Assessment of cancer immunotherapy outcome in terms of the immune response time features

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Abstract

A cytokine based periodic immunotherapy treatment is included in a model of tumor growth with a delay. The effects of dose schedule are studied in the case of a weak immune system and a growing tumor. We find the existence of *metastable* states (that may last for tens of years) induced by the treatment, and also of potentially adverse effects of the dosage frequency on the stabilization of the tumor. These two effects depend on the delay between tumor growth and the immune system response, the cytokine dose burden and other parameters considered in the model.

Keywords tumor growth, delay differential equations, immunotherapy, immunodepression

1 Introduction

The development of a cancer tumor under the influence of the immune system deploys a very rich dynamics with some aspects that must be highlighted. The effector mechanism by which immune systems attacks an immunogenic tumor is cell-mediated cytotoxicity. Innate immunity cells, as macrophages and natural killers (NK) but, above all, adaptive immunity cells, as T lymphocytes, are the effector cells in the anti-tumor immune response. T-lymphocytes become activated in the presence of certain cytokines and are leaded by the presence of antibodies attached to cancerous cells (Tortora and Grabowski, 1999; Dezfouli et al., 2005; Saleh et al., 2005). Antibodies are produced by B-lymphocytes whose growth and activation, in turn, are stimulated by the cytokines produced by T-lymphocytes in contact with the tumor-associated antigens (TAA). A delay between the detection of the "strange cells" (antigen) and the attack of the T-lymphocytes exists, being a (destabilizing) control parameter of the immune response (Asachenkov et al., 1994; Marchuk, 1997; Byrne, 1997; Nani and Freedman, 2000; Galach, 2003).

However, when the tumor size increases lymphocyte effectivity may decrease. Various mechanisms have been proposed to account for this effect: i) antigenic heterogeneity: cells from one tumor may lose their specific tumor-associated antigens (TAA) and acquire unrelated TAA (each TAA requires a specific immune response) (Schuster et al., 2006); or ii) immunodepression: deactivation of lymphocytes that enter the tumor region (de Boer et al., 1985; Whiteside, 2002).

As far as we know, the existence of the immune response delay just described between the stimulus (antigen) and the triggering of the defenses of the immune system, has not been considered enough in tumor models found in the literature, despite the reports by Forys (2002) and Galach (2003). In these papers, time delay is introduced in the tumor-immune system model proposed by Kuznetsov et al. (1994) following the Marchuk approach to immunological response (see Marchuk (1997) or Asachenkov et al. (1994), for example). Marchuk has shown that time delay is a very important factor to take into account in the modelling of the immune system and the reaction of living organisms to diseases. Consideration of this factor may also shed light on the failure of therapies, not only in the much more complicated clinical context, but also in laboratory models as the murine adenoviral-vectored immunotherapy reported by Liu et al. (2002).

Another delay times have been shown to be of importance. For example, Villasana and Radunskaya (2003) introduce a delay time in a model adapted from that of Kirschner and Panetta (1998) to take into account the cell cycle, leading to a linear delayed term in the equations. A similar approach is adopted by Byrne (1997); Forys and Bodnar (2003) to multicellular spheroidal tumors in the framework of tumour growth under nutrient diffusion and consumption (Greenspan, 1972).

Oncological immunotherapy uses self immune system to prevent, treat and control the population of tumoral cells. In its simplest form, immunotherapy attempts to boost immune response to cancerous antigens by means of exposing

T-cells to these proteins. The immune response against the tumor can be modulated by unspecific stimulation with adjuvants (e.g., cytokines) (Schuster et al., 2006). Cytokines are a group of proteinic compounds involved in inter-cellular communication, particularly relevant in the immune response coordination (Tortora and Grabowski, 1999). The first cytokine treatment aproved by FAA was interferon-α (Horton et al., 1999; Brassard et al., 2002; Berman et al., 2006). The role played by IF-α consists not only in immune stimulation but also in increasing TAA expression by the cancerous cells. Another cytokine treatments currently in use are IL-2 and IL-12 (Szymanska, 2003; Schuster et al., 2006; Berman et al., 2006) which are mainly immune modulators. These treatments are not entirely effective because high doses (needed to obtain an apreciable increase in the lymphocite growth rate) also produce negative side effects. The current trends in immunology try to avoid these decreasing peak levels (using gene therapy, reviewed by Cross and Burmester (2006)) and combining cytokines in naturally occurring proportions (Schuster et al., 2006).

The study presented in the this paper is based on a previous model introduced by some of the authors (Sotolongo-Costa et al., 2003) to describe the effects of tumor immunodepression. That model is modified to include a "time delay effect" in the stimulation of the the immune system through its interaction with the tumor, thus giving a set of delay ordinary differential equations (Beretta and Kuang, 2002; Hale, 1977; Gopalsamy, 1992; Driver, 1977). The influence of this time delay effect is studied both with and without immunotherapy. Sample values for the model parameters will be taken within the range of biological significance, estimated below.

2 Model

The model introduced by Sotolongo-Costa et al. (2003), accounting for immune system interaction and immunotherapy stimulation was

$$\begin{array}{rcl} \frac{dX}{dt} & = & aX - bXY \\ \frac{dY}{dt} & = & dXY - fY - kX + u + F\cos^2 wt \end{array} \tag{1}$$

where $X(t)$, and $Y(t)$ denote the populations of cancer cells and immune cells (lymphocytes and NK), respectively.

The first equation describes the rate of change for the number of tumoral cells X. As in other models (see d'Onofrio (2005) for an unifying review), the tumor growth rate is assumed proportional to X, while the tumoral cells mortality is taken proportional to the frequency of random interaction with the effector cells Y.

