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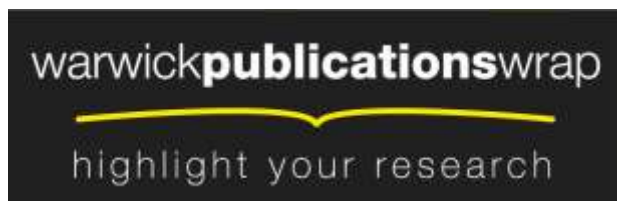
Modelling of tumour growth and cytotoxic effect of docetaxel in xenografts

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Abstract

One of the major sources of information on physiological and pathophysiological effects in pre-clinical oncology studies is the xenografted tumour animal model. However, measurement of tumour volume over time potentially masks a range of biological changes that the xenograft is undergoing. In this paper a mechanistic model of tumour growth in xenografts is presented that can be used to investigate the mode of drug action with respect to phenotypic changes. The model encapsulates key histological biomarkers and spatial constraints. The unknown model parameters are first shown to be uniquely identifiable from the proposed experimental studies, and then estimated from the resulting data using the anti-cancer agent docetaxel.

Keywords: Biomedical systems, structural identifiability, parameter estimation, xenograft tumour models, tumour growth models, cytotoxic agents

1. Introduction

Quantitative pharmacology techniques are used to understand the various steps between dosing a drug and changes to the pathophysiology of the disease ("efficacy"). Pre-clinically, in oncology, one of the major sources of information on physiological and pathophysiological effects is the xenografted tumour animal model, in which a human tumour is implanted into a host

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animal. Such models are now being supplemented by the primary tumour tissue explant animal model. Measurement of tumour volume over time potentially masks a range of biological changes that the xenograft is undergoing as a result of tumour cell biology, host animal and anti-cancer agent effects. Application of different modelling approaches are therefore required to decode these signals. Thus in this paper a mechanistic model of tumour growth in xenografts is presented that can be used to investigate the mode of drug action with respect to phenotypic changes.

Currently there are two principal approaches for modelling tumour growth: Empirical models that seek to represent data from tumour growth studies without attempting to elucidate the mechanisms involved [1]; and mechanistic models that attempt to reproduce tumour growth data [2]. The mechanistic tumour modelling literature contains many models that are so mechanistically complete that they are too complex for parameter identification and hence for robust prediction. However, even relatively complex mechanistic models have limitations resulting from simplifying assumptions. For example, models that acknowledge spatial and rheostatic effects typically assume that tumours are spherical and homogeneous. In order to cope with the challenges of modelling tumour growth in a heterogeneous environment some authors have adopted more computational approaches like cellular automata [3].

The approach adopted in this paper is to combine simple empirical models with focused mechanistic modelling of pathways important for elucidating the steps between dosing and efficacy, which will combine the computational benefits of empirical modelling with the knowledge/understanding benefits of mechanistic modelling. A model for solid tumour growth is proposed and considered with respect to treatment with docetaxel, a semisynthetic taxane analogue, which is a mitotic spindle poison that promotes microtubule assembly and inhibits depolymerisation to free tubulin [4, 5].

Few tumour growth modelling studies consider the problem of structural identifiability that relates to the uniqueness of model parameters with respect to model simulations that are to be compared with experimental data [6]. For example, it may be possible for different combinations of parameters to give rise to identical simulations and thus identical fits to data, but entirely different predictions of unmeasured variables. This property has important implications for elucidating the exact effect a drug is having on the tumour biology and for the interpretation of combination data.

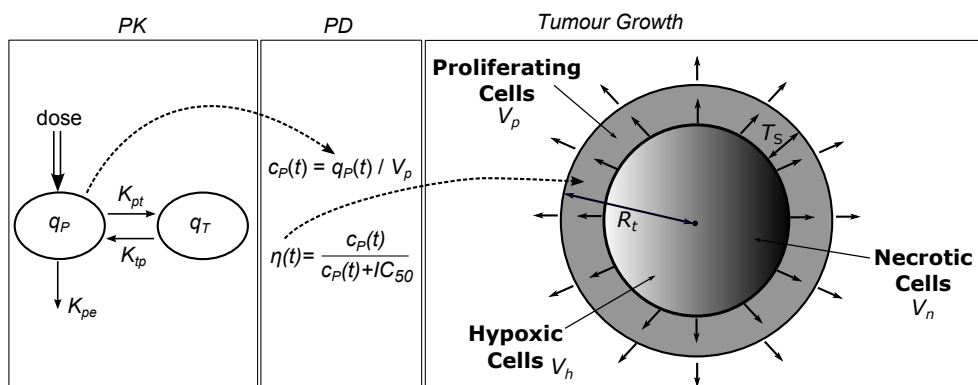There are a number of well-established techniques for performing a struc-

Figure 1: Schematic of full model for cytotoxicity of docetaxel comprising agent pharmacokinetics (PK) and pharmacodynamics (PD), coupled with tumour growth dynamics.

tural identifiability analysis for linear systems (see, for example, the tutorial paper by Godfrey and DiStefano III [7] and other works in the same volume). In comparison, greater care has to be taken over choice of approach for nonlinear systems since the form of any inputs to the system generally limits applicable techniques. For systems with a single impulsive input, such as a bolus injection of drug, perhaps the conceptually simplest approach is the Taylor series approach [8]. This approach considers uniqueness of the parameters with respect to the uniqueness of the coefficients of a Taylor series expansion (generally about $t = 0$) of the output. More recently there has been considerable effort applying techniques based on differential algebra [9–11]. A related algebraic approach to determine a canonical output equation for a rational system, the uniqueness of which can then be used to determine identifiability of the parameters, will be applied in this paper [12].

2. Combined Model of Cytotoxic Effect

A model of the effect of docetaxel, a cytotoxic agent typically used in the treatment of breast, ovarian, prostate and non-small cell lung carcinomas, in xenografted tumour animals comprises sub-models of the agent's pharmacokinetics and pharmacodynamics, coupled with a suitable tumour growth model (see, for example, Figure 1).

