A Mathematical Model of Gene Therapy for the Treatment of Cancer

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1 Introduction

Cancer is a major cause of death worldwide, resulting from the uncontrolled growth of abnormal cells in the body. Cells are the body's building blocks, and cancer starts from normal cells. Normal cells divide to grow in order to maintain cell population equilibrium, balancing cell death. Cancer occurs when unbounded growth of cells in the body happens fast. It can also occur when cells lose their ability to die. There are many different kinds of cancers, which can develop in almost any organ or tissue, such as lung, colon, breast, skin, bones, or nerve tissue. There are many known causes of cancers that have been documented to date including exposure to chemicals, drinking excess alcohol, excessive sunlight exposure, and genetic differences, to name a few [37]. However, the cause of many cancers still remains unknown. The most common cause of cancer-related death is lung cancer. Some cancers are more common in certain parts of the world. For example, in Japan, there are many cases of stomach cancer, but in the United States, this type of cancer is pretty rare [49]. Differences in diet may play a role. Another hypothesis is that these different populations could have different genetic backgrounds pre-disposing them to cancer. Some cancers also prey on individuals who are either missing or have altered genes as compared to the mainstream population. Unfortunately, treatment of cancer is still in its infancy, although there are some successes when the cancer is detected early enough. To begin to address these important issues, in this work we will focus solely on genetic issues related to cancer so that we can explore a new treatment area known as gene therapy as a viable approach to treatment of cancer.

Genes are located on chromosomes inside all of our cells and are made of DNA. Humans have approximately 35,000 genes. Gene therapy is an experimental treatment currently being tested in clinical trials that involves introducing additional genetic material (either DNA or RNA) into cells to fight cancer in a few different ways. There are several gene therapy approaches that are being explored. First, scientists are attempting to use gene therapy to replace missing or mutated genes with healthy

genes (for example, p53, [41]). Second, scientists are attempting to put genes into tumors that act like suicide bombs once they are turned on by drugs that are administered to the patient [53]. Similar to the suicide genes, a third approach is to insert genes that make tumors more susceptible to treatments such as chemotherapy and radiotherapy. And finally, gene therapy is being used to improve the immune response to cancers by enhancing the ability of immune cells, such as T cells, to fight cancer cells [42]. Figure 1 summarizes these different gene therapies. Not surprising, gene therapy research has continued to includes other genetics manipulations of gene expression through delivery of modified genes, short pieces of RNA known as siRNA (see [5]), naked plasmid DNA, and even viruses as vectors for delivery of genetic material into cells. To reduce the risks of side effects, specific tissues and cell types must be targeted. Not only is the type of gene therapy best suited for each case not known *a priori*, the choice of which gene to target, the dose and timing of treatments all must be determined. A recent review summarizes advances in gene therapy and also highlights opportunities for systems biology and mathematical modeling to synergize efforts with experimentalists and clinicians to push cancer research forward [22].

Mathematical modeling has been instrumental in the past 50 years in helping decipher different aspects of complex systems in biology. In particular, mathematical modeling has had an impact on our understanding of cancer biology and treatment (cf. [4,24,60] for excellent reviews). We begin by briefly reviewing existing models designed specifically for capturing tumor-immune dynamics, one of which forms the basis for our current work. As a first step to exploring the use of gene therapy on the tumor-immune interaction during cancer, we will apply a simple mathematical model to explore the dynamics of these different types of gene therapies, with the goal of predicting optimal combinations of approaches leading to clearance of a tumor. We present the model and its analysis (both analytical and numerical) and offer some conclusions.

2 Brief Review of Mathematical Models Describing Tumor-Immune Dynamics

For the past 40 years, mathematical models have been developed describing many aspects of cancer from tumor growth dynamics (cf. [11,21,31]), angiogenesis and vascularization (cf. [35,39,52]), to the topic of immune response to tumors. Since the work herein will be focused solely on tumor-immune dynamics, we briefly review work in this area.

Tumor-immune models have been around since the early 1990s and have evolved to capture very complex aspects of the immune response as knowledge of the molecular dynamics of immunity has increased. For example, an important aspect of immunity is the recognition of non-self, or foreign antigens. Specialized antigen presenting cells (known as macrophages and dendritic cells) present foreign antigen to immune cells, such as T cells, to train them to respond and clear the foreign material (like bacteria and viruses). Of course, since tumor cells began as self, or non-foreign

