Humoral and Cell-mediated responses on Cancer Immune system - a study based on Mathematical Modeling

Sumana Ghosh · Sandip Banerjee · Hien Tran · Alexei Tsygvintsev

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Abstract We analyse the mathematical model of interaction between cancer cells and immune system, where we have considered both humoral mediated and cell-mediated Immune response. Our model includes 5 ordinary differential equations and can be analysed analytically. We have done the sensitivity analysis of our system and estimate the model parameter values using least square method. We study its equilibrium points and find the conditions for global stability for the cancer-free equilibrium point and also discuss the bifurcation analysis of the system . We illustrate our results by numerical simulations.

Keywords Cancer cells · Antibodies,B cells · Plasma cells · Cytotoxic T lymphocytes · Global stability · Transcritical bifurcation.

1 Introduction

Cancer is still a leading cause of death worldwide. The world health organization(WHO) has predicted rise in the number of new cancer cases by 70 over the next two decades (WHO (2015)). Cancer is not a single disease; it comprises more than 200 diseases (Gabriel (2007); Schulz (2007)), which share common characteristics, that is, they are abnormal cells, where the normal processes which regulate normal cell proliferation, differentiation and death (cell apoptosis) are interrupted. When certain genes are activated or deacti-
vated because of mutation and environmental factors then a normal cell can be transformed into cancerous cells.

The immune system, the body’s natural defence mechanism, helps the host in fighting against the cancerous cells. As the cancerous cells grow and reach a detectable threshold number, the body’s own immune system is triggered into a search and destroy mode. The defence mechanisms of the human body against external invaders and other pathogens have two major components, namely, the innate and the adaptive immune-systems. Lymphocytes are the principal components of the adaptive immune system, consisting of a class of white blood cells. Adaptive immune system response against external invaders and pathogens are mediated by lymphocytes, either through humoral (antibody) mediated or cell mediated immune responses (Adam and Bellomo (1997)). B-cells are the most important elements for humoral response. Millions of different B-cells are produced by the human body, which secrete antibodies against antigens, thus performing the role of immune surveillance. The antigen binds to the immunoglobulin receptors, which are present on the surface of un-stimulated B-cells, resulting in the cell division of B-cells into non-dividing antibody secreting plasma cells. They are the key elements for humoral (antibody) mediated immune responses. These B-lymphocytes are stimulated by antigens (pathogenic agents), which in turn release antibodies in the blood. These antibodies bind to the antigens and mark them as foreign objects, so that they can be eliminated by other components of the immune system, like macrophages. Antibodies are the key players in humoral immune responses. The antibodies neutralize the cancerous cells by forming a coat over them, thus preventing them from invading the target cells. The major breakthrough came from the research article by Ivanov et al. (2009), who have shown in their latest study that antibodies can kill cancerous cells directly. When the antibodies bind with a cancerous cell, it causes lysosomes (small acid containing sacs) inside the cell to swell and burst rapidly releasing their toxic contents with fatal results for cancerous cells, which is non-apoptotic in nature. T-lymphocytes that is, T Cells are the key element for cell mediated immune response. There are two types of T cells, they are CD8+ T cells and CD4+ T cells. CD8+ T cell take a major role in cell mediated immune response, it also known as cytotoxic T-lymphocytes (CTL).

One of the most important and challenging questions in immunology and cancer research is to understand how the immune system affects on cancer development and progression. The theoretical study of cancer-immune system interaction has a long history in mathematical biology, which has been done by many authors (Adam and Bellomo (1997); Banerjee et al. (2015); Banerjee and Tsygvintsev (2015); Foryś (2002); Kolev (2003a); Khajanchi and Banerjee (2014); Preziosi (1996); Szymanska (2003); Tsygvintsev and Banerjee (2014); Kuznetsov et al. (1994); Kirschner and Panetta (1998); Nani and Freedman (2000); Kuznetsov and Knott (2001); Bodnar and Forys’ (2000); de Pillis and Radunskaya (2003); Moore and Li (2004); Sarkar and Banerjee (2005); Mallet and de Pillis (2006); Chaplain and Matzavinos (2006); Banerjee and Sarkar (2008); Kirschner and Tsygvintsev (2009); de Pillis et al. (2006); Kolev
(2003b); Dillman and Koziol (1987); Dubey et al. (2008)). In Kuznetsov et al. (1994), the authors presented a mathematical model of the cytotoxic T-lymphocyte and responses to the growth of an immunogenic tumor. They studied the immune-stimulation of tumor growth, ”sneaking through” of the tumor and the formation of a tumor dormant state. Kirschner and Panetta (1998) have illustrated the dynamics between tumor cells, immune effector cells and interleukin-2 (IL-2) through mathematical modeling. Their efforts explain both short -term tumor oscillations in tumor size as well as long-term tumor relapses. Nani and Freedman (2000) proposed a general type mathematical model to study cancer immunotherapy. Mathematical analysis of the model equations with regard to dissipativity, boundedness of solutions, invariance of non-negativity, nature of equilibria, persistence, extinction and global stability were analyzed. Kuznetsov and Knott (2001), proposed a deterministic model describing the mechanisms underlying tumor growth, suppression and regrowth and fit to data on B-cell lymphoma. They demonstrated that either a modest change in the effectiveness of killer suppression or the existence of a variant non immunogenic clone of the tumor cells can explain the regrowth of a tumor after initial suppression. Bodnar and Forýs’ (2000) studied the periodic dynamics in the mathematical model of the immune system. Based on the experimental results of Diefanbach et al. (2001), de Pillis and Radunskaya (2003) developed a mathematical model of tumor growth to describe the mechanisms involved in the immune response to a tumor challenge using a system of differential equations. In their model they mainly focus on the interaction of NK-cells and $CD^8$ T-cells with various tumor cells and proposed new forms for the tumor-immune competition terms, and validate these forms through comparison with the experimental data of Diefanbach et al. (2001). In (Moore and Li (2004)), the authors proposed and analyzed a mathematical model for the interaction between naive T cells, effector T cells, and CML cancer cells in the body, using a system of ordinary differential equations. From the analysis they observed that the parameters, namely, the growth and death rates of CML, plays great role to the outcome of the system. In Sarkar and Banerjee (2005), the authors expressed the spontaneous regression and progression of a malignant tumor as a prey-predator like system, where the tumor (cancerous cells) is treated as prey and the cytotoxic T-lymphocytes as predator. The deterministic model is extended to a stochastic one, allowing random fluctuations around the positive interior equilibrium point. The stochastic stability properties of the model are investigated both analytically and numerically. Mallet and de Pillis (2006) have presented a hybrid cellular automaton partial differential equation model of moderate complexity, to describe the interaction between tumor and the immune systems of the host. Chaplain and Matzavinos (2006) have explained the effect of time and space in tumor immunology using a mathematical model, that is, the spatio-temporal phenomena. The role of interleukin-2 (IL-2) in tumor dynamics is illustrated through mathematical modeling (a modified version of the Kirshner-Panetta model) in Banerjee and Sarkar (2008), where the authors have shown that interleukin-2 alone can cause the tumor cell population to regress. In Kirschner and Tsygvintsev...
(2009), the global dynamics of the Kirshner-Panetta model was explored and the conditions under which tumor clearance can be achieved, were obtained.

