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ORIGINAL ARTICLE

A Mathematical Model of the Enhancement of Tumor Vaccine Efficacy by Immunotherapy

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Abstract TGF-β is an immunoregulatory protein that contributes to inadequate antitumor immune responses in cancer patients. Recent experimental data suggests that TGF-β inhibition alone, provides few clinical benefits, yet it can significantly amplify the anti-tumor immune response when combined with a tumor vaccine. We develop a mathematical model in order to gain insight into the cooperative interaction between anti-TGF-β and vaccine treatments. The mathematical model follows the dynamics of the tumor size, TGF-β concentration, activated cytotoxic effector cells, and regulatory T cells. Using numerical simulations and stability analysis, we study the following scenarios: a control case of no treatment, anti-TGF-β treatment, vaccine treatment, and combined anti-TGF-β vaccine treatments. We show that our model is capable of capturing the observed experimental results, and hence can be potentially used in designing future experiments involving this approach to immunotherapy.

Keywords Immunotherapy · Cancer

1 Introduction

Current cancer therapies predominantly focus on surgery, chemotherapy, and radiotherapy; each of which carries major side-effects. The immune system is not always efficient in providing an adequate response to cancer, since cancer cells may not be easy to identify, and they use various immunosuppression techniques to avoid the immune response. Recently, there has been an increased interest in improving the ability

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of the autologous immune response to target tumors, an approach that is generally being referred to as "immunotherapy" (Blattman and Greenberg 2004; Ribas et al. 2003; Rosenberg 2001).

Although animal models have demonstrated that humoral mechanisms may be relevant to immunotherapy, much of the promising work in tumor immunotherapy has been focused on T-cell-mediated, antigen-specific vaccines. Previous research has shown that through cellular immunotherapy, T cells can destroy large, established tumors (Rosenberg et al. 2004). Over the years, researchers have taken various approaches to tumor immunotherapy. Among these approaches are tumor cell-based vaccines, peptide-based vaccines, virus-based vaccines, DNA-based vaccines, and Dendritic cells vaccines; each of which have met varying degrees of success at reducing or eliminating tumors (see the review papers Dermime et al. 2002; Rosenberg et al. 2004 and the references therein).

The primary cell-mediated immune response is the process by which the human immune system responds to a foreign antigen. As a part of this process, cytotoxic T cells (CTLs) must activate, proliferate, and induce apoptosis in infected cells. To prevent damage to healthy cells, cell-mediated immune responses must be closely regulated following antigenic stimulation. There are a number of mechanisms by which the immune system self-regulates. Among these are various regulatory cells (such as regulatory T cells and natural suppressor cells) and proteins (such as TGF-β, CTLA-4, and IL-6) (Murphy et al. 2008).

This work highlights how immunotherapy might be used to overcome the effects of two such regulatory agents exploited by cancer: regulatory T cells and the Transforming Growth Factor (TGF)-β protein. TGF-β is a protein that controls proliferation, cellular differentiation, and other functions in most cells. It acts as an antiproliferation factor in normal epithelial cells (Cerwenka and Swain 1999). Experimental evidence has shown that TGF-β can act as both a tumor suppressor and stimulator (Reiss 1999). In early stages, it acts directly on cancer cells to suppress their growth. As the tumor progresses, TGF-β stimulates tumor progression by suppressing immune cells and promoting factors that contribute to tumor metastasis. High levels of TGF-β dampen the function and frequency of antigen presenting cells, cytotoxic T cells, and helper T cells. Also, TGF-β (in combination with IL-2) has been implicated in inducing an increased number of CD4+CD25+Fox3p+ regulatory T cells seen in tumors (Flavell et al. 2010). These regulatory T cells (Tregs) play a critical role in suppressing excessive immune responses. They modulate the function of effector cells rendering them unable to continue their cytotoxic activity, leading to a weak or nonexistent immune response to cancerous cells (Beyer and Schultze 2006; Sakaguchi et al. 2010).

The immunosuppressive effects of TGF-β on immune cells strongly support the development of TGF-β inhibitors to treat cancer (Derynck et al. 2001; Llopiz et al. 2009). Several inhibitors of TGF-β are in various stages of development (see Flavell et al. 2010 and the references therein). Several clinical trials have evaluated TGF-β inhibition in cancer patients with some promising results. Unfortunately, while a few studies have shown the beneficial effects of anti-TGF-β in tumor treatment (see Baylor College of Medicine 2006, 2009), Terabe et al. demonstrate that depletion of TGF-β is not always sufficient to elicit an effective immune response against cancerous cells (Flavell et al. 2010; Terabe et al. 2009). Using a mouse model, Terabe

et al. showed that treatment with anti-TGF-β alone does not enhance the immune response. However, an anti-TGF-β treatment did appear to facilitate an enhanced immune response when combined with an immune-boosting vaccine.

The goal of our present study is to understand part of the complex interplay between cancer, the immune system, and the immunoregulatory mechanisms that lead to ineffective immune responses. More specifically, we are interested in quantifying the effects that anti-TGF-β and vaccine treatments might have on the stability of the tumor-immune dynamic and how the combined treatment might contribute to tumor clearance as opposed to tumor escape. In order to understand how the suppression of regulatory mechanisms might affect a cancer vaccine, we develop a mathematical model to analyze the effects of anti-TGF-β treatment when used in conjunction with a vaccine as treatments for tumor growth. This is viewed as a step in developing a framework within which experimentalists may test treatment protocols prior to conducting their experiments. Our work is based on the experiments of Terabe et al. (2009).

A number of mathematical models have been developed to describe tumor-immune dynamics. A review of nonspatial tumor-immune models can be found in Eftimie et al. (2011). ODE models provide a framework within which one can explore the interactions among tumor cells and the alternate agents (such as immune cells, healthy tissue cells, cytokines, etc.). A general, nonspatial tumor-immune model considers an effector cell population (CTLs, NK cells, etc.) interacting with tumor cells. In the earliest models, these interactions are described by two equations, where the immune cells play the role of the predator, while the tumor cells are the prey (Kuznetsov et al. 1994). A framework for all such models is developed and analyzed in d'Onofrio (2005). Many models incorporate different immunotherapeutic strategies such as injection of cytokines (Cappuccio et al. 2006; de Pillis et al. 2006; Kirschner and Panetta 1998), transfer of effector cells (Kirschner and Panetta 1998), or immunization with dendritic cells (Castiglione and Piccoli 2006).