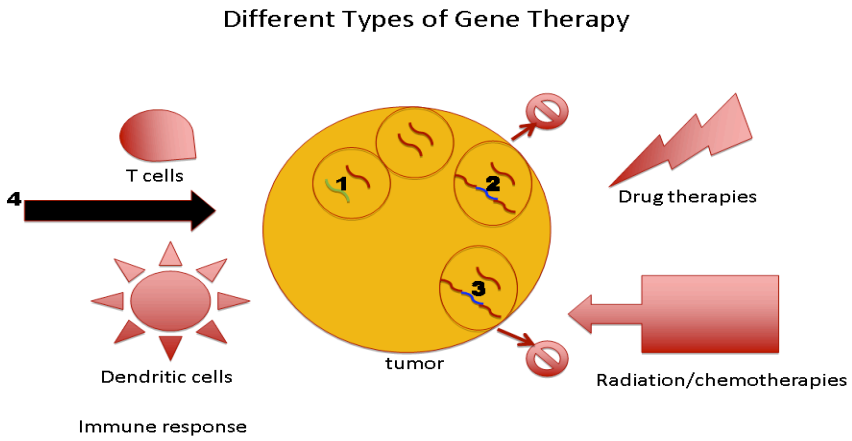

Fig. 1. Gene therapy and immunotherapy treatments. As denoted by the numbers in the figure: (1) Replace missing or mutated genes with healthy genes. (2) Insert genes into tumors that act like suicide bombs once they are turned on by drugs (3) Insert genes that make tumors more susceptible to treatments such as chemotherapy and radiotherapy. (4) Augment the immune response to cancers by enhancing the ability of immune cells, such as T cells and dendritic cells, to fight cancer cells.

host cells, the level of antigenicity of the tumor may be weak as the human immune system is trained to not kill self. Mathematical models of tumor-immune interactions that have explored dynamics at this scale are [25,33,40,47,51]. Recently, Joshi et al. [30] develop a new mathematical model to capture immunotherapy that involves the antigen presentation pathway and its role in tumor-immune dynamics. Other models focus only on therapy as well as on boosting immunity [3,6,8–10,15–17,20,28,48]. Immune competition models have been studied [14,33,34], which focus on the dynamics between host immune cells and tumors. These type of studies have their origin in the Lotka-Volterra models established almost 100 years ago.

2.1 Lotka-Volterra Models for Tumor-immune Interactions

The idea to use the qualitative theory of ordinary differential equations in mathematical biology reaches back to 1920's when Lotka and Volterra formulated a simple mathematical model in population dynamics theory. A good summary published in 1997 by Adam and Bellomo [1] presents a summary of early work regarding this approach to tumor-immune dynamics, and much of the original work on this was done by Kutznetsov [29] and colleagues. We review it briefly.

Let $y(t)$ be the population of predator and $x(t)$ is population of its prey (for example, one can imagine populations of wolves and rabbits in a forest). Assuming that numbers $x(t), y(t)$ are big enough and that the predator and prey populations are homogeneous, one can view them as continuous functions of time. Let $\Delta x(t) = x(t+\Delta t) - x(t)$ and $\Delta y(t) = y(t+\Delta t) - y(t)$ be small variations of populations during a certain period of time Δt. Taking $\Delta t = 1$ (for example 1 day) one can replace $\Delta x(t), \Delta y(t)$ by their derivatives, i.e. write $\dot{x}(t), \dot{y}(t)$ instead. The Lotka–Volterra equations are given by

$$\begin{cases} \dot{x} = ax - bxy \\ \dot{y} = -cy + dyx \end{cases} \tag{1}$$

where a, b, c, d are some positive numbers.

The linear positive term ax in the first equation (prey) corresponds to exponential growth; the negative predation term, $-bxy$, describes the rate prey are lost and is proportional to number of prey and predators in mass action form. In the second equation (predator), the negative linear term $-cy$ corresponds to natural death, as prey will not survive without prey, and $+dxy$ describes the growth of the predator population proportional to prey and number of predators. The simple form of Lotka-Volterra (LV) system is remarkable. It allows for investigation of the quantitative and qualitative behavior for all of its solutions both analytically and numerically. First, no chaotic behavior is possible according to Poincaré-Bendixon theorem, and, asymptotically, every non-periodic solution either goes to a fixed point or approaches a limit cycle. Simple analysis shows that most solutions of LV system are periodic, i.e. the population numbers $x(t), y(t)$ are oscillating around a certain equilibrium state $x(t) = x^*, y(t) = y^*$. The stable, stationary solution is (0,0).

In 1994 Kuznetsov et al. [29] applied Lotka–Volterra ideas to cancer modeling, where $E(t)$ represents the effector immune cells (predators) and $T(t)$ the tumor cells (prey). The equations, which are similar to the LV system, are written as follows:

$$\begin{cases} \dot{E} = s + p\dfrac{ET}{g+T} - mET - dE \\ \\ \dot{T} = aT(1-bT) - nET \end{cases} \tag{2}$$

where s, p, g, m, d, a, n, b are positive parameters.

Here the exponential growth of T in the second equation, was replaced by a more realistic one in logistic form: $aT(1-bT)$ (originally due to Verhulst, 1838), where b^{-1} is the maximal carrying capacity for tumor cells and a is the maximal growth rate. The term $-nET$ describes the loss of tumor cells due to the presence of immune cells. In the first equation s is normal immune cell growth, which is n cell death with d the loss rate; $-mET$ describes the decay of E cells due to interacting with tumor cells in a mass action way. The term $p\dfrac{ET}{g+T}$ represents Mchaelis-Meten growth of the immune response in response to tumors.