Most authors have studied the dynamics of cancer with cell mediated immune responses, consisting of the so called T-lymphocytes. There are a few who have concentrated on humoral immune responses to cancer (Kolev (2003b); Dillman and Koziol (1987); Dubey et al. (2008)). In this paper, the aim is to consider a mathematical model with both humoral as well as cell mediated immune responses to cancer and explore various aspect of the dynamical behavior. This work will apply techniques from mathematics to gain insight into the complexities of immune-cancer dynamics.

Schematic diagram and model formulation have been discussed in section 2. In section 3 we study the sensitivity analysis of the model parameters along with the subset selection and consequently the estimation of the parameters. Quantitative analysis of the mathematical model is done in section 4. In section 5 we numerically simulate the model to observe how the dynamics of the system changes with the change of parameter values. the paper ends with a discussion.

2 Model Formulation

The host immune system has the ability to produce some significant anticancerous immune responses, one of which is the B-cells. The human body produces millions of different B-cells each day that circulates in the blood and lymphatic system performing the role of immune surveillance. The principal function of B-cells is to secrete antibodies against antigens. Prior to stimulation by either antigen or mitogen, B-cells are morphologically small cells. The binding of antigen to antigen receptors (that is, the antibodies) on B-cells can result in the activation and differentiation of small B-cells into large B-cells. A set of immunoglobulin molecules is present on the surface of unstimulated B-cells (Warner (1974)). By binding to these immunoglobulin receptors and with a possible second signal from an accessory cell such as the T-cell, the antigen stimulates the B-cell to differentiate and mature into terminal (non-dividing) antibody secreting cells called plasma cells. The plasma cells are most active in secreting antibodies at a much faster rate but large B-lymphocytes, which proliferate rapidly, also secrete antibody, albeit at a lower rate (Eisen (1973)). Some of the large B-cells eventually revert back to small B-cells where they probably function as memory cells, which can respond vigorously to subsequent antigenic challenges (Perelson et al. (1976)). There is two types on immune system in our body they are humoral mediated and cell mediated immunity. Antibody is the key elements of humoral immunity and Cytotoxic T lymphocyte(CTL) pay a major role in cell mediated immunity. The secreted antibodies then circulate in the blood and lymphatic system, and bind to the original antigen, marking them for elimination by several mechanisms, including activation of the complement system, promotion of phagocytosis via
Opsonization and mediation of antibody dependent cell-mediated cytotoxicity (ADCC) with effector cells such as macrophage, NK cells and neutrophils. Also, the antibodies have the ability to kill the cancerous cells through direct contact while binding to them and causing lysosomes inside the cancerous cells, which then swells and burst resulting in the death of cancerous cells. Antibodies, being a protein only decays. CTL and cancer cells both killed each other due to direct interaction and this interaction will enhance the secretion of CTL. Based on the above biological scenario, we present a schematic diagram to describe the interaction between the cancerous cells, large B-cells, plasma cells and antibodies (see figure 1). We now propose a mathematical model to describe the interaction between the host immune system considering both humoral and cell mediated immune responses.

If $B$, $P$, $A$, $T_c$ and $T_u$ be the number of large B-cells, plasma cells, antibodies and cytotoxic T-lymphocytes and the cancerous cells respectively at any time $t$, then the governing equations are given by:

\[
\begin{align*}
\frac{dB}{dt} &= a_0B \left( 1 - \frac{B}{k_1} \right) - b(1 - u)B, \\
\frac{dP}{dt} &= b(1 - u)B - \mu_1P, \\
\frac{dA}{dt} &= r_1B + r_2P - \mu_2A, \ (r_1 < r_2), \\
\frac{dT_c}{dt} &= s + pT_cT_u - \beta_1T_cT_u - \mu_3T_c, \\
\frac{dT_u}{dt} &= rT_u \left( 1 - \frac{T_u}{k_2} \right) - \beta_2T_cT_u - \beta_3AT_u,
\end{align*}
\]

with the following initial conditions:

\[
B(0) = B_0 \geq 0; P(0) = P_0 \geq 0; A(0) = A_0 \geq 0; T_c(0) = T_{co} \geq 0 \text{ and } T_u(0) = T_{uo} \geq 0
\]

