2 - E_{\text{Gem}} \times K
\]

(8)

**Gemcitabine and carboplatin**

The model for combination treatment incorporates the effect of carboplatin (\(E_{\text{Carbo}}\)), through a decrease in the nutrient supply, therefore impairing tumour progression, as indicated in equation 9:

\[
\frac{dK}{dt} = -\lambda_2 \times K + B \times T - D \times K \times T^2 - (E_{\text{Gem}} + E_{\text{Carbo}}) \times K
\]

(9)

Where \(E_{\text{Carbo}}\) has the general form of \(h(Cp_{\text{Carbo}})\), where \(Cp_{\text{Carbo}}\) is the predicted carboplatin concentration in plasma, and \(h()\) represents a linear or non-linear expression of \(Cp_{\text{Carbo}}\).

The initial conditions for the tumour volume (\(T_0\)) and carrying capacity (\(K_0\)) compartments were estimated during the modelling analysis, while the corresponding values of \(TR_{1-3}\) were set to 0. The model is represented schematically in figure 2 using the example of ovarian cancer, including the combination therapy.

**Model selection**

Selection between models was mainly based on the minimum value of the objective function (MOFV) provided by NONMEM which is approximately equal to \(-2 \times \log (\text{likelihood})\) (-2LL).

On the other hand, non-nested models were compared using the Akaike Information Criteria (AIC) (Ludden et al., 1994) calculated as \(-2LL + 2 \times NP\), where NP is the number of parameters in the model. The model with the lowest value of AIC, given the precision of model parameters and an adequate description of the data, was selected.

It was also made through a visual exploratory analysis of the goodness of fit plots, and the meaningfulness of the parameter estimates, as well as their precision.

**Model evaluation**

Simulation-based diagnostics were used to evaluate model performance. Prediction corrected visual predictive checks (pc-Vpc) (Bergstrand et al., 2011) were performed as follows: For each tumour cell line, five hundred datasets of the same characteristics of the original one were simulated using the selected model and its parameter estimates. Then for each simulated dataset the 2.5th, 50th and 97.5th of the tumour volume were calculated per
time interval, binning by count automatically with PsN with 5 or 6 bins depending on the cell line, and the 95 % prediction intervals of the calculated percentiles were superimposed onto the 2.5\textsuperscript{th}, 50\textsuperscript{th}, and 97.5\textsuperscript{th} raw data percentiles. In addition, the precision of the parameter estimates was evaluated from the analysis of 500 simulated bootstrap data sets for each tumour cell line (Efron and Tibshirani, 1993).

Model exploration

As it is described in the results section, the PKPD models selected showed a certain degree of complexity. It is therefore difficult to visualize how model elements and characteristics interact based just on the model equations and parameter estimates. Deterministic simulations were performed to provide graphical information that allows a better interpretation and understanding of the modelling results. The doses and dosing regimens chosen in the simulations were part of those used during the experiments. These simulations were performed with the software Berkeley Madonna (version 8.3.18) (Macey and Oster, 2010).

The software Perl-speaks-Nonmem (PsN v4.48) (Lindbom et al., 2004) and Xpose v.4.53 (Jonsson and Karlsson, 1998) were used to perform the required calculations for the bootstrap analysis, prediction-corrected visual predictive checks and graphical representation.
Results

General description of the data

Figure 1 shows the raw tumour dynamic data used during the current evaluation. For each of the four pancreas tumour cell lines tested, treatment with gemcitabine slowed down tumour progression with respect to untreated groups. A more pronounced response to treatment was observed in KP4 and PANC1 cell lines compared to ASPC1 and MIA PACA2 cell lines. For the case of the ovarian xenografts, similar results could be observed when gemcitabine was administered as single agent. In combination with carboplatin, treatment effects appear to be augmented and tumour shrinkage becomes more apparent. For the SKOVx3luc ovarian tumour cell lines, data from two experiments were pooled. As can be seen in the corresponding panel in figure 1, the tumour growth dynamics differed quite a lot between the two experiments. Additional data characteristics relevant for the model building process are the (i) high degree of variability between individual profiles, (ii) delayed onset of tumour response with respect to time of dosing, and (iii) oscillatory profiles, with pronounced decrease in tumour growth at initial time after cell inoculation. That phenomenon was especially noticeable, in the PANC1 tumour cell line.