2.1. Pharmacokinetic-Pharmacodynamic Model

The pharmacokinetics (PK) of docetaxel in mice is generally observed to be biphasic in nature with widely different half-lifes for the distribution and

3

elimination phases [4, 13]. However, the PK in humans in typically modelled using a three-compartment model [14]. Therefore, a linear two-compartment model is assumed for docetaxel PK (as illustrated in the 'PK' sub-model in Figure 1) following intravenous bolus administration. The corresponding model equations are given by:

$$\begin{aligned}
\dot{q}_P(t) &= -\left(K_{pe} + K_{pt}\right) q_P(t) + K_{tp} q_T(t) \\
\dot{q}_T(t) &= K_{pt} q_P(t) - K_{tp} q_T(t)
\end{aligned} \tag{1}$$

where q_P and q_T denote the quantities of drug per kilogram body weight in plasma and tissue, respectively. The plasma drug concentration, c_P, is therefore given by

$$c_P(t) = q_P(t)/v_p \tag{2}$$

where v_p denotes the mean plasma volume of distribution per kilogram animal body weight. Prior to administration of drug both variables are 0 and the bolus injection is modelled via an instantaneous addition of the dose to the plasma compartment.

The pharmacodynamics (PD) of the agent (as illustrated in the 'PD' sub-model in Figure 1) are modelled in a similar manner to Jumbe et al. [15] using the following Michaelis-Menten nonlinear term:

$$\eta(t) = \frac{c_P(t)}{IC_{50} + c_P(t)} \tag{3}$$

where $0 \leq \eta(t) \leq 1$ is the efficacy of the agent and IC_{50} is the concentration of drug giving 50% efficacy. Friberg et al. [16] used a similar nonlinear PD component in a human PK-PD model for docetaxel that successfully described chemotherapy-induced myelosuppression. It should also be noted that a linear PD component was also considered and was similarly able to characteristise myelosuppression.

The combined PK-PD model is coupled to a tumour growth model via the efficacy, η, which will be applied to the rate constant for a cytotoxic term applied to proliferating cells.

2.2. Tumour Growth Model

In the absence of chemotherapeutic agent the tumour is modelled as an expanding sphere consisting of a proliferating shell surrounding a hypoxic and necrotic core (see the 'Tumour Growth' sub-model in Figure 1). Since

this shell represents the part of the tumour with adequate oxygen supply exponential growth is assumed. The purpose of the basic tumour model is to represent the observed exponential followed by linear growth [1] in a mechanistic manner rather than employing logistic-type growth (such as done by Ribba et al. [2]). Hypoxic cells can also be assumed to proliferate [2], in which case the proliferation rate constant for hypoxic cells, k_P, is non-zero. The system equations are given by the following:

$$\dot{V}_t(t) = k_G V_p(t) + k_P V_h(t) - k_{DN} V_n(t)$$
$$\dot{V}_n(t) = k_N V_h(t) - k_{DN} V_n(t) \qquad (4)$$
$$\dot{T}_s(t) = k_A V_h(t)$$

for the following volumes (mm^3): V_t, the total tumour volume, V_n the necrotic part of the tumour core, V_h the hypoxic part of the core, V_p the proliferating (non-hypoxic) part of the shell; and T_s denotes the thickness (mm) of the proliferating shell. The growth rate constant for proliferating cells is k_G, the destruction rate constant for necrotic cells is k_{DN} and the rate constant for transfer from hypoxic to necrotic states is k_N. The term $k_A V_h$ represents angiogenesis driven by the hypoxic tumour volume, which results in an increase in the profilerating shell thickness.

If V_c denotes the total core volume then the following additional equations complete the system description:

$$V_t = \frac{4}{3}\pi R_t^3, \quad V_c = \begin{cases} 0 & R_t < T_s \\ \frac{4}{3}\pi(R_t - T_s)^3 & R_t \geq T_s \end{cases}$$
$$V_h = \begin{cases} 0 & V_c < V_n \\ V_c - V_n & V_c \geq V_n \end{cases}, \quad V_p = V_t - V_h - V_n \qquad (5)$$

where R_t denotes the radius (mm) of the tumour.

In order to consider pharmacodynamic experiments the tumour growth model (4)–(5) must be extended to include the effect of the agent under investigation. Typically agents have one or more of the following effects:

AP An anti-proliferative effect can be included by replacing the growth parameter, k_G, by a drug-mediated term, $u_{AP}k_G$, where $0 \leq u_{AP} \leq 1$ represents the effect of the drug. If the agent has an effect beyond the perfused shell then a similar modification of the hypoxic proliferation parameter, k_P, can also be included.

AA An anti-angiogenesis effect can be included as an inhibition term, in which the angiogenesis parameter, k_A, is replaced by a drug-mediated term, $u_{AAi}k_A$ ($0 \leq u_{AAi} \leq 1$); and/or a disruption term, in which an additional drug-induced term, $-u_{AAd}T_s$, is included in the final equation in (4).

CT To include a cytotoxic effect, such as that of docetaxel, the basic tumour model (4) must be extended to include an apoptotic class of cells in the perfused shell.

2.3. Growth Model with Cytotoxicity

In the presence of a cytotoxic agent the system equations (4) are extended to give the following:

$$
\begin{aligned}
\dot{V}_t(t) &= k_G V_p(t) + k_P V_h(t) - k_{DN} V_n(t) - k_{DA} V_a(t) \\
\dot{V}_n(t) &= k_N V_h(t) - k_{DN} V_n(t) \\
\dot{T}_s(t) &= k_A V_h(t) \\
\dot{V}_a(t) &= \eta(t) k_K V_p(t) - k_{DA} V_a(t) \\
\dot{V}_{ac}(t) &= \phi_A(t) - k_{DA} V_{ac}(t)
\end{aligned}
\tag{6}
$$

where V_a is the total volume of apoptotic cells (and V_{ac} the volume in the core), η is the efficacy of the agent (from the PK-PD model), k_K is the kill rate constant for the initiation of apoptosis by the agent and k_{DA} is the destruction rate constant for apoptotic cells. The term ϕ_A denotes the rate of change of apoptotic volume distribution across the shell/core boundary. Since docetaxel is a mitotic spindle poison it is assumed only to act on proliferating cells and, for simplicity, only such cells in the adequately perfused shell (V_p).

