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A delay differential equation model for tumor growth

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Abstract. We present a competition model of tumor growth that includes the immune system response and a cycle-phase-specific drug. The model considers three populations: Immune system, population of tumor cells during interphase and population of tumor during mitosis. Delay differential equations are used to model the system to take into account the phases of the cell cycle. We analyze the stability of the system and prove a theorem based on the argument principle to determine the stability of a fixed point and show that the stability may depend on the delay. We show theoretically and through numerical simulations that periodic solutions may arise through Hopf Bifurcations.

1. Introduction

According to the American Cancer Society, every year more than one million people in the United States are diagnosed with cancer and each year over 500000 die from cancer. It comes as no surprise that scientists around the world have been trying to successfully model the disease. The idea is to gain understanding of the process, and to design better treatment strategies or improve existing strategies to eradicate the disease or at least to improve the patient's quality of life. Different types of models have already been constructed, and each one contributes in its own way to a better understanding of cancer and the dynamics that determine the patient's outcome.

In this paper we are interested in modeling the effects and interactions between tumor cells and immune cells, clearly differentiating between phases for subsequent treatment with a cycle-phase-specific drug, i.e. a drug that acts on a specific phase of the cell cycle. In many instances most of these drugs (for example Hydroxy Ara-C, Paclitaxel and others) interfere with mitosis, disabling the cell from continuing in the cell cycle, thus stopping proliferation and allowing natural death of cells and the immune system to kill these arrested cells (see [1]). To model this situation it is most natural to subdivide the tumor population into its different stages or cycle

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phases to properly account for cycle-specificity. In this way it is simpler to control and model how the drug acts on the different stages of the cycle.

Previously, authors such as Adam and Panetta [2], have modeled cycle specific chemotherapy. In their paper they consider two compartments, the cycling (proliferating) cells and the resting (quiescent) cells and study the effects that different dosing intervals have on the populations considered. The inclusion of the immune system in a mathematical model was studied by Kirschner and Panetta ([3]). In their paper they study immunotherapy as an alternative to chemotherapy. In [4] the authors model the cytotoxic T lymphocyte response to an immunogenic tumor, and find that the model exhibits phenomena that are seen in patients but that had previously remained unexplained.

The model we propose is an extension of the models above, but the approach that we will take differs from the works cited in that we will subdivide the cycling tumor population into phases but we will not consider the quiescent phase (commonly denoted as G_0). We are interested however in the interaction of the tumor cells and drug with the immune system: we use the size of the immune population as a first representation of the patient's health, and we include its ability to fight cancer in the model. One major difference between this work and that of others is the use of Delay Differential Equations (DDE). They appear naturally when one considers the cell cycle. Also, the importance of DDE, is well explained by Baker et al. [5]. In their work, Baker and colleagues fitted the models to existing data using least squares estimates for the parameters and compared different models, to find that the delayed versions provided a much better fit by calculating the euclidean norm of the errors.

The paper is organized as follows: In section 2 we present the governing equations of our system, section 3 provides estimates for some of the parameter values involved in the model, section 4 assesses the stability of the fixed points that arise in the model, and shows the occurrence of a Hopf Bifurcation, and section 5 contains a brief discussion of the results.

2. The model

The cell cycle is the process between two cell divisions (or mitosis) ([6], [7]), and it can be divided into 4 phases: the G_1 phase is a resting phase (or gap period) called pre-synthetic phase. G_1 could last as long as 48 hours and is the longest phase of the cycle. The next phase is the S phase or synthetic period, where the replication of DNA occurs. This phase may last between 8 and 20 hours. The cells complete the DNA replication and enter another gap period G_2 called the post-synthetic phase. G_2 is a preparation phase for mitosis. The last phase is mitosis M in which the cells segregate the duplicated sets of chromosomes between daughter cells. Mitosis is the shortest phase of all, lasting up to one hour. The duration of the cell cycle is very much dependent on the type of cell and their growth conditions. The most typical normal cell will have a cell cycle duration of approximately 24 hours, with various exceptions (e.g. liver cells can take up to a year to complete their cycle). However a study made by Tubiana and Malaise ([8]) on 30 solid human tumors reveal that the median duration of these phases can be even higher with a cell cycle duration

lasting a median time of 2 days and distributed as 1 day for G_1 , 18 hours for S , 6 hours for G_2 , leaving just approximately 1 hour for mitosis, M . These values are median values and one must be cautioned to the fact that different cell lines have different cell cycle times (normal and cancerous cells). They give evidence that the cell cycle time is approximately twice as large in man than in animals.

There are many checkpoints throughout the cell cycle that prevent the cell from completing the cycle if it detects an abnormality. A cancerous cell does not necessarily divide more rapidly than their normal counterparts, but they lose the ability to regulate the cell cycle, thus proliferation of these cells is not controlled. Once mitosis is completed each daughter cell can enter the cycle again or shift into a quiescent phase, commonly denoted as G_0 , during which cells do not divide for long periods. Phase-specific drugs alter the natural course of action for the active or cycling cells. Many chemotherapeutic agents acting on the S phase aim to suppress mitosis, and therefore have no visible effect until the M phase.

In this work we exclude the quiescent phase. This assumption is a simplification of the model whose effect will be worth exploring in more detail in future work since it is known that the cells in this phase are resistant to most cytotoxic agents and that approximately only 20% of the cells are cycling ([9]). We assume that the drug is cytotoxic to both the immune system (which interacts with tumor cells at any stage of their cycle) and the tumor cells. This assumption comes from the fact that cycle specific drugs are more cytotoxic to rapidly proliferating cells including those in the bone marrow, among others. We will assume that the drug arrests tumor cells in mitosis, and that the concentration of drug decays exponentially with time. Since mitosis is short in comparison with the rest of the cycle we will assume that mitosis is an instantaneous process.

Let $T_I(t)$ denote the population of tumor cells during interphase at time t , where interphase is the pre-mitotic phase, namely $G_1 + S + G_2$. Let $T_M(t)$ be the tumor population during mitosis at time t , $I(t)$ be the immune system population at time t , $u(t)$ the amount of drug present at time t , and τ be the resident time of cells in interphase. The governing equations for the system are:

$$\begin{aligned} T_I' &= 2a_4T_M - (c_1I + d_2)T_I - a_1T_I(t - \tau) \\ T_M' &= a_1T_I(t - \tau) - d_3T_M - a_4T_M - c_3T_MI - k_1(1 - e^{-k_2u})T_M \\ I' &= k + \frac{\rho I(T_I + T_M)^n}{\alpha + (T_I + T_M)^n} - c_2IT_I - c_4T_MI - d_1I - k_3(1 - e^{-k_4u})I \\ u' &= -\gamma u \end{aligned} \tag{1}$$

with initial data given by:

$$\begin{aligned} T_I(t) &= \phi_1(t) \quad \text{for } t \in [-\tau, 0] \\ T_M(t) &= \phi_2(t) \quad \text{for } t \in [-\tau, 0] \\ I(t) &= \phi_3(t) \quad \text{for } t \in [-\tau, 0] \\ u(0) &= u_0 \end{aligned}$$

In reality we only need T_I determined on the interval $[-\tau, 0]$, but we may specify the complete history of the system in the interval mentioned above. The terms d_2T_I , d_3T_M and d_1I in the model equations represent proportions of natural cell death or

apoptosis, a_1 and a_4 represent the different rates at which cells cycle or reproduce, the c_i terms represent losses from encounters of tumor cells with immune cells and the term $\frac{\rho I(T_I + T_M)^n}{\alpha + (T_I + T_M)^n}$ represents the nonlinear growth of the immune population due to stimulus by the tumor cells. We have chosen a Michaelis-Menten form for this term following other models in the literature, (see, for example, [10], [3] and [4]). We feel that this form is reasonable since proliferation of tumor-specific effector cells is stimulated by the presence of tumor cells, but reaches a saturation level at tumor populations. Hence, the recruitment function should be zero when there are no tumor cells, and should increase monotonically towards a horizontal asymptote: this rational form reflects these characteristics in a simple, smooth function. The parameters ρ , α , and n depend on the type of tumor being considered and the health of the immune system, specifically its ability to produce certain cytokines. In the absence of tumor cells ($T_I = T_M = 0$), the immune cells grow at a constant source rate k . The tumor cells reside in interphase for a certain period of time τ , before continuing in the cycle to M . Assuming that cells reside in interphase τ units of time, then the cells that enter mitosis at time t are those cells that entered interphase τ units of time before. This explains the terms $T_I(t - \tau)$ in system (1).

It is worthwhile noting that the usual growth term encountered in other growth models such as, for example, logistic, gompertz, and exponential functions (see references [10], [4], [11], [12], [13], and [14]) is absent here, since the growth of the tumor cell population is obtained through mitosis and is given by the constants a_1 , a_4 , and τ which regulate the pace of cell division.