There are several mathematical models that specifically incorporate the effects of TGF-β on tumor development (Byrne and Gourley 1997; Clarke and Liu 2008; Kolev 2005; Michelson and Leith 1991; Ribba et al. 2006; Wang et al. 2009). One such model that considers the effects of TGF-β on tumor growth, while also including a treatment that consists of constant infusion of exogenous CTLs, is developed in Kogan et al. (2010). The model developed in Kirschner et al. (2003) specifically considers disrupting TGF-β production as a method of tumor treatment. Their mathematical model describes tumor growth, immune escape, and anti-TGF-β treatment. In contrast, this work mathematically studies a combined therapy through TGF-β inhibition and CTL vaccine.

The structure of this paper is as follows: In Sect. 2.1, we describe the experimental background that was used as a basis for this work. In Sect. 2.2, we present an ordinary differential equations (ODEs) model of tumor growth that is then used to investigate the effects of vaccinations and TGF-β inhibition. Model simulations and a stability analysis are included in Sect. 3. The main results for the four treatment regimes are shown in Fig. 2. Closing remarks and directions for future work are given in Sect. 4.

2 A Model of Tumor Vaccine Enhancement by TGF-β Inhibition

2.1 Biological Background

Our mathematical model is based on the experimental data presented in Terabe et al. (2009). In this study, Terabe et al. examined whether TGF-β neutralization can potentiate immune responses caused by a CTL-inducing vaccine. Their goal was to determine the conditions under which this enhanced immune response inhibits and/or eliminates tumor growth in a TC1 mouse tumor model. This particular tumor line expresses the human papilloma virus (HPV) E6 and E7 genes (i.e., the tumor is slightly immunogenic) and manifests in lung epithelial cells. Twenty thousand cancer cells were injected into the right flank of the mouse and four days after tumor challenge, mice were immunized with HPV peptide. The TGF-β inhibitor used for experiments was 1D11; a murine anti-TGF-β monoclonal antibody that neutralizes all three isoforms of TGF-β. This antibody was shown to have minimal side effects in normal, tumor-free animals. The main results of Terabe et al. (2009) can be summarized as follows:

1. Blocking TGF-β enhances the effects of an antitumor peptide vaccine. In the case where both treatments were given, the tumor burden was significantly lower than any other treatment option tested; and 40 % of mice remained tumor-free for at least 55 days after tumor challenge.
2. Anti-TGF-β enhances the quantity and the quality of the vaccine-induced CD8+ CTL responses.
3. The enhancement of the immune response was shown to *not* be due to:
   - suppression of CD4+CD25+ Tregs.
   - suppression of IL-17 producing T cells.
   - Natural Killer T cell-induced TGF-β production by Myeloid-derived Suppressor Cells.

The conclusion of the experimental study in Terabe et al. (2009) was that monotherapy with anti-TGF-β did not have a significant impact on tumor growth. The anti-TGF-β did, however, significantly enhance the efficacy of the peptide vaccine by inducing an increased number of tumor antigen-specific CTLs, which is critical for the effective elimination of tumors.

2.2 A Mathematical Model

In order to quantitatively study the experimental setup of Terabe et al. (2009), we developed a mathematical model. In this model, we follow the dynamics of the tumor size, denoted $T(t)$; TGF-β concentration, denoted $B(t)$; activated cytotoxic effector cells, denoted $E(t)$; regulatory T cells, denoted $R(t)$; and vaccine-induced cytotoxic effector cells, denoted $V(t)$. A diagram of the different interactions between these elements is shown in Fig. 1.

Our mathematical model is written as the following system of ODEs:

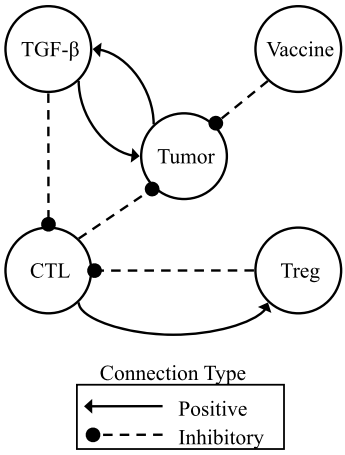


Fig. 1 A diagram of the interactions between the different populations in the mathematical model

$$\frac{dT}{dt} = a_0 T(1 - c_0 T) - \delta_0 \frac{ET}{1 + c_1 B} - \delta_0 TV, \tag{1}$$

$$\frac{dB}{dt} = a_1 \frac{T^2}{c_2 + T^2} - dB, \tag{2}$$

$$\frac{dE}{dt} = \frac{fET}{1 + c_3 TB} - rE - \delta_0 RE - \delta_1 E, \tag{3}$$

$$\frac{dR}{dt} = rE - \delta_1 R, \tag{4}$$

$$\frac{dV}{dt} = g(t) - \delta_1 V. \tag{5}$$

Equation (1) describes the tumor size measured in mm^2. The tumor follows logistic growth dynamics with growth rate, a_0, and carrying capacity, $1/c_0$. The second term on the RHS of (1) describes the ability of immune cells to induce apoptosis of tumor cells. This clearance rate is inversely related to the amount of TGF-β present in the system (i.e., TGF-β diminishes CTL ability to induce apoptosis in tumor cells). The last term defines the action of vaccine cells on tumor cells. Since vaccine cells are considered to be fully differentiated, they are assumed to be unaffected by the inhibitory effects of TGF-β. Vaccine cells induce the death of tumor cells at a rate δ_0.

The dynamics of the concentration of TGF-β cytokine, measured in ng/ml, are described in Eq. (2). Experimental evidence has shown that TGF-β production by tumor cells is low for small tumors but "switches" on as the tumor grows; promoting immune evasion (Paillard 2000). The use of Eq. (2) as a model for TGF-β production is described in Kirschner et al. (2003). As in Kirschner et al. (2003), the maximum rate of TGF-β production is represented by the parameter a_1; c_2 is the critical tumor size at which the switch occurs; and the decay rate of the protein is d.

Equation (3) describes the dynamics of the number of effector T cells in the system. The first term represents immune recruitment. Effector cells are activated proportionally to the number of interactions with tumor cells. This term is multiplied by $(1 + c_3 TB)^{-1}$ to account for the combined negative effect of tumor growth and TGF-

β production on immune recruitment and proliferation. The parameter c_3 represents the magnitude of the inhibition associated with tumor growth and TGF-β. A proportion, r, of effector cells differentiate into regulatory T cells (a process that is further discussed in the next paragraph). The final term of this equation models the removal of effector T cells from the system. These cells have both a natural death rate; assumed to be the natural death rate for all effector cells, δ_1; and a death/removal rate that is proportional to the mass action interaction with regulatory T cells, δ_0. These magnitudes are assumed to be the same.