The Kuznetsov equations describe several important features and allow us to make predictions that are relevant for understanding cancer immunotherapy. The

paper by Kuznetsov et al. [29] establishes existence of long period oscillations of tumor that agrees with recurrent clinical manifestations of certain human leukemias. In addition, the model predicts the existence of a critical level of E-cells in the body below which the tumor growth cannot be controlled by the immune response. It describes qualitatively the "escape" phenomena in which low doses of tumor cells can escape immune defenses and grow into a large tumor, whereas larger doses of tumor cells are eliminated.

The Kuznetsov model was generalized by Kirschner and Panetta in 1998 [26]. The idea was to introduce a third population (concentration) of effector molecules known as *cytokines*, which are information signaling molecules used extensively in intercellular communication by the immune system. Below we describe briefly the Kirschner-Panetta equations. Tumor cells are tracked as a continuous variable as they are large in number and are generally homogeneous; their concentration is denoted by $T(t)$. Immune cells (called effector cells) are also large in number and represent those cells that have been stimulated and are ready to respond to the foreign matter (known as antigen); their concentration is denoted by $E(t)$. Finally, effector molecules are represented as a concentration $C(t)$. These are self-stimulating, positive feedback proteins for effector cells that produce them. The equations that describe the interactions of these three state variables are referred herein as the Kirschner–Panetta (KP) system:

$$\begin{cases} \dfrac{dE}{dt} = cT - \mu_2 E + \dfrac{p_1 EC}{g_1 + C} + s_1 & (3a) \\ \dfrac{dT}{dt} = r_2 T(1 - bT) - \dfrac{aET}{g_2 + T} & (3b) \\ \dfrac{dC}{dt} = \dfrac{p_2 ET}{g_3 + T} + s_2 - \mu_3 C & (3c) \end{cases} \tag{3}$$

In equation (3a), the first term represents stimulation by the tumor to generate effector immune cells. The parameter c is known as the antigenicity of the tumor. Since tumor cells begin as self, c represents how different the tumor cells are from self cells (i.e., how foreign). The second term in (3a) represents natural death and the third is the proliferative enhancement effect of the cytokine IL-2. In equation (3b), the first term is a logistic growth term for tumor growth and the second is a clearance term by the immune effector cells. In the final equation (3c), IL-2 is produced by effector cells (in a Michaelis-Menten fashion) and decays via a known half-life (third term).

To capture a novel treatment approach (still in use in some clinical settings), KP introduced three terms into their models. The first one is *Adoptive cellular immunotherapy* (ACI), representing the introduction of immune cells into cancer patients that have been stimulated to have specific anti-tumor activity [42, 44–46]. T cells, also known as lymphocytes, produce cytokines that are either self-stimulating or can stimulate (or shut down) other cells. ACI is usually performed in conjunction with large amounts of IL-2. There are two types of immune cells that are cultured for this purpose: 1. LAK-(lymphokine-activated killer cells): cells taken from host and then stimulated with activating factors. These cells are then injected back to patient.

2. TIL-(tumor infiltrating lymphocytes): Immune cells are taken from patient, and grown with high concentrations of IL-2 before injected back to the patient.

In the KP model, s_1 represents the treatment terms of introducing LAK and TIL cells to the tumor site of a patient. The second term, s_2, is a treatment term that represents administration of the cytokine IL-2 by a physician to a patient, to again stimulate effector cell growth and proliferation.

The KP system can exhibit chaotic behavior. The typical example of chaotic behavior is the system of Lorenz (1963) representing a so called strange attractor. The complete qualitative analysis of KP equations is much harder than the conventional Kuznetsov model. Nevertheless, Kirschner and Panetta, using stability analysis and modern bifurcation theory, classified representative behaviors of solutions and stability of cancer-free equilibrium states. Description of oscillations with long time dormant periods of illness were described in order to complete the studies by Kuznetsov.

Arciero and colleagues [5] extended the KP equation by including a suppressive cytokines known as TGF-β and also a simple type of gene therapy known as siRNA [38]. The use of siRNA is an early type of gene therapy where short-interfering RNA fragments interfere with the expression of a specific gene and modify behaviors in a cell. In addition, they added TGF-β to the model, which is a cytokine that acts to suppress immunity by inhibiting activation of effector cells and reducing antigenicity of tumors. It also stimulates tumor growth by promoting tumor vascularization. Their model predicts that increasing the rate of TGF-β production for reasonable values of tumor antigenicity enhanced tumor growth and its ability to escape host detection. siRNA treatment focused on the gene expression for TGF-β: it acts to suppress TGF-β production by targeting the messenger RNA that codes for TGF-β. Reducing TGF-β helped to rescue these negative effects to the host. Another group also recently explored the development of a microRNA-mRNA for the purpose of gene network regulation in tumors [2]. In an additional paper, Burden and colleagues [12] explored optimal control methods for determining the best treatment strategy based on the KP equations. They designed a control functional to maximize numbers of effector cells and interleukin-2 concentration while minimizing numbers of tumor cells. In 2008, S. Banerjee [7] proposed a delay version of KP equations where the equation (3a) was replaced by a new one:

$$\frac{dE(t)}{dt} = cT(t) - \mu_2 E(t) + \frac{p_1 E(t-\tau)C(t-\tau)}{g_1 + C(t)} + s_1 \tag{4}$$

with the rest of the KP system (equations 3) remaining the same. The introduction of a time delay, $\tau > 0$, corresponds to the delay that occurs between the production of a cytokine production, and its downstream binding and activation action on host effector cells. In that work, Banerjee analyzed the local stability of the cancer free equilibrium in the presence of the delay using semi-numerical bifurcation methods.