The terms $T_M I$ and $T_I I$ are standard competition terms that in our model will represent losses due to encounters among the different cell types. For high concentrations of drug we know that the drug arrests tumor cells in mitosis where they die naturally when they fail to continue in the cycle. One way to view this is by assuming that once the drug encounters the tumor cell, the tumor cell is taken out of the cycle and can no longer proliferate. This can be modeled by the term $-k_1(1 - e^{-k_2 u})T_M$, but there are other curves that describe a similar feature (see [11]). High drug concentration also impairs the immune system by either destroying cells or diminishing their ability to attack the tumor cells; we model this by a term similar to the tumor drug-kill term, but with different coefficients.

The drug decay is assumed to be exponential, and the coefficient γ incorporates both the elimination and absorption effects. We further assume that application of the drug ceases at time $t = 0$ (so that $u(0) = u_0 > 0$); in [15] the effect of multiple applications of the drug is considered as well as a more accurate description of elimination and absorption of the drug (modeled as a sum of exponentials). Given that treatment options are beyond the scope of this paper and for the sake of simplicity we will consider these two effects together.

3. Parameter values

The issue of selecting appropriate parameter values is an important one, since they determine the dynamics of the system (number of fixed points and stability of each). As one varies one or two parameters we can see dramatically different behaviors

in the solutions of the equations. Baker et al. ([5]) found suitable parameter values by minimizing an error function (least squares fit to data). Birkhead et al. ([9]) obtained their parameter estimates for a breast cancer model by using values corresponding to some known values for leukemic cells. By analyzing the biological meaning of the different parameter values one might make an educated guess as to an appropriate range for them.

The description of the growth of a tumor is defined in terms of three principal parameters: the cell cycle time of proliferating cells, the proportion of cells proliferating and the extent of cell loss ([16]). It is possible through the percentage of labelled mitosis curves (FLM) to determine the growth fraction as well as cycle times T_c . The potential doubling time, T , is the time tumor cells would take to double in the absence of cell loss and is defined as $T = \lambda \frac{T_s}{L.T.}$, where λ is an artificial parameter that takes into account the rate in which proliferating cells are produced. λ is often taken to be 0.75 [16]. If we have at least two observations of the volume of the tumor $((t_0, v_0), (t_1, v_1))$ then we can obtain the actual doubling time T_d .

There are many different types of cells that comprise the immune system and each one with different functions. Some very important cell types are the lymphocytes. A subset of the lymphocytes are the T cells which are divided into two subcategories, the helper T cells, and the cytotoxic T cells (CTL) which actively fight or disable tumor cells. We will focus our attention on CTL (cytotoxic T Lymphocytes) as the primary representation of the immune system given its importance in the battle against cancer, however we are well aware that the immune system is much more complex. We will focus on one to one bindings of CTL to tumor cells, based on studies which show that this configuration is more frequent than others ([17]), and disregard all other possible forms of interactions between these cell lines (two to one bindings, etc.).

The values for losses of CTL due to encounters with tumor cells, as well as the source rate for CTL and the natural death rate, d_1 were obtained from [4]. The parameter n , which determines the shape of the response term, was determined by examining qualitative aspects of data in several theoretical and *in vitro* studies, for example, [18], [19], [20], and [21]. While $n = 1$ has been used in the literature, ([4], [3]), we felt that the infinite derivative of the resulting function at zero was not biologically realistic. The studies mentioned show that, in fact, there is a threshold of antigen necessary to stimulate a noticeable immune response, and that the steepness of the response best matches the value of the exponent $n = 3$. The parameters ρ and α were determined by requiring a tumor-free fixed-point and a saturation level consistent with those given in the literature. Table 1 summarizes the parameter values.

Before we begin with the analysis it is important to non-dimensionalize and scale our variables. Consider the following change of variables $T = \frac{t}{\text{day}}$, $x = \frac{T_I}{T_I(0)}$, $y = \frac{T_M}{T_M(0)}$, $z = \frac{I}{I(0)}$ and $w = \frac{u}{PPC}$, where PPC is the peak plasma concentration, and $T_I(0) = T_M(0)$ initial reference values (taken to be 10^6 cells). With this change of variables our system can be written in our new dimensionless variables (x, y, z, w) as:

Table 1. Parameter Values and Range.

Parameter	Estimated value	Source and Comments
τ	22 hr. (0.9167 days)	[22] Mitosis is considered instantaneous so $\tau=T_c$
a_1	0.8470 day ⁻¹	[22] Regress on equations with no drugs, and no immune
a_4	0.9159 day ⁻¹	[22] Same as above
d_2	0.1145 day ⁻¹	[22], [16] Consider the discrepancy between T_d and T
d_3	0.6641 day ⁻¹	[22], [16] Same as above
$c_1 = c_3$	2.16×10^{-7} cell ⁻¹ day ⁻¹	[17] Similar estimates in [4]
$c_2 = c_4$	3.422×10^{-10} cell ⁻¹ day ⁻¹ day ⁻¹	Use parameters in [4] as reference due to lack of data
k	1.3×10^4 cell day ⁻¹	Use parameter in [4]
d_1	0.04 day ⁻¹	[4] Consider $\frac{dI}{dt} = k - d_1 I$
n	3	See [18], [19], [20] and [21]
ρ	0.2 day ⁻¹	Suggested value from biological meaning.
α	$(0.3 \times 10^6 \text{cell})^3$	Same as above

$$\begin{aligned}
 \frac{dx'}{dT} &= 2a_4y - c_1 \times 3.5 \times 10^5zx - d_2x - a_1x(T - \tau) \\
 \frac{dy'}{dT} &= a_1x(T - \tau) - d_3y - a_4y - c_3 \times 3.5 \times 10^5zy - k_1(1 - e^{-k_2PPCw})y \\
 \frac{dz'}{dT} &= k/(3.5 \times 10^5) + \frac{\rho z(x + y)^3}{\alpha/10^{18} + (x + y)^3} - c_2 \times 10^6zx - c_4 \times 10^6yz - d_1z \\
 &\quad - k_3(1 - e^{-k_4PPCw})z \\
 \frac{w'}{dT} &= -\gamma w
 \end{aligned}
 \tag{2}$$

by renaming the variables $T, x, y, z,$ and w to $t, T_I, T_M, I,$ and $u,$ and the parameter values as $c_1 = c_1 \times 3.5 \times 10^5, c_3 = c_3 \times 3.5 \times 10^5, c_4 = c_4 \times 10^6, c_2 = c_2 \times 10^6, k_2 = PPCk_2, k_4 = PPCk_4, k = k/(3.5 \times 10^5),$ and $\alpha = \alpha/10^{18},$ then all the parameter values and variables do not have dimensions. We will work with non-dimensionalized parameters from this point on. However, we would like to point out that the parameters will vary between tumor types and from person to person. Therefore there is no unique set of parameters values for any given model. The new parameter values are summarized below and they will provide a base point for the exploration of the entire parameter space. In what follows we have allowed ourselves to vary these values for purpose of analysis leading to a deeper

understanding of the behavior of the model, and for a clearer illustration of different behaviors.

$$a_1 = 0.84 \quad a_4 = 0.91 \quad c_1 = c_3 = 7.56 \times 10^{-2} \quad d_1 = 0.04 \quad d_2 = 0.11$$

$$d_3 = 0.66 \quad c_2 = c_4 = 3.422 \times 10^{-4} \quad k = 0.037$$

In later works ([15]) the parameter estimation for the drug terms are included (since we do not make specific use of them here).

4. Stability results

The long-term behavior of the system is crucial to the outcome of therapy. Depending on the initial conditions, a trajectory can either converge to an attractor, or diverge to infinity. In our system the attractor may be an equilibrium point, a limit cycle, or a higher dimensional subset of phase space. Knowing the conditions for which we can obtain all these possibilities enables us to better understand the long term behavior of our system. We first study the stability of the fixed points of the system described in the previous section.

4.1. Drug-free system

We first determine the type of dynamics that can arise in the system without the presence of the drug. The rationale behind this is to use the information about the drug-free system when designing chemotherapeutic protocols: when we stop treatment, we would like the patient to be ‘cured’, or to be inside the basin of attraction of the tumor free fixed point. We therefore begin by eliminating all drug terms in the model, computing the fixed points of this new drug-free system, and analyzing their stability. It is also of interest to study how the delay τ affects the behavior of our system and how each element contributed to the overall stability. So we begin by analyzing the simplest case: a drug-free model in a non-delay case in absence of the immune response.