Equation (4) describes the number of Tregs in the regulatory T cell compartment. Though regulatory T cells originate from both CD4+ and CD8+ T cells (Sakaguchi et al. 2008), this model follows the principles of minimal design by considering only CD8+ effector T cells as precursors to Tregs. The feedback mechanism in this model applies as long as CTLs induce the production and/or recruitment of Tregs. A similar approach to simplifying the modeling adaptive regulation was taken in Kim et al. (2010) and Wilson et al. (2010).

In the model, Tregs differentiate from (or are recruited by) effector T cells at a rate r. The second term is the rate at which Tregs die. These cells provide negative feedback to the effector T cell population. Regulatory T cells should be considered as removing effector T cells from the system rather than killing them. While it is possible that effector T cells die upon interaction with regulatory T cells, that is not necessarily the only explanation. As suggested in Kim et al. (2007), it is also possible that some effector cells might turn into memory cells, some might lose their effector function, and others might migrate away from the lymph node and carry out effector functions in the periphery. For the purposes of the model, suppressed cells, cells that have migrated, and dead cells are irrelevant to the dynamics, so we consider them all to be removed from the system.

Equation (5) describes the vaccine. The vaccine is modeled as an influx of activated tumor-specific cytotoxic T cells. These cells are impulsively introduced into the system at day 3 and are considered to be fully differentiated (i.e., no longer dividing). If the vaccine is given,

$$g(t) = g_0 \delta(t - 3),$$

where $g_0 = 5{,}000$ and $\delta(t)$ is the Dirac delta function. If the vaccine is withheld,

$$g(t) \equiv 0.$$

Vaccine cells have a natural death rate of δ_1. This aspect of the model deviates from the experimental setup. In the experiment, a peptide vaccine is given to induce the production and proliferation of CTLs, while here, we model the vaccine as a direct injection of CTLs. The model vaccine is more in line with vaccination through adoptive T cell transfer and, therefore, might make the model more adaptable to experiments involving less antigenic tumors. Some of the consequences of this design decision will be discussed in the Sects. 3 and 4.

3 Results

In our simulations, we consider the following four scenarios:

Table 1 The baseline control parameter values used in the simulations

Parameter	Units	Description	Estimate	Source
a_0	day^{-1}	tumor growth rate	0.1946	fit to data (Terabe et al. 2009)
$1/c_0$	mm^2	tumor carrying capacity	369	fit to data (Terabe et al. 2009)
δ_0	$\#^{-1}\,\text{day}^{-1}$	effector T-cell induced tumor death rate/removal rate of CTLs by Tregs	1×10^{-5}	estimated
c_1	ml/ng	TGF-β inhibitory parameter for CTL induction of tumor death	100	estimated
a_1	days^{-1} ng/ml	maximal production rate of TGF-β	0.3	Kirschner et al. (2003)
c_2	$(\text{mm}^2)^2$	steepness coefficient of TGF-β production	300	Kirschner et al. (2003) or estimated
d	day^{-1}	degradation rate of TGF-β	7×10^{-4}	Kirschner et al. (2003)
f	$\#^{-1}\,\text{day}^{-1}$	tumor antigenicity	0.62	estimated
c_3	ml / (ng mm^2)	combined tumor growth and TGF-β inhibitory parameter for activation of CTLs	300	estimated
r	$\#^{-1}$	rate of effector T cells that become regulatory T cells	0.01	Kim et al. (2010)
δ_1	day^{-1}	natural death of CTLs, Vaccine cells, and Tregs	1×10^{-5}	estimated

(a) no treatment
(b) vaccine treatment
(c) anti-TGF-β treatment
(d) combined anti-TGF-β and vaccine treatment.

The list of the parameters used in our simulations is given in Table 1. The parameters a_0 and c_0 were approximated using a nonlinear least squares fit to the control data presented in Terabe et al. (2009). Baseline values for a_1, c_2, and d were obtained from Kirschner et al. (2003). The value for the immuno-suppressive effects of TGF-β, c_1, was estimated based on data presented in Terabe et al. (2009). The rate of effector cells that differentiate into regulatory cells, r, was given in Kim et al. (2010) and falls in accordance with the range presented in Sakaguchi et al. (2008). A parameter sensitivity analysis was performed on the model parameters.

3.1 Simulations

Numerical solutions of (1)–(5) were obtained using Matlab (version $R2010a$) $ODE23$ solver. Starting with the initial measurements presented in Terabe et al. (2009), we begin our simulations at day 3 after tumor presentation and conclude all simulations on day 30. At the initial time point, we assume that tumor antigenicity has led approximately 100 activated effector T cells to be present at the site of the tumor. This is consistent with the number of mouse precursor CTLs presented in Blattman et al. (2002) in which they estimated the number of D^b GP33-specific CD8 T cells to be 2×10^2.

Figure 2 shows results of our simulations in the four treatment regimes along with the corresponding experimental data. The simulation and the control data to which it