Here, $a$ is the rate at which large B-cells are proliferating , $b$ is the differentiation rate of large B-cells into plasma cells, a fractional part $u$ of large B-cells remains as proliferating large B-cells at fraction $u$ ($0 < u < 1$) (Perelson et al. (1976)) and $(1 - u)$ is the fraction, that differentiates into plasma cells. $k_1$ is the carrying capacity of large B-cells, $\mu_1$ is the decay rate of the plasma cells. Both B-cells and plasma cells secrete antibodies at a rate $r_1$ and $r_2$ respectively, however, plasma cells secrete at a higher rate ($r_2 > r_1$) and $\mu_2$ is the decay rate of antibodies. $s$ is the constant source term of cytotoxic T lymphocytes, $p$ is the rate at which the cytotoxic T lymphocytes are recruited in the vicinity of cancer cells and $\beta_1$ is the rate at which cytotoxic T lymphocytes are killed due to interaction with cancer cells. $\mu_3$ being natural death rate of cytotoxic T lymphocytes. The intrinsic growth rate and carrying capacity of cancerous cells are $r$ and $k_2$ respectively. Lastly, cytotoxic T lymphocytes and antibodies kill the cancerous cells by direct interactions at a rate $\beta_2$ and $\beta_3$ respectively.
3 Sensitivity Analysis, Subset selection and parameter estimation

3.1 Initial Parameter Values

We refer to few published work with proper justifications to get appropriate parameter values for the first two model equations. The mean generation time for large B lymphocytes is approximatly to be 6 to 48 hours (Davis et al. (1973)). Hence, the growth rate $a_1$ of large B lymphocytes is estimated to be between 0.02 and 0.2 $hr^{-1}$ (Perelson et al. (1976)). Since the immune response to a T-independent antigen generally lacks any detectable immunological memory (Miranda (1972); Paul et al. (1974)), the conversion of large lymphocytes back into small lymphocytes and the possible subsequent recycling of memory cells back into large lymphocytes have not been explicitly considered. The life time of plasma cells ranges from few days to few weeks. Thus, the natural death rate $\mu_2$ of the plasma cell is approximated to vary between 0.002 to 0.02 $hr^{-1}$ (Perelson et al. (1976)). The plasma cells and large B-cells secrete antibodies at different rates. It is known that plasma cells may be even 100 fold more active in immunoglobulin synthesis than the large B cells (Melchers and Anderson (1973)). Thus, the rate ($r_2$) at which plasma cells secrete antibodies can be estimated to be 2 to 100 times that of large B cells ($r_1$). The secretion rate of antibodies by a single cell also varies considerably. Different authors have estimated the absolute rate of antibody secretion by a single cell. For example, Nossal and Makela (1962) have been found in vitro that the rate at which a single cell secretes antibodies ranges from 100 to 1500 antibodies $cell^{-1}sec.^{-1}$.

To determine the other parameter values of the system, we look into the experiment by Tutt et al. (2002), where, an investigation has been made in the treatment of three syngeneic mouse B cell lymphomas, $BCL_1$, A31, and A20 on a panel of rats antimouse B cell mAb, including Ab directed at surface IgM Id, CD19, CD22, CD40, CD74 and NHC class II. It is noticed that only three mAb, namely, anti Id, anti CD19 and anti CD40 are therapeutically active in vivo. They are interested in the immunotherapy of $BCL_1$, A31 and A20 cells from the experiments by monoclonal antibody Anti-CD40. Groups of age-matched mice were injected $10^5$ i.v (intravenously) with different tumor cells ($BCL_1$, A31, and A20) on day 0, followed by mAb treatments daily for 4 days at the times indicated for each experiments. In vivo T cell depletion was described by Cobbold (1990), using i.p injection of 0.5 mg of anti-CD8 mAb and/or 1 mg of anti-CD4 mAb. The injections were repeated every 4-5 days.

The data shown in Tutt et al. (2002) (figure 4A, pg No.2723) gives the growth of tumor in presence of control IgG mAb and anti-CD40 mAb. As seen in the figure, control IgG is failed to control the tumor growth whereas anti-CD40 eradicates tumor cells between 8-10 days. Since control IgG mAb does
not have any impact on the growth on tumor cells, we assume the effect of control IgG mAb as the normal growth of tumor cells in absence of antibody therapeutic effect. In that case, the fourth equation of the model reduces to

$$\frac{dT}{dt} = rT \left( 1 - \frac{T}{K_2} \right)$$

To estimate the parameters $r$ and $K_2$, we obtain 8 data points from Tutt et al. (2002) (figure 4A, pg No.2723). Using least square method, the estimated values of $r$ and $K_2$ are respectively $r = 0.9889 \, \text{day}^{-1}$ and $K_2 = 1.336 \times 10^9 \, \text{cells}$, that closely approximate the data (see Fig.4).

3.2 Sensitivity Analysis and subset selection

Nonlinear dynamical models derived from basic principles often contain parameters whose values cannot be accurately predicted from theory. Missing parameters can be estimated using information from available data by solving a parameter estimation problem. Parameter estimation problems are often formulated as optimization problems in which the unknown parameters are determined by minimizing the least squares error between the data and the model output subject to constraints imposed by the model equations and known bounds on the parameters. However, due to the model structure and possible lack of measurements, some parameters may not be identifiable. Parameters may have a very weak effect on the measured outputs (sensitivity), or the effect of certain parameters on the measured outputs may be nearly linearly dependent (i.e., statistically correlated).

**Local Sensitivity Analysis.**

A first step before rigorous parameter estimation methods can be applied to an ODE model is to determine which parameters contribute most to the output of the system. In the past, sensitivity analysis was primarily used in the analysis of the forward, or simulation, problem when one needed to understand the effects of parameter perturbation on the output. Now, in recent years sensitivity analysis has become a de-facto part of inverse problem analysis, partly because it directly aids in uncertainty analysis. When physiologically based mathematical models are developed, the parameters typically have biological relevance, and thus one may wish to estimate these parameters from data. However, if these parameters are insensitive then they could be difficult to estimate despite their relevance. Sensitivity analysis determines which state outputs are sensitive to given parameters and whether you can expect to estimate the parameter from available data, as well as understanding which model parameters most influence the model. Sensitivity analysis can also aid in the optimal design of experiments and data collection.