4.1.1. Drug-free model in a non-delay case in absence of immune response

In this case the equations are a simple set of ordinary differential equations:

$$\begin{aligned} T_I' &= 2a_4T_M - (d_2 + a_1)T_I, & T_I(0) &= \phi_0 \\ T_M' &= a_1T_I - dT_M, & T_M(0) &= \phi_1 \end{aligned}$$

This is a linear system with the only fixed point being $(T_I, T_M) = (0, 0)$ and $d = d_3 + a_4$. The trace of the corresponding matrix representation of the system is $tr = -(d + d_2 + a_1) < 0$. and the determinant Δ , is given by: $\Delta = d(d_2 + a_1) - 2a_1a_4$. The eigenvalues associated with the matrix are given by

$$\lambda_{\pm} = \frac{tr \pm \sqrt{tr^2 - 4\Delta}}{2}.$$

Notice that if $\Delta < 0$, then $tr^2 - 4\Delta > 0$, and the fixed point results in a saddle (one positive eigenvalue and one negative eigenvalue), which will result in an unstable

fixed point. If $\Delta > 0$, then we are in the presence of a stable fixed point (a stable spiral if $tr^2 - 4\Delta < 0$, and a stable node if $tr^2 - 4\Delta > 0$).

So the necessary condition for tumor growth is $\Delta < 0$, i.e.

$$d(d_2 + a_1) < 2a_1a_4$$

and the tumor-free state will be stable otherwise. In other words, if the growth rates dominate the death rates, the tumor will grow.

4.1.2. *Drug-free model when $\tau > 0$, in the absence of immune response*

When we add the effect of the delay in the simple system of section 4.1.1 we get the system

$$\begin{aligned} T_I' &= 2a_4T_M - d_2T_I - a_1T_I(t - \tau), & T_I(t) &= \phi_0(t) \quad t \in [-\tau, 0] \\ T_M' &= a_1T_I(t - \tau) - dT_M, & T_M(t) &= \phi_1(t) \quad t \in [-\tau, 0] \end{aligned}$$

Note that the system in section 4.1.1 corresponds to the special case when $\tau = 0$. We are interested in studying how the conditions for tumor growth are varied for a positive value of the delay τ . As before the only fixed point of this system is the point $(0, 0)$.

The determination of stability in the case of a DDE is analogous to the ODE case: we linearize the system around the fixed point and consider exponential solutions which are characterized by the eigenvalues or exponents of these solutions. These eigenvalues are the roots of the *characteristic equation* of the system, which in general has infinitely many solutions (for more details on DDE refer to Bellman and Cooke [23] or Hale and Lunel [24]). The fixed point in question is stable if, as in the case of ordinary differential equations, all the eigenvalues have negative real parts.

The characteristic equation for this system around the fixed point $(0, 0)$ is given by

$$F(\lambda) = P(\lambda) + e^{-\tau\lambda}Q(\lambda) = (\lambda + d)(\lambda + d_2) + e^{-\tau\lambda}(a_1\lambda + (a_1d - 2a_1a_4)).$$

There are many ways in which we can determine if there is a root λ of the characteristic equation with positive real part. Geometric arguments can be used to establish the stability of a given fixed point, such as those used by Mahaffy in [25], where the argument principle is used to count the number of zeros of $F(\lambda)$ on the right hand side of the complex plane. In this case we will resort to some results by Cooke and van den Driessche in Theorem 1 of [26].

They define the function

$$F_1(y) = |P(iy)|^2 - |Q(iy)|^2 \tag{3}$$

and analyze the function F_1 , giving conditions under which equation $F(\lambda)$ is stable as a function of τ . They also give conditions under which stability changes may occur as the delay is increased and show that in these cases the fixed point is unstable for large enough τ . In summary, if F_1 has no positive real roots then the stability of $F(\lambda, \tau)$ is the same as the $\tau = 0$ case. Otherwise, if F_1 has positive real roots

(and they are simple) then stability switches may occur as τ increases, and there is a positive number T^* such that for all $\tau > T^*$, $F(\lambda, \tau)$ is unstable. The interested reader is referred to [26] for more details.

Following the steps in this theorem it is straightforward to check the stability of the fixed point and find the conditions for tumor growth. In this case $F_1(y)$ is found to be:

$$F_1(y) = y^4 + (d_2^2 + d^2 - a_1^2)y^2 - a_1^2(d - 2a_4)^2 + d_2^2d^2$$

by letting $x = y^2$, the equation is transformed into a quadratic with roots given by:

$$x = \frac{\left(-(d_2^2 + d^2 - a_1^2) \pm \sqrt{(d_2^2 + d^2 - a_1^2)^2 - 4d_2^2d^2 + 4a_1^2(d - 2a_4)^2} \right)}{2}$$

From the above expression we can obtain conditions for tumor growth, tumor decay and stability switching. We can summarize these results as follows:

1. if

$$\Delta < 0$$

then $(0, 0)$ is unstable (this is the condition for tumor growth), where Δ is defined as before.

2. if

$$\begin{cases} d > 2a_4 \\ d(a_1 - d_2) > 2a_1a_4 \\ \Delta > 0 \end{cases}$$

or

$$\begin{cases} \text{the quantity inside the radical is positive} \\ d_2^2 + d^2 - a_1^2 < 0 \\ \Delta > 0 \end{cases}$$

then there are crossings of eigenvalues from the left hand side of the plane to the right hand side and therefore there is a switch (or change) in the stability, from stable to unstable.

The complement of these regions is where the system converges towards the $(0, 0)$ fixed point, i.e., the fixed point is stable. All these conditions are better expressed in pictures. Say we fix the parameters a_4 and d_2 , and we plot the curves that define the regions of growth, decay and stability switching. Setting a_4 to 0.5 and d_2 to 0.3, the resulting curves are seen in Figure 1. There are five clearly identified regions labelled R-I, R-II, R-III, R-IV, and R-V. In the case when $\tau = 0$, the region of tumor growth is given by R-I and the complement is the region in the parameter space $a_1 - d$, where we get tumor decay. When we increase τ , the region of tumor decay is subdivided to include regions of possible stability switching. In the case $\tau > 0$, R-I remains the region of tumor growth. The decay region in the case for $\tau = 0$, is now smaller when $\tau > 0$. When $\tau = 0$, the decay region is

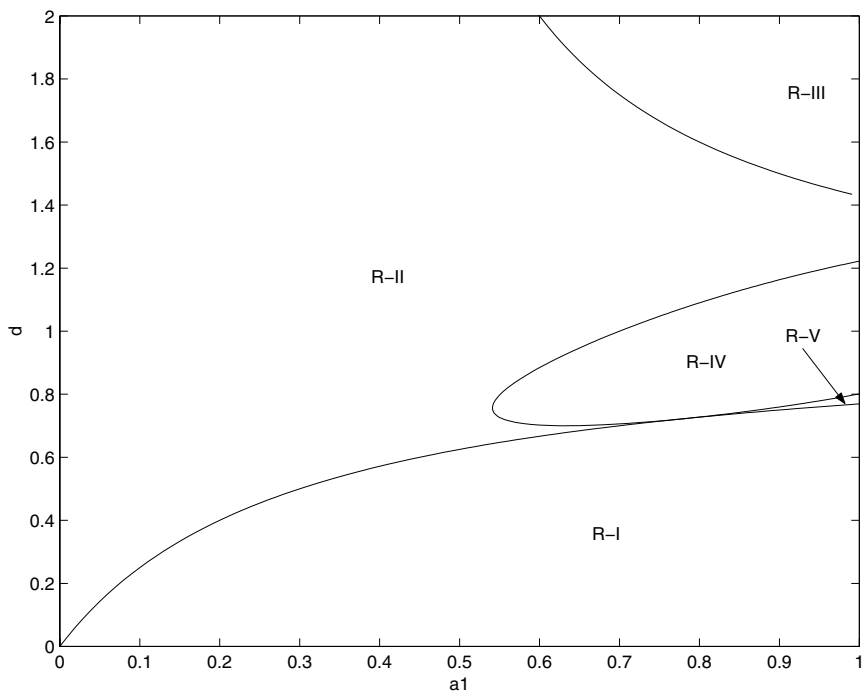


Fig. 1. Setting our parameter to $a_4 = 0.5$ and $d_2 = 0.3$ we obtain a stability map in a_1 and d . Depending on the parameter values we may have up to 5 different regions. In the case when $\tau = 0$, the region of tumor growth is given by R-I and in the complement we get tumor decay. When we increase τ , the pure growth region is essentially unaltered, but when $\tau > 0$ the decay region is given by R-II \cup R-IV, while in R-III \cup R-V there is stability switching as τ increases. Region R-V is shown in the graph with an arrow pointing to its center.

given by R-II \cup R-III \cup R-IV \cup R-V, but when $\tau > 0$ the decay region is given by R-II \cup R-IV, while in R-III \cup R-V there is stability switching as τ increases but eventually for sufficiently large $\tau > T$ it will remain unstable. This fact can be illustrated in figure 2. In the upper portion of the graph we have set our parameter values in region R-III for $\tau = 1$ and in this case we see that the tumor decays. This situation will prevail up to $\tau = 14.25$, after which the tumor grows. This switching of the stability of the fixed point from stable to unstable and vice versa may continue, but eventually (according to the theorem) the tumor will grow for large enough τ . In his publication ([27]), Bo gives precise information on the τ values for which the fixed point is stable or not.