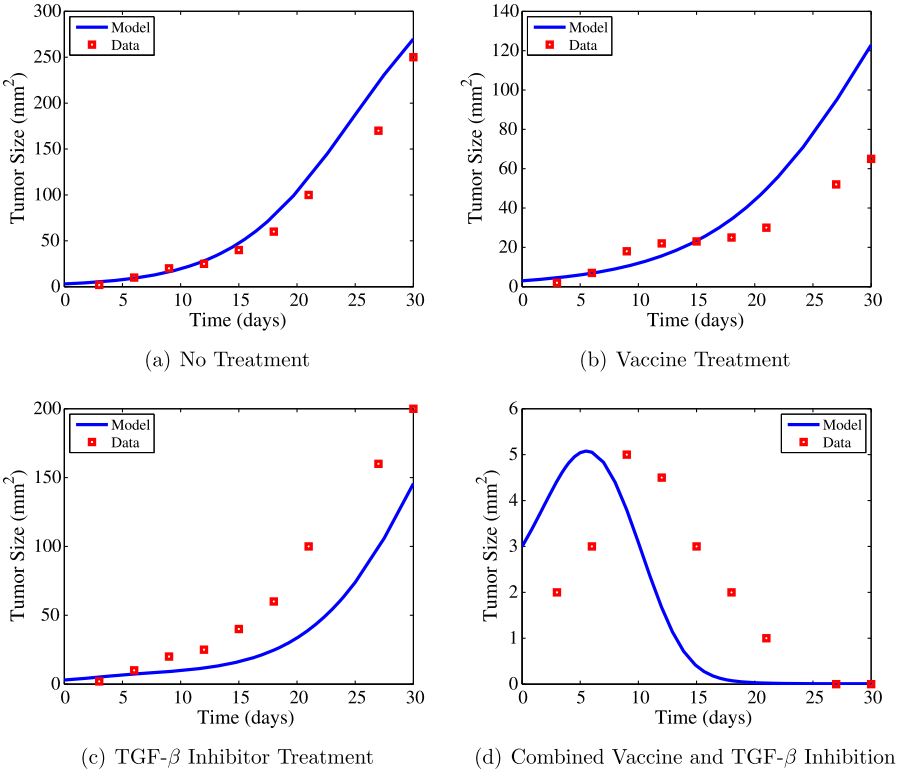


Fig. 2 The dynamics of the tumor size in four treatment regimes. Shown are the results of the numerical simulations on top of the experimental data from Terabe et al. (2009)

was fit are shown in Fig. 2(a). As previously mentioned, the control data set was used to approximate some of the model parameters. We calibrated the no-treatment model to follow the growth trend of the experimental data. While the precise timing of the observed phenomena is not captured by the present model, it is the qualitative aspects of the increase and decrease in the tumor size that we are seeking. The goal of this model is to capture the phenomena of tumor escape with monotherapy, and the peak tumor size and tumor eradication in the case of a combined therapy. These biological aspects are clearly captured by the current model.

In Fig. 2(b), the vaccine treatment is modeled as an addition of 5,000 effector T cells to the vaccine equation at day 3 of simulation. These cells are assumed to be resistant to TGF-β. In this case, there is a steady growth of the tumor throughout the simulation. The vaccine facilitates conditions that lead to a smaller tumor at the final time step. These cells do not multiply once added to the system, and hence the benefit of the vaccine slowly diminishes at the natural death rate for vaccine cells. This means that if the initial size of the vaccine is not large enough to overpower the tumor growth, then the tumor will always escape immunosurveillance.

We model TGF-β inhibition as an increase of c_2 from 300 to 7,000. This effectively delays the "switch" of TGF-β production by approximately 8 days. The results

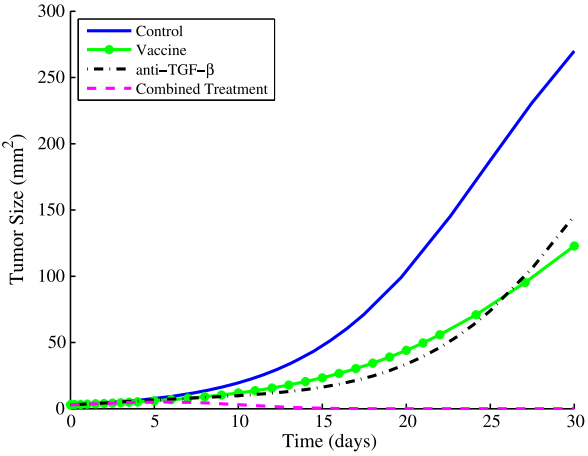


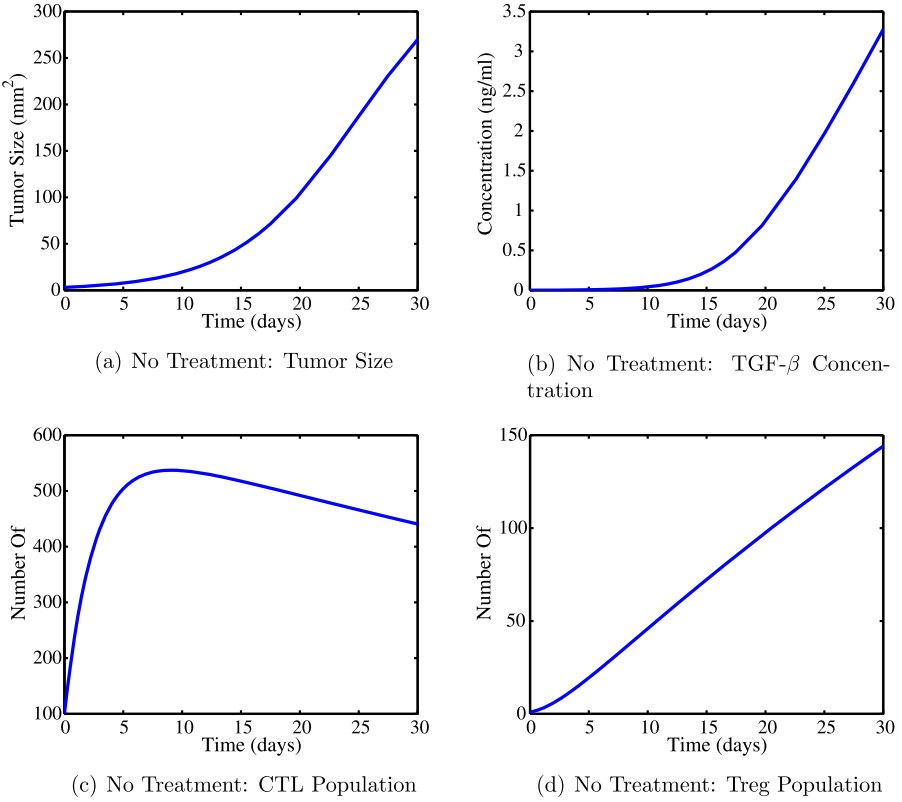
Fig. 3 A comparison of the dynamics of the tumor size for all treatment regimes

of this simulation are shown in Fig. 2(c). In this case, we see that the tumor remains small for the duration of TGF-β inhibition. However, soon after the TGF-β levels begin to recover, tumor growth quickly becomes uncontrolled. Similar results were seen for other values of c_2. In simulation, we see that singular TGF-β inhibition leads to a reduction in final tumor load at 30 days of simulation. This initial delay of tumor growth differs from the original data in Terabe et al. (2009), however, the final result of uncontrolled tumor growth remains similar.

The final case, Fig. 2(d), shows the predictions of the model when both TGF-β and vaccine treatments are administered. Similar to the experimental results in Terabe et al. (2009), we see that a combined treatment is sufficient to induce tumor eradication. Model simulations lead to agreement with experiments concerning the peak tumor size. The timing of this maximum tumor size will be the addressed in the discussion. Simulations show an initial phase of tumor growth, but at approximately day 21, the immune system is able to clear the tumor. This suggests that such an outcome is the result of long-term presence of CTLs provided by the vaccine, in combination with the TGF-β inhibitor that provides an initial boost to the host's native immune system.