All of the above applications of dynamical system theory were studied using a similar approach: investigation of local stability of solutions by linear approximation (i.e., non-linear equations are replaced by linear ones in a suitable regions of phase space). However, non-linear phenomena have a much greater complexity and

require analysis on a global level. Very few generalized methods have been developed; as such, usually each non-linear system must be studied individually. The main difficulty is the presence of free, non-numerical parameters in the system as clearly exemplified in the KP equations. Parameters (13 of them for KP model) such as antigenicity, c, or maximal growth ratio, a, are not known experimentally. These parameters are mostly composite parameters that phenomenologically represent a set of biological mechanisms in a simple way. To describe different qualitative scenarios of the model when performing a stability analysis without using numerical values for the model parameters is a critical task. Thus, we consider the pairing of numerical and analytical methods as the best approach to gain as much information as possible. Finally, In 2009, Kirschner and Tsygvintsev [27] performed a global analysis of the KP system using the generalized Lyapunov method. They derived sufficient conditions that guarantee asymptotic convergence as $t \to +\infty$ of $T(t)$ to 0. For a "virtual" patient, that would imply complete clearance of cancer once a corresponding therapy is adopted. Another result of [27] was to analytically prove the existence of host self-regulation of cytokine levels that never exceed certain critical values. See also [13,19] for further discussion.

3 A Gene Therapy Model

The problem with the use of LAK and TIL cells as described above is that only about half of the TILs that are typically generated are reactive to tumors [50]. Thus, the ability to genetically engineer TIL cells that are directed against tumor specific antigens is a key objective. Recently, this was attempted in a small clinical trial [43] and a small percentage of patients had complete tumor regression. In this study, Rosenberg and colleagues took a blood sample from each patient and transferred genes into T cells inducing each cell to produce specialized T-cell receptors (TCR). These cells are then transferred back into the patient. In the body, T cells produce TCRs on their outer membrane and the TCRs recognize and attach to certain molecules found on the surface of the tumor cells. Finally, signaling through the TCRs activates T cells to attack and kill the tumor cells. To explore these studies further we will build on the KP model.

First, to simplify the model we can remove the IL-2 equation (3c). We replace the IL-2 saturation term in equation (3a) with a self-proliferation term, i.e. $p_1 E/(E+f)$. The idea that the proliferation rate of effectors may be a decreasing function of effectors has been explored by d'Onofrio et al. [18]. To capture the effects of gene therapy (see Figure 1) we must allow for the immune parameters of the model, i.e. a and c, to be step functions. Antigenicity, c, will signal stronger to the immune system during gene therapy and the clearance of tumor cells, a, will be strongly enhanced after gene therapy. Finally the source term representing TIL cells, $s_1(t)$ should be time dependent. We can also combine this with a self-limiting gene therapy treatment for tumors, which affects the growth rate of the tumor, r_2, by allowing it to be a step function that decreases its growth rate. The new equations are:

$$\begin{cases} \dot{E} = c(t)T - \mu_2 E + p_3 \dfrac{E}{E+f} + s_1(t), \\ \\ \dot{T} = r_2(t)T(1-bT) - a(t)\dfrac{ET}{T+g_2}. \end{cases} \tag{5}$$

It is this model that we analyze both analytically and numerically in the next sections. We define the parameters of system (5), their values as well as their ranges of variation in Table 1. They are mostly based on previously published data (cf., [5,7]).

Table 1. Parameter values for the model (5)

Name	*Definition*	*Baseline(units)*	*Range*
μ_2	Half-life of effector cells E	0.03 (1/time)	0.03
p_3	Proliferation rate of E	0.1245 (1/time)	0.1245
f	Half-sat for E proliferation term	10^{-3} (cells)	$[10^{-5}, 1]$
$s_1(t)$	Immunotherapy term	1 (cells/time)	$[10^{-2}, 10^2]$
$c(t)$	Cancer antigenicity	0.05 (1/time)	$[10^{-3}, 0.5]$
$r_2(t)$	Cancer growth rate	0.18 (1/time)	$[10^{-1}, 2]$
b	Cancer cell capacity (logistic growth)	10^{-9} (1/cells)	10^{-9}
$a(t)$	Cancer clearance term	1 (1/time)	$[10^{-2}, 10^2]$
g_2	Half-saturation, for cancer clearance	10^5 (cells)	10^5

4 Stability Conditions for Non-Autonomous Gene Therapy Model

In this section we derive conditions for global stability of the cancer free state ($T = 0$) for the Gene Therapy model (5). First, we investigate the second equation of system (5) independently from the first equation. Thus, we consider $r_2(t)$, $a(t)$ and $e(t) = E(t)$ as *arbitrary* positive data functions:

$$\dot{T} = r_2(t)T(1-bT) - a(t)\frac{e(t)T}{T+g_2}. \tag{6}$$

The only biological plausible solutions should satisfy the condition $T(t) \in [0, b^{-1}]$. Moreover, as easily seen from the second equation of system (5), the interval $[0, b^{-1}]$ is dynamically invariant under the flow. Our first aim is to derive conditions on the functions $r_2(t), a(t)$ and $e(t)$, which would imply asymptotical global stability of the cancer free equilibrium state $T = 0$.