Modelling tumour profiles

Model for tumour progression model

From the numerous alternatives available to describe tumour volume data in the absence of treatment (Claret et al., 2009; Frances et al., 2011; Simeoni et al., 2004; Wang et al., 2009), a reduced version of the model proposed by Hahnfeldt (Hahnfeldt, 1999) provided the lowest value of AIC (see Table I) and the best data description for the six different tumour cell lines.

The $\lambda_2$ parameter describing the first order rate constant of the degradation of K could not be estimated with precision from any control group and was therefore removed from the model with negligible effects on -2LL. The same happened with the D parameter describing the negative feedback of the tumour volume profile on the dynamics of K for the MIA PACA2 and PANC1 tumour cell lines. Table II lists the estimates of the model parameters and their
corresponding precision represented by the 95% confidence intervals computed from the 
bootstrap analysis.

Typical estimates of parameters were in general of the same order of magnitude among the 
different cell lines, with the exception of $K_0$ which ranged from $3 \times 10^{-5}$ (PANC1) to $1.16 \times 10^2$ 
(SKOV3xluc). The covariate ‘study’ was tested for significant effects on all the model 
parameters and was found to be significant ($p < 0.001$) for the SKOV3xluc ovarian tumour 
cell line on the $T_0$ and $\lambda_1$ parameters (see table I).

Data supported the estimation of inter-animal variability on (i) $\lambda_1$ for all the six tumour cell 
lines with a magnitude ranging from 31 to 79% (MIA PACA2), and (ii) $T_0$ and $K_0$ for just the 
four pancreas tumour cell lines, where the magnitude was low-moderate except for MIA 
PACA2 (44%, $T_0$; 85% $K_0$). For the rest of parameters the incorporation of inter-animal 
variability did not reduce the value of -2LL significantly ($p > 0.05$).

Models for drug effects

Once the model for tumour progression was established, model building was focused on the 
following main aspects: (i) selection of the mechanism(s) of action, (ii) characterisation of the 
onset of drug action and (iii) description of the concentration effect relationship. Table II lists 
the estimates of the parameters (and their precision) obtained from the selected model (see 
figure 2) describing drug effects as single agent or as combination therapy.

Single agent

Data were best described considering two different effects of gemcitabine: promoting tumour 
shrinkage through an apoptosis mechanism and decreasing the carrying capacity. By 
including both types of drug effects, the AIC value was significantly reduced (Table I). The 
two mechanisms of action differed on the time of onset; whereas the effect of the carrying 
capacity was linked directly with the change in drug exposure, apoptotic response appeared 
to be delayed with respect to dosing. The mean transit time calculated as $(n+1)/K_{TR}$, where $n$ 
is the number of transit compartments, varied from 0.8 (KP4) to 7 (SKOV3xluc) days. The 
exposure effect relationship was best characterised with a linear model for the case of all 
pancreas tumour cell lines, which showed a sensitivity represented by the $\theta_{SLOPE}$ parameter.
within the same range and between $3.26 \times 10^{-2}$ and $4.2 \times 10^{-1}$ L.mg$^{-1}$.days$^{-1}$. As it was mentioned before, the same value of the slope parameter was estimated to describe both types of effects.

The $E_{\text{MAX}}$ model was selected to describe the apoptotic effects in the case for the two ovarian tumour cell lines; despite the same model structure, parameter estimates showed high difference between the two cell lines with regard to both efficacy ($E_{\text{MAX}}$) and potency ($C_{50}$). However the ratio between $E_{\text{MAX}}$ and $C_{50}$ was similar between the A2780 and SKOV3xluc cell lines (8.65 vs 5.25 L.mg$^{-1}$.day$^{-1}$, respectively), and higher than any of the slope parameters estimated from the pancreas xenografts. This last result is clearly seen in the raw data represented in figure 1 where higher tumour effects are shown for ovarian xenografts. The effect on the carrying capacity was described with a linear model and appears to be lower than the apoptotic effect and of the same range as in the case of pancreas tumours.