The second equation describes the dynamics of the immune cell population. It is assumed to exist a constant flux rate u of mature lymphocytes and NKs towards the tumor region. Moreover, lymphocytes are recruited through the term dXY accounting for the presence of the tumor and its interaction with lymphocytes (Kirschner and Panetta, 1998). Constant factor d is usually

termed "antigenicity" of the tumor and represents the number of T lymphocyte precursors that can be stimulated upon introduction of the antigen (de Boer et al., 1985). Tumor induced immunodepression (the decrease in the effectivity of T-lymphocytes) is assumed proportional to the tumor size (Sotolongo-Costa et al., 2003) through a term $-kX$ which represents a decrease in the number of activated lymphocytes arriving to the tumor due to the induced T-suppressor cells (de Boer et al., 1985) and has been judged as of great interest by d'Onofrio (2005) (although this author disagrees with the linear functional form). Lymphocytes are also affected by natural death or spontaneous deactivation, $-fY$, proportional to the number of effector cells.

Immunodepression term $-kX$ allows to account for general immunosuppression, which is mathematically expressed as $Y(t) = 0$. Under these circumstances immune system evolution is not well described by the system of equations (1) anymore, and a fatal end (tumor has escaped immune control) is assumed. This effect of the term $-kX$ is considered by d'Onofrio (2005) as "physically inconsistent", however it only represents a limitation of the model to "moderate" conditions where appreciable quantities of both populations do exist. From the practical point of view, this only means that the numerical integration of the system of equations cannot proceed any further.

On the other hand, immunotherapy with cytokines has two main effects (Schuster et al., 2006): it increases the number of locally activated immune cells and also enhances the cancerous cells recognisement by the immune cells. As in other models we will neglect this latter effect, and include it in the model as a periodically scheduled stimulation (Sotolongo-Costa et al., 2003) of the form $F\cos^2 wt$, where w is the therapy frequency and F the peak dose value. Although somewhat unrealistic, this is a commonly used model because it only involves two parameters and is amenable to analytical study (d'Onofrio, 2005).

Following Asachenkov et al. (1994); Galach (2003) we consider the time delay introducing in the recruitment term, dXY, the values $X(t-T)$ and $Y(t-T)$, where T is the average time leading from the detection of tumour growth by the immune system to the arrival of new activated T-lymphocytes. The evolution equations (1) become now:

$$\begin{array}{rcl} \frac{dX}{dt} = & aX - bXY \\ \frac{dY}{dt} = & dX(t-T)Y(t-T) - fY - kX + u + F\cos^2 wt \end{array} \tag{2}$$

For $F = k = u = 0$, the classical delayed Lotka-Volterra model (Driver, 1977) is recovered having a dynamics time scale $t_c = 1/\sqrt{af}$. This time t_c, called in what follows characteristic time, gives the time scale on which oscillations around the equilibrium would be brought about by the population competition (in our complete system, this oscillation may or may not be present, depending on the values of the linearized system eigenvalues). Recasting system (2) in terms of this new time unit t_c, we obtain the following simplified expressions:

$$\begin{array}{rcl} \frac{dx}{dt} = & \alpha x - xy \\ \frac{dy}{dt} = & x(t-\tau)y(t-\tau) - \frac{1}{\alpha}y - \kappa x + \sigma + V\cos^2 \beta t \end{array} \tag{3}$$

where we have defined the dimensionless variables and parameters

$$x = dX/\sqrt{af}, \quad y = bY/\sqrt{af}, \quad \tau = T/t_c, \quad V = Fb/af$$
$$\alpha = \sqrt{a/f}, \quad \beta = w/\sqrt{af}, \quad \kappa = kb/d\sqrt{af}, \quad \sigma = ub/af$$

The assumption of t_c as time unit will allow us to discuss the system temporal behavior independently of the absolute value of the tumoral cells and lymphocytes populations and growth/death/interaction rate coefficients.

3 Parameter estimation

To get some insight about the numerical range of the parameters included in the model we give some estimates of the values of a, f, T, b, d, Y_0, u, X_0, k as well as F and w , using days and cells as basic units.

- a **(intrinsic tumor growth rate)**
  It is known that a "representative" value for the duplication time of a solid tumor is about 70 days (Begg and Steel, 1977). Obviously, there are tumors that duplicate their volume in 20 days, whereas others do it in 100 days, and tumors like those of colon or rectal cancer take a few years to duplicate. We are assuming this growth in the (almost) absence of lymphocytes so $a = \log 2/T_d$ (days)$^{-1}$ may be bounded to the interval $a \in [10^{-4}, 10^{-2}]$ days^{-1}, a conservative estimation, compared to others found in the literature (de Pillis et al., 2005).

- f **(inverse of lymphocite mean life)**
  T **(lymphocite proliferation and maturation time)**
  The lifetime of lymphocytes can be measured, being about 30 days (Kuznetsov et al., 1994), hence the estimation $f \in [10^{-2}, 10^{-1}]$ days^{-1}. The time needed to proliferate ranges from 2 to 12 days (Byrne, 1997) which can be taken as the delay time T of the immune system, $T \in [1, 20]$ days.

- b **(cancerous cells killed per effector cell and per unit time)**
  d **(T-lymphocytes recruited upon interaction of one effector cell with one cancerous cell in the tumor)**
  Following Kuznetsov et al. (1994); Kirschner and Panetta (1998), these parameters can be guessed as b, $d \in [10^{-9}, 10^{-7}]$ (cells day)$^{-1}$.

- Y_0 **(initial immune cells population around the tumor)**
  u **(constant influx of effector cells from the immune system in the absence of the tumor)**
  In the absence of tumor, the second equation in (2) shows the existence of an initial steady state, $u - fY = 0$. From data about the number of cytotoxic lymphocytes produced by the spleen taken from the literature (Forys, 2002; Galach, 2003; Sotolongo-Costa et al., 2003) we estimate the number of lymphocytes involved in the initial immune response as $Y_0 \simeq 3 \cdot 10^5$ cells. From these data we obtain $u \simeq 1.2 \cdot 10^4$ cells $\cdot$ day^{-1}.

- X_0 **(initial tumor cell population)**
  k **(immunodepression coefficient)**
  We assume the existence of an initial population X_0 of about 10^6 cells (correspoding to a tumour of about 1 mg, i.e. in the order of 1 mm^3, well under the clinical detection threshold). In the absence of lymphocytes k represents the fraction of tumor cells that block the flow of lymphocytes to the region occupied by the tumor. From this, we make an initial appreciation of $k \simeq 1.2 \cdot 10^{-2}$ $days^{-1}$.