Consider the following dynamical system:

$$\frac{dx(t)}{dt} = f(x(q), t; q), \quad x(t_0) = x_0,$$  \hspace{1cm} (7)
with observation function

\[ y(t) = g(x(t)). \] (8)

Here, \( x \in \mathbb{R}^n \) is a vector of the state variables and \( y \in \mathbb{R}^p \) is the output vector (to be compared with the data). We assume the parameter vector \( q \in \mathbb{R}^m \) to be constant over time.

The sensitivity equations are formed by implicitly differentiating both sides of (8) with respect to \( q \).

\[ \frac{\partial y}{\partial q} = \frac{\partial g}{\partial x} \frac{\partial x}{\partial q}. \] (9)

These are the output sensitivities, and are in terms of the state sensitivities \( \frac{\partial x}{\partial q} \). These equations are obtained by differentiating (7) also with respect to \( q \) and then exchanging the order of differentiation. The \( mn \) sensitivity equations are then given by

\[ \frac{d}{dt} \frac{\partial x(t)}{\partial q} = \frac{\partial f}{\partial x} \frac{\partial x}{\partial q} + \frac{\partial f}{\partial q}. \] (10)

In addition, if the initial model states are unknown, we must also estimate the initial conditions for the model. Doing so requires the formulation of initial condition sensitivity equations, which quantify how the states respond to perturbations in initial condition. To construct these \( n^2 \) equations we differentiate (7) with respect to \( x_0 \). After exchanging the order of integration, we have

\[ \frac{d}{dt} \frac{\partial x(t)}{\partial x_0} = \frac{\partial f}{\partial x} \frac{\partial x}{\partial x_0}. \] (11)

These equations are coupled with the original sensitivity and state equations yielding a system of \( n + mn + n^2 \) differential equations,

\[ \frac{dx(t)}{dt} = f(x(q), q) \] (12)

\[ \frac{d}{dt} \frac{\partial x(t)}{\partial q} = \frac{\partial f}{\partial x} \frac{\partial x}{\partial q} + \frac{\partial f}{\partial q} \] (13)

\[ \frac{d}{dt} \frac{\partial x(t)}{\partial x_0} = \frac{\partial f}{\partial x} \frac{\partial x}{\partial x_0}. \] (14)

which would then be integrated in forward time giving the sensitivity functions.

We assume a zero initial condition for the parameter sensitivity equations (10) at time \( t = t_0 \), as the initial conditions for model states are not parameter dependent. In addition, if the initial conditions are to be estimated, a unity initial condition for the initial condition sensitivities (11) is assumed, in general. The sensitivity functions can ultimately be calculated using several different methods, including automatic differentiation (AD) or finite differencing.
AD techniques are based on the fact that any function is executed on a computer as a well-determined sequence of elementary operations like additions, multiplications and calls to elementary functions such exponential, sine, cosine, etc. By repeatedly applying the chain rule to the composition of these elementary operations one can compute (completely automatically) derivatives of a function correct to machine epsilon. In our work, we used the MATLAB AD code, myAD, developed by our collaborator Martin Fink. Another AD code, which has also been widely available, is the ADIFOR Fortran library developed by Argonne National Laboratory. It has been shown that ADIFOR can produce exact sensitivity information up to machine epsilon, and can reduce computer CPU time requirements by up to 57% compared with brute-force methods.

Sensitivity functions can also be computed by using finite difference formulas. In our work, we have used a central finite difference approximation,

\[ \frac{\partial x}{\partial q_i} = \frac{x(q_i + h e_i) - x(q_i - h e_i)}{2h} + O(h^2). \]  

(15)

The advantage of using finite difference is its relative ease of implementation versus the tedium of direct calculation. Using a central difference approximation is computationally more expensive than standard forward differences, however the approximation is accurate to \( O(h^2) \) versus \( O(h) \). The selection of step size \( h \) is important; it has been shown that a good choice for \( h \) in central differencing schemes is \( h = (\text{macheps})^{1/3} \), where macheps is the relative error in computing \( f(x) \).

To compare the sensitivity functions, we non-dimensionalize the functions and then compute the norm of the resulting function, referred to as the sensitivity coefficients, given by

\[ C_{ij} = \left\| \frac{\partial x_i}{\partial q_j} \right\|_{\text{max}x_i}^2 = \int_{t_0}^{t_f} \left| \frac{\partial x_i}{\partial q_j} \right|_{\text{max}x_i}^2 dt \]  

(16)

After ranking the functions, the sensitivities can be sorted from least to most sensitive. Many identifiability techniques have been derived around the sensitivity coefficients. The simplest techniques state that the larger the coefficients, then the more dramatic the system output with respect to that parameter. In this case, a parameter is more likely to be identifiable when there is a greater sensitivity. Finally, because the sensitivity functions are evaluated at some nominal values for the parameters, the analysis is only local.

Now in our case we calculate the \( L_2 \) norm of the corresponding parameter of the system by using MATLAB and plotting them we see that the \( L_2 \) norm of the parameter \( p, \beta_1, r, \beta_3, r_2, \mu_2, \mu_3, \beta_3 \) is large compare to the other parameter of the system(see figure3). So \( p, \beta_1, r, \beta_3, r_2, \mu_2, \mu_3, \beta_3 \) are more sensitive parameter for the system(1-5).

Local Identifiability Analysis.
The presence of parameters with weak and/or nearly linearly dependent effects is manifested by the lack of a unique solution to the optimization problem for different initial parameter values. Estimation of such parameters can lead to significant degradation in the predictive capability of the model. A parameter is said to be practically identifiable if a unique estimate can be obtained from different initial values using the available data.