To complete the system description the additional equations (5) are modified to account for the apoptotic volume to give the following:

$$
V_t = \frac{4}{3}\pi R_t^3, \quad V_c = \begin{cases} 0 & R_t < T_s \\ \frac{4}{3}\pi(R_t - T_s)^3 & R_t \geq T_s \end{cases}
$$

$$
V_h = \begin{cases} 0 & V_c < V_n + V_{ac} \\ V_c - V_n - V_{ac} & V_c \geq V_n + V_{ac} \end{cases},
\tag{7}
$$

$$
\text{and} \quad V_p = V_t - V_h - V_n - V_a.
$$

The apoptotic volume transfer rate, ϕ_A, is dependent on whether the core is increasing (flow from shell to core) or shrinking (flow from core to shell). If ϕ denotes the rate of volume change from shell to core then it is given by:

$$\phi(t) = \dot{V}_c(t) + k_{DN}\left(V_c(t) - V_{ac}(t)\right) + k_{DA}V_{ac}(t) - k_P V_h(t). \tag{8}$$

It can be seen that ϕ incorporates terms relating to the growth of the core, replacement of removed necrotic (in the core) and apoptotic cells, and proliferation of hypoxic cells within the core. If ϕ is positive then there is net flow of cells from the shell to the core, and vice-versa when it is negative. Therefore, assuming homogeneous mixing of cells in the core and shell, the apoptotic flow rate, ϕ_A, is given by:

$$\phi_A(t) = \begin{cases} \frac{V_a(t) - V_{ac}(t)}{V_p(t) + V_a(t) - V_{ac}(t)}\phi(t) & \phi(t) \geq 0 \\ \frac{V_{ac}(t)}{V_h(t) + V_{ac}(t)}\phi(t) & \phi(t) < 0 \end{cases} \tag{9}$$

where in the latter expression if $V_{ac} = 0$ then $\phi_A = 0$.

3. Structural Identifiability

Suppose that $p \in \Omega \subset \mathbb{R}^m$ denotes a vector comprising the unknown parameters in the model (as an ordered list), which belongs to an open set of admissible vectors, Ω. The output of the model, which corresponds to the function of the model variables that will be compared with experimental data, depends on the choice of $p \in \Omega$ and so is denoted $y(t, p)$.

Two parameter vectors, $p, \bar{p} \in \Omega$, are *indistinguishable*, written $p \sim \bar{p}$, if they give rise to identical outputs:

$$y(t, p) = y(t, \bar{p}) \qquad \text{for all } t \geq 0.$$

For generic $p \in \Omega$, the parameter p_i is *locally identifiable* if there is a neighbourhood, N, of p such that

$$\bar{p} \in N, \ p \sim \bar{p} \quad \text{implies that} \quad \bar{p}_i = p_i.$$

In particular, if $N = \Omega$ in the above definition then p_i is *globally identifiable*, otherwise it is *nonuniquely (locally) identifiable*. Notice that, for a given output, a locally identifiable parameter can take any of a distinct (countable) set of values. If there does not exist a suitable neighbourhood N then p_i is

unidentifiable and, for a given output, can take an (uncountably) infinite set of values.

A parameterised model is *structurally globally identifiable* (SGI) if all parameters are globally identifiable; it is *structurally locally identifiable* (SLI) if all parameters are locally identifiable and at least one is nonuniquely identifiable; and the model is *structurally unidentifiable* (SU) if at least one parameter is unidentifiable.

Suppose that a Taylor series expansion of the output about $t = 0$ is taken to give

$$y(t, p) = y(0, p) + \dot{y}(0, p)t + \cdots + y^{(k)}(0, p)\frac{t^k}{k!} + \ldots$$

where $y^{(k)}(0, p) = \lim_{t \to 0} \frac{\mathrm{d}^k y}{\mathrm{d}t^k}(t, p)$. For a given output the coefficients of the Taylor series expansion are unique and can therefore be used to determine the uniqueness of the unknown parameters [8]. More explicitly, if $p, \overline{p} \in \Omega$ are indistinguishable then

$$y(0, p) = y(0, \overline{p}), \quad y^{(k)}(0, p) = y^{(k)}(0, \overline{p}) \quad \text{for all} \quad k > 0. \tag{10}$$

If a finite number of coefficients ensures that $\overline{p} = p$ then the model is SGI. Otherwise it is necessary to establish dependence between coefficients so that an upper bound on the number sufficient to characterise structural identifiability.

Alternatively, following the approach taken by Evans et al. [12], an output equation can be derived for the model, which only involves the output and its derivatives up to order n (the number of model variables). Then (following a similar approach as Denis-Vidal et al. [17]) any indistinguishable parameter vector, $\overline{p}$, must also satisfy the same equation with p replaced by $\overline{p}$ and a polynomial of the following form can be generated:

$$\sum_{k=1}^{l} c_k(p, \overline{p})\Psi_k(y(t, p), \dot{y}(t, p), \ldots, y^{(n-1)}(t, p)) = 0$$

for all $t \geq 0$, where $\Psi_k(y(t, p), \dot{y}(t, p), \ldots, y^{(n-1)}(t, p))$ are monomials in $y(t, p)$ and its derivatives. Moreover, the $\phi_k(\cdot)$ are linearly independent and so it must be the case that

$$c_k(p, \overline{p}) = 0 \qquad k = 1, \ldots, l.$$

In addition, the initial conditions for the output equation are equal for p and $\bar{p}$ and so the relationship between $\bar{p}$ and p can be determined. If the only solution is $\bar{p} = p$ then the model is SGI, if there is a set of distinct solutions then the model is SLI and it is SU otherwise.

3.1. Pharmacokinetic Model

For the Pharmacokinetic Model in (1) the vector of unknown parameters, p, is given by

$$p = \begin{pmatrix} K_{pe} & K_{pt} & K_{tp} & v_P \end{pmatrix}^T.$$

The experimental data to be used for parameter estimation were obtained following an intravenous bolus administration of drug followed by measurement of the plasma concentration. With respect to this experiment the initial conditions are given by:

$$q_P(0) = d \quad \text{and} \quad q_T(0) = 0$$

where d denotes the known dose, and the model output is given by

$$y(t, p) = c_P(t, p) = q_P(t, p)/v_P.$$

If $\bar{p} \in \Omega$ is any parameter vector such that $p \sim \bar{p}$ then consideration of the first four Taylor series coefficients, that is (10) for $k = 1, \ldots, 3$, shows that $\bar{p} = p$. Since all parameters are globally identifiable the model is SGI. An example of the analysis performed in the symbolic computation system MATHEMATICA is provided in Appendix A.1.)

3.2. Tumour Growth Model

Analysis of the tumour growth model (4) is complicated by the conditional equations in (5). To overcome these complications the model is considered in distinct phases. A structural identifiability analysis is performed for the model in the absence of angiogenesis (i.e., $k_A = 0$ so that $T_s(t) = t_S$, a constant) since only tumour volume data are available whereas one would expect to require further biomarker data to validate the full model. The model output corresponding to the experiment is

$$y(t, p) = V_t(t, p).$$

Without loss of generality analysis of the identifiability of the model in each phase will be done with respect to (different) initial conditions corresponding to the first time point in the respective phase.