Theorem 4.1. *Let one of the following two conditions holds*

Condition 1: There exist $t_0 > 0$ *and* $\varepsilon > 0$ *such that*

$$\frac{a(t)e(t)}{r_2(t)} > g_2 + \frac{(1-bg_2)^2}{4b} + \frac{\varepsilon}{r_2(t)}, \quad \forall t \geq t_0 \tag{7}$$

or

Condition 2: $g_2 > b^{-1}$ *and there exist* $t_0 > 0$ *and* $\varepsilon > 0$ *such that*

$$\frac{a(t)e(t)}{r_2(t)} > g_2 + \frac{\varepsilon}{r_2(t)}, \quad \forall t \geq t_0. \tag{8}$$

Then every solution of (6) *satisfies* $\lim_{t\to+\infty} T(t) = 0$ *with exponential convergence*.

Proof. We write equation (6) in the form:

$$\dot{T} = \frac{r_2(t)T}{T+g_2}V(T), \tag{9}$$

where V is a quadratic polynomial with respect to T given by:

$$V(T) = -bT^2 + (1-bg_2)T + g_2 - \frac{a(t)e(t)}{r_2(t)}, \tag{10}$$

with discriminant D as follows:

$$D = (1-bg_2)^2 + 4b\left(g_2 - \frac{a(t)e(t)}{r_2(t)}.\right) \tag{11}$$

Condition 1 is equivalent to $D < -4b\varepsilon/r_2(t)$. Thus,

$$r_2(t)V(T) < -\varepsilon, \tag{12}$$

for all T, since the leading coefficient of $V(T)$ is negative (i.e., $-b < 0$, see Fig. 2). The equation (9) can be written, for every fixed solution $T(t)$, in the form:

$$\dot{T} = -\delta_T(t)T, \tag{13}$$

where $\delta_T(t) = -r_2(t)V(T)/(T+g_2)$. Since $T(t)$ is bounded above by b^{-1} and because of (12), the inequality $\delta_T(t) > \delta_0 > 0$ begins from a moment of time. That completes the proof of the exponential convergence of $T(t)$ to 0 as $t \to +\infty$.

Now, let us assume that (10) have two real roots A and B, $A < B$. The quadratic polynomial function $V(T)$ has unique extremum given by $C = \dfrac{(1-bg_2)}{2b} < b^{-1}$, corresponding to maximal value of $V(T)$. Also, $V(b^{-1}) = -\dfrac{a(t)e(t)}{r_2(t)} < 0$. Both roots are negative, i.e. $A, B < 0$ if and only if $C < 0$ and $V(0) < 0$. We assume that $r_2(t)V(0) < -\varepsilon$ for certain positive ε. The same arguments of Condition 1 can be applied.

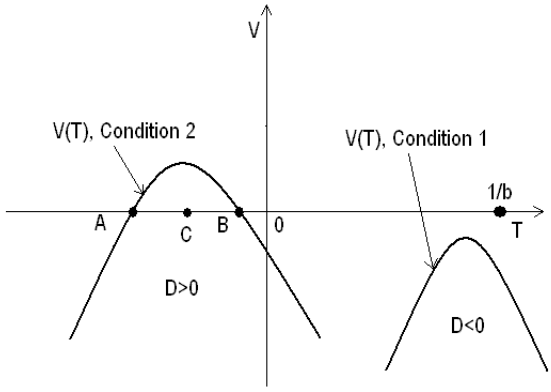

Fig. 2. The quadratic function $V(T)$

We note that Conditions 1 and 2 are also necessary for *global* stability of the state $T = 0$: if both are not satisfied, it is always possible to choose parameter values that return a solution for which $T(t)$ is not converging to 0. As it follows from the analysis above, key parameters governing the stability of cancer free equilibrium state $T = 0$ are included in the function:

$$S(t) = \frac{a(t)E(t)}{r_2(t)} \tag{14}$$

In order to stabilize or completely eliminate the cancer, we suggest the choice of functions in system (5) that force $S(t)$ to be uniformly bounded from zero for all values of time. Here, different paths can be proposed. We can adjust the external source of effector cells $s_1(t)$ every time $S(t)$ starts to decrease or, alternatively, the functions $r_2(t), a(t)$ can be made E-dependent in a way that the stability condition $S(t) > S_0$ holds until complete eradication of the tumor is achieved. Below we propose conditions which do not involve effector cells, $E(t)$ explicitly. In the next theorem, T_0 plays a key role: if T falls below T_0, the cancer is assumed cleared.