In cancer chemotherapy stability switching is a very important issue in the design of a drug protocol. We must keep in mind that in many cases the drugs prevent cells from continuing through their cell cycle, thus trapping them at some point during interphase, where the cells die from natural causes. This effect can be interpreted as an increase in the delay τ . But as we have seen here this trapping may have an adverse effect, since it may cause the tumor free fixed point to become unstable when it was stable initially. On the other hand, the same properties can be

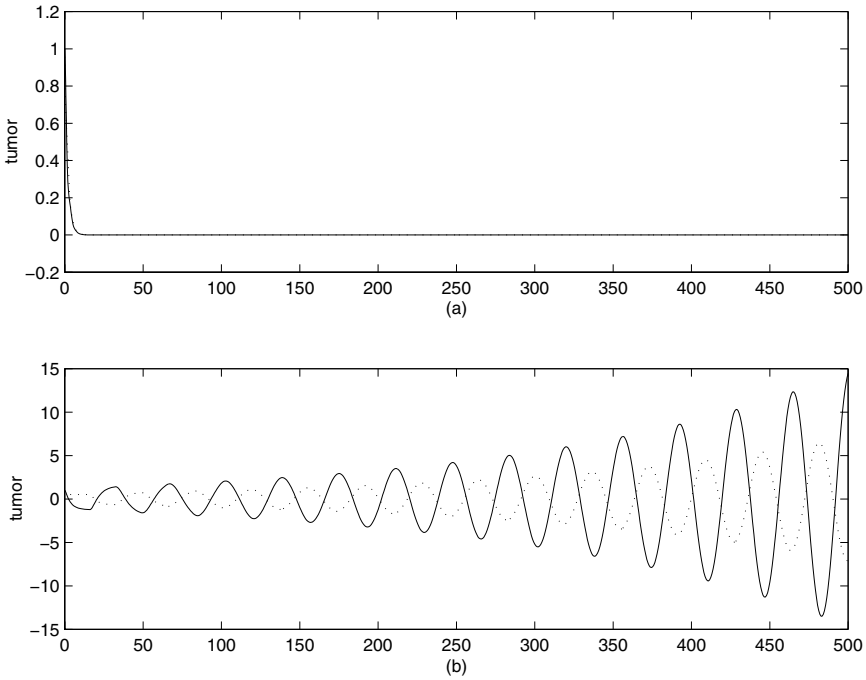


Fig. 2. (a) shows tumor decay when we set $\tau = 1$ (days) and the parameter values in region R-IV of figure 1. Theorem 1 of [26] indicates that for $\tau = 9.5$ a pair of eigenvalues cross the imaginary axis, thus rendering the fixed point unstable. (b) is the result of the numerical integration for $\tau = 11$ (days). The oscillations in (b) are predicted by a pair of purely imaginary eigenvalues.

used to the clinicians advantage, if we are certain that our parameters are in region R-V of figure 1 and the fixed point is unstable. In this case, it may be possible to use the same trapping mechanism to stabilize of the tumor-free equilibrium.

An important characteristic that we can extract from figure 1 is the de-stabilizing effect that the delay has on our system. Note that the region of tumor decay is much smaller when $\tau > 0$, compared to the case $\tau = 0$. This means that the duration of the cell cycle is a determining factor in the outcome of the disease and theoretically be affected by the administration of a particular drug. Therefore, it is possible that the size of these regions might influence the choice of drug, and the eventual cure.

4.1.3. Drug-free model in a non-delay case with immune suppression

We now add the effect of immune suppression when $\tau = 0$. In this case the system considered is:

$$\begin{aligned}
 T_I' &= 2a_4T_M - (c_1I + d_2)T_I - a_1T_I \\
 T_M' &= a_1T_I - d_3T_M - a_4T_M - c_3T_MI \\
 I' &= k + \frac{\rho I(T_I + T_M)^n}{\alpha + (T_I + T_M)^n} - c_2IT_I - c_4T_MI - d_1I
 \end{aligned}$$

$(0, 0, k/d_1)$ is a fixed point of this system with zero tumor level and a positive immune level. In general, there will be other fixed points, but this fixed point is of particular interest since it represents a tumor-free state. The linearization about $(0, 0, k/d_1)$ is

$$\begin{pmatrix} T_I \\ T_M \\ I \end{pmatrix}'(t) = \begin{pmatrix} -d_2 - a_1 - c_1k/d_1 & 2a_4 & 0 \\ a_1 & -d - c_3k/d_1 & 0 \\ -c_2k/d_1 & -c_4k/d_1 & -d_1 \end{pmatrix} \begin{pmatrix} T_I \\ T_M \\ I \end{pmatrix}$$

Let $\hat{d}_1 = k/d_1$, and $d = d_3 + a_4$, and for notational convenience we will drop the hat on \hat{d}_1 . Clearly $\lambda = -d_1$ is an eigenvalue, the remaining eigenvalues are given as the solutions to the characteristic equation

$$\lambda^2 + \lambda(a_1 + d_2 + (c_1 + c_3)d_1 + d) + (a_1 + d_2 + c_1d_1)(d + c_3d_1) - 2a_1a_4$$

Let $\bar{tr} = -(a_1 + d_2 + (c_1 + c_3)d_1 + d)$, and $\bar{\Delta} = (a_1 + d_2 + c_1d_1)(d + c_3d_1) - 2a_1a_4 = \Delta + (c_1 + c_3)d_1$. Notice that the trace \bar{tr} is always negative (as in the system without immune response). Also note that if $\Delta > 0$, then $\bar{\Delta} > 0$. This means that in the regions of the parameter space where $(0, 0)$ was stable, then $(0, 0, d_1)$ is also stable. If $\Delta < 0$, then $\bar{\Delta}$ can be either positive or negative so we have $\bar{\Delta} < 0 \Leftrightarrow \Delta < -d_1(c_1 + c_3)$, giving the condition for tumor growth.

As before we can make a plot in the $a_1 - d$ parameter space (Figure 3), comparing the regions of tumor growth and tumor decay in the case $\tau = 0$ with and without immune suppression. From Figure 3 we see that the region of tumor growth is reduced in size when compared to the case with no immune. The parameter values were set to clearly differentiate the two curves for pedagogical purposes.

4.1.4. Drug-free model when $\tau > 0$, with immune suppression

Again $(0, 0, k/d_1)$ is a fixed point and its analysis is similar to the case in section 4.1.2 though computations are complicated by more terms. This system has the same fixed points as the system described in section 4.1.3, but again we focus on the tumor free fixed point.

In the case of a positive delay, the characteristic equation for the linearized equation around a fixed point $(\hat{T}_I, \hat{T}_M, \hat{I})$ is given by:

$$\begin{aligned} F(\lambda) &= P(\lambda) + e^{-\tau\lambda}Q(\lambda) \\ &= \lambda^3 + p_2\lambda^2 + p_1\lambda + p_0 + e^{(-\tau\lambda)} \left[q_2\lambda^2 + q_1\lambda + q_0 \right] \end{aligned} \tag{4}$$

with coefficients

$$\begin{aligned} p_2 &= p + q + r, & p_1 &= pq - c_e\hat{T}_Mw + r(p + q) + z, \\ p_0 &= rpq - c_3\hat{T}_M(wr - 2za_4) + pz \end{aligned}$$

$$q_2 = a_1, \quad q_1 = a_1(p + q - 2a_4), \quad q_0 = a_1(pq - c_3\hat{T}_Mw - 2a_4q + c_1\hat{T}_Iw)$$

$$p = d_3 + a_4 + c_1\hat{I}, \quad q = c_2\hat{T}_I + c_4\hat{T}_M + d_1 - \frac{\rho(\hat{T}_I + \hat{T}_M)^3}{\alpha + (\hat{T}_I + \hat{T}_M)^3}, \quad r = d_2 + c_1\hat{I}$$