Phase I ($R_t < t_S$): In this phase $V_h = V_c = 0$, $V_p = V_t - V_n$ and the model is linear. The initial conditions are unknown and given by

$$V_t(0) = v_{T0} \quad \text{and} \quad V_n(0) = v_{N0}$$

so that the vector of unknown parameters is given by

$$p = \begin{pmatrix} k_G & k_{DN} & v_{T0} & v_{N0} \end{pmatrix}^T.$$

Using the approach of Evans et al. [12] (illustrated using MAPLE in Appendix A.2) the output equation is found to be

$$\ddot{y}(t, p) + (k_{DN} - k_G)\,\dot{y}(t, p) - k_{DN}k_G y(t, p) = 0$$

and then k_{DN} and k_G determined to be globally identifiable. From consideration of the initial conditions for the output equation $(y(0, p), \dot{y}(0, p))$ it is found that v_{T0} and v_{N0} are also globally identifiable. Thus in this phase the reduced model is SGI.

Phase II ($R_t \geq t_S$, $V_c < V_n$): In this phase $V_h = 0$, $V_p = V_t - V_n$ and $V_c(t) = \frac{4}{3}\pi R_t(t)^3$. The system equations are identical to Phase I and so the model is also SGI in this phase.

Phase III ($R_t \geq t_S$, $V_c \geq V_n$): This phase involves the most general dynamics and is the only phase for this model in which the important parameter t_S appears. The initial conditions are unknown and given by

$$V_t(0) = v_{T0} \quad \text{and} \quad V_n(0) = v_{N0}$$

so that the vector of unknown parameters is given by

$$p = \begin{pmatrix} k_G & k_P & k_{DN} & k_N & t_S & v_{T0} & v_{N0} \end{pmatrix}^T.$$

Determination of the lengthy output equation (consisting of 239 terms) followed by consideration of a parameter vector $\bar{p}$ such that $p \sim \bar{p}$ enables the following relations between p and $\bar{p}$ to be found: Either $\bar{p} = p$ or

$$\bar{k}_G = k_G, \bar{t}_S = t_S, \bar{k}_P = k_P, \bar{k}_{DN} = k_N - k_P,$$

$$\bar{k}_N = k_P + k_{DN}, \bar{v}_{T0} = v_{T0}, \bar{v}_{N0} = \frac{v_{N0}(k_P + k_{DN})}{k_N}.$$

Therefore, k_G, t_S, k_P and v_{T0} are globally identifiable, while k_{DN}, k_N and v_{N0} are nonuniquely (locally) identifiable. Hence in this phase the model is SLI.

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If the experiment corresponds to dynamics in Phase III only then the model is SLI, but if the dynamics includes Phase I and/or Phase II then, since k_{DN} is globally identifiable in these phases, the model becomes SGI. The control data used for parameter estimation in the next section includes Phase I and III dynamics and so the model is SGI.

3.3. Growth Model with Cytotoxicity

The tumour growth model with a cytotoxic agent included (6) has similar complications to the drug-free case, which arise from the conditional equations in (7). However, it can be seen from the experimental data to be considered during parameter estimation that the dynamics remain in the exponential growth phase at administration of agent and immediately following. Thus the structural identifiability analysis is only considered for Phase I when $R_t < t_S$. Since control tumour growth data are available covering enough phases to ensure that the model (4) is SGI the analysis is performed in the case when all parameters from (4) are uniquely determined.

Without loss of generality (via a transformation of the time variable) suppose that the agent is applied at $t = 0$. Since the agent is administered in Phase I and there is no apoptotic cells prior to administration the unknown initial condition is given by

$$V_t(0) = v_{T0} \quad V_n(0) = v_{N0} \quad \text{and} \quad V_a(0) = 0.$$

Note that in Phase I there is no core ($V_c = 0$), or hypoxic cells ($V_h = 0$) and so $V_p = V_t - V_n - V_a$. Therefore the model in Phase I is governed entirely by the variables V_t, V_n and V_a.

The vector of unknown parameters is given by

$$p = \begin{pmatrix} k_{DA} & k_K & IC_{50} & v_{T0} & v_{N0} \end{pmatrix}^T$$

with output given by

$$y(t, p) = V_t(t, p).$$

Since the input to the tumour model is the drug plasma concentration, which is the impulse response of a two-compartment linear model, in order to apply the approach of Evans et al. [12] the output equation of a five-state model would be needed, which results in increased computational burden even though only three new parameters (and two initial conditions) have been added.

To remove the need for additional computational burden resulting from inclusion of the pharmacokinetic submodel, the drug plasma concentration can be treated as a known input to the tumour model. The input-output equation for the tumour model can then be generated using the methods of Forsman [18] to yield the following relationship:

$$(u(t) + ic_{50})^2 \left[y^{(3)}(t) + (k_{DA} + k_{DN} - k_G) \ddot{y}(t) \right.$$
$$\left. + (k_{DA}(k_{DN} - k_G) - k_{DN}k_G) \dot{y}(t) - k_{DA}k_G k_{DN}y(t) \right]$$
$$+ k_K (u(t) + ic_{50}) (k_{DA} + k_G) (\dot{y}(t) + k_{DN}y(t)) u(t)$$
$$+ k_K ic_{50} (k_{DA} + k_G) y(t)\dot{u}(t) = 0$$

where $y(t) = y(t, p)$ and $u(t) = c_P(t)$. However, since $u(t)$ is not an arbitrary input but the response from a linear model, it is not straightforward to determine whether the coefficients of this equation are necessary and sufficient to characterise the uniqueness of the parameters.

From the first five coefficients of the Taylor Series Approach, that is (10) for $k = 1, \ldots, 5$, two possible solutions can be found for an indistinguishable parameter vector $\bar{p}$. However, one of these solutions for $\bar{p}$ does not reduce the sixth coefficient to 0. Therefore it can be concluded that the only solution is the first one, which is $\bar{p} = p$, and so the additional parameters for the model including cytotoxic agent is SGI.

4. Parameter Estimation

The models were applied to experimental data in order to determine relevant unknown parameters and rate constants. In each case the model was implemented in FACSIMILE for Windows (MCPA Software, UK), which employs a robust predictor-corrector ODE solver able to accomodate highly stiff systems and a hybrid parameter fitting method combining features from the Newton-Raphson, Steepest descent and Marquardt methods (VA05 routine from Harwell Subroutine Library). Parameter estimation is performed with respect to the minimisation of the residual sum of squares (RSS) given by

$$RSS = \sum_{j=1}^{N} \frac{(D_j - y(t_j, p))^2}{\sigma_j^2} \tag{11}$$

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where $y(t_j, p)$ is the model output at sampling time t_j $(j = 1, \ldots, N)$, D_j is the corresponding data point and σ_j the standard error. Estimation in FACSIMILE is actually performed with respect to internal parameters, P, that are the natural logarithms of the model parameters. During the fitting process the singular values of the sensitivity matrix of the residuals with respect to the parameters are used to detect parameters that are not well determined (NWD) by the data (where the singular value is less than $0.357\sqrt{RSS/d}$, for d degrees of freedom). Once detected the values of such parameters are fixed and then treated as unknown in subsequent analyses. The standard deviation of the natural logarithm (SDLN) of each of the remaining fitted parameters, P_0, is estimated from the variance-covariance matrix of $P - P_0$ using a Singular Value Decomposition of the sensitivity matrix [19]. Assuming a normal distribution for the natural logarithms of the well-determined parameters, the 5% and 95% confidence limits are estimated for each.