Theorem 4.2 (Main Stability Theorem). *Let the following condition be satisfied for all $t \geq t_0$ with some constants $t_0 \geq 0$, $\varepsilon > 0$, $\sigma > 0$, $\beta > 0$ and $T_0 \in (0, b^{-1})$*

$$\begin{cases} \mu_2(\varepsilon + \theta r_2(t))^2 + (\mu_2 f - p_3 - s_1(t) - c(t)T_0)(\varepsilon + \theta r_2(t))a(t) \\ \qquad -(s_1(t) + fc(t)T_0 - \beta)a^2(t) < 0 \\ \dfrac{\varepsilon + \theta r_2(t)}{a(t)} \quad \text{is a non} - \text{increasing function of time} \\ fc(t)T_0 + s_1(t) > \sigma > 0 \end{cases} \tag{15}$$

where

Case a: $\theta = g_2 + \dfrac{(1-bg_2)^2}{4b}$

or

Case b: $\theta = g_2$ *and* $g_2 > b^{-1}$

The following statements hold:

Case I (partial clearance). For every solution $(T(t),E(t))$ *of* (5) *given by initial condition* $(T(t_1),E(t_1))$, $t_1 \geq t_0$ *with* $T(t_1) > T_0$ *the function* $T(t)$ *will reach in finite time the value* T_0.

Case II (complete clearance). If condition (15) *is satisfied with* $T_0 = 0$ *then for all solutions of the system* (5)

$$\lim_{t\to+\infty} T(t) = 0 \tag{16}$$

Proof. We write the first equation of the system (5) as follows

$$\dot{E} = \frac{K(E)}{E+f} \tag{17}$$

where K is quadratic polynomial with respect to E given by

$$K = \tilde{\alpha}E^2 + \tilde{\beta}E + \tilde{\gamma} \tag{18}$$

and $\tilde{\alpha} = -\mu_2, \tilde{\beta} = c(t)T(t) + p_3 + s_1(t) - \mu_2 f, \tilde{\gamma} = fc(t)T(t) + fs_1(t)$.

The conditions (7) and (8) can be all expressed, for suitable real positive number θ in the following form

$$E(t) - h(t) > 0 \tag{19}$$

with

$$h(t) = \frac{\varepsilon + \theta r_2(t)}{a(t)} \tag{20}$$

Checking the discriminant of quadratic polynomial $K(E)$ one proves that $K(E) = 0$ has always two real roots $A < 0, B > 0$ of opposite signs such that $\tilde{\gamma} > 0$. Since the leading coefficient $\tilde{\alpha} < 0$ the quadratic polynomial $K(E)$ is positive in the interval (A,B) and negative outside. The first inequality of (15), in the case $T > T_0$, is equivalent to condition $K(h(t)) > \beta \Rightarrow h(t) \in [0,B)$ (see Fig. 3).

At the same time $K(0) = \tilde{\gamma} = fc(t)T(t) + s_1(t)f > \sigma$. This implies that one will have $\dot{E}(t) > \beta_0/(E+f)$ with

$$\beta_0 = \min(\sigma,\beta) \tag{21}$$

once the inequality (19) is violated, forcing $E(t)$ to increase. Since $h(t)$ is non increasing function, the inequality (19) will be satisfied for certain $t = t^*$ and will hold

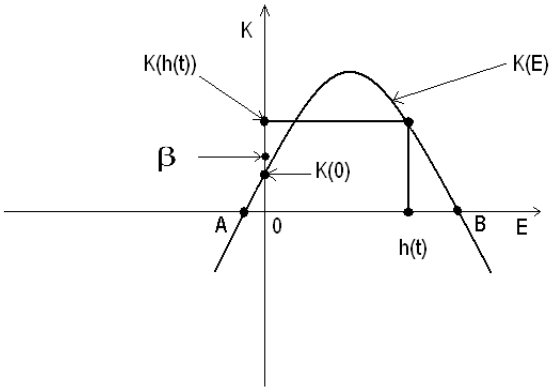

Fig. 3. The quadratic function $K(E)$

then for all $t \geq t^*$. As follows from the proof of Theorem 4.1 and (13), for $t \geq t^*$ the function $T(t)$ will decrease till it takes the value $T = T_0$. If $T_0 = 0$ (Case II) then one uses Theorem 4.1 again to derive (16).

One can interpret the significance of the inequality $fc(t)T_0 + s_1(t) > \sigma > 0$ in (15) as follows. Once antigenicity is switched off, i.e. $c(t) = 0$, treatment, $s_1(t)$, should be non-zero and vice versa. In the case of complete tumor clearance ($T_0 = 0$), the treatment term should be always positive above a certain level. Indeed, partial clearance does not exclude future regressing via the "escape" effect.

5 Numerical Simulations

Because the analytical results hold only for very small regions of parameter space,we would like to explore the gene therapy model more fully. To this end, we will apply statistical sampling techniques and numerical analysis to the system.