Figure 3 compares the tumor growth in all four treatment regimes. It is clear that while monotherapy results in a slowing down of the tumor growth, the tumor is still able to escape immunosurveillance and grow uncontrolled. Only in the case of dual therapy is the immune system able to eradicate the tumor.

We show the dynamics of the individual populations in the control case and the combined treatment case in Fig. 4 and Fig. 5. Figures 4(a) and 5(a) show how the tumor population changes over time. Figures 4(b) and 5(b) demonstrate the dynamics of the TGF-β concentration. In the no-treatment scenario 4(b), we see that the TGF-β levels are increasing with the tumor size. These high levels of TGF-β, particularly at later time points, contributes to the suppression of the effector T cell concentration as seen in Fig. 4(c). In the combined treatment scenario, TGF-β levels are kept very low (see Fig. 5(b)). This contributes to a robust immune response peaking on day 16 with just under 140,000 CTLs present in the system (Fig. 5(c)). The regulatory T cell populations are shown in Figs. 4(d) and 5(d). Though these regulatory cells are effective at ending the immune response in both cases, the maximum ratio of regulatory



(a) No Treatment: Tumor Size

(b) No Treatment: TGF-β Concentration

(c) No Treatment: CTL Population

(d) No Treatment: Treg Population

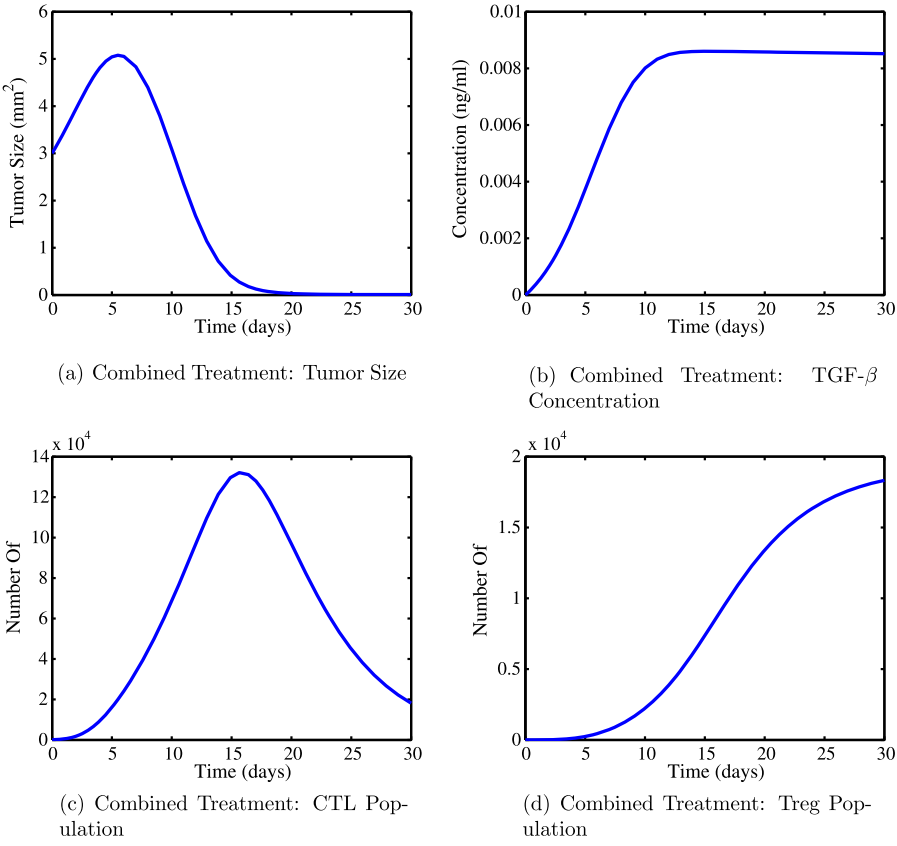
Fig. 4 Simulated population dynamics of the individual populations in the control case: (**a**) Tumor size (mm^2), (**b**) TGF-β concentration (ng/ml), (**c**) CTL population (number of), (**d**) Treg population (number of)

cells to T cells is 0.075 in the no-treatment case and 0.062 in the case of a combined treatment. This aligns with the results of Terabe et al. (2009) which indicated that TGF-β inhibition does not suppress Treg production, but it does increase the ratio of effectors to Tregs in each of the treatment scenarios.

3.2 Stability Analysis

In order to analyze the stability of the system, we begin by considering the Jacobi matrix of (1)–(4) with $V \equiv 0$. It is as follows:

$$\begin{pmatrix} a_0(1-2c_0T) - \frac{\delta_0 E}{1+c_1B} & \frac{\delta_0 c_1 ET}{(1+c_1B)^2} & \frac{-\delta_0 T}{1+c_1B} & 0 \\ \frac{2Ta_1c_2}{(c_2+T^2)^2} & -d & 0 & 0 \\ \frac{fE}{(1+c_3TB)^2} & \frac{-fc_3ET^2}{(1+c_3TB)^2} & \frac{fT}{1+c_3TB} - r - \delta_0 R - \delta_1 & -\delta_0 E \\ 0 & 0 & r & -\delta_1 \end{pmatrix}.$$



(a) Combined Treatment: Tumor Size

(b) Combined Treatment: TGF-β Concentration

(c) Combined Treatment: CTL Population

(d) Combined Treatment: Treg Population

Fig. 5 Simulated population dynamics of the individual populations with combined treatment: (**a**) Tumor size (mm^2), (**b**) TGF-β concentration (ng/ml), (**c**) CTL population (number of), (**d**) Treg population (number of)

A steady state analysis of the system reveals two feasible (nonnegative) steady states. The solution including maximum tumor capacity, $T = 1/c_0$, $B = \frac{a_1}{d(1+c_2c_0^2)}$, $E = R = 0$, is stable while the all zero, $T = B = E = R = 0$, solution is unstable. This implies that even in the case of successful treatment, simulations will eventually lead to a nonzero tumor equilibrium. Hence, we consider treatment to be successful if the size of the tumor is reduced to less than the size of one cell or if the tumor is reduced to a "manageable" size for the duration of simulation. As previously mentioned, all other steady states contain at least one negative component, implying that they are not feasible for the given biological system. This implies that there is no "small-tumor" equilibrium in which a tumor is maintained at a nonzero, nonlethal size by immune cells. In Fig. 6, we present a phase portrait displaying the relation between tumor size and effector cells when combined treatment is simulated. Here, we see that for tumors with high antigenicity, the tumor load is reduced to near zero for a period of time before the immune response is no longer able to control the tu-