In order to provide a more global parameter search, optimisation of the initial estimates for the parameters (i.e., those initial estimates that give rise to the fits in FACSIMILE with lowest residual sum of squares) was performed using the Genetic Algorithm solver in the MATLAB (R2011a) Global Optimization Toolbox (The MathWorks, Inc). Default settings were used together with a population size of 100, tournament selection involving 4 individuals and a uniform (random) initial population between the limits. Therefore, the following approach was adopted to perform the parameter estimation:

1. For positive vectors $a, b \in \mathbb{R}^m$ let $\mathcal{X} = \{x \in \mathbb{R}^m : a_i \leq x_i \leq b_i\}$ and define a function $\Psi : \mathcal{X} \longrightarrow \mathbb{R}^+$ as follows:

   - $\Psi(x)$ is the RSS obtained when performing parameter fitting in FACSIMILE using 10^{x_i} as the initial estimate for parameter p_i.

2. Apply the Genetic Algorithm solver in the MATLAB Global Optimization Toolbox to determine x^* $(a_i \leq x_i^* \leq b_i)$ that minimises Ψ.

3. For x^* (and other vectors returning an RSS within 1% of the minimum) perform iterative parameter fitting in FACSIMILE to refine the estimate for p^* (where $p_i^* = 10^{x_i^*}$).

The above procedure is repeated with narrowing bounds a_i and b_i; for example, when fitting the pharmacokinetic model initial bounds were set at $a_i = -6$ and $b_i = 6$ (for all i) and then refined through further iterations.

Table 1: Parameter estimates for pharmacokinetic model. SDLN is the estimated standard deviation of the natural logarithm of the parameter. If parameter estimates are log-normally distributed then corresponding 5% and 95% confidence limits are provided.

	Value	SDLN	5%	95%
K_{pe} (h^{-1})	0.382	0.49	0.169	0.860
K_{pt} (h^{-1})	0.523	0.80	0.140	1.950
K_{tp} (h^{-1})	0.196	0.48	0.090	0.430
v_P (l/kg)	1.30	0.57	0.508	3.323

4.1. Pharmacokinetic model

Data were provided from a preclinical oncology study at AstraZeneca for the plasma concentration for four mice over a period of 24 hours following intravenous administration of 24.75 μmol per kilogram (body weight) of docetaxel. Blood samples were taken at times 1, 2, 4, 6 and 24 hours after administration. The model was fit to the mean of the plasma concentrations at each time point with the standard errors for the data used as the weights, σ_j, for the sum of squares. One of the individual mouse plasma concentration time series exhibited a second peak at 4 minutes of almost equal magnitude to the first peak, which resulted in a skew of the respective mean and poor estimation of the apparent plasma volume, v_P. Since the data did not support a detailed analysis of the pharmacokinetics and possible "double peak" phenomenum the second peak was omitted from the calculation of the mean for fitting.

The three unknown rate constants and the apparent volume for plasma were estimated using these averaged data (see Table 1). The fitted model response is shown with the corresponding experimental data in Figure 2. There is a reasonable visual correspondence between model and data in Figure 2. Due to the sparseness of the sampling for the data all of the parameters have estimated standard deviations of their natural logarithms (SDLN)—a measure of the accuracy of estimation—above 0.48. From the estimated correlation matrix there is seen to be strong correlation between K_{pe} and K_{pt} (0.93), K_{pe} and v_P (-0.99), K_{pt} and v_P (-0.95), and moderate correlation between K_{pt} and K_{tp} (0.77). It is worth noting that the purpose here is not to determine robust estimates for the model parameters but to predict the average plasma concentration time course in a population of mice, since it is this latter response that is necessary in order to determine the docetaxel pharmacodynamics.
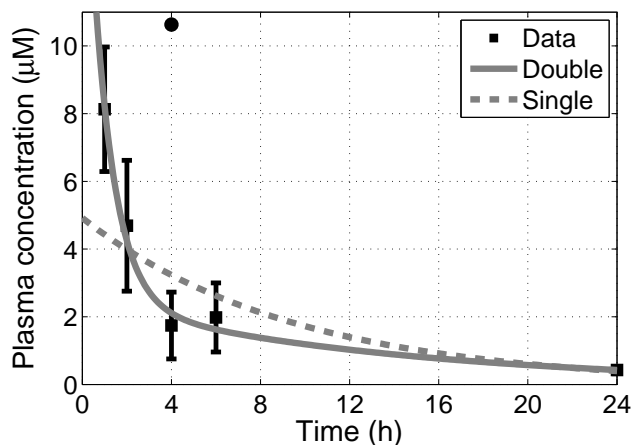
Figure 2: Fitted two compartment pharmacokinetic model response (solid line) together with corresponding experimental data (squares) and corresponding single compartment fit (dashed line). Error bars indicate plus/minus one standard error (calculated from data). Single circle point corresponds to second peak data value that was omitted from averaged data used in fitting.

In Figure 2 a corresponding one compartment fit is provided for comparison and to confirm the multi-phasic nature of docetaxel murine PK. The fit suggests a terminal half-life of 9.5 h, which is only slightly higher than that observed by Bradshaw-Pierce et al. [5] (8.6 h) for comparable doses (20 mg/kg) in mice. The large apparent plasma volume, compared to a physical volume of around 0.06 l/kg [20], suggests high protein binding that is supported by the literature [4, 13] (76–89% and above 84%, respectively).

4.2. Tumour Model

Data were provided for tumour growth in twelve xenografted mice both in the presence and absence of docetaxel (i.e., 12 animals in both the control and drug groups). Data were collected from 648 hours after tumour implant until 720 hours after implant in the control arm of the study. In the drug arm data were again collected from 648 hours after tumour implant until 1224 hours after implant, with a single dose of 24.75 μmol per kilogram (body weight) of docetaxel administered intravenously 672 hours after tumour implant. In the control group one animal was removed from the study from a time of 648 hours after tumour implant (at time 1296 h) and a further four animals at 720 hours after implant (time 1368 h). It can be seen in Figure 3 that animal

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Table 2: Parameter estimates for tumour growth model with no angiogenesis (**I** No hypoxic proliferation; **II** With hypoxic proliferation). Estimated SDLNs given in brackets.