The correlation between parameters can be analyzed and handled through the analysis of the sensitivity function matrix. This matrix is defined for outputs \( y_1(t), \ldots, y_k(t) \) with respect to parameters \( q = [q_1, \ldots, q_m]^T \) at the time points \( t = (t_1, \ldots, t_n) \) by the \( kn \times m \) matrix

\[
S(q, t) = \begin{pmatrix}
  s_{1,1}(q, t_1) & \cdots & s_{1,m}(q, t_1) \\
  s_{1,1}(q, t_2) & \cdots & s_{1,m}(q, t_2) \\
  \vdots & \ddots & \vdots \\
  s_{1,1}(q, t_n) & \cdots & s_{1,m}(q, t_n) \\
  s_{2,1}(q, t_1) & \cdots & s_{2,m}(q, t_1) \\
  \vdots & \ddots & \vdots \\
  s_{k,1}(q, t_n) & \cdots & s_{k,m}(q, t_n)
\end{pmatrix}
\]

where \( s_{i,j}(q, t_k) = \frac{\partial y_i(t_k)}{\partial q_j} \) denotes the sensitivity of output \( y_i \) to parameter \( q_j \) at time \( t_k \). The sensitivity matrix \( S \) represents the classical mechanism of sensitivity analysis. A nominal parameter value is required for the evaluation of the sensitivity matrix, thus identifiability analysis based on sensitivity methods is evaluated with respect to a specific point in the parameter space. Consider the first order Taylor series expansion of the system response near a nominal parameter value \( q^* \),

\[
y(q) = y(x(t_k), q) \\
\approx y(x(t_k), q^*) + \frac{\partial y(x(t_k), q)}{\partial q}|_{q=q^*} (q - q^*),
\]

where in our notation \( S = \left[ \frac{\partial y(x(t_k), q)}{\partial q} \right]_{q=q^*} \). Let \( \hat{y}_k \) denote a model observation, e.g. a measurement at time \( t_k \) and \( \Delta q = q - q^* \). The residual sum of squares between the model observations and the linear approximation (17) is

\[
\text{RSS}(\Delta q) = \sum_{k=1}^{N} [\hat{y}_k - y_k(q^*) - \left. \frac{\partial y(x(t_k), q)}{\partial q} \right|_{q=q^*} \Delta q]^2
\]

where we have assumed that the residuals \( r_k = \hat{y}_k - y_k(q^*) \) are small and negligible. Rewriting (18) in terms of the sensitivity function matrix \( S \), we
have

$$RSS(\Delta q) = (S\Delta q)^T S\Delta q,$$

(19)

which has a minimum when $S^T S \Delta q = 0$. The matrix $F = S^T S$ is referred to as the Fisher Information Matrix, and is a first order approximation to the Hessian of the cost function in an ordinary least squares sense. It follows that the parameters $q$ are locally identifiable if and only if the column rank of the matrix $S$ is equal to $m$, or equivalently $\det(S^T S) \neq 0$. If $S^T S$ is full rank, then $(S^T S) \Delta q = 0$ has a unique solution, and $q = q^*$ implying that $q$ is locally identifiable at $q^*$. If $S^T S$ is singular, then there exists a nontrivial solution $\hat{q} \neq q^*$, and the model parameters are not identifiable at $q^*$.

It is clear that identifying $q$ is contingent on $F$ being full rank. It is typically the case, especially when working with complex compartment models the matrix $F$ is not exactly singular. However $F$ is often nearly singular, in the sense that its smallest singular value is much smaller than the largest singular value of $F$. The numerical rank of a matrix offers a measure of linear independence in the columns of a matrix. To compute the numerical rank of the matrix, we use the following measure with $\varepsilon = \varepsilon \|F\|$, the floating point accuracy at $\|F\|$.

$$\text{rank}(A, \varepsilon) = \max \{ \text{is.t.} \frac{|\sigma_i|}{\sigma_1} > \varepsilon \|A\| m \}$$

(20)

where $\sigma_i$ are the singular values of the matrix, with $\sigma_1 \geq \sigma_i \forall i > 1$, and $m$ is the number of columns. The calculation of the numerical rank is highly dependent on the tolerance used, especially when the spectrum is relatively smooth with no obvious breaks or gaps.

When the matrix $F$ is of numerical rank $k$, we say that only $k$ parameters are identifiable, as $k$ parameters form a mostly linearly independent spanning set. The question then becomes which $k$ parameters are identifiable, and this is answered through subset selection.

**Subset Selection.** A number of approaches have been developed and used to find an identifiable subset of parameters. In this work we utilize the QR factorization with column pivoting proposed by Golub (1965) and is implemented in the MATLAB routine `qr`, `[Q,R,E]=qr(A)`. It is computationally more efficient than the singular value decomposition method and is useful in many applications including the rank-deficient least squares problems. The method consists in using a column pivoting strategy to determine a permutation matrix $E$ so that $AE = QR$ is the QR factorization of $AE$. The indices in the first $k$ columns of $E$ identify the $k$ parameters that are most estimable. Our case yields the ordering $p, \beta_2, \beta_3, \beta_1, \mu_1, r, \mu_3, \mu_2$, which means that these 8 parameters are most estimable from the given data Tutt et al. (2002)(figure 4A, pg No.2723).