- F **(immunotherapy peak dose)**
  w **(immunotherapy dossage frequency)**
  We will consider dosage frequency within a wide range from fractions of day to months based on protocols for IL-2 and IFN-alpha administration found in the literature. The peak dose F (taken as the increase in the flux of lymphocytes towards the tumor) is more difficult to estimate and we will assume a peak capable to double the flux of lymphocytes (that is, of the order of u).

All these estimates are in good agreement with particular values collected from other models in the literature (see de Pillis et al. (2005) and references therein). In particular, the characteristic time of the system t_c, with respect to which adimensionalization is performed, ranges from 30 to 1000 days. The estimated values of the corresponding dimensionless quantities are summarized in table 1.

	min	max	typical
α	$\sim 10^{-2}$	~ 1	1
κ	$\sim 10^{-2}$	~ 10	0.3
σ	$\sim 10^{-2}$	$\sim 10^3$	0.5
τ	$\sim 10^{-3}$	$\sim 10^2$	1
x_0	$\sim 10^{-3}$	$\sim 10^2$	0.1

Table 1: Ranges for the orders of magnitude of the dimensionless parameter values of equation (3) as well as initial condition for tumor cell population. In the last column, the typical values used in the simulations reported in this work.

4 Results without therapy

As usual, in this model two fixed points of the dynamics exist corresponding to the clinical situation of tumor regression towards a tumorless state and also that of a dormant tumor, in which an equilibrium is established between the tumor growth and its control by the immune system. Related to this last state are tumor remissions; these are periodic tumor outgrowths immediately controlled by an immune system acute reaction (Steel, 1993; Kuznetsov et al., 1994).

The linear stability analysis of the non-delayed, non-stimulated system, performed by Sotolongo-Costa et al. (2003), identifies the positions of these two critical points together with their stability conditions. The results are summarized in what follows.

Tumorless state: $L_0 = (0,\, \alpha\sigma)$

- stable (node), $\sigma > 1$
- unstable (saddle), $\sigma < 1$ (E1)

Controlled growth state (or dormant tumor): $L_1 = \left(\frac{1-\sigma}{\alpha-\kappa},\, \alpha\right)$

- unstable (saddle), if $\frac{\kappa}{\alpha} > \sigma > 1$ or $\sigma > \frac{\kappa}{\alpha} > 1$ (E2)
- stable (node or focus), if $\frac{\kappa}{\alpha} < \sigma < 1$ (E3)
- unstable (node or focus), if $\sigma < \frac{\kappa}{\alpha} < 1$ (E4)

An analogous analytical study for the delayed system (but without immune-stimulation with cytokines) can be performed using current techniques of delay differential equations (Hale, 1977; Gopalsamy, 1992; Beretta and Kuang, 2002) concerning changes in the stability of the solutions of the nondelayed system (see appendix for the detailed proofs of the following afirmations). The main results are as follows:

Tumorless state L_0: remains stable or unstable for any dimensionless delay τ, so (E1) remains valid for any $\tau \geq 0$.

Controlled growth state (or dormant tumor) L_1:

- remains unstable for any $\tau \geq 0$ in the condition of (E2)
- becomes unstable for some critical value τ_c of τ in the conditions of (E3)
- remains unstable for any $\tau \geq 0$ in the conditions of (E4)

Figure 1 shows the effects of this delay induced instability. It can be seen there, that this $\tau_c(\alpha)$, which destabilizes L_1, involves the appearance of a stable limit cycle, resembling much the tumoral remissions. This behavior is similar to the one described in (E4) of the nondelayed system, however the global behavior there was unstable: the $x - y$ cycles increased their amplitude and eventually ended in the suppression of the lymplocytes. Now, however, the limit cycle is stable and the cycles can last forever with constant amplitude. Moreover, there exists a second critical delay $\tau_{c2}(\alpha)$ for which the limit cycle stability is lost eventually arriving to a state of complete immunosupression. This value has been obtained numerically (using the same method and assuming the same initial conditions explained in the next section 5).

On the other hand, figure 2 shows the critical value of τ for which the controlled growth state, L_1, becomes unstable, as a function of the parameter

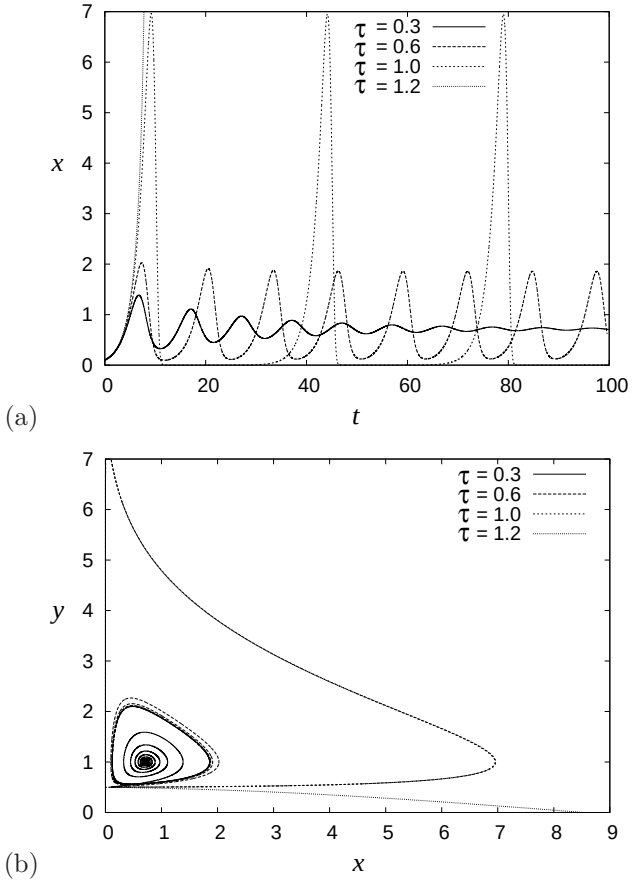


Figure 1: (a) Temporal evolution of tumor population, and (b) phase portrait for different delays ($\tau = 0.3, 0.6, 1.0, 1.2$), for initial conditions $x_0 = 0.1$ and $y_0 = 0.5$. Other parameter values are taken as $\alpha = 1$, $\sigma = 0.5$, $\kappa = 0.3$.