Inter-animal variability in drug effect parameters could only be quantified for the case of pancreas xenografts on the parameters $K_{\text{TR}}$ and SLOPE, showing low to moderate magnitude, with the exception of $K_{\text{TR}}$ from MIA PACA2 (90%).

**Combination**

Different alternatives were explored to characterise the contribution of carboplatin to tumour shrinkage in ovarian xenografts. Carboplatin showed significant model improvement ($p<0.001$) when it was incorporated into the apoptotic (only for A2780 cell line) or the inhibitory carrying capacity effects; however it was not possible to keep both carboplatin effects in the model; and the effect on the carrying capacity was preferred based on the visual exploration of the goodness of fit plots (not shown) and on the reduction of the AIC (Table I). The effect of carboplatin was greater for the A2780 than for SKOV3xluc, and in general of the same magnitude that gemcitabine showed on the same mechanism of action. Inter-animal variability could not be estimated for the effects of carboplatin.
Individual model predicted profiles are displayed in figure 3, showing that for several mice chosen at random from the different control groups, the tumour progression and tumour response model performed very well at the individual level for every dosing group.

Figure 4A, B, C shows the results from the prediction corrected visual predictive checks where the full model selected describes the general tendency and the dispersion of the data quite well for the six tumour cell lines studied both for the unperturbed tumour growth profile as well as the effects of gemcitabine administered as single agent and in combination with carboplatin.

**Model exploration**

The results from the current modelling exercise indicate that (i) tumor growth depends on a self-proliferation mechanism and a carrying capacity, (ii) gemcitabine exerts its drug action through two mechanisms of action, (iii) there is a contribution of carboplatin to the tumor shrinkage elicited by gemcitabine, (iv) drug effects appear to be exposure-dependent, and (v) different cell lines shows different degree of sensitivity to gemcitabine and carboplatin effects. Deterministic simulations were performed to provide a better interpretation and understanding of the modelling results. The profiles that resulted from this simulation exercise are displayed in figures 5 and 6. Pancreatic cell lines show similar drug effects on tumour size and carrying capacity except for MIA PACA2 cell line for which higher drug effect on carrying capacity was seen. For the case of the ovarian cell lines, the apoptosis-related mechanism of action is greater than the action exerted on carrying capacity.

The typical simulated profiles of the control groups that appear in each of the panels in figures 5 and 6 represent the profiles corresponding to the reduced version of the Hahnfeldt model with $\lambda_2$ fix to 0 (all cell lines) and D fix to 0 in the case of MIA PACA2 and PANC1 cell lines.
Discussion

In this analysis, a semi-mechanistic population PKPD model for the tumour shrinkage effects of gemcitabine given as a single agent or in combination on human xenografts was developed showing consistency in its structure across different tumour cell lines representing two tumour types where gemcitabine treatment is approved. Simulation-based evaluation diagnostics showed that the model performed well in all the different design scenarios including dose, schedule, and tumour type.

Several mechanisms have been reported to describe tumour response to treatment with gemcitabine. Its main effect is apoptotic caused by the integration in the DNA of the malignant cell, eliciting masked chain termination and anti-proliferative activity as a consequence of DNA synthesis inhibition (Mini et al., 2006). In the same work, Mini et al described several pharmacodynamic activities of the pro-drug decreasing the competing natural metabolite pools necessary for DNA synthesis by mechanisms of self-potentiation. Those self-potentiation mechanisms in which gemcitabine inhibits different enzymes resulting in the decrease of cellular deoxynucleotides (metabolite pools), have also been reported in several publications (de Sousa Cavalcante and Monteiro, 2014; Gesto et al., 2012; Plunkett et al., 1996). That composite mechanism of action has been captured in the model selected in the current analysis through the $E_{Gem1}$ and $E_{Gem2}$ effects represented in equation 7 & 8, and associated to apoptosis by masked chain termination and self-potentiation, respectively. The two mechanisms could be identified and integrated into the model, since interestingly, the dynamics shown by all control groups regardless of the type of cell lines quantified two different but interconnected processes implicated in tumour growth: the tumour proliferation, and the nutrient supply or metabolite pools required for tumour growth represented by the ‘carrying capacity’ compartment.