As we have already estimated the parameters $a, u, b, r_1, r_2, \mu_1, s, r$ and $k_2$ (see subsection 3.1), we now estimate rest of the parameter values $\mu_2, \mu_3, p, \beta_1, \beta_2, \beta_3$. 
From Tutt et al. (2002) (figure 4A, pg No.2723) we obtain 8 data points showing the growth of cancer cells in presence of the immune system. Using these 8 data points we estimate the parameters $\mu_2, \mu_3, p, \beta_1, \beta_2, \beta_3$. To start the estimation process, the initial values of the parameters are chosen arbitrarily (within meaningful biological range). We use least square method, which minimize the sum of the residuals to obtain the estimated values of the system parameters $\mu_2, \mu_3, p, \beta_1, \beta_2, \beta_3$. In practice we use MATLAB function fminsearch to estimate the parameter values. The estimated values of the parameters are as follows:

$\mu_2 = 1.3666 \text{ sec}^{-1}$, $p = 7.2641 \times 10^{-8} \text{ Ab}^{-1} \text{ hr}^{-1}$, $\beta_1 = 6.8891 \times 10^{-8} \text{ Ab}^{-1} \text{ hr}^{-1}$, $\mu_3 = 0.1605 \text{ sec}^{-1}$, $\beta_2 = 1.9169 \times 10^{-10} \text{ Ab}^{-1} \text{ hr}^{-1}$, $\beta_3 = 1.6530 \times 10^{-11} \text{ Ab}^{-1} \text{ hr}^{-1}$.

Figure 5 shows the best fit estimate for the model parameters $\mu_2, \mu_3, p, \beta_1, \beta_2, \beta_3$. All the values of the system parameters are given in tabular form in Table 1.

4 Basic properties of the mathematical model

4.1 Positivity

In this section, we study the positivity of the solutions of the system (1-5). We start with a theorem which assures the system is positive in the interval $[0, \infty)$.

**Theorem 1** Every solution of the system (1-5) with respect to the initial conditions (6) exist in the interval $[0, \infty)$ and remain positive for all future time $t > 0$.

**Proof.** Consider the system of equation (1-5) in vector form

$$X(t) = \text{col}(B(t), P(t), A(t), T_c(t), T_u(t)) \in \mathbb{R}_+^5,$$

and

$$\mathcal{H} = \begin{pmatrix}
\mathcal{H}_1(X(t)) \\
\mathcal{H}_2(X(t)) \\
\mathcal{H}_3(X(t)) \\
\mathcal{H}_4(X(t)) \\
\mathcal{H}_5(X(t))
\end{pmatrix} = \begin{pmatrix}
auB \left(1 - \frac{B}{k_1}\right) - b(1 - u)B \\
b(1 - u)B - \mu_1P \\
r_1B + r_2P - \mu_2A \\
s + pT_uT_u - \beta_1T_uT_u - \mu_3T_c \\
rT \left(1 - \frac{T}{k_2}\right) - \beta_2T_uT_u - \beta_3AT
\end{pmatrix},$$

which can be written as

$$\dot{X} = \mathcal{H}(X(t)),$$

where $\frac{d}{dt}$ with initial conditions $X(t) = \text{col}(B(0), P(0), A(0), T_c(0), T_u(0)) \in \mathbb{R}_+^5$. Consider the mapping $\mathcal{H} : \mathbb{R}_+^5 \to \mathbb{R}^5$ and $\mathcal{H} \in C^\infty(\mathbb{R}_+^5)$. It is easy to verify
that $\mathcal{H}(X)|_{X_i=0} = \mathcal{H}_i(0) \geq 0, \forall i = 1, 2, 3, 4, 5$. Due to the well known theorem by Nagumo (1942), the solution of (1-5) with initial conditions $X_0 \in \mathbb{R}_+^5$, is such that $X(t) \in \mathbb{R}_+^5$, for all $t > 0$, that is, it remains non-negative throughout the region $\mathbb{R}_+^5, \forall t > 0$.

4.2 Boundedness

**Theorem 2** The non negative solution of the system (1−5) with respect to the initial conditions (6) are bounded in the region $\Omega$ provided $q_0 = au - b(1-u) > 0$ and $\mu_3 > pk_2$ holds.

From equation (1) we get,

$$B(t) = \frac{q_0}{k_1} \left(1 - e^{-q_0 t}\right) + \frac{q_0}{\mu_0} e^{-\mu_1 t},$$

where \(q_0 = au - b(1-u)\).

Now if $au - b(1-u) > 0$, then $\limsup_{t \to \infty} B(t) = \frac{k_1}{\mu_1} q_0 = \bar{B}$ (say).

From equation (2),

$$\frac{dP(t)}{dt} + \mu_1 P(t) \leq b(1-u)\bar{B}$$

$$\Rightarrow P(t) \leq \frac{b(1-u)\bar{B}}{\mu_1} (1 - e^{-\mu_1 t}) + C_1 e^{-\mu_1 t}$$

$$\Rightarrow \limsup_{t \to \infty} P(t) \leq \frac{b(1-u)\bar{B}}{\mu_1} = \bar{P} (say).$$

Now, considering equation (3) we get,

$$\frac{dA(t)}{dt} + \mu_2 A(t) \leq r_1 \bar{B} + r_2 \bar{P}$$

$$\Rightarrow \limsup_{t \to \infty} A(t) \leq \frac{r_1 \bar{B} + r_2 \bar{P}}{\mu_2} = \bar{A} (say).$$

From equation (5) we get,

$$\frac{dT_u(t)}{dt} \leq cT_u(t) \left(1 - \frac{T_u(t)}{k_2}\right)$$

which implies

$$T_u(t) \leq \frac{C_2 k_2}{C_2 + e^{-\pi}}, \quad C_2 \text{ being an arbitrary constant.}$$

By using standard kamke’s comparison theory (Kamke (1932)), we get,

$$\limsup_{t \to \infty} T_u(t) \leq k_2 = T_u.$$
From equation (4) we get,
\[
\frac{dT_c(t)}{dt} \leq s + pT_c k_2 - \mu_3 T_c \\
\leq s - (\mu_3 - pk_2)T_c \\
\Rightarrow \frac{dT_c(t)}{dt} + (\mu_3 - pk_2)T_c \leq s \\
\therefore T_c(t) \leq \frac{s}{\mu_3 - pk_2} \left(1 - e^{-(\mu_3 - pk_2)t}\right) + C_3 e^{-(\mu_3 - pk_2)t}
\]

Now, if \( \mu_3 > pk_2 \), then \( \lim_{t \to \infty} T_c(t) \leq \frac{s}{\mu_3 - pk_2} = \bar{T}_c \)

Hence the region
\[
\Omega = \{(B, P, A, T_c, T_u) \in \mathbb{R}_+^5/ 0 \leq B \leq \frac{k_1q_0}{au}, 0 \leq P \leq \frac{b(1-u)k_1}{\mu_3}, 0 \leq A \\
\leq \frac{k_1 (r_1q_0\mu_1 + r_2au(1-u))}{au \mu_1 \mu_2}, 0 \leq T_c \leq \frac{s}{\mu_3 - pk_2}, 0 \leq T_u \leq k_2\}
\]
is bounded, provided \( q_0 = au - b(1-u) > 0 \) and \( \mu_3 > pk_2 \).