α. These values of $\tau_c(\alpha)$ can be computed analytically (see the appendix, or also the original work of Beretta and Kuang (2002); Villasana and Radunskaya (2003)) within the range of parameters for which L_1 has positive abscissa and where L_1 is stable for $\tau = 0$. In figure 3 it is also shown that $\tau_{c2}(\alpha) > \tau_c(\alpha)$ which marks the end of the existence (for the particular initial conditions x_0, y_0 of figure 1) of the limit cycle, which ends in total immunosupression.

We must emphasize that the delay τ does not affect the stablity of L_0, its nature being determined by the same previous condition (**E1**) on σ. In particular, the limit cycle behavior around L_1 takes place when L_0 is a saddle point, and is embraced by a homoclinic trajectory departing and ending at this latter point; this structure is usually called a "saddle-loop".

5 Results with delay and therapy

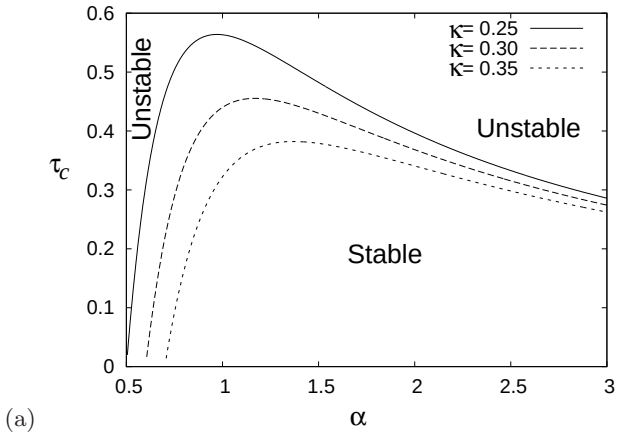
With the introduction of the immunotherapy term, system (3) cannot be studied analytically in a simple manner. Following Sotolongo-Costa et al. (2003) we perform numerical integration of (3) and explore the values of β and τ for which, values of the other parameters fixed, the system behaves as stable or unstable with respect to the size of the tumor.

Numerical integration has been performed using the steps method (Driver, 1977). The method requires the storage of the values of x and y at least from $t-\tau$ up to t and the precise value in $t-\tau$ has been computed using a simple linear interpolation between the two nearest values. Integration has been carried out up to a time $t_{\max}$ corresponding to a real time of around 10 years (from typical values estimated in 3, it can be obtained $t_{\max} \in [30, 10^3]$; we will consider $t_{\max} = 100$ dimensionless time units in what follows). However, whenever the condition $y \leq 0$ is met, that is, when the immune system becomes annihilated due to tumor aggressiveness, integration stops before $t = t_{\max}$.

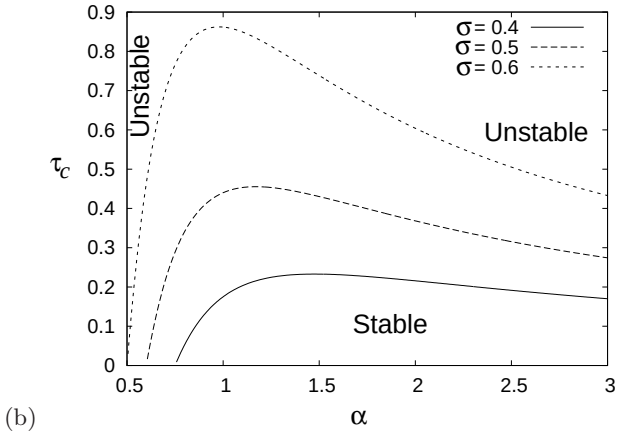
As a realistic initial condition for the integration of the delay system we take that corresponding to an exponentially growing tumor, and an unaware immune system (τ-delayed response), *i.e.*, $x(t) = x_0(0)e^{\alpha(1-\sigma)t}$, $y(t) = \alpha\sigma$, in $t \in [-\tau, 0)$. This simplifying election allows us to determine the initial conditions just giving $x_0(0)$.

Integration of (3) with these initial and stop conditions ($y > 0$ and $t < t_{\max}$) classifies the (β, τ) plane into two regions: (asymptotically) stable and unstable. However the unstable region can be divided in subregions depending on the time to immunosupression. We call them metastability regions, i.e. where the growth is controlled up to a certain large enough time. Examples of these behaviors are depicted in figure 4 for some representative pairs (β, τ), and are summarized in figure 5. Although this "phase diagram" outlined in terms of the stability outcome is not the usual mathematical representation, we will use it as a simple means to depict the "prognostic" information of our model. Thus its medical interpretation in the discussion will be clearer.

The region marked as "asymptotically stable" in figure 5a denotes those cases where the tumoral size is kept controlled by the cytokine treatment for an arbi-



(a)



(b)

Figure 2: L_1 stability regions for different values of (a) κ, around 0.30 (with fixed $\sigma = 0.5$) and (b) σ, around 0.5 (with fixed $\kappa = 0.30$), within the physiological estimated range. These regions are delimited by the graph of the function $\tau_c(\alpha)$, the critical delay above which the critical point L_1 of system (3) becomes unstable (for $V = 0$: no immunological treatment). Parameter values were taken such that the system is stable for $\tau = 0$ and $\alpha > \kappa/\sigma$ (the range of stability without the delay).