The relationship between the carrying capacity and tumour mass allows to describe the initial decrease or slow tumour growth detected in most of the individual profiles (figure 5) which might be explained by an initial lack of metabolite pool required for DNA synthesis. For cell lines with a $K_0$ estimate lower than $T_0$, the initial behaviour of tumour growth will be
regressive (KP4, ASPC1 and PANC1 cell lines, see figure 5). Once the carrying capacity reaches tumour volume, the vasculature will be optimal to provide nutrients to allow the tumour to grow.

Estimates of the parameters governing tumour progression are within the range of those published in literature (Ouerdani et al., 2015). In this work, Ouerdani and co-authors reported values for $\lambda_1$ of 0.166 days$^{-1}$ (with IIV of 53%), $K_0$ of 543 mm$^3$ (IIV= 36%) and $B$ parameter of 0.0183 days$^{-1}$.

From the parameter estimates listed in table 1 and the profiles shown in figures 5 and 6 it can also be appreciated that ASPC1 and MIA PACA2 cell lines are less sensitive to gemcitabine treatment, in accordance with recent previous results (Prasad et al., 2016). Those differences in drug efficacy are explained by cell-type specific genetic and phenotypic characteristics (Deer et al., 2010; Mitra et al., 2012). For example, as Deer et al indicated in their work, ASPC1, MIA PACA2 and PANC1 cell lines present different mutations in KRAS, TP53, CDKN2A and SMAD4 genes, mutations that might contribute to the different growth characteristics, tumourigenicity and chemosensitivity (Deer et al., 2010).

Figure 6 explores the contribution of the two mechanisms of action to the tumour drug effects. In all cases as expected the apoptotic effect showed greater contribution, although for the case of ASPC1 and MIA PACA2 the differences were marginal.

The current model was developed using gemcitabine pharmacokinetics after a scale-down exercise from the pharmacokinetic model developed in humans (Zhang et al., 2006). In their clinical study performed in non-small cell lung cancer patients, Tham et al., 2008 also linked gemcitabine drug effects with its concentration levels in plasma, as well as in the rest of gemcitabine models that are mentioned below (Hamed et al., 2013; Miao et al., 2016; Yuan et al., 2015; Zhu et al., 2015). However, the active metabolite, dFdCTP, is the main agent responsible for tumour effects after gemcitabine administration, presenting also different cellular pharmacokinetics among cell types. In order to capture the process of the active metabolite development, a signal transduction model, represented by transit compartments between the plasmatic and the effective concentration, was included in this work.
characterizing different $K_{TR}$ between cell lines. Therefore, this effective concentration ($TR_{3}$) responsible of driven the cytotoxic effect in the model, would represent the active metabolite.

The PKPD characteristics of gemcitabine have been studied to some extent in vitro and in in vivo pre-clinical experiments. In vitro models developed on pancreatic cells estimated two types of effects for gemcitabine, inhibiting cell cycle progression and inducing apoptosis (Hamed et al., 2013; Miao et al., 2016; Zhu et al., 2015). Those analyses were performed at concentrations of gemcitabine ranging from 0 to 100 mg/L while in the present work, the simulated concentrations of gemcitabine range from 50 to 700 mg/L. In those in vitro experiments, drug combinations were also considered; the interactions of gemcitabine with trabectecin and birinapant were described as synergistic. The model presented in this work is able to reproduce in vivo the two different effects for gemcitabine described in vitro, albeit with a different model structure. Yuan et al developed a semi-mechanistic PKPD model for gemcitabine in combination with dexametasone in breast cancer in mice (Yuan et al., 2015). They could identify a single mechanism of action for the case of gemcitabine, and reported pharmacodynamic parameters that were in accordance with those found in our study for the A2780 ovarian xenograft ($K_{max}=3.23 \text{ days}^{-1}$, $K_{C50}=0.176 \mu g/mL$).

With respect to clinical studies, Wendling and co-authors have recently characterised the change in tumour size in patients with pancreatic cancer treated with gemcitabine (Wendling et al., 2016), linking tumour size with overall survival. However, the model establishing the relationship between exposure to gemcitabine and tumour effects was not developed, making therefore the comparison with our results difficult. Tham et al have investigated the pharmacodynamics of gemcitabine given in combination with carboplatin in NSCL cancer patients (Tham et al., 2008), and were not able to discriminate between the effect of both drugs, resulting in the use of a gemcitabine dose-driven model to describe tumour shrinkage.