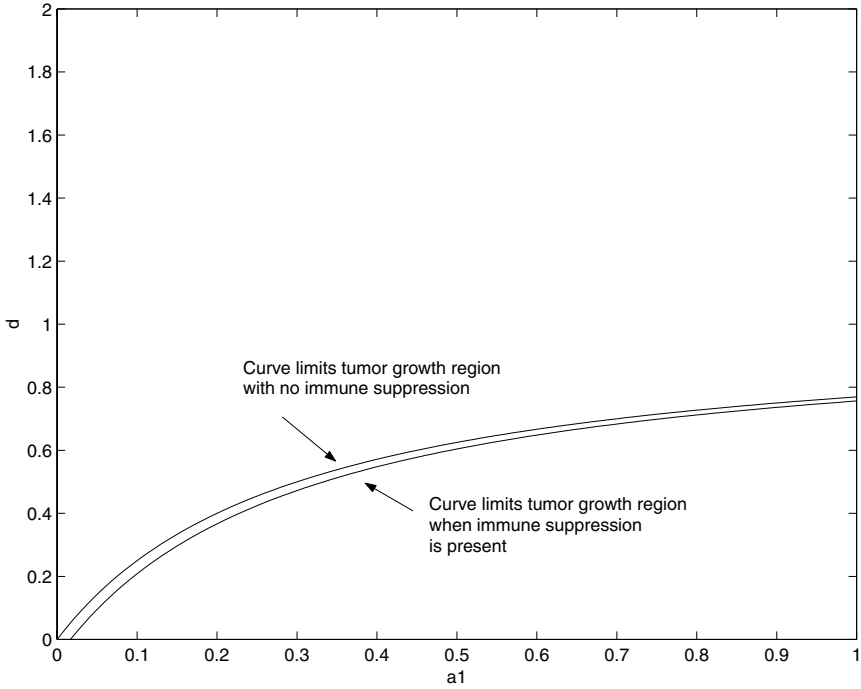


Fig. 3. Setting our parameter to $a_4 = 0.6, d_2 = 0.3$ we obtain a stability map in a_1 and d plane. In the case when $\tau = 0$, the region of tumor growth with no immune suppression is limited by the upper curve; below this curve, there is tumor growth and above this curve there is tumor decay. The corresponding curve when immune suppression is added is shown to lie consistently below the first. In generating this figure parameter values were set to $k = 0.1, d_1 = 0.6, c_1 = c_3 = 0.05$.

$$w = c_4 \hat{I} - \frac{3\alpha \hat{I}(\hat{T}_I + \hat{T}_M)^2}{[\alpha + (\hat{T}_I + \hat{T}_M)^3]^2}, \text{ and } z = c_2 \hat{I} - \frac{3\alpha \hat{I}(\hat{T}_I + \hat{T}_M)^2}{[\alpha + (\hat{T}_I + \hat{T}_M)^3]^2}.$$

Geometric arguments using the argument principle can be used to establish the stability of a given fixed point by counting the number of zeros of $F(\lambda)$ on the right hand side of the complex plane ([25]). The argument is based on the relative orientation of F when compared to P as we traverse a given contour. Unfortunately, the theorem developed in [25] cannot be used directly in our case because the hypotheses are not satisfied, but the argument can be modified and we can thereby deduce conditions on parameter space which ensure stability. These are given in the following theorem.

Theorem 1. Let $P(\lambda) = (\lambda + \beta_1)(\lambda + \beta_2)(\lambda + \beta_3)$ and $Q(\lambda) = (\lambda + \alpha_1)(\lambda + \alpha_2)$, such that

$$d := \prod_{i=1}^3 \beta_i^2 - \prod_{i=1}^2 \alpha_i^2 > 0, \quad \prod_{i=1}^3 \beta_i > 0, \quad \prod_{i=1}^2 \alpha_i < 0,$$

$$\sum 1/\beta_j > \sum 1/\alpha_k, \text{ and } \sum \alpha_j > 0.$$

Assume that (4) is stable for $\tau = 0$.

Let $b := \sum \beta_i^2 - 1$, $c := \sum_{i \neq j} \beta_i^2 \beta_j^2 - \sum \alpha_i^2$ and $R := (-b + \sqrt{b^2 - 3c})/3$. Then if:

1. $c < 0$ and if $R(-b^2 + 6c + b\sqrt{b^2 - 3c}) + 9d < 0$, then there exists a $\tau^* > 0$, such that for $\tau > \tau^*$ are at least two roots of (4) on the right hand side of the complex plane.
2. $c > 0$ and $b > 0$, then there are no roots on the right hand side of the complex plane.
3. $c > 0$, $b < 0$, $b^2 - 3c > 0$ and $R(-b^2 + 6c + b\sqrt{b^2 - 3c}) + 9d < 0$, then there exists a $\tau^* > 0$, such that for $\tau > \tau^*$ there are at least two roots of (4) on the right hand side of the complex plane.

The proof of Theorem 1 can be found in the appendix. The idea of Theorem 1 is very general and can be applied to any equation of the form (4). In case 3, stability switching can occur, and we would like to find those values of the delay, τ , at which this happens. We resort again to Theorem 1 of [26]. Following the steps in this theorem it is straightforward to check the stability of each fixed point, given a specific set of parameters.

For example, let $a_1 = 1$, $a_4 = 0.4$, $c_1 = 0.02$, $c_2 = 0.008$, $c_3 = 0.02$, $c_4 = 0.008$, $d_1 = 0.028$, $d_2 = 0.28$, $d_3 = 0.28$, $\alpha = 0.2$, $\rho = 0.2$, $k = 0.036$. For these parameter values there is one fixed point, namely the tumor-free fixed point at $(0, 0, 0.9)$. A simple calculation reveals that for $\tau = 0$ the fixed point is stable and Theorem 1 in [26] determines that, when $\tau = 4.24293$, a pair of conjugate eigenvalues cross the imaginary axis from left to right thus making the fixed point unstable. As τ is increased to $\tau = 28.6675$ this pair of conjugate eigenvalues cross from right to left and so the fixed point becomes stable again. This stability switching can continue as τ is increased, but eventually, for large enough τ the fixed point is unstable.

These results can be corroborated using the techniques described in Theorem 1 of this paper. If we chose $\tau = 1$, we should see that there are no encirclements of the origin by the image of a contour Γ under F . However if we chose $\tau = 6$, we should see two encirclements of the origin, and in fact this is the situation shown in Figures 4 and 5.

Figure 6 provides a way to view the changes in the stability of the system. In Figure 6 we have set our parameters identical to those used in the previous example. On the x -axis we plot the constant immune source rate k , and on the y -axis is the delay τ . Setting $a_4 = 0.452$, we have only one fixed point for the range of k considered (from 0 to 0.2), therefore it refers to the tumor-free fixed point. Region II depicts those (k, τ) values for which the unique fixed point is unstable. Region I corresponds to (k, τ) values for which the fixed point is stable. Notice that this agrees with previous results and also that here we can see the stability switching for a fixed parameter set as we vary the delay τ . If we fix a value of $k = 0.036$, then the fixed point starts out stable (region I), but as τ increases to approximately 29 we encounter region II indicating that the fixed point switches from being stable to unstable, and if τ is further increased, the fixed point becomes stable again (not shown in figure). This pattern repeats itself many times until eventually the fixed

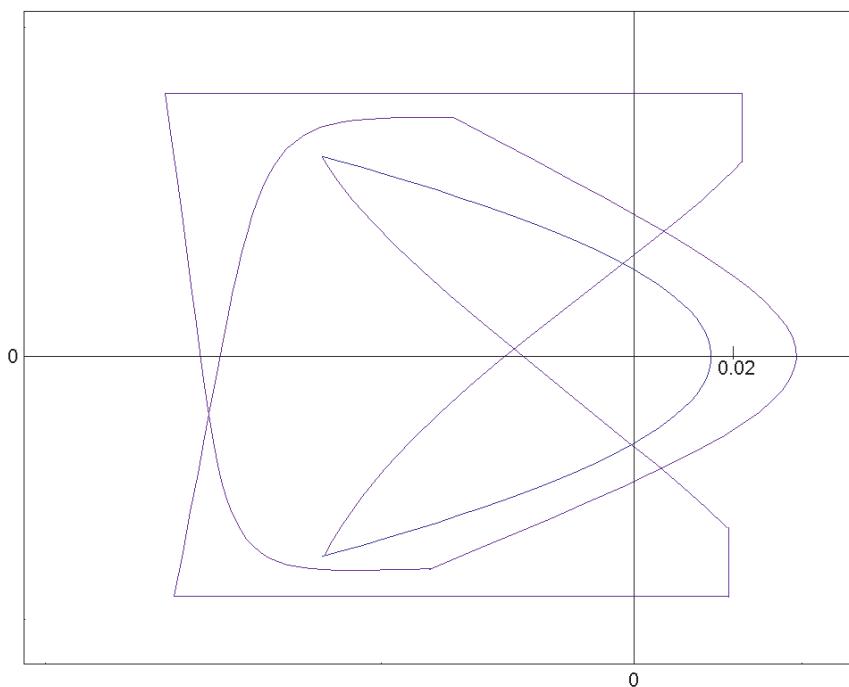


Fig. 4. $F \circ \gamma$ for $\tau = 1$. The parameter values have been set to yield a stable fixed point, and this is seen as $F \circ \gamma$ gives a net of 0 encirclements as we traverse the contour γ .

point remains unstable for all τ . It is important to mention that this particular phenomenon did not depend on the values for the parameters that we have not been able to estimate, namely ρ and α .