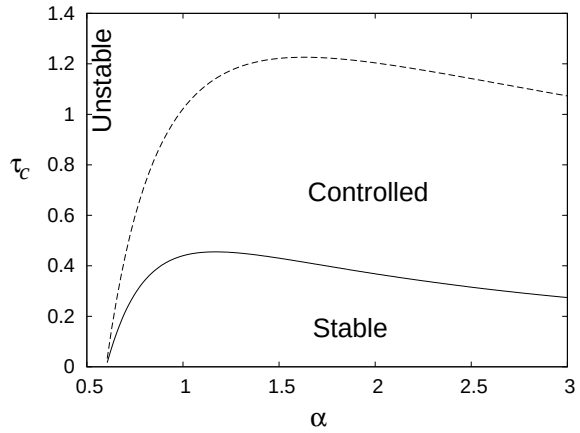


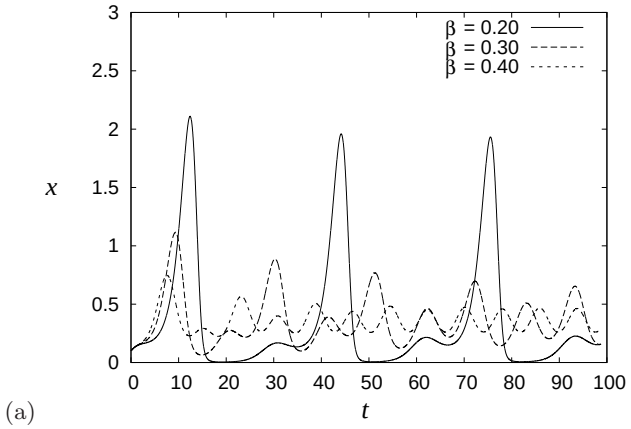
Figure 3: L_1 stability region (below solid line, representing the graph of $\tau_c(\alpha)$) and limit cycle existence threshold (below dashed line). $V = 0$, i.e. no immunological treatment is considered.

trarily long time. The one marked as "unstable" denotes, for the chosen values of the parameters, those values of the frequency β and the delay τ for which the system becomes unstable in a finite time, smaller than a reasonable treatment time, leading to immunosuppression and eventually to unlimited tumor growth (Sotolongo-Costa et al., 2003). The *metastable* region of 5b corresponds to unstable states where instabilities (i.e. immunosupression) manifest themselves after a given time shorter than $t_{\max}$, but long enough to allow for an external intervention, and hence they are indicative of the short term immunotherapic success.

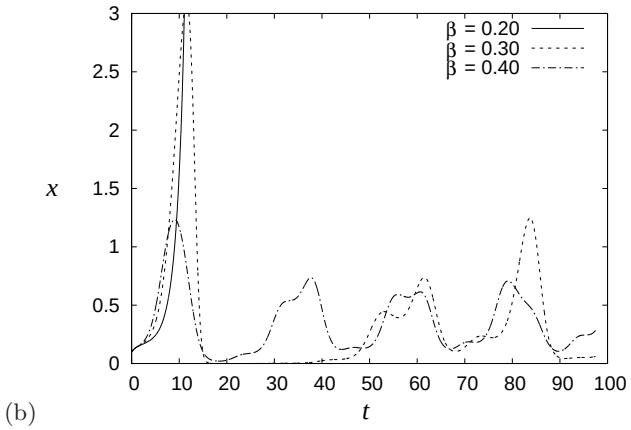
We want to point out that the limit between the *metastable* region and the unstable one in figure 5b depends not only on the time cutoff, but also on the initial conditions (x_0, y_0, as well as on the hypotheses about their history) used to initialize the numerical calculation, and on the degree of instability determined by the values of the other parameters. In fact there can be seen "islands" of more unstable states within the region marked as *metastable*. This sort of islands are produced by the interplay between treatment dossage and the proper limit cycle dynamics.

6 Discussion and Conclusions

We have studied the effect of the immune response time delay on cancer tumor growth. This delay is introduced, following Asachenkov et al. (1994); Galach (2003), to approximate missing dynamical components such as the chemical signal and B-lymphocytes mediated maturation and activation of (T-)lymphocytes, and is a body characteristic time. It can be estimated, together with other particular dynamical system parameter values, as shown in section 3,



(a)



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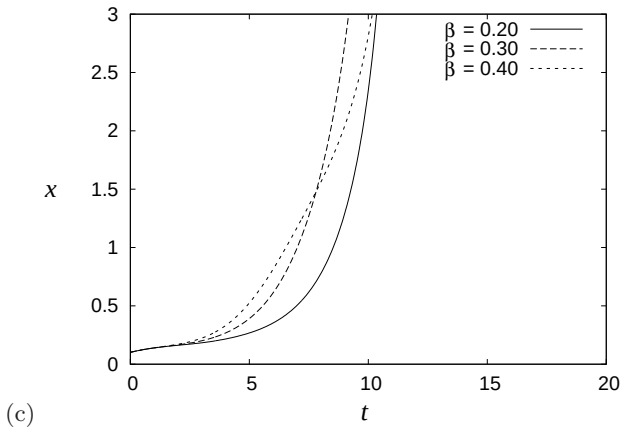
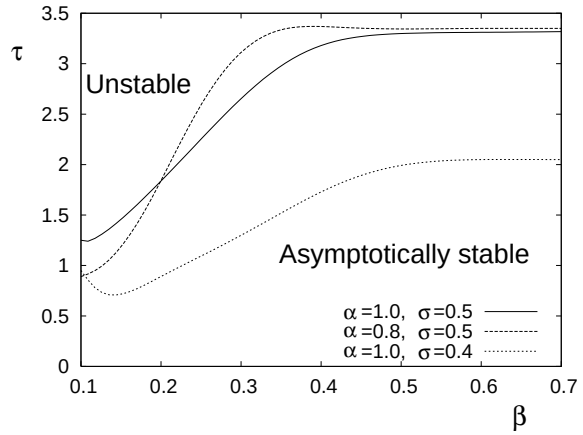
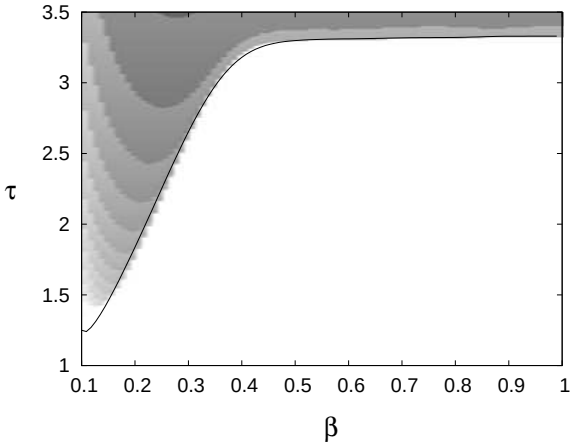