Sufficient conditions (15) of the Main Stability Theorem 4.2 imply large ranges for the four *treatment parameters* $c(t)$, $s_1(t)$, $a(t)$, $r_2(t)$, which are directly related to the four treatment strategies in Figure 1. The values of initial conditions have been varied between 1 and 10^5 for the populations of effector and tumor cells, $E(0)$ and $T(0)$. Global stability conditions (15) hold for any initial conditions (we use $E(0) = C(0) = 10^3$ for our baseline run). Sufficient conditions (15) are tested numerically by solving system (5) in Matlab using *ode15s* (a solver for stiff systems). Since conditions (15) are sufficient, we combine techniques from uncertainty and sensitivity analysis (see [32] for a review) to efficiently and comprehensively investigate treatment combinations and how they might affect cancer progression.

Regions of the parameter space where cancer is cleared are searched by sampling the parameter space in the ranges defined in Table 1. We only vary the 4 *treatment parameters*, while all others are kept constant at their baseline values (see *Baseline* column in Table 1). Samples are generated from uniform distributions and the sampling scheme used is known as Latin hypercube sampling (LHS) [36]. LHS scheme comprises three main steps: i) definition of probability density functions to use as *a priori* distributions for the parameters under analysis, ii) number N of samples to perform and iii) independent sampling of each parameter. The last step assumes that each parameter distribution is divided into N subintervals of equal probability and that the sampling is preformed without replacement. The accuracy of LHS is comparable to simple random sampling schemes but more efficient (i.e., with a significant reduction in the number of samples needed). In our study we use a sample size of 10,000 and tested numerically the impact of combining only constant treatment strategies, although conditions (15) are also valid for time-varying inputs.

5.1 Sensitivity Analysis as a Way to Determine Optimal Parameters for Treatment

In conjunction with uncertainty analysis, we use a generalized correlation coefficient (partial rank correlation coefficient, PRCC) to guide our understanding of which treatment parameter(s) contribute most to drive cancer proliferation or clearance (our model outcomes). PRCC is one of the most popular sensitivity indexes used for the analysis of deterministic models [32]. PRCCs results can be interpreted as a degree of correlation between input and output variability: PRCCs vary between -1 and 1 and can be applied to any nonlinear monotonic relationship. A test of significance is also available: only PRCCs that are significantly different from zero are shown in this study. In order to select an optimal combination of treatments, a pairwise comparison between PRCCs has been performed by a generalized z-test (see page 183 in [32]) and a ranking of the treatments is generated. Uncertainty and sensitivity analysis results are shown in Table 2. We review these techniques and others in [32]; our Matlab scripts to perform LHS, PRCC as well as other uncertainty and sensitivity analysis techniques are available online at http://malthus.micro.med.umich.edu/lab/usanalysis.html.

Table 2 shows how all four parameters have PRCCs that are statistically different from zero (with $p < 0.01$): not surprisingly they are all negatively correlated to cancer cell count (i.e., increasing their values from the baseline, decreases cancer cell count). Two treatment parameters, $a(t)$ and $s_1(t)$ (cancer clearance and immunotherapy terms, respectively), have the highest impact on reducing cancer cells. The other two parameters (i.e., $c(t)$ and $r_2(t)$) have similar PRCCs (they share the same ranking since they are not statistically different from each other), so they are equally effective in reducing cancer cell count. Figure 4 shows scatter plots of parameters versus cancer-cell counts, resulting from our extensive uncertainty analysis with an LHS scheme of 10,000 samples. We classify the outputs in four groups: complete clearance (green dots, no cancer cells), partial clearance (blue dots, cancer cell count below the initial condition $T(0) = 10^3$), small growth (red dots, cancer cell count

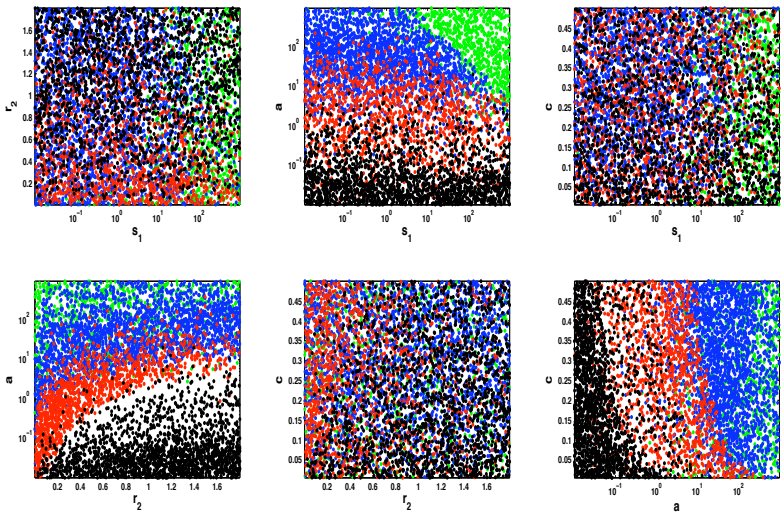

Fig. 4. Treatment combinations. Scatter plot of the numerical solution of system (5). The values plotted correspond to the cancer cell counts at day 2000 and they are classified by color: green-clearance (no cancer), blue-partial clearance (cancer below the initial condition of 10^3), red-small growth (from 10^3 to 10^6), black-large growth (above 10^6). The x and y axis represent the six combinations of the 4 *treatment parameters* $c(t)$, $s_1(t)$, $a(t)$, $r_2(t)$ as sampled in the LHS described in the *Numerical simulations* section. We vary all four inputs simultaneously (sampling from uniform distributions within their respective ranges) and keep the rest of the parameters constant to the baseline values shown in Table 1 (*Baseline* column).