As mentioned before, in the context of cancer models stability switching as the delay is varied is very important since many cycle-phase-specific drugs retain the cells or trap them in a given phase, thus increasing the time a cell spends in a particular compartment. This analysis shows that care must be taken when trapping the cells in a compartment since the ultimate effect may be adverse: the tumor-free fixed point may switch from a stable equilibrium to an unstable one. This would mean that when treatment is stopped, the system would not move towards the tumor-free state. On the other hand, it is possible to increase or decrease the resident time during the interphase to ‘unlock’ a fixed point from its instability and to push it towards the stable range.

4.2. Hopf bifurcation

With the aid of Theorem 1 in the work by Cooke and van den Driessche [26], it is also straightforward to check for possible Hopf bifurcations as we increase the delay, τ . The importance of Hopf bifurcations in this context is that at the bifurcation point a limit cycle is formed around the fixed point, thus resulting in stable

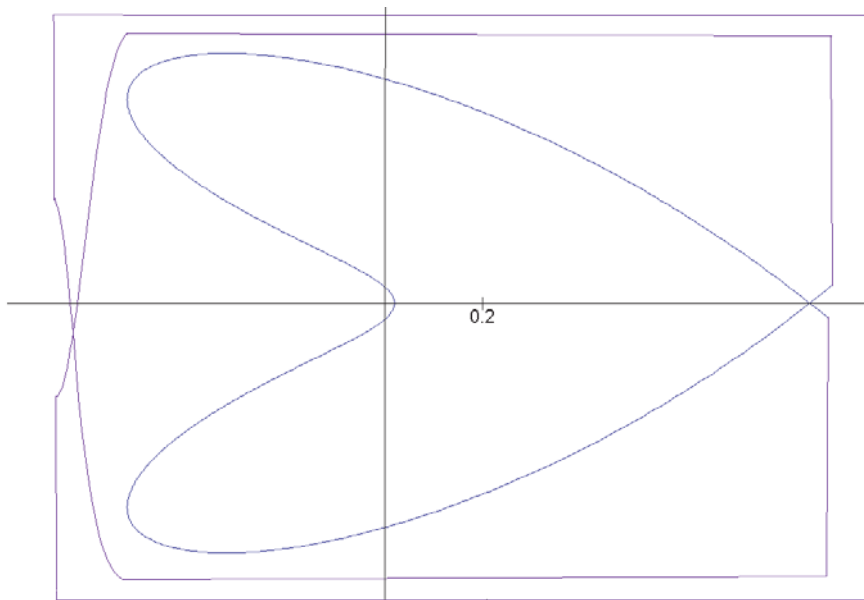


Fig. 5. $F \circ \gamma$ for $\tau = 6$. As we increase the delay τ , the fixed point changed its stability from being stable (as in the case $\tau = 1$) to unstable. Theorem 1 predicts this change of stability as there is a net of 2 encirclements as we traverse the contour.

periodic solutions. The existence of periodic solutions is relevant in cancer models, because it implies that the tumor levels may oscillate around a fixed point even in the absence of any treatment. Such a phenomenon has been observed clinically and is known as “Jeff’s Phenomenon” ([28]). In this section we will show an example of parameters that exhibit this behavior.

For example, with the parameter values set at: $a_1 = 1$, $a_4 = 0.5$, $c_1 = 0.02$, $c_3 = 0.008$, $c_2 = 0.02$, $c_4 = 0.008$, $d_1 = 0.04$, $d_2 = 0.11$, $d_3 = 0.28$, $\alpha = 0.5$, $\rho = 0.2$, and $k = 0.036$, we can use Theorem 1 in [26] to check the stability of the fixed points of the model described in section 4.1.4. In this case there are three fixed points: $x_1 = (0, 0, 0.9)$, $x_2 = (9.7829, 11.5293, 3.4261)$, and $x_3 = (0.21272, 0.25069, 3.4261)$. By computing the roots of $F(\lambda)$ as given by equation (4), it can be seen that x_1 and x_2 are unstable fixed points for all values of the delay. However, x_3 has some stability switching, and they satisfy the conditions of the Hopf Bifurcation Theorem [24]: the stability of the fixed point switches from stable to unstable at some $\hat{\tau}$ and the transversality condition ($\frac{\partial \Re \lambda(\hat{\tau})}{\partial \tau} \neq 0$) is satisfied. This equilibrium (x_3) corresponds to a steady state of the system at which a tumor is present. Oscillations around this equilibrium would therefore correspond to a tumor which grows and shrinks with no application of treatment. Figures 8 and 7 have been generated using the Matlab subroutines developed by Shampine and Thompson for solving delay differential equations, `dde23` (see [29]). We see that there are clear oscillations in the tumor. Many other sets of parameter values have been found which give rise to periodic solutions through Hopf bifurcations

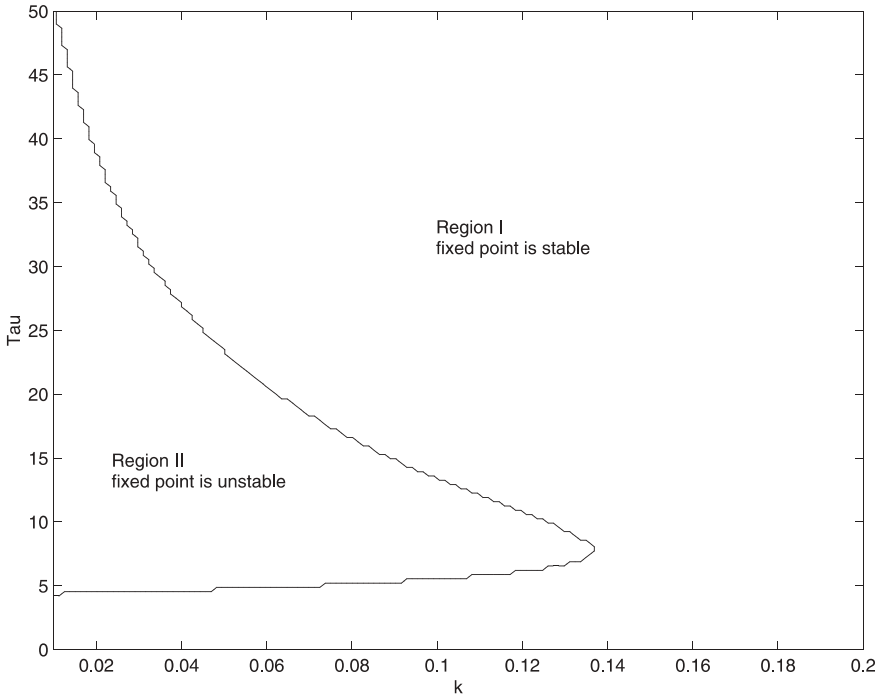


Fig. 6. Stability Map setting $a_4 = 0.4$. The tumor-free fixed point is stable in Region I, and is unstable in Region II. This change in stability is due to an increase in the delay τ . τ has a destabilizing effect and we may see many stability switchings (passage from a region type I to a region type II) before the fixed point becomes unstable as we increase τ .

in a feasible parameter range. It is important to mention that in all the numerical experiments the existence of the Hopf bifurcation did not depend on the specific values for ρ or α .