(c)

Figure 4: Tumor population temporal evolution for different pairs (β, τ) to illustrate the effect of the dose period, as well as the delay of the immune system response. The values of the other parameters in all cases are: $\kappa = 0.3$, $\sigma = 0.5$, $\alpha = 1$, $V = 0.5$, all of them within the physiological range estimated in section 3. The initial conditions were taken as $x_0 = 0.1$, $y_0 = \sigma\alpha = 0.5$. Values of $\beta = 0.2$, 0.3, 0.4 are taken along $\tau =$ const. lines with and without (meta)stability change with β. In particular: (a) $\tau = 1.25$ changes from *metastable* to asymptotically stable, (b) $\tau = 2.5$ changes from *metastable* to asymptotically stable, and (c) $\tau = 3.5$ changes from *metastable* to unstable.



(a)



(b)

Figure 5: (a) Stability (β, τ) portrait for different values of α and σ, showing asymptotically stable (up to $t_{\max} = 100$) and unstable regions. The values of x_0, y_0, κ, σ, α and V are the same as in figure 4. (b) *Survival time* map with the shadowed region corresponding to *metastable* regions (for $\alpha = 1.0$), where tumor grows in an uncontrollable way only after a time represented by the grayscale (the smaller, i.e. the more unstable, the darker). Also the continuous line marks the limit of the marginally stable region. Constant gray profiles aparent from the image (b) have the meaning of "survival isolines".

from available data.

Furthermore, the dimensionless form of the equations (3) reflects the existence of dimensionless groups (or "cancer indexes" as σ or κ/α, so common in other branches of the medicine), which capture the essence of the dynamics, and are thus more suitable to describe and classify treatment outcomes. These dimensionless groups that describe qualitatively the dynamics, have the meaning of relative magnitudes. For instance, $\sigma = \frac{u/f}{a/b}$ is the ratio of the number of "non specific" effector cells around the tumor, previous to the moment of its detection by the immune system (u/f), to the number of effector cells needed to stop tumoral growth at any time (a/b). It is evident now the meaning of the stability condition $\sigma > 1$. Similarly, when we write $\kappa/\alpha = \frac{k/d}{a/b}$, it is read as the ratio of the number of lymphocytes around the tumor needed to compensate the local immunodepression caused by cancerous cells (k/d), to the number of effector cells needed to stop tumoral growth (a/b). Even in the case when a patient has $\sigma < 1$, there is a chance of tumor control if $k/d < u/f$ because there are still activated effector cells left to fight cancer.

The destabilizing effect of the inherent delay τ is responsible of the appearance of controlled tumor remission and, as it becomes larger, of the eventual anihilation of the immune system, as shown in figures 1 and 2. It is informative to rewrite this delay in terms of the original dimensional parameters, $\tau = T\sqrt{af}$, as well as $\alpha = \sqrt{a/f}$. It becomes apparent then, that an increase in lymphocyte mortality ($\sim f$) or an increase in cancer malignancy ($\sim a$) may lead from a controlled growth situation (stable or controlled around L_1) to a periodic tumor remission or, upon further deterioration, to immunosupression, as shown in figure 3. Figure 2, also shows the effects that the variation of parameters κ (immunodepression) and σ (constant flux of lymphocytes from the immune system) have on these thresholds. This is more dramatic for $\alpha \gtrsim \frac{\kappa}{\sigma}$, that is, for slightly stable systems.

When immunotherapy is considered, the analysis of the solutions of our model classifies cancer situations into stable/curable (that is controllable by the stimulated immune system) and unstable (those which cannot be controlled by the stimulated immune system) in terms of the delay (τ) and dossage (β^{-1}) characteristic times. However, we have also included in our classification those *metastable* states which can be kept controlled for a time in which another treatment (radiotherapic or surgical procedure, for instance) can be applied. In both *metastable* and asymptotically stable conditions, immunotherapy does not lead to the annihilation of cancer cells, but keeps tumor size in a controlled state, allowing for another therapeutic approach.

For a fixed time delay τ (patient dependent) an increase of the dossage frequency β usually stabilizes tumor growth. This is observed as an initial gradual increase of the *survival time*, that ends in a fast transition to the asymptotically stable region. These survival times are schematically depicted in figure 5b as a grey-map from which "survivance isolines" can be obtained. However, there exists a threshold τ-delay for which β variation alone is not enough to control tumoral growth. Thresholds and isolines will change as α and σ are varied, as

can be seen in figure 5a. A larger σ (stronger immune system) will increase the limiting τ (will raise the threshold $\beta \to \infty$ asymptote), and an increase of α will decrease the lower stabilizing β (for a fixed τ). However the qualitative behavior just described will remain valid.

Our investigation has dealt with the effects of the immune system response time (represented by τ in this work) on the temporal evolution of a tumoral mass with and witout immunotherapic stimulation. The model employed considers the simplest models of tumor growth, tumor-immune system interaction, immune response delay, immunodepression and immunotherapic treatment. These simplest models also involve the minimum number of parameters to be estimated. The mathematical analysis has evidenced the existence of several dimensionless parameters or "cancer indexes" that, in turn, suggest a new quantitative way to evaluate clinical history records and define new criteria about the suitability of immunotherapy, based on a given patient immunological state characterized by the σ, κ/α and τ defined in this work.

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A Stability analysis of the delayed system (3) without therapy ($V = 0$)

Proposition 1: The stability around the steady state L_0, does not change with the inclusion of the delay.