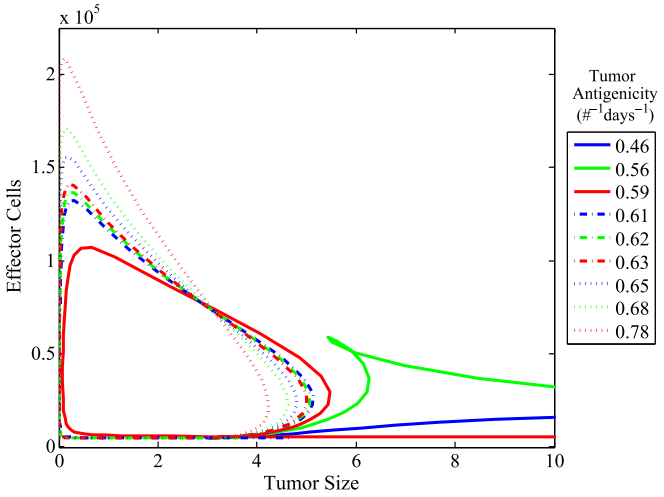
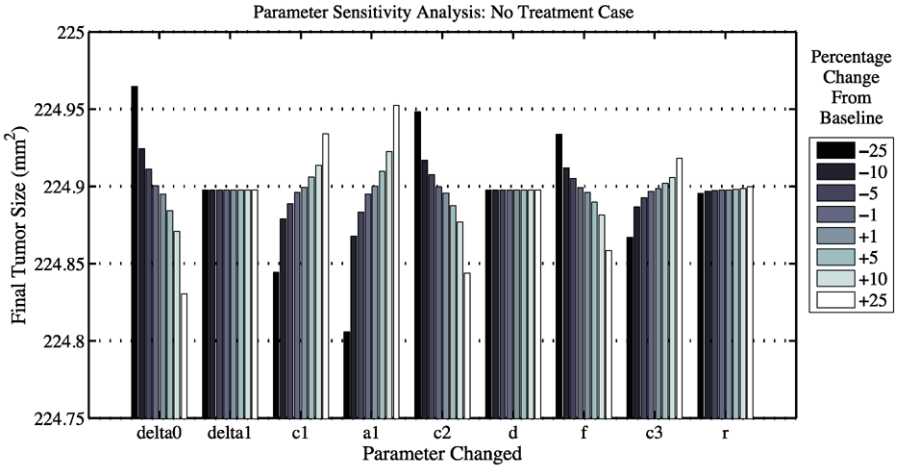


Fig. 6 Effector T cell versus tumor size phase portrait when combined treatment is simulated with different levels of tumor antigenicity. Depending on the antigenicity, the tumor load is reduced to near zero for a period of time before the immune response is no longer able to control the tumor (Color figure online)

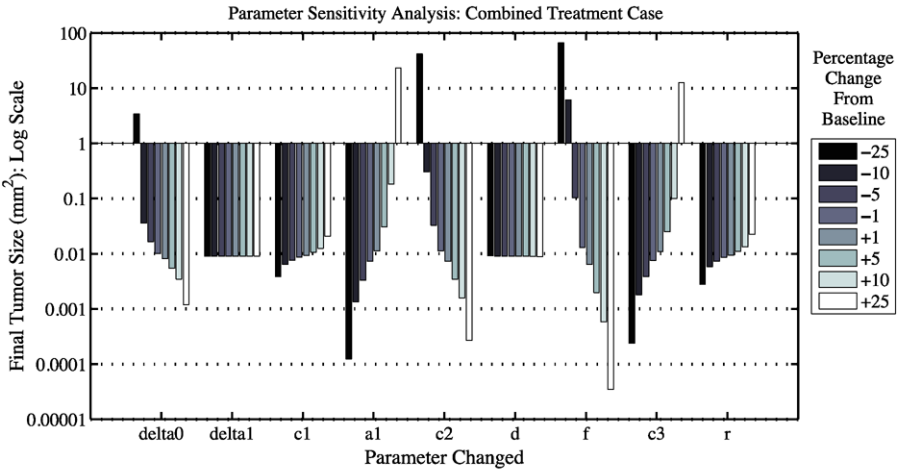
mor. For mildly antigenetic tumors, effector cells are only mildly stimulated by the presence of the tumor and cannot impose tumor shrinkage to manageable levels.

To determine the parameters to which the model is most sensitive, we performed a sensitivity analysis. This was done by varying each parameter over a range of values centered around a baseline value and observing the size of the tumor at the end of 30 simulated days. Figure 7 shows the results of this parameter sensitivity analysis with Fig. 7(a) and Fig. 7(b) displaying the results for the no treatment case and the combined treatment case, respectively. In the no treatment case, variations of parameters leads to very little changes in the final tumor size. This shows that for a wide range of cases, a lack of treatment will lead to uncontrolled tumor growth. In the case of combined treatment, the system was found to be sensitive to a_1, the parameter quantifying the maximal production rate of TGF-β, c_2, the quantity describing the size at which a tumor begins to produce TGF-β, and f, the quantification of a tumor's antigenicity. The system is most sensitive to the parameter f which aligns with the results concerning the corresponding parameters in de Pillis et al. (2005) and Kirschner et al. (2003).

Due to the expression of the HPV E6 and E7 genes, the type of tumor considered for the model is considered to be reasonably antigenic. What happens if a less antigenic tumor is considered? This case is considered in Fig. 8. Here, we reduce the value of the tumor antigenicity parameter, f. As previously mentioned, our sensitivity analysis suggests that the final tumor size is sensitive to this parameter. Figure 8 shows the results of reducing f from 0.62 by 10 % to 0.56. In this case, there is a mild immune reaction, peaking around day 20 after tumor presentation. This immune response is capable of reducing the size of the tumor. However, the reduction of antigenicity causes the immune response to be unsustainable, leading to the eventual unbounded growth of the tumor.