### 4.3 Existence and Local Stability Analysis of the equilibrium points

The system (1) has four feasible equilibrium points, namely, 
(i) the boundary equilibrium \( E(0, 0, 0, \frac{s}{\mu_3}, 0) \), which always exist.
(ii) the antibody free equilibrium point \( \tilde{E}(0, 0, 0, \frac{s}{\mu_3+(\beta_1-p)\bar{T}_u}, \tilde{T}_u) \)
where \( \tilde{T}_u \) is the root of the quadratic equation
\[
\frac{r(\beta_1-p)}{k_2} \tilde{T}_u^2 + \left(\frac{r \mu_3}{k_2} + (p - \beta_1)r\right) \tilde{T}_u + s\beta_2 - \mu_3 r = 0 \tag{22}
\]
\( \tilde{E} \) exists if equation (22) has positive real root and as well as \( \mu_3+(\beta_1-p)\tilde{T}_u > 0 \).
(iii) the cancer free equilibrium point \( \hat{E}(\bar{B}, \bar{P}, \bar{A}, \bar{T}_c, 0) \)
where
\[
\bar{B} = \frac{k_1}{au}(au - b(1-u)), \quad \bar{P} = \frac{b(1-u)\bar{B}}{\mu_1} \\
\bar{A} = \frac{r_1\bar{B} + r_2\bar{P}}{\mu_2}, \quad \bar{T}_c = \frac{s}{\mu_3}
\]
(iv) the interior equilibrium $E^*(B, P, A, \frac{s}{\mu_3 + (\beta_1 - p)T_u^*}, T_u^*)$,

where, $T_u^*$ is obtained from

$$\frac{r(\beta_1 - p)}{k_2}T_u^2 + \left( \frac{r\mu_3}{k_2} + (\beta_1 - p)(\beta_3A - r) \right) T_u^* + s\beta_2 + \mu_3(\beta_3A - r) = 0 \quad (23)$$

The interior equilibrium point exist when equation (23) has a positive real root and $(\mu_3 + (\beta_1 - p)T_u^*) > 0$.

The local behavior of the system (1 - 5) around each of the equilibrium points are obtained by computing the jacobian matrix corresponding to each equilibrium point.

At the boundary equilibrium point $E(0, 0, 0, \frac{s}{\mu_3}, 0)$, the variational matrix $J_E$ is given by

$$J_E = \begin{pmatrix}
au - b(1 - u) & 0 & 0 & 0 & 0 \\
b(1 - u) & -\mu_1 & 0 & 0 & 0 \\
r_1 & r_2 & -\mu_2 & 0 & 0 \\
0 & 0 & 0 & -\mu_3(p - \beta_1) & 0 \\
0 & 0 & 0 & 0 & r - \frac{s\beta_2}{\mu_3}
\end{pmatrix}$$

The eigen values are $\lambda_1 = au - b(1 - u) > 0, \lambda_2 = -\mu_1, \lambda_3 = -\mu_2, \lambda_4 = -\mu_3, \lambda_5 = r - \frac{s\beta_2}{\mu_3}$, which implies that the point is a saddle and is unstable.

The jacobian matrix calculated at antibody free equilibrium point $\tilde{E}$ is

$$J_{\tilde{E}} = \begin{pmatrix}
au - b(1 - u) & 0 & 0 & 0 & 0 \\
b(1 - u) & -\mu_1 & 0 & 0 & 0 \\
r_1 & r_2 & -\mu_2 & 0 & 0 \\
0 & 0 & 0 & -\beta_3\tilde{T}_u & (p - \beta_1)\tilde{T}_c \\
0 & 0 & 0 & -\beta_2\tilde{T}_u & r - \frac{s\tilde{T}_u}{\tilde{T}_c} - \beta_2\tilde{T}_c
\end{pmatrix}$$

Among the five eigen values, one of them is $\lambda_1 = au - b(1 - u) > 0$, hence the antibody free equilibrium point $E_3$ is unstable.

Variational matrix calculated at the disease free equilibrium point $\hat{E}$ is

$$J_{\hat{E}} = \begin{pmatrix}
-(au - b(1 - u)) & 0 & 0 & 0 & 0 \\
b(1 - u) & -\mu_1 & 0 & 0 & 0 \\
r_1 & r_2 & -\mu_2 & 0 & 0 \\
0 & 0 & 0 & -\mu_3(p - \beta_1) & \frac{s}{\mu_3} \\
0 & 0 & 0 & 0 & r - \frac{s\beta_2}{\mu_3} - \beta_3A
\end{pmatrix}$$
The eigen values are
\[ \lambda_1 = -(au - b(1 - u)) < 0, \lambda_2 = -\mu_1, \lambda_3 = -\mu_2, \lambda_4 = -\mu_3, \lambda_5 = r - \frac{sT_u}{\mu_3} - \beta_3A. \] So if \( r < \frac{sT_u}{\mu_3} + \beta_3A \), then the system is locally asymptotically stable about the equilibrium point \( \hat{E} \) and if \( r > \frac{sT_u}{\mu_3} + \beta_3A \), then the system becomes unstable. For \( r = \frac{sT_u}{\mu_3} + \beta_3A \), we get one zero eigen value of \( J_{\hat{E}} \). Hence no immediate conclusion about the local stability of \( \hat{E} \) can be obtained. The foregoing argument establishes the growth rate of tumor cells, \( r \) as a threshold parameter value that characterizes the local stability of the ‘disease free equilibrium \( \hat{E} \).