Proof: Around $L_0 = (0, \alpha\sigma)$, the associated variational lineal system (Hale, 1977) is

$$\begin{aligned} x'(t) = &\quad \alpha(1-\sigma)x(t) \\ y'(t) = &\quad \alpha\sigma x(t-\tau) - \kappa x(t) - \tfrac{1}{\alpha}y(t) \end{aligned}$$

whose characteristic equation is

$$D(\lambda,\tau) = \begin{vmatrix} \lambda - \alpha(1-\sigma) & 0 \\ \kappa - \alpha\sigma e^{-\lambda\tau} & \lambda + \frac{1}{\alpha} \end{vmatrix} = 0$$

having the same eigenvalues as in the non delay case

$$\lambda(L_0) = \begin{cases} \alpha(1-\sigma) \\ \frac{-1}{\alpha} \end{cases}$$

Then it follows that the stability of the solution around L_0 remains the same as in non delay case. $\lhd$

Proposition 2: When $\sigma > 1$, the solution keeps its unstable behavior around $L_1 = \left(\frac{1-\sigma}{\alpha-\kappa}, \alpha\right)$, for every $\tau > 0$.

Proof: The associated variational linear system and the characteristic equation have now more complicated expressions.
The system linearized around L_1 becomes

$$\begin{aligned} x'(t) = &\quad -\tfrac{1-\sigma}{\alpha-\kappa} y(t) \\ y'(t) = &\quad \alpha x(t-\tau) + \tfrac{1-\sigma}{\alpha-\kappa} y(t-\tau) - \kappa x(t) - \tfrac{1}{\alpha} y(t) \end{aligned}$$

whose characteristic equation that can be written as,

$$D(\lambda, \tau) = M(\lambda) + N(\lambda) e^{-\lambda\tau} = \lambda^2 + \frac{1}{\alpha}\lambda - \kappa\frac{1-\sigma}{\alpha-\kappa} + \frac{1-\sigma}{\alpha-\kappa}(\alpha - \lambda) e^{-\lambda\tau} \tag{4}$$

$D(0, \tau) = 1 - \sigma < 0$, under the assumption that $\sigma > 1$, the expression (4) tends to ∞, when $\lambda \to \infty$. Then for every $\tau > 0$, there exists some $\lambda \in \mathbb{R}$, $\lambda > 0$, such that $D(\lambda, \tau) = 0$. We can then conclude that, since there always exists a positive eigenvalue, the delayed system remains unstable around L_1. $\lhd$

Proposition 3: In the range $\frac{\kappa}{\alpha} < \sigma < 1$, of stable solutions around L_1 for the nondelayed system, there exists $\tau_i > 0$, such as for every $\tau \geq \tau_i$, the delayed system solution becomes unstable around L_1. For $\sigma < \frac{\kappa}{\alpha} < 1$, there exists no τ for which solutions around L_1 change their stability.

Proof: We must prove that a stable state becomes unstable upon the introduction of the delay. We want to find the values of τ for which the stability change takes place. Following the criterium exposed by Beretta and Kuang (2002), we look for roots of (4) of the form $\lambda = \imath y$.
For every $y \neq 0$ we can construct the function,

$$\Phi(y) = |M(\imath y)|^2 - |N(\imath y)|^2 \tag{5}$$

The necessary condition for the stability change is that Φ has some real root, $y \in \mathbb{R}$, and then we can apply the criterium. In our case,

$$\Phi(y) = y^4 + Ay^2 + B = 0 \tag{6}$$

where,

$$A = 2\kappa\tilde{x} + \frac{1}{\alpha^2} - \tilde{x}^2 \qquad B = \tilde{x}^2(\kappa^2 - \alpha^2) \qquad \tilde{x} = \frac{1-\sigma}{\alpha-\kappa}$$

The sufficient condition for the stability change from the stable system ($\frac{\kappa}{\alpha} < \sigma < 1$) is that there must exist a positive root of (5) such as $\frac{dRe(\lambda)}{d\tau} > 0$. We know that,

$$\text{Sign}\left(\frac{dRe(\lambda)}{d\tau}\right) = \text{Sign}\left[Re\left(\frac{d\lambda}{d\tau}\right)^{-1}\right] \tag{7}$$

and following Beretta and Kuang (2002) we obtain,

$$\sin y\tau = \frac{-M_R N_I + M_I N_R}{|N(\imath y)|^2} \qquad \cos y\tau = -\frac{M_R N_R + M_I N_I}{|N(\imath y)|^2} \tag{8}$$

where

$$\begin{array}{llll} M_R(\imath y) = & -(\kappa\tilde{x} + y^2) & M_I(\imath y) = & \frac{y}{\alpha} \\ N_R(\imath y) = & \tilde{x}\alpha & N_I(\imath y) = & -y\tilde{x} \end{array}$$

Then, taking into account that when stability changes $D(\lambda, \tau) = 0$, computing implicitly the derivative $d\lambda/d\tau$, and comparing it with $d\Phi(y)/dy$, we obtain,

$$\text{Sign}\left[Re\left(\frac{d\lambda}{d\tau}\right)^{-1}\right] = \text{Sign}\left[\frac{1}{y\,|N(\imath y)|^2} \cdot \frac{d\Phi(y)}{dy}\right]$$

and finally, using (6) and the expression for the roots of $\Phi(y)$,

$$\text{Sign}\left[Re\left(\frac{d\lambda}{d\tau}\right)^{-1}\right] = \text{Sign}(\Lambda^{1/2})$$

where, $\Lambda = A^2 - 4B > 0$, since $B < 0$ when $\frac{\kappa}{\alpha} < 1$.
It can be immediately inferred that for y fulfilling (6), and in the range of parameters for which the non delay system is stable ($\frac{\kappa}{\alpha} < \sigma < 1$), there exists some $\tau > 0$, such that the system becomes unstable. However, in the range of parameters for which the system is unstable ($\sigma < \frac{\kappa}{\alpha} < 1$), we can see that there is no change of stability since (7) remains positive.
Let θ be a principal argument of $y\tau$ in $[0, 2\pi]$. Then we can find the values of τ such that the system changes into instability from the equations (8). These values are plotted in Fig. 2 as a function of α. Figure 2 shows the threshold τ_c for which a solution around L_1 becomes unstable. For delay values above this threshold the system becomes unstable while for those below τ_c solutions around L_1 remain stable. $\lhd$

References

Asachenkov, A. L., Marchuk, G. I., Mohler, R. R., and Zuev, S. M. (1994). Immunology and disease control: a systems approach. *IEEE Trans. Biomedical Eng.*, 41(10):943–953.