(a) No Treatment Sensitivity Analysis. Baseline values: $c_1 = 100$, $a_1 = 0.3$, $c_2 = 300$, $d = 7 \times 10^{-4}$, $f = 0.62$, $c_3 = 300$, $r = 0.01$



(b) Combined Treatment Sensitivity Analysis. Baseline values: $c_1 = 100$, $a_1 = 0.3$, $c_2 = 7 \times 10^3$, $d = 7 \times 10^{-4}$, $f = 0.62$, $c_3 = 300$, $r = 0.01$

Fig. 7 Model sensitivity analysis. Done by varying each parameter over a range of values centered around a baseline value and observing the size of the tumor at the end of 30 simulated days. (**a**) No treatment case: variations of parameters leads to very little changes in the final tumor size. (**b**) Combined treatment: the system was found to be sensitive to a_1, the parameter quantifying the maximal production rate of TGF-β, c_2, the quantity describing the size at which a tumor begins to produce TGF-β, and f, the quantification of a tumor's antigenicity

4 Discussion

The qualitative aspects of the simulations align with the data described in Terabe et al. (2009). Obtaining precise quantitative matches with the data proved difficult as the data was presented as averages without error estimates or statistical measurements.

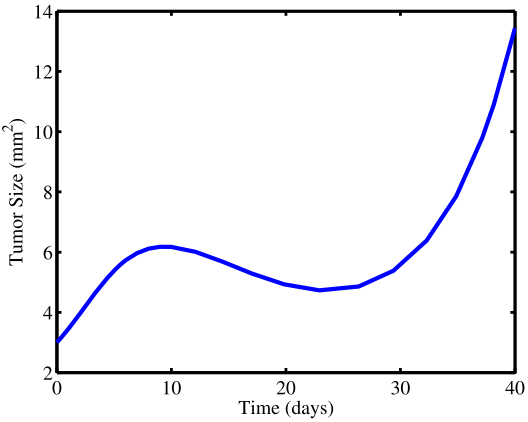


Fig. 8 A simulated tumor growth for a mildly antigenic tumor ($f = 0.56$)

However, the general characteristics of each of the four cases has been captured by the present model. For instance, in the case where both vaccine and TGF-β inhibitors were given, the model predicts that the tumor size will reach its peak on day 5 and tumor eradication will occur on day 21. The data suggests that these events occur respectively on days 15 and 27. Also, unlike the data presented by Terabe et al., in which TGF-β inhibition lead to no significant delay in tumor growth, the model displays a slowed down (yet uncontrollable) tumor growth in the case of a TGF-β treatment. Modifying the model to better capture the timing of these events will be considered in future work. The choice of modeling the vaccine as an adoptive T cell transfer as opposed to a peptide vaccine could be one of the causes for the discrepancy in timing. In the model, T cells are immediately available to begin killing tumor cells, where as in the case of a peptide vaccine there would be a delay between the time of the vaccine and the time that newly recruited CTLs would be activated and available. This design choice contributes to the lack of need of delay differential equations and makes the model amendable to study questions regarding adoptive T cell transfer.

The means by which tumors evolve is nontrivial and all aspects of tumor treatment cannot be included in a single model. Our mathematical model highlights just one possible way of combining tumor treatments to promote tumor eradication through an immune response. A number of biological experiments and mathematical models have highlighted the fact that immunotherapy alone is not always effective in eradicating a tumor (Akhurst and Derynck 2001; Cappuccio et al. 2006; Currie 1972; Dermime et al. 2002; Flavell et al. 2010; Kirschner et al. 2003; Terabe et al. 2009). Here, we show how combined immunotherapy treatments might work through different mechanisms to promote tumor clearance. Simulations of the model (1)–(5) show qualitative agreement with the data in Terabe et al. (2009). In the case of administering either the vaccine or the TGF-β inhibitor, we see a temporary delay in tumor growth; but this delay is not sustainable over time. The vaccine alone is not enough to eradicate the tumor, and though TGF-β is inhibited in the initial days of tumor presentation, the protein level recovers soon thereafter, regaining its immunosuppressive effects. Tumor eradication requires a combination of therapy approaches. Our results suggest that the vaccine allows for the development of a significant and long-term immune response that is minimally affected by the TGF-β that

is present at later time points. The TGF-β inhibitor provides conditions that help the populations of immune cells to expand during the initial phases of tumor presentation. One very pertinent follow up question is: Does one treatment amplify the other or do they act independently of each other? The data collected in Terabe et al. (2009) seems to support the notion that one treatment amplifies the other, but further study is required in order to reach a conclusive understanding.

The results of this work provide an initial analytical framework for studying immunotherapy via TGF-β inhibition in combination with vaccine treatment. Optimally, future studies should be conducted in combination with experiments. Control of nonlinear processes will play a vital role in determining the effectiveness of treatments and in obtaining a protocol for their administration.

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References

Akhurst, R., & Derynck, R. (2001). TGF-β signaling in cancer—a double-edged sword. *Trends Cell Biol.*, *11*(11), S44–S51.

Baylor College of Medicine. (2006). *Safety study of injections of autologous/allogeneic TGFBeta-resistant LMP2A-specific cytotoxic T lymphocytes (CTL)*. Bethesda: National Library of Medicine. Available from http://clinicaltrials.gov/ct/show/NCT00368082.

Baylor College of Medicine. (2009). *Her2 and TGFBeta in treatment of Her2 positive lung malignancy (HERCREEM)*. Bethesda: National Library of Medicine. Available from http://clinicaltrials.gov/ct/show/NCT00368082.

Beyer, M., & Schultze, J. L. (2006). Regulatory T cells in cancer. *Blood*, *108*(3), 804–811.

Blattman, J. N., & Greenberg, P. D. (2004). Cancer immunotherapy: a treatment for the masses. *Science*, *305*(5681), 200–205.

Blattman, J. N., Antia, R., Sourdive, D. J. D., Wang, X., Kaech, S. M., Murali-Krishna, K., Altman, J. D., & Ahmed, R. (2002). Estimating the precursor frequency of naive antigen-specific CD8 T cells. *J. Exp. Med.*, *195*(5), 657–664.

Byrne, H., & Gourley, S. (1997). The role of growth factors in avascular tumour growth. *Math. Comput. Model.*, *26*(4), 35–55.

Cappuccio, A., Elishmereni, M., & Agur, Z. (2006). Cancer immunotherapy by interleukin-21: potential treatment strategies evaluated in a mathematical model. *Cancer Res.*, *66*(14), 7293–7300.

Castiglione, F., & Piccoli, B. (2006). Optimal control in a model of dendritic cell transfection cancer immunotherapy. *Bull. Math. Biol.*, *68*(2), 255–274.

Cerwenka, A., & Swain, S. L. (1999). TGF-β1: immunosuppressant and viability factor for T lymphocytes. *Microbes Infect.*, *1*(15), 1291–1296.