	I	II
k_G (h^{-1})	$2.52{\times}10^{-3}$ (0.08)	$2.52{\times}10^{-3}$ (0.08)
k_P (h^{-1})	**0**	$1.11{\times}10^{-3}$ (NWD)
k_{DN} (h^{-1})	$5.27{\times}10^{+3}$ (NWD)	$5.26{\times}10^{+3}$ (NWD)
k_N (h^{-1})	$2.11{\times}10^{+4}$ (NWD)	$2.10{\times}10^{+4}$ (NWD)
k_A (h^{-1})	**0**	**0**
k_{DA} (h^{-1})	$1.65{\times}10^{-3}$ (0.18)	$1.65{\times}10^{-3}$ (0.18)
k_K (h^{-1})	$1.64{\times}10^{-2}$ (0.10)	$1.64{\times}10^{-2}$ (0.10)
IC_{50} (μM)	$7.23{\times}10^{-5}$ (NWD)	$7.23{\times}10^{-5}$ (NWD)
$R_t(0)$ (mm)	$4.25{\times}10^{-1}$ (0.01)	$4.25{\times}10^{-1}$ (0.01)
t_S (mm)	$6.28{\times}10^{-1}$ (0.02)	$6.28{\times}10^{-1}$ (0.02)
$V_n(0)$ (mm^3)	$3.35{\times}10^{-2}$ (NWD)	$3.35{\times}10^{-2}$ (NWD)
RSS	9.8860	9.8860

dropout from the study results in apparently strange dynamics towards the end of the control group experiment. In the drug cohort, one animal was removed from the study from a time of 1152 hours after tumour implant (time 1800 h). To enable the most robust estimation of growth parameters both the control and drug versions of the tumour model were fit simultaneously with appropriate parameters common to both. The fitted model response is shown with the corresponding experimental data in Figure 3 while the estimates for the unknown parameters are provided in Table 2. Since the model in the absence of angiogenesis was found to be structurally globally identifiable this version of the model was fit to the data, both with the assumption that hypoxic cells profilerate ($k_P \neq 0$) and without this assumption ($k_P = 0$).

Both the control and drug-effect responses capture the essential dynamics in Figure 3, but some care must be exercised when interpreting the latter time points for the control cases due to loss of subjects from the study. For the drug-effect data it can be seen that the latter time points also exhibit the greatest variability in responses and hence corresponding standard errors. The two versions of the tumour model fit equally well, both in terms of visual fit to data, residual sum of squares (RSS) and estimated SDLNs. Comparison of the two model fits suggests that true discrimination between them requires histology data to improve sensitivity of parameter estimates and fits. It is noticeable from the estimated correlation matrix for the well-determined
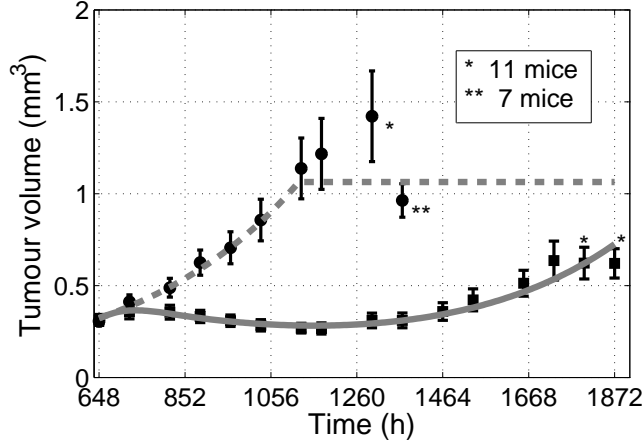
Figure 3: Fitted tumour growth model (**I:** $k_A = k_P = 0$) response in the absence of agent (dashed line) and in presence of agent (solid line) together with corresponding experimental data (circles and squares, respectively). Error bars indicate plus/minus one standard error (calculated from the data).

parameters (those in Table 2 with a numerical SDLN) that the growth rate constant parameter, k_G, is highly correlated with k_{DA} (-0.75) and k_K (0.96) for both fits. Therefore it appears that the tumour growth aspects of the model and data are dominating the fitting process. In addition, the well-determined parameters that relate to the effect of the agent, k_{DA} and k_K, are also highly correlated (-0.82).

A graphical investigation of the residuals from the fits are shown in Figure 4. A plot of the residuals from the fits versus model prediction (Figure 4(a)) does not seem to indicate any systematic errors, while a plot of residual r_{i+1} versus r_i (Figure 4(b)) does not seem to indicate autocorrelation between residuals. Finally a normal probability plot (Figure 4(c)) provides evidence that the residuals are normally distributed providing partial justification for the least squares approach to parameter fitting used in this paper. Results for the model that includes hypoxic proliferation are comparable.

Fits of the full model, including angiogenesis, both with and without proliferation of hypoxic cells are comparable in terms of RSS with the versions excluding angiogenesis. However, analysis of the identifiability of these models is still a subject of investigation.
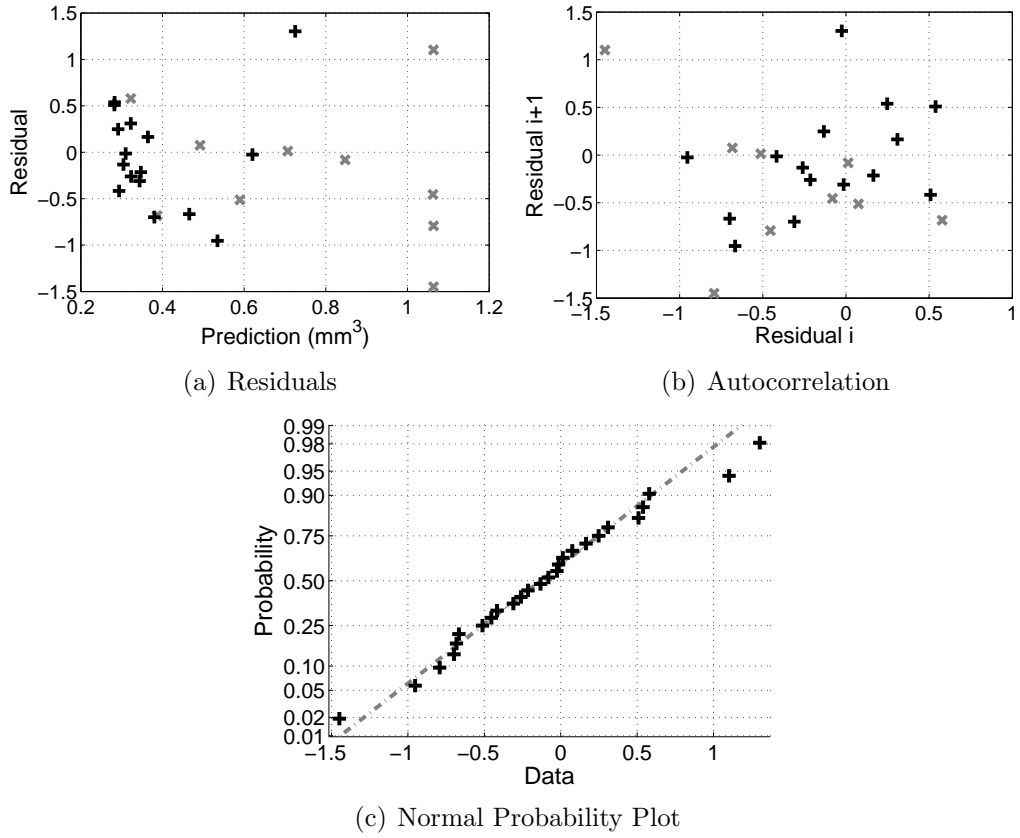
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(a) Residuals