Begg, A. and Steel, G. G. (1977). *Cell proliferation and growth rate*. Oxford.

Beretta, E. and Kuang, Y. (2002). Geometric stability switch criteria in delay differential systems with delay dependent parameters. *SIAM J. Math. Analysis*, 33:1144–1165.

Berman, B., Perez, O. A., and Zell, D. (2006). Immunological strategies to fight skin cancer. *Skin Therapy Letter*, 11(5).

Brassard, D. L., Grace, M. J., and Bordens, R. W. (2002). Interferon-alpha as an immunotherapeutic protein. *Journal of Leukocyte Biology*, 71:565–581.

Byrne, H. M. (1997). The effect of time delays on the dynamics of avascular tumor growth. *Math. Biosciences*, 144:83–117.

Cross, D. and Burmester, J. K. (2006). Gene therapy for cancer treatment: Past, present and future. *Clinical Medicine & Research*, 4(3):218–227.

de Boer, R., Hogeweg, P., and Dullens, H. (1985). Macrophage t lymphocite interactions int the anti-tumor immune response: a mathematical model. *Journal of Immunology*, 134:2748–2758.

de Pillis, L., Radunskaya, A. E., and Wiseman, C. L. (2005). A validated mathematical model of cell-mediated immune response to tumor growth. *Cancer Research*, 65:7950–7958.

Dezfouli, S., Hatzinisiriou, I., and Ralph, S. (2005). Use of cytokines in cancer vaccines/immunotherapy: recent developments improve survival rates for patients with metastatic malignancy. *Curr. Pharm. Des.*, 11:3511–30.

d'Onofrio, A. (2005). A general framework for modeling tumor-immune system competition and immunotherapy: Mathematical analysis and biomedical inferences. *Physica D*, 208:220–235.

Driver, R. D. (1977). *Ordinary and delay differential equations*. Springer.

Forys, U. (2002). Marchuk's model of immune system dynamics with application to tumor growth. *J. Theor. Med.*, 4:85–93.

Forys, U. and Bodnar, M. (2003). Time delays in proliferation process for solid avascular tumor. *Math. Comp. Modelling*, 37:1201–1209.

Galach, M. (2003). Dynamics of the tumor-immune system competition: the effect of time delay. *Int. J. Appl. Math. Comput. Sci.*, 13:395–406.

Gopalsamy, K. (1992). *Stability and oscillations in delay differential equations of populations dynamics*. Kluwer Academic Publishers.

Greenspan, H. P. (1972). Models for the growth of a solid tumour by diffusion. *Stud. Appl. Math.*, 52:317–340.

Hale, J. (1977). *Introduction to functional differential equations*, volume 99. Springer-Verlag.

Horton, H. M., Anderson, D., Hernandez, P., Barnhart, K. M., Norman, J. A., and Parker, S. E. (1999). A gene therapy for cancer using intramuscular injection of plasmid dna encoding interferon alpha. *Proc. Natl. Acad. Sci. USA*, 96(4):1553–1558.

Kirschner, D. and Panetta, J. (1998). Modelling immunotherapy of the tumor-immune system interaction. *J. Math. Biol.*, 38:235–252.

Kuznetsov, V. A., Makalkin, I., Taylor, M. A., and Perelson, A. S. (1994). Nonlinear dynamics of immunogenic tumors: parameter estimation and global bifurcation analysis. *Bull. Math. Biology*, 56:295–321.

Liu, Y., Huang, H., Saxena, A., and Xiang, J. (2002). Intratumoral coinjection of two adenoviral vectors expressing functional interleukin-18 and inducible protein-10, respectively, synergizes to facilitate regression of established tumors. *Cancer Gene Therapy*, 9:533–542.

Marchuk, G. (1997). *Mathematical Modelling of Immune Response in Infectious Diseases*. Kluwer Academic Publishers.

Nani, F. and Freedman, H. I. (2000). A mathematical model of cancer treatment by inmunotherapy. *Mathematical Biosciences*, 163:159–199.

Saleh, F., Renno, W., Klepacek, I., Ibrahim, G., Asfar, S., Dashti, H., Romero, P., Dashti, A., and Behbehani, A. (2005). Melanoma immunotherapy: past, present and future. *Curr. Pharm. Des.*, 11:3459–60.

Schuster, M., Nechansky, A., Loibner, H., and Kircheis, R. (2006). Cancer immunotherapy. *Biotech. J.*, 1:138–147.

Sotolongo-Costa, O., Morales Molina, L., Rodríguez-Pérez, D., Antoranz, J., and Chacón Reyes, M. (2003). Behavior of tumors under nonstationary therapy. *Physica D*, 178:242–253.

Steel, G. G., editor (1993). *Basic clinical radiobiology for radiation oncologists*. Edward Arnold Publishers.

Szymanska, Z. (2003). Analysis of immunotherapy models in the context of cancer dynamics. *Int. J. Appl. Math. Comp. Sci.*, 13(3):407–418.

Tortora, G. and Grabowski, S. (1999). *Principles of Anatomy and Physiology*. Oxford.

Villasana, M. and Radunskaya, A. (2003). A delay differential equation model for tumor growth. *J. Math. Biol.*, 47:270–294.

Whiteside, T. L. (2002). Apoptosis of immune cells in the tumor microenvironment and peripheral circulation of patients with cancer: implications for immunotherapy. *Vaccine*, 20:A46–A51.