Clarke, D. C., & Liu, X. (2008). Decoding the quantitative nature of TGF-beta/Smad signaling. *Trends Cell Biol.*, *18*(9), 430–442.

Currie, G. (1972). Eighty years of immunotherapy: a review of immunological methods used for the treatment of human cancer. *Br. J. Cancer*, 141–153.

de Pillis, L. G., Radunskaya, A., & Wiseman, C. L. (2005). A validated mathematical model of cell-mediated immune response to tumor growth. *Cancer Res.*, *65*(17), 7950–7958.

de Pillis, L. G., Gu, W., & Radunskaya, A. E. (2006). Mixed immunotherapy and chemotherapy of tumors: modeling, applications and biological interpretations. *J. Theor. Biol.*, *238*(4), 841–862.

Dermime, S., Armstrong, A., Hawkins, R. E., & Stern, P. L. (2002). Cancer vaccines and immunotherapy. *Br. Med. Bull.*, *62*, 149–162.

Derynck, R., Akhurst, R. J., & Balmain, A. (2001). TGF-β signaling in tumor suppression and cancer progression. *Nat. Genet.*, *29*(2), 117–129.

d'Onofrio, A. (2005). A general framework for modeling tumor-immune system competition and immunotherapy: mathematical analysis and biomedical inferences. *Physica D, Nonlinear Phenom.*, *208*(3–4), 220–235.

Eftimie, R., Bramson, J., & Earn, D. (2011). Interactions between the immune system and cancer: a brief review of non-spatial mathematical models. *Bull. Math. Biol.*, *73*, 2–32.

Flavell, R. A., Sanjabi, S., Wrzesinski, S. H., & Lixon-Limon, P. (2010). The polarization of immune cells in the tumour environment by TGFβ. *Nat. Rev. Immunol.*, *10*(8), 554–567.

Kim, P., Lee, P., & Levy, D. (2010). Emergent group dynamics governed by regulatory cells produce a robust primary t cell response. *Bull. Math. Biol.*, *72*, 611–644.

Kim, P. S., Lee, P. P., & Levy, D. (2007). Modeling regulation mechanisms in the immune system. *J. Theor. Biol.*, *246*(1), 33–69.

Kirschner, D., & Panetta, J. C. (1998). Modeling immunotherapy of the tumor-immune interaction. *J. Math. Biol.*, *37*(3), 235–252.

Kirschner, D., Jackson, T., & Arciero, J. (2003). A mathematical model of tumor-immune evasion and siRNA treatment. *Discrete Contin. Dyn. Syst., Ser. B*, *4*(1), 39–58.

Kogan, Y., Forys, U., Shukron, O., Kronik, N., & Agur, Z. (2010). Cellular immunotherapy for high grade gliomas: mathematical analysis deriving efficacious infusion rates based on patient requirements. *SIAM J. Appl. Math.*, *70*(6), 1953–1976.

Kolev, M. (2005). A mathematical model for single cell cancer immune system dynamics. *Math. Comput. Model.*, *41*, 1083–1095.

Kuznetsov, V., Makalkin, I., Taylor, M., & Perelson, A. (1994). Nonlinear dynamics of immunogenic tumors: parameter estimation and global bifurcation analysis. *Bull. Math. Biol.*

Llopiz, D., Dotor, J., Casares, N., Bezunartea, J., Díaz-Valdés, N., Ruiz, M., Aranda, F., Berraondo, P., Prieto, J., Lasarte, J. J., Borrás-Cuesta, F., & Sarobe, P. (2009). Peptide inhibitors of transforming growth factor-β enhance the efficacy of antitumor immunotherapy. *Int. J. Cancer*, *125*(11), 2614–2623.

Michelson, S., & Leith, J. (1991). Autocrine and paracrine growth factors in tumor growth: a mathematical model. *Bull. Math. Biol.*, *53*(4), 639–656.

Murphy, K., Travers, P., Walport, M., et al. (2008). *Immunobiology*. New York: Garland Science.

Paillard, F. (2000). Immunosuppression mediated by tumor cells: a challenge for immunotherapeutic approaches. *Hum. Gene Ther.*, *11*(5), 657–658.

Reiss, M. (1999). TGF-β and cancer. *Microbes Infect.*, *1*(15), 1327–1347.

Ribas, A., Butterfield, L. H., Glaspy, J. A., & Economou, J. S. (2003). Current developments in cancer vaccines and cellular immunotherapy. *J. Clin. Oncol.*, *21*(12), 2415–2432.

Ribba, B., Colin, T., & Schnell, S. (2006). A multiscale mathematical model of cancer, and its use in analyzing irradiation therapies. *Theor. Biol. Med. Model.*, *3*, 7.

Rosenberg, S. A. (2001). Progress in human tumour immunology and immunotherapy. *Nature*, *411*(6835), 380–384.

Rosenberg, S. A., Yang, J. C., & Restifo, N. P. (2004). Cancer immunotherapy: moving beyond current vaccines. *Nat. Med.*, *10*(9), 909–915.

Sakaguchi, S., Yamaguchi, T., Nomura, T., & Ono, M. (2008). Regulatory T cells and immune tolerance. *Cell*, *133*(5), 775–787.

Sakaguchi, S., Miyara, M., Costantino, C. M., & Hafler, D. A. (2010). FOXP3+ regulatory T cells in the human immune system. *Nat. Rev. Immunol.*, *10*(7), 490–500.

Terabe, M., Ambrosino, E., Takaku, S., O'Konek, J. J., Venzon, D., Lonning, S., McPherson, J. P., & Berzofsky, J. A. (2009). Synergistic enhancement of CD8+ T cell-mediated tumor vaccine efficacy by an anti-transforming growth factor-β monoclonal antibody. *Clin. Cancer Res.*, *15*(21), 6560–6569.

Wang, S. E., Hinow, P., Bryce, N., Weaver, A. M., Estrada, L., Arteaga, C. L., & Webb, G. F. (2009). A mathematical model quantifies proliferation and motility effects of TGF-β on cancer cells. *Comput. Math. Methods Med.*, *10*(1), 71–83.

Wilson, S. N., Lee, P., & Levy, D. (2010). A mathematical model of the primary T cell response with contraction governed by adaptive regulatory T cells. In K. E. Herold, W. E. Bentley, & J. Vossoughi (Eds.), *Proceedings IFMBE* (Vol. 32, pp. 209–212). Berlin: Springer.