(b) Autocorrelation

(c) Normal Probability Plot

Figure 4: Analysis of weighted residuals for fitted tumour growth model (**I:** $k_A = k_P = 0$) where in (a) and (b) 'x' denote residuals from control case and '+' from drug case.

4.3. Sensitivity to PK fits

The nested nature of the full model, in the sense that the tumour model fits depend on the predicted output of the fitted PK model, means that the behaviour of the fitted model depends on the robustness of the PK model fits. To assess the impact of the uncertainty of the PK model fits on the observed behaviour of the full model a simulation study was performed. In this study 2000 random parameter vectors for the PK model were generated where it was assumed that the fitted values represent the means and the SDLNs the standard deviations of corresponding lognormal distributions. The PK model was simulated for each of the 2000 parameter vectors and only those vectors producing an output within one standard error (calculated from the data) of each of the experimental data points was retained (52 in total; see Figure 5(a)).

The full model was simulated with the fitted values from Table 2 (with $k_A = k_P = 0$) and the PK model parameters taking those from the 52 sets generated previously. The simulated output of the model is shown in Figure 5(b). These results show that as time progresses the uncertainty in the PK model fits results is quite widely varying behaviour for the full model. Considering Figure 5(a) it is seen that it is in the early stages where the greatest variability is seen, which suggests that the full tumour fits are relatively sensitive to these early kinetics. Although more frequent samples, with some earlier in the experiment, might improve the robustness of the PK model fits it should be noted that docetaxel PK exhibits wide inter-subject variability. A population approach, using nonlinear mixed effects, might provide a more robust approach. It is perhaps also worth noting that a number of the parameters in the full model were not well determined by the data and so it is not clear whether the sensitivity of the model with respect to the drug plasma concentration is a true feature of the model. Estimation problems could be improved for the full model using data that include histology information.

5. Conclusions

A tumour growth model has been considered that includes the effect of a cytotoxic agent. The model can be readily extended to include other pharmacodynamic effects such as anti-angiogenic and anti-proliferative ones. The intravenous pharmacokinetics of docetaxel were modelled using a linear
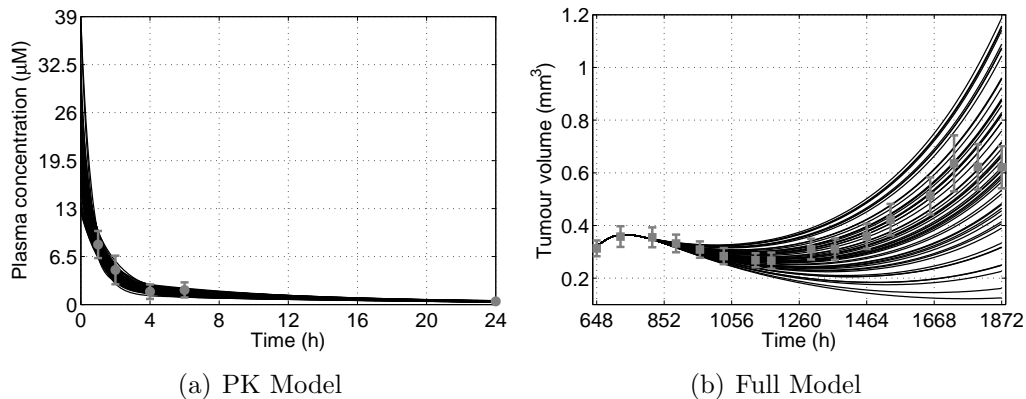
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Figure 5: An analysis of the sensitivity of the tumour volume prediction of the fitted full model (**I:** $k_A = k_P = 0$) with respect to the uncertainty of the PK model fits. Experimental data (with error bars indicating plus/minus one standard error) are also shown as grey points.

two-compartment model, while the pharmacodynamic effects were modelled using a simple Michaelis-Menten type Imax equation.

The tumour growth model includes important histological biomarkers and essential spatial characteristics in a relatively simple mechanistic manner. Thus the model attempts to strike an important balance between applicability to data and predictive capacity.

The pharmacokinetic model was determined to be structurally globally identifiable, while the drug-free tumour growth model, in the absence of angiogenesis, was shown to be structurally globally identifiable provided data are available for both the exponential growth phase ($R_t < t_S$) and continued growth phase ($R_t \geq t_S$). For the drug-effect tumour growth model, provided the growth parameters are known to be uniquely determined then exponential growth phase data are sufficient to uniquely determine the additional drug-related parameters.

The models were found to give good fits to experimental data from intravenous pharmacokinetic studies, xenografted tumour growth and drug-effect xenografted tumour growth studies. In order to maximise the applicability of the full model proposed in this paper further studies are necessary in which additional biomarker data are collected.

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Appendix A. Example structural identifiability analyses

Due to the symbolic nature of a structural identifiability analysis, and the computational complexities that can arise from such an analysis, a computer algebra system such as MAPLE (Waterloo Maple Inc) or MATHEMATICA (Wolfram Research) is an invaluable tool. To illustrate the use of different symbolic software to perform a structural identifiability analysis MATHEMATICA is employed to implement the Taylor series approach and MAPLE to implement the algebraic approach of Evans et al. [12]. All analyses were performed using MAPLE (version 16.01) or MATHEMATICA (version 8.01) on a dual core (2×2.66 GHz) Windows XP PC with 4 GB memory.