In addition, Tate et al have successfully characterised the tumour growth inhibitory effects of gemcitabine in combination with paclitaxel in metastatic breast cancer (Tate C et al., 2013). Finally, Zecchin et al have also studied the change in tumour size in patients with metastatic ovarian cancer treated with carboplatin or carboplatin plus gemcitabine (Zecchin et al.,...
2016). In their work, both drugs had additive effects (driven by drug exposure) promoting apoptosis, making the tumour more sensitive to gemcitabine effects. Those results are also supported by the current model as in Figure 6, showing that contribution of carboplatin to tumour effects is lower than the response exerted by gemcitabine. However, it has to be taken into account that mouse data receiving carboplatin alone were not available.

We do believe this study provides a robust preclinical PKPD model that could be used in future analysis in terms of translational approaches. Pharmacodynamic parameters tend to be independent between species (Mager et al., 2009), and that line, Wong and colleagues (Wong et al., 2012) demonstrated the predictive value of xenograft/allograft PKPD models, relating simulated %TGI in mice and overall response from clinical trials.

We have already performed the first translational attempts following the same methodology as Wong and co-authors, relating simulated tumour growth inhibition with patients overall survival (Garcia-Cremades et al., 2016). Although further analyses are currently on-going, those primary results are encouraging, and outline the benefit of properly characterized preclinical data under the population PKPD framework.

In summary, a comprehensive semi-mechanistic model to account for pancreas and ovarian tumour progression in mice as well as the pharmacodynamic effects of gemcitabine alone and in combination has been developed under different in vivo experimental conditions. This work contributes timely to the recent, but still scarce, quantitative information available on gemcitabine, with a robust and consistent model across cell lines and tumour types, and is expected to be useful for the translational approach and in predicting tumour responses in humans from xenograft models as shown by others (Wong et al., 2012).
Acknowledgments

The authors would like to thank Sonya Tate for providing assistance during the writing process and Sophie Callies for sharing the pharmacokinetic model for gemcitabine.
Authorship Contributions

Performed data analysis: Garcia-Cremades, Pitou, Iversen, Troconiz

Wrote or contributed to the writing of the manuscript: Garcia-Cremades, Pitou, Iversen, Troconiz
References


Macey RI and Oster GF. (2010) Berkeley madonna, version 8.3.18, in (University of California ed).


Footnotes

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IFT is an employee of the University of Navarra and MG-C is a Phd student from University of Navarra. CP and PWI are employees of Eli Lilly and Company.
Figure legends

Figure 1. Data summary. In plots, points represent individual tumour growth profile. Each profile corresponds to the dosing group with the same colour. Lines represent the median tumour growth profile by dosing group. Gem=Gemcitabine. Carbo=Carboplatin, d=days of drug administration. TCB=Tumour Cell Burden, n=number of mice.

Figure 2. Schematic representation of the tumour growth inhibition model for ovarian after treatment with Gemcitabine and/or Gemcitabine plus Carboplatin. The yellow compartments correspond to the pharmacokinetics of gemcitabine, being \( Cp_{\text{Gem}} \) the plasmatic concentration of gemcitabine in the central compartment and \( P_2 \) and \( P_3 \) the concentration of gemcitabine in the peripheral compartments. The blue compartment, \( Cp_{\text{Carbo}} \) corresponds to the plasmatic concentration of Carboplatin. \( K \) represents the carrying capacity or vasculature. \( TR_1, TR_2 \) and \( TR_3 \) are the transit compartment between Gemcitabine plasma concentration and effective concentration. \( E_{\text{Gem1}} \) and \( E_{\text{Gem2}} \) correspond to the effects exerted by Gemcitabine on the tumour (1) and on the vasculature (2) respectively. \( E_{\text{Carbo}} \) represents the effect driven by Carboplatin on the carrying capacity. A description of the rest of the parameters can be found on the Supplemental Table 1 and under Material and Methods.