between 10^3 and 10^6) and large growth (black dots, cancer cell count above 10^6). Cases where conditions (15) are satisfied are included in the "green" region of Figure 4. There is clearly a synergy between immunotherapy and cancer clearance terms ($s_1(t)$ and $a(t)$): both must be large to achieve complete clearance (green). High values for $s_1(t)$ or $a(t)$ are always associated with lower cancer cell counts, but no correlation can be inferred between either of these two parameters and antigenicity ($c(t)$) or cancer growth rate ($r_2(t)$). Below we show two examples of numerical simulations leading to complete clearance, when conditions (15) are either satisfied or not (Fig. 5). Clearance is usually achieved fast when conditions (15) are satisfied.

6 Conclusion and Discussion

Using mathematical models to explore important problems in biology is an ever-increasing tool towards shedding light on these complex systems. Cancer modeling has had a recent and successful history of making predictions that can assist in hypothesis generation leading to experimental and perhaps clinical verification. For ex-

Table 2. Uncertainty and Sensitivity Analysis Results. PRCC values significantly different from 0 (with $p < 0.01$). PRCCs ranking based on generalized z-test ($p < 0.05$).

Name	*Definition*	*PRCC*	*Ranking*
$a(t)$	Cancer clearance term	-0.1993	1^{st}
$s_1(t)$	Immunotherapy term	-0.1061	2^{nd}
$c(t)$	Cancer antigenicity	-0.0814	3^{rd}
$r_2(t)$	Cancer growth rate	-0.0791	3^{rd}

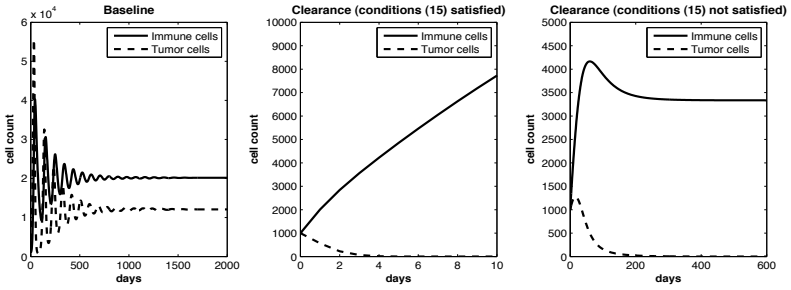

Fig. 5. Numerical solutions of system (5). Shown are plots for the baseline simulation (Parameters are chosen from the *Baseline* column in Table 1), for clearance when conditions (15) are satisfied (values of the treatment parameters are: $s_1(t)$=764.5072, $r_2(t)$=0.0023, $a(t)$=38.0040, $c(t)$=0.3710), and clearance where conditions (15) are not satisfied (values of the treatment parameters are: $s_1(t) = 10^2$,$r_2(t)$=0.0523, $a(t)$=2, $c(t)$=0.05). The rest of the parameters are set to baseline values shown in Table 1.

ample, gene therapy is a relatively young idea in treatment of diseases, the practice of which is even younger. As with the development of any therapy, questions relating to which gene to target, or what combination of therapies can be used (immunotherapy plus gene therapies) is important. A recent paper reviewed the importance of pairing high-throughput experimental studies together with computational systems biology studies to help determine the optimal answers to these questions [22]. Excitingly, these types of studies can lead to personalized medical treatment, which one would expect from medicine in the 21st century.

In this work, we begin by offering a small step in using mathematical models to make predictions that could be useful to experimentalists and clinicians working in the area of tumor-immune interactions and the development of treatment protocols. To this end, we simplified an existing model describing tumor-immune dynamics [7] by merging the effector molecule equation (for IL-2) into the effector cell equation, and allowing for time-varying inputs representing several options for immunotherapy and gene therapy. Sufficient global stability conditions of the cancer-free state were derived and tested numerically. Since the conditions are sufficient, further numerical analysis was performed to investigate regions of the parameter space where the system clears the cancer, even when sufficient conditions are not satisfied. Our results suggest that the source term of TIL cells, s_1, in combination with the cancer

clearance term, a, provide the optimal treatment combination: high levels of both will clear the tumor. Further investigation is necessary to establish whether this is a viable immunotherapy/gene therapy option in the clinical setting. We are working now on deriving necessary conditions for the stability of the cancer-free state for the model system (5) in the general time dependent therapy case.

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