Appendix A.1. Taylor series approach: PK model

The first stage in applying the Taylor series approach to perform a structural identifiability analysis is to define the system equations in MATHEMATICA:

```
qP'[t_] := -(Kpe + Kpt) qP[t] + Ktp qT[t]
qT'[t_] := Kpt qP[t] - Ktp qT[t]
cP[t_] := qP[t] / vP
```

with corresponding initial conditions:

```
qP[0] = d ; qT[0] = 0 ;
```

The next stage is to determine a list, `coeffs`, of a number of the Taylor series coefficients (in this case the first four):

```
y1 = D[y[t], t];
coeffs = y[t], y1 /. t -> 0;
y2 = D[y1, t];
coeffs = Union[coeffs, y2 /. t -> 0];
y3 = D[y2, t];
coeffs = Union[coeffs, y3 /. t -> 0];
```

Since the first coefficient ($y(0, p) = d$) is a known constant (the dose, d) it is omitted from the computation. The code above successively differentiates the output (using the `D[ , ]` operator) and sets $t = 0$ (via the substitution `/. t -> 0`).

If $\overline{p} = \left(K_{pe}, K_{pt}, K_{tp}, v_P \right)^T$ denotes a parameter vector with identical output then it must give rise to identical Taylor series coefficients. Therefore

the next stage of the analysis is to set up equations, `eqns`, that equate coefficients of the Taylor series expansions of the outputs for p and $\bar{p}$ (`coeffs` and `coeffsPbar`, respectively):

```
coeffsPbar = coeffs /. {Kpe->Kpeb,Kpt->Kptb,Ktp->Ktpb,vP->vPb};
eqns = coeffs - coeffsPbar ;
soln = Simplify[Solve[eqns == 0, { Kpeb, Kptb, Ktpb, vPb }]]
```

The list of solutions, `soln`, returned by MATHEMATICA is a list of relations that must hold between the components of the two parameter vectors p and $\bar{p}$. In particular, if these relations force $\bar{p} = p$ then the model is structural globally identifiable. This situation is the case for this analysis where MATHEMATICA returns the following list for `soln`:

```
{ { Kpeb -> Kpe, Kptb -> Kpt, Ktpb -> Ktp, vPb -> vP } }
```

It should be noted that if these relations do not force $\bar{p} = p$ then further coefficients of the Taylor series must be considered (if computationally possible) or it must be established that enough coefficients have been considered so that further coefficients will not yield any additional information.

Appendix A.2. Algebraic approach: Tumour growth (Phase I)

To implement the algebraic approach of Evans et al. [12] a slightly modified and updated version of the function `outptEqn1` (here implemented as `iorel`) and related subfunctions must be defined:

```
lieDer := proc (H, F, vars)
  local V:
  V := map( (a, b) -> diff(b, a) , vars, H):
  DotProduct(Vector(F), Vector(V), conjugate = false)
end:

listLieDer := proc (H, F, k)
  local L, i, tmp, N, vars:
  L := [y[0]-H]: tmp := H :
  N := nops(F);
  vars := [seq(x[t], t = 1 .. N)];
  for i to k do
    tmp := lieDer(tmp, F, vars);
    L := [op(L), y[i]-tmp]
```

```
    od;
end:

xlistLieDer := proc (H, F, k, uvars)
  local L, i, f, h, tmp, N, var, vars, duvars:
  N := nops(F);
  f := F; h := H;
  for var in uvars do
    f := subs(var = var[0], f);
    h := subs(var = var[0], h);
  od;
  duvars := [];
  vars := [seq(x[t], t = 1 .. N)];
  for var in uvars do
    vars := [op(vars), seq(var[i], i = 0 .. 10)];
    duvars := [op(duvars), seq(var[i], i = 1 .. 11)];
  od;
  f := [op(f), op(duvars)];
  L := [y[0]-h]; tmp := h;
  i := 1;
  for i to k do
    tmp := lieDer(tmp, f, vars);
    L := [op(L), y[i]-tmp];
  od;
end:

iorel := proc (f, h, uvars)
  local n, L;
  n := nops(f);
  if _params['uvars'] = NULL then
    L := listLieDer(h, f, n)
  else
    L := xlistLieDer(h, f, n, uvars)
  fi;
  L := map(expand, numer(L));
  UnivariatePolynomial(y[n], L, [seq(x[t], t = 1 .. n), y[n]])
end:
```

The command `iorel` determines the input/output equation using the sub-command `listLieDer` (or `xlistLieDer` in the case with inputs) that creates a list of the first n Lie derivatives of the output function along the vector field defined by the right-hand side of the system equations (calculated using the subfunction `lieDer`). Since the above functions use short command names the `LinearAlgebra` and `Groebner` packages must be loaded first. Once these packages and the above functions have been defined in MAPLE then the system equations can also be defined (though note that components of the state are denoted by `x`) and the output equation, `outptEqn`, determined:

```
F := [k[G]*(x[1]-x[2])-k[DN]*x[2], -k[DN]*x[2]]:
H := x[1]:
outptEqn := iorel(F, H)
```

The next stage in the analysis is to create a list of the coefficients of the output equation:

```
uA := {coeffs(collect(outptEqn,[y[0],y[1],y[2]],'distributed'),
                [y[0], y[1], y[2]]) }
```

In a similar fashion to the Taylor series approach an alternative parameter vector is defined (by adding a 'b' after the `K`, or `v`, of the parameter name) that has the same output and a corresponding list of coefficients constructed:

```
B := eval(uA, [k[G] = kb[G], k[DN] = kb[DN]])
```

Since these coefficients (`uA` and `uB`) correspond to the same output they must be equal. Therefore, equations equating these coefficients are created and solved as follows:

```
eqns := convert(uA, list) - convert(uB, list):
solve(eqns, [kb[G], kb[DN]])
```

This result shows that the parameters k_G and k_{DN} are identical for any parameter vectors with identical behaviour (they are globally identifiable). To complete the analysis it only remains to consider uniqueness of the initial conditions of the output equation ($y(0, p)$ and $\dot{y}(0, p)$):

```
ics := [ v[T0] - vb[T0], k[G]*(v[T0] - vb[T0]) - k[DN]*vb[T0]
        - k[G]*(vb[T0] - vb[T0]) + k[DN]*vb[T0]]:
solve(ics, [vb[T0], vb[N0]])
```

The result shows that the initial conditions are also unique for a given output (i.e., globally identifiable). In more complicated examples it is often more useful to solve the coefficients and initial conditions simultaneously.