Figure 3. Evaluation of model performance at the individual level. Tumour size observation (points) and individual model predictions (lines) for different mice receiving different doses schedules by cell line. ID= mouse identification per cell line. G= Gemcitabine. C=Carboplatin

Figure 4. Prediction corrected visual predictive check (Pc-vpc) per cell line. (A) Pc-vpc for pancreas cell lines for control groups (upper panels) and for the groups receiving Gemcitabine (lower panels). (B) Pc-vpc for ovarian cell lines for control group (upper panels) and for the groups treated with Gemcitabine (lower panels). (C) Pc-vpc for ovarian cell lines receiving Gemcitabine in combination with carboplatin. In the plots, solid lines represent the 50\(^{th}\) percentile of the observed data while dashed lines correspond to the 2.5\(^{th}\) and 97.5\(^{th}\)
percentile of the observed data. Shaded areas are the 95% confidence intervals based on simulated data (n = 500) for the corresponding percentiles.

**Figure 5. Typical simulated profiles of tumour volume and carrying capacity under different scenarios per cell line.** Ln tumour volume versus time is represented by the continuous line (left axis) whereas the carrying capacity profiles correspond to the discontinuous line (right axis). For all the cell lines, simulations of unperturbed growth of tumour volume and carrying capacity (dark grey lines) and after receiving 30 mg/kg (green profiles) or 80 mg/kg (red profiles) on days 18, 21, 24 and 27 after randomisation are represented. For ovarian cell lines, tumour volume and carrying capacity profiles, are also simulated under the effect of the combination therapy of Gemcitabine 50 mg/kg plus Carboplatin 10 mg/kg (blue lines) or 50 mg/kg (yellow lines) given on days 18, 25 and 32.

**Figure 6. Contribution of the different Gemcitabine mechanisms of actions on tumour growth inhibition by cell line.** Tumour growth profile was simulated after i.p administration of 80 mg/kg of gemcitabine on days 18, 21, 24 and 27 for the six tumour cell lines and plus i.p carboplatin 50 mg/kg administration, given at the same schedule, in ovarian xenografts. As an example, in KP4 xenograft, the grey line represents the typical unperturbed tumour growth. The green profile represents tumour profile after gemcitabine treatment. The yellow line is the same gemcitabine effect only driven by the apoptotic mechanism and the red profile only accounts for the effect under the carrying capacity. In ovarian xenografts, for gemcitabine alone is the same as in pancreas, being the blue line the tumour profile after treatment with the combination therapy. Tumour profile only driven by carboplatin effect under the carrying capacity is represented in purple.
Table I. Summary of model development based on AIC values