5. Discussion

The model presented is very simple and some might argue that it does not contain many important interactions, cell types, etc. We must keep in mind that a complicated model may simply be more complicated, and may not necessarily give more information about the overall dynamics. However, there are a few features we consider significant that have not been included in this simple model. For example the inclusion of another delay in the cell cycle might be pertinent as we separate the phases of the cycle to be more precise about the mode of action of the drugs on the different phases, or that these the delays may be a function of the drug. Often tumor cells can become resistant to cytotoxic agents, thus entering a quiescent phase in which they are not affected by cycle-specific drugs. Including drug resistance in the model would call for the addition of the quiescent phase, and it would help us understand how the resistant population contributes to or delays the eradication of the disease. The immune system is a very complicated entity, and

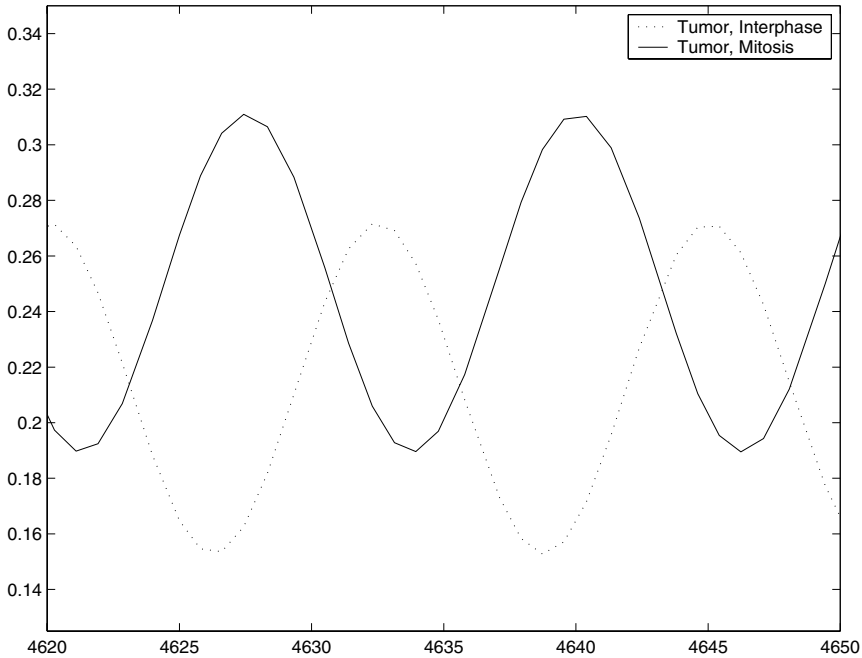


Fig. 7. Periodic Solutions, Tumor. Periodic Solutions of the tumor. The solid line corresponds to The first time series (upper graph) corresponds to T_S and the lower graph corresponds to T_M . The oscillatory behavior of the signal is due to a Hopf Bifurcation as we vary the delay τ .

in this work we feel that we have merely touched the surface of the interactions and processes involved in the system's immune response. There is still much to be learned about the immune system and how it affects the course of the disease. A more careful study and detailed modeling of this interaction is advisable. This can be done by including more immune cell types in our model, for example different T cells, Natural Killer cells (NK), various cytokines, etc., and investigating more precisely the interactions among the cells that comprise the immune system and between immune and tumor cells.

This model is very general and it is not intended to fit every type of cancer, since each cancer type presents different difficulties and challenges. For example, a patient with leukemia, a cancer of the immune system, has virtually no natural defense against the disease, so the consideration of the immune system as a mechanism of defense against leukemia is not realistic ([30]). In this case, the more important characteristics determining the outcome of the disease are the time of diagnosis (the earlier the cancer is detected, the better the chances for survival), and the effects of the antineoplastic agents used. In other cancers, such as melanoma, spatial characteristics are an important feature to consider, allowing for the characterization of the way tumor cells spread out and compete for surrounding resources.

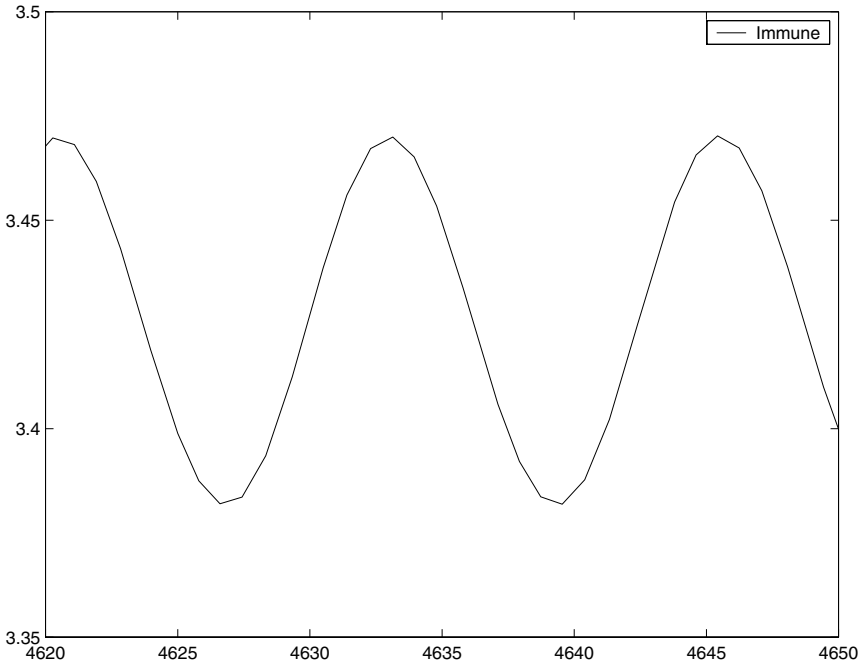


Fig. 8. Periodic Solutions, Immune System. The oscillatory behavior of the signal is due to a Hopf Bifurcation as we vary the delay τ .

There are many components in this model that may be regarded as stochastic, rather than deterministic. For example, the dwelling time or resident time in each phase is not a fixed amount but varies slightly around an average value. These variations may significantly alter the dynamics of the system, as seen in the examples of stability switching. In this work we considered average amounts, or expected value of these quantities: average resident time, average population size and, average interaction rates for example.

Another consideration is to allow the delay to be a function of the drug concentration, $\tau = \tau(u)$. This would give a more accurate description of the effect of the drug on the system.

For this model we proved Theorem 1 based on the argument principle which gives conditions under which all the roots of the characteristic equation have negative real parts. We saw through the work of Cook and van den Driessche [26] that in some cases increasing the delay can stabilize or destabilize the system. This is particularly important, since the effect of the drug Paclitaxel and others can be described as ‘delaying’ or trapping the cells in the M phase of the cell cycle. Hence the conclusion of this theorem gives useful information for a model in which the delay is a function of the drug. For example, one can say that the more drug present in the system, the more cells will be trapped in the cycle. We also observed periodic solutions for some parameter values through a Hopf bifurcation. Periodic tumors

arise from time to time in patients. This mystifies clinicians, so being able to observe periodic solutions in our system may explain periodic tumors whose growth is uncorrelated with the administration of chemotherapy, more commonly referred to as Jeff’s Phenomenon.

The main objective when applying the drug is to drive the system into the basin of attraction of the tumor-free equilibria (assuming that the equilibria is stable). In a further report ([15]) the basin of attraction for the tumor free fixed point is computed and shows how this information can be used in designing optimal drug scheduling.

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6. Appendix

In this section we give the proof of Theorem 1.

Let $P(\lambda) = (\lambda + \beta_1)(\lambda + \beta_2)(\lambda + \beta_3)$ and $Q(\lambda) = (\lambda + \alpha_1)(\lambda + \alpha_2)$, such that

$$d := \prod_{i=1}^3 \beta_i^2 - \prod_{i=1}^2 \alpha_i^2 > 0, \quad \prod_{i=1}^3 \beta_i > 0, \quad \prod_{i=1}^2 \alpha_i < 0,$$

$$\sum 1/\beta_j > \sum 1/\alpha_k, \quad \text{and} \quad \sum \alpha_j > 0.$$

Assume that (4) is stable for $\tau = 0$.

Let $b := \sum \beta_i^2 - 1$, $c := \sum_{i \neq j} \beta_i^2 \beta_j^2 - \sum \alpha_i^2$ and $R := (-b + \sqrt{b^2 - 3c})/3$. Then if:

1. $c < 0$ and if $R(-b^2 + 6c + b\sqrt{b^2 - 3c}) + 9d < 0$, then there exists a $\tau^* > 0$, such that for $\tau > \tau^*$ are at least two roots of (4) on the right hand side of the complex plane.
2. $c > 0$ and $b > 0$, then there are no roots on the right hand side of the complex plane.
3. $c > 0$, $b < 0$, $b^2 - 3c > 0$ and $R(-b^2 + 6c + b\sqrt{b^2 - 3c}) + 9d < 0$, then there exists a $\tau^* > 0$, such that for $\tau > \tau^*$ there are at least two roots of (4) on the right hand side of the complex plane.

Proof. To prove this theorem we will use techniques similar to those given in [25]. Consider the contour Γ which consists of the union of the line segments:

$$\Gamma = [0, i2\pi/\tau] \cup [i2\pi/\tau, \mu^* + i2\pi/\tau] \cup [\mu^* + i2\pi/\tau, \mu^* - i2\pi/\tau] \\ \cup [\mu^* - i2\pi/\tau, -i2\pi/\tau] \cup [-i2\pi/\tau, 0].$$

for sufficiently large μ^* .