<table>
<thead>
<tr>
<th>Description</th>
<th>KP4</th>
<th>ASPC1</th>
<th>MIA PACA2</th>
<th>PANCl</th>
<th>SKOV3xLuc</th>
<th>A2780</th>
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<tr>
<td><strong>Disease progression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simeoni</td>
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<td>-718.093</td>
<td>-256.786</td>
<td>-282.99</td>
<td>-163.86</td>
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<td>-</td>
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<td>416.606</td>
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<tr>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Gemcitabine effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptotic effect on tumour volume</td>
<td>-625.927</td>
<td>-234.289</td>
<td>-1232.49</td>
<td>-527.143</td>
<td>-480.371</td>
<td>345.295</td>
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<tr>
<td>+ Effect on carrying capacity</td>
<td>-632.2</td>
<td>-250.761</td>
<td>-1297.358</td>
<td>-567.945</td>
<td>-570.865</td>
<td>211.292</td>
</tr>
<tr>
<td>+ EMAX model on apoptotic effect</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-927.887</td>
<td>-77.724</td>
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<td><strong>Carboplatin effects</strong></td>
<td></td>
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<td></td>
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<tr>
<td>No Carboplatin effect (all driven by</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-585.679</td>
<td>158.691</td>
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<td>gemcitabine)</td>
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<tr>
<td>Apoptotic effect on tumour volume</td>
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<td>-</td>
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<td>-583.63</td>
<td>-110.775</td>
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<tr>
<td>Effect on carrying capacity</td>
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<td>-</td>
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<td>-594.739</td>
<td>-346.857</td>
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Table II. Population model parameter estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KP4</th>
<th>ASPC1</th>
<th>MIA PACA2</th>
<th>PANC1</th>
<th>SKOV3xLuc</th>
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<tr>
<td>Disease progression</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>$T_{c}$ (mm$^{-1}$)</td>
<td>8.73x10$^{-1}$</td>
<td>[2.9-2.9] x10$^{-1}$</td>
<td>7.47x10$^{-1}$</td>
<td>[6.0-8.7] x10$^{-1}$</td>
<td>7.4x10$^{-1}$</td>
</tr>
<tr>
<td>$K_{p}$ (mm$^{-1}$)</td>
<td>1.13x10$^{2}$</td>
<td>[0.0001-1.6] x10$^{-1}$</td>
<td>3.86x10$^{-5}$</td>
<td>[0.1-5.3] x10$^{-5}$</td>
<td>4.91x10$^{-6}$</td>
</tr>
<tr>
<td>$\lambda_{i}$ (day$^{-1}$)</td>
<td>1.48x10$^{-1}$</td>
<td>[0.1-1.9] x10$^{-1}$</td>
<td>1.82x10$^{-3}$</td>
<td>[0.4-4.6] x10$^{-3}$</td>
<td>3.45x10$^{-3}$</td>
</tr>
<tr>
<td>$B$ (day$^{-1}$)</td>
<td>7.73x10$^{-1}$</td>
<td>[5.2-9.2] x10$^{-1}$</td>
<td>2.98x10$^{-1}$</td>
<td>[0.2-1.1] x10$^{-1}$</td>
<td>4.17x10$^{-2}$</td>
</tr>
<tr>
<td>$D$ (day$^{-1}$ mm$^{-2}$)</td>
<td>1.2x10$^{3}$</td>
<td>[0.6-1.6] x10$^{3}$</td>
<td>3.1x10$^{3}$</td>
<td>[1.5-5.1] x10$^{3}$</td>
<td>0*</td>
</tr>
<tr>
<td>$\lambda_{d}$ (day$^{-1}$)</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>IVV $\lambda_{c}$ (%)</td>
<td>31 (0.3%)</td>
<td>[21-40]</td>
<td>35 (3.6%)</td>
<td>[11-78]</td>
<td>79 (17.2%)</td>
</tr>
<tr>
<td>IVV $T_{c}$ (%)</td>
<td>9 (31.9%)</td>
<td>[1-12]</td>
<td>15 (18.4%)</td>
<td>[1-22]</td>
<td>44 (3.2%)</td>
</tr>
<tr>
<td>IVV $K_{p}$ (%)</td>
<td>14 (100%)</td>
<td>[1-90]</td>
<td>29 (40.6%)</td>
<td>[1-39]</td>
<td>85 (28.1%)</td>
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<tr>
<td>Gemcitabine effect</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$K_{m}$ (day$^{-1}$)</td>
<td>5.02</td>
<td>[3.4-5.1]</td>
<td>1.8x10$^{-1}$</td>
<td>[1.5-1.9] x10$^{-1}$</td>
<td>1.93x10$^{-1}$</td>
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<tr>
<td>SLOPE$_{P}$ (L/mg·day)</td>
<td>4.2x10$^{-1}$</td>
<td>[1.6-4] x10$^{-1}$</td>
<td>1.86x10$^{-1}$</td>
<td>[0.4-3.3] x10$^{-1}$</td>
<td>3.26x10$^{-1}$</td>
</tr>
<tr>
<td>Emax (day$^{-1}$)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ce50 (mg/L)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SLOPE$_{P}$ (L/mg·day)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IVV $K_{m}$ (%)</td>
<td>19 (97.5%)</td>
<td>[30-60]</td>
<td>10 (55.4%)</td>
<td>[1-11]</td>
<td>90 (80%)</td>
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<tr>
<td>IVV SLOPE$_{P}$ (%)</td>
<td>28 (35.8%)</td>
<td>[28-90]</td>
<td>45 (43.8%)</td>
<td>[36-300]</td>
<td>28 (49.7%)</td>
</tr>
<tr>
<td>Carboptin effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SLOPE$_{P}$ (L/mg·day)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Residual error</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$W$ (log(mm$^{-1}$))</td>
<td>1.73x10$^{-1}$</td>
<td>[1.7-2.1] x10$^{-1}$</td>
<td>1.49x10$^{-1}$</td>
<td>[1.5-2.3] x10$^{-1}$</td>
<td>1.81x10$^{-1}$</td>
</tr>
</tbody>
</table>

Shrinkage % is indicated in (). *Parameter fixed. SLOPEp corresponds to gemcitabine effect constant in pancreas cancer that drives both mechanisms; under tumour volume and under carrying capacity. Emax and Ce50 are the maximum effect and the potency of gemcitabine responsible of promoting the apoptosis mechanism in ovarian cancer. SLOPEp is gemcitabine effect constant that drives the effect under the carrying capacity in ovarian cancer. SLOPEp is carboplatin effect constant under the carrying capacity.
in ovarian cancer. IIV represents the interindividual variability of the indicated parameter. A description of rest of the parameters can be found on the Supplemental Table 1 and under Material and Methods.
<table>
<thead>
<tr>
<th>CANCER</th>
<th>PANCREAS</th>
<th>OVARIAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>CELL LINE</td>
<td>KP4</td>
<td>ASPC1</td>
</tr>
<tr>
<td>TCB</td>
<td>5x10⁰</td>
<td>5x10⁰</td>
</tr>
<tr>
<td>N° STUDIES</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>N° MICE</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>MICE STRAIN</td>
<td>Athymic nude</td>
<td>Athymic nude</td>
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<tr>
<td>MICE GENDER</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>RAW DATA</td>
<td>Log Tumour volume (mm&lt;sup&gt;3&lt;/sup&gt;) vs Time (days)</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1**
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Characterising Gemcitabine effects administered as single agent or combined with carboplatin in mice pancreatic and ovarian cancer xenografts. A semi-mechanistic pharmacokinetic/pharmacodynamics tumour growth-response model

Maria Garcia-Cremades, Celine Pitou, Philip W Iversen, Iñaki F Troconiz

Journal of Pharmacology and Experimental Therapeutics

Supplemental Table 1

Description of the PKPD model parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease progression</strong></td>
<td></td>
</tr>
<tr>
<td>$T_0$ (mm$^3$)</td>
<td>Tumour volume at baseline.</td>
</tr>
<tr>
<td>$K_0$ (mm$^3$)</td>
<td>Carrying capacity at baseline</td>
</tr>
<tr>
<td>$\lambda_1$ (day$^{-1}$)</td>
<td>First order rate constant of tumour proliferation</td>
</tr>
<tr>
<td>$B$ (day$^{-1}$)</td>
<td>Drives the stimulatory feedback mechanism of tumour volume on the dynamics of carrying capacity</td>
</tr>
<tr>
<td>$D$ (day$^{-1}$ mm$^{-2/3}$)</td>
<td>Drives the negative feedback mechanism of tumour volume on the dynamics of carrying capacity</td>
</tr>
<tr>
<td>$\lambda_2$ (day$^{-1}$)</td>
<td>First order rate constant for the spontaneous loss of the carrying capacity.</td>
</tr>
<tr>
<td><strong>Gemcitabine effect</strong></td>
<td></td>
</tr>
<tr>
<td>$K_{TR}$ (day$^{-1}$)</td>
<td>First order rate constant of transfer between transit compartments.</td>
</tr>
<tr>
<td>$\text{SLOPE}_p$ (L/mg·day)</td>
<td>Gemcitabine effect constant in pancreas cancer that drives both mechanisms; under tumour volume and under carrying capacity.</td>
</tr>
<tr>
<td>$E_{max}$ (day$^{-1}$)</td>
<td>Maximum effect of gemcitabine responsible of promoting the apoptosis mechanism in ovarian cancer.</td>
</tr>
<tr>
<td>$Ce_{50}$ (mg/L)</td>
<td>Potency of gemcitabine responsible of promoting the apoptosis mechanism in ovarian cancer.</td>
</tr>
<tr>
<td>$\text{SLOPE}_o$ (L/mg·day)</td>
<td>Gemcitabine effect constant that drives the effect under the carrying capacity in ovarian cancer.</td>
</tr>
<tr>
<td><strong>Carboplatin effect</strong></td>
<td></td>
</tr>
<tr>
<td>$\text{SLOPE}_c$ (L/mg·day)</td>
<td>Carboplatin effect constant under the carrying capacity in ovarian cancer</td>
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