We compare the orientation of F and P with respect to the origin as we traverse the contour Γ and count the times F encircles the origin clockwise. If F does not

encircle the origin, the argument principle implies that F has no roots inside Γ , and we conclude that, in fact, it has no roots with positive real part (See [23], [31], [25]). Here are the details: Let $\lambda = \mu + i\nu$, and write F as:

$$\begin{aligned}
 F(\lambda) &= P(\lambda) + Q(\lambda)e^{-\lambda\tau} \\
 &= |P(\lambda)|e^{i\theta_p(\lambda)} + |Q(\lambda)|e^{i\theta_q(\lambda)}e^{-\lambda\tau}
 \end{aligned}$$

where

$$\theta_p(\lambda) = \sum \arctan\left(\frac{\nu}{\beta_j + \mu}\right)$$

$$\theta_q(\lambda) = \sum \arctan\left(\frac{\nu}{\alpha_j + \mu}\right)$$

$$|P(\lambda)| = \prod_{j=1}^3 \left[\nu^2 + (\beta_j + \mu)^2 \right]^{1/2}$$

$$|Q(\lambda)| = \prod_{j=1}^2 \left[\nu^2 + (\alpha_j + \mu)^2 \right]^{1/2}$$

As we traverse the contour Γ , we consider the image of Γ under F , $F(\Gamma)$, and the number of encirclements of $F(\Gamma)$ around the origin. We first look at the alignments of $P(\lambda)$ and $F(\lambda)$.

Note that $\arg [F(\lambda) - P(\lambda)] = \theta_q(\lambda) - \nu\tau$ and alignment occurs when

$$\theta_p(\lambda) - \arg [F(\lambda) - P(\lambda)] = k\pi$$

For $\nu = \frac{i2\pi}{\tau}$, $\arg [F(\frac{i2\pi}{\tau}) - P(\frac{i2\pi}{\tau})] = \theta_q(\frac{i2\pi}{\tau}) - 2\pi$. Therefore,

$$\theta_p\left(\frac{i2\pi}{\tau}\right) - \arg \left[F\left(\frac{i2\pi}{\tau}\right) - P\left(\frac{i2\pi}{\tau}\right) \right] > \pi,$$

so there exists a ν_0 such that as λ traverses γ_1 the alignment condition is satisfied:

$$\theta_p(i\nu_0) - \arg [F(i\nu_0) - P(i\nu_0)] = \pi$$

other alignments, ν_k may exist, in which case we have

$$\theta_p(i\nu_k) - \arg [F(i\nu_k) - P(i\nu_k)] = (k + 1)\pi$$

One can prove that if $|P(i\nu_0)| > |Q(i\nu_0)|$, then F does not encircle the origin and if $|P(i\nu_0)| < |Q(i\nu_0)|$, then F encircles the origin and so by the argument principle we can show that there are at least 2 roots inside Γ . The proof of this result is the same as the proof of Proposition 3.1 in [31], hence it will not be reproduced here.

We have shown that alignment of F and P must occur as we traverse the contour Γ . The proof of the theorem proceeds as follows:

1. Find the region where $|P(i\nu)| < |Q(i\nu)|$
2. Pick a contour so that the above condition is possible (in many cases the contour Γ is sufficient).
3. Show that the alignment condition holds in the region where $|P(i\nu)| < |Q(i\nu)|$.

Step 1. Given P and Q , we compute $|Q(i\nu)|$ and $|P(i\nu)|$ and after some calculations we get that

$$|Q(i\nu)| - |P(i\nu)| = 0 \Leftrightarrow \nu^6 + b\nu^4 + c\nu^2 + d = 0$$

Making the change of variables $y = \nu^2$, we obtain

$$y^3 + by^2 + cy + d = 0 \tag{5}$$

We want the curves for $|P(i\nu)|$ and $|Q(i\nu)|$ to intersect at a real ν , i.e. a positive root (y) of (5). Since $d > 0$, the only way that we can obtain a positive root for (5) is that the derivative has a positive root, and that this root evaluated on the third order polynomial above is negative, i.e. (5) has a negative local minimum at a positive y value. The derivative of (5) is given by

$$3y^2 + 2by + c = 0$$

with roots at

$$R_{1,2} = \frac{-b \pm \sqrt{b^2 - 3c}}{3}$$

There are three cases to consider:

1. If $c < 0$, then $R_1 > 0$ and $R_2 < 0$; evaluating (5) at R_1 we get that $R_1(-b^2 + 6c + b\sqrt{b^2 - 3c}) + 9d$ must be negative in order that $|Q(i\nu)| > |P(i\nu)|$ for some real ν .
2. If $b < 0, c > 0$ and $b^2 > 3c$ we will have 2 positive roots for the derivative and with $R_1(-b^2 + 6c + b\sqrt{b^2 - 3c}) + 9d < 0$, we are assured that there will be positive root for the cubic.
3. In the case $b > 0$ and $c > 0$, there are no positive roots, which implies no encirclements.

Step 2. Let $k \geq 2$ be an integer such that $\nu^* < k\pi/\tau$, with $\nu^* = \sqrt{R_1}$, where R_1 is the biggest root of the cubic. Consider the contour

$$\Gamma_1 = [0, ik\pi/\tau] \cup [ik\pi/\tau, \mu^* + ik\pi/\tau] \cup [\mu^* + ik\pi/\tau, \mu^* - ik\pi/\tau] \cup [\mu^* - ik\pi/\tau, -ik\pi/\tau] \cup [-ik\pi/\tau, 0].$$

for sufficiently large μ^* This choice of k ensures that the condition $|P(i\nu)| < |Q(i\nu)|$ holds for $0 \leq \nu < \nu^*$ and that there is alignment at some $0 \leq \nu_0 \leq \nu^*$, as described below.

Step 3. To obtain alignment of $P(i\nu)$, $F(i\nu, \tau)$ and the origin as we traverse the segment $[0, ik\pi/\tau]$, consider $P(-i\nu)$ and $F(-i\nu, \tau)$ with ν from 0 to $-ik\pi/\tau$. For alignment, the following must hold.

$$\arg [F(-i\nu, \tau) - P(-i\nu)] - \arg [P(-i\nu)] = k\pi$$

$$\iff g(v) = v\tau + \arg(Q(-iv)) - \arg(P(-iv)) = k\pi \tag{6}$$

If $\arg(Q(-iv)) < \arg(P(-iv))$ for any $v < v^*$, then (6) cannot hold since then:

$$\begin{aligned} 0 &= -k\pi + v\tau + \arg(Q(-iv)) - \arg(P(-iv)) \\ &< -k\pi + v\tau \Rightarrow k\pi < v\tau \\ &\Rightarrow v > \frac{k\pi}{\tau} > v^* \rightarrow \leftarrow \end{aligned}$$

We can expand the expression for the arguments as

$$\begin{aligned} \arg(Q(-iv)) &= \sum \arctan\left(\frac{-v}{\alpha_j}\right) \\ \arg(P(-iv)) &= \sum \arctan\left(\frac{-v}{\beta_j}\right) \end{aligned}$$

The expansion around 0 for $\arctan x$ is

$$\arctan x \approx x - \frac{x^3}{3} + \frac{x^5}{5} - \dots \text{ for } |x| < 1.$$

Given $\epsilon > 0$, there exists $\tau_1 \gg 1$ such that if $\tau > \tau_1$, $v < k\pi/\tau < \epsilon$. We can therefore approximate $\arctan x \approx x$. Since $\prod \beta_j > 0$, we have $\arg(P(-iv)) \approx \sum \arctan\left(\frac{-v}{\beta_j}\right) = \sum\left(\frac{-v}{\beta_j}\right) = -v \sum 1/\beta_j$. Similarly, $\prod \alpha_i < 0$ gives,

$$\arg(Q(-iv)) = \pi + \sum \arctan\left(\frac{-v}{\alpha_j}\right) \approx \pi + \sum\left(\frac{-v}{\alpha_j}\right) = \pi - v \sum 1/\alpha_j.$$

From $\sum 1/\beta_j > \sum 1/\alpha_k$, $\arg(Q(-iv)) > \arg(P(-iv))$ for all $v > 0$, and therefore by continuity of $g(v)$ (6) holds for some $v_0 \in \gamma_1$.

So far we have that for τ large enough P and Q may align on $[0, ik\pi/\tau]$ and we also have found conditions for which $|Q(-iv)| > |P(-iv)|$.

As we mentioned before, it is shown in [31] that if

$$|Q(-iv_0)| > |P(-iv_0)|,$$

where v_0 is the first alignment of F, P and the origin, then $F(\lambda, \tau)$ encircles the origin as λ goes around Γ , and that the argument principle may be used to demonstrate that at least 2 roots of F lie inside Γ .

Let τ^* , be the solution of (6) where $v = v^*$, then for $\tau > \tau^*$, we are certain that $|Q(-iv_0)| > |P(-iv_0)|$ will hold, and the theorem is proved. \square

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