Jacobian matrix calculated at interior equilibrium point \( E^* \) takes the form

\[
J_{E^*} = \begin{pmatrix}
-(au - b(1 - u)) & 0 & 0 & 0 & 0 \\
b(1 - u) & -\mu_1 & 0 & 0 & 0 \\
r_1 & r_2 & -\mu_2 & 0 & 0 \\
0 & 0 & 0 & -(p - \beta_1)T_u^* - \mu_3 (p - \beta_1)T_c^* \\
0 & 0 & -\beta_3T_u^* & -\beta_2T_u^* & -\frac{rT_u^*}{k_2}
\end{pmatrix}
\]

The characteristic equation is

\[
(\lambda + au - b(1 - u))(\lambda + \mu_1)(\lambda + \mu_2)(\lambda^2 + \sigma_1\lambda + \sigma_2) = 0
\]

where

\[
\sigma_1 = \mu_3 + (\beta_1 - p)T_u^* + \frac{rT_u^*}{k_2},
\]

\[
\sigma_2 = \frac{rT_u^*}{k_2}(\mu_3 + (\beta_1 - p)T_u^*) + \beta_2(p - \beta_1)T_c^* T_u^*
\]

The first three eigen value of the system are \( \lambda_1 = -(au - b(1 - u)) < 0, \lambda_2 = -\mu_1, \lambda_3 = -\mu_2 \), which are negative and the last two eigen value is negative or negative real part if \( \sigma_1 > 0 \) and \( \sigma_2 > 0 \). So, the interior equilibrium \( E^* \) is locally asymptotically stable if \( \sigma_1 > 0 \) (which is true by existence condition of endemic equilibrium point) and \( \sigma_2 > 0 \).

4.4 Global stability of disease free equilibrium point

**Theorem 3** The cancer-free equilibrium point \( \hat{E} \) is globally asymptotically stable, when the system does not contain any other stable equilibrium point and the following condition holds

\[
r - \frac{s\beta_2}{\mu_3} - \beta_3 \frac{[\mu_1 r_1 + br_2(1 - u)][au - b(1 - u)]}{au\mu_1\mu_2} < 0
\]
we obtain \( \eta \) where we put \( \frac{b_1}{\mu_1} \beta_1 b_1 \alpha_1 \beta_2 \alpha_2 \left( 1 - u \right) \left( a u - b \left( 1 - u \right) \right) \) \( \left( a u - b \left( 1 - u \right) \right) \) \( \left( a u - b \left( 1 - u \right) \right) \) \( < 0 \) implies that the cancer-free equilibrium point \( E_2 \) is locally asymptotically stable. Now we have to study the global stability of the disease free equilibrium point when the system does not contain any other equilibrium point. For that we solve the first equation of the system and get

\[
B(t) = \frac{q_0}{r_0} \left( 1 - e^{-q_0 t} \right) + \frac{q_0}{r_0} e^{-q_0 t}.
\]

Hence, \( \lim_{t \to +\infty} B(t) = \frac{b_1}{\mu_1} \left( a u - b \left( 1 - u \right) \right) = \bar{B} \), and for all \( t \geq 0 \) we have \( B(t) < \bar{B} \), assuming that the initial condition \( B(0) < \bar{B} \). Since \( B \) is an increasing function for \( t \geq 0 \), for arbitrary small \( \epsilon_1 > 0 \), there exists \( T_{\epsilon_1} > 0 \) such that \( B(t) \geq \bar{B} - \epsilon_1 \) for all \( t \geq T_{\epsilon_1} \). Thus, one has \( \bar{B} - \epsilon_1 \leq B(t) < \bar{B}, \forall t \geq T_{\epsilon_1} \).

From the second equation of the system we get, for any \( \epsilon \),

\[
b(1 - u)(\bar{B} - \epsilon_1) - \mu_1 P \leq \frac{dP}{dt} \leq b(1 - u)\bar{B} - \mu_1 P,
\]

which can be written as

\[
b(1 - u)(\bar{B} - \epsilon_1)e^{\mu_1 t} \leq (e^{\mu_1 t} P(t))' \leq b(1 - u)\bar{B}e^{\mu_1 t}
\]

Integrating the last inequality in the interval \([T_{\epsilon_1}, t]\), one obtains

\[
\frac{\eta(\bar{B} - \epsilon_1)}{\mu_1} (e^{\mu_1 t} - e^{\mu_1 T_{\epsilon_1}}) \leq e^{\mu_1 t} P(t) - e^{\mu_1 T_{\epsilon_1}} P(T_{\epsilon_1}) \leq \frac{\eta\bar{B}}{\mu_1} (e^{\mu_1 t} - e^{\mu_1 T_{\epsilon_1}}), \tag{27}
\]

or, after division by \( e^{\mu_1 t} \)

\[
\frac{\eta(\bar{B} - \epsilon_1)}{\mu_1} (1 - e^{\mu_1 (T_{\epsilon_1} - t)}) \leq P(t) - e^{\mu_1 (T_{\epsilon_1} - t)} P(T_{\epsilon_1}) \leq \frac{\eta\bar{B}}{\mu_1} (1 - e^{\mu_1 (T_{\epsilon_1} - t)}), \tag{28}
\]

where we put \( \eta = b(1 - u) \).

If \( t \to +\infty \), according to the last inequality and since \( \epsilon_1 > 0 \) is arbitrary, we obtain

\[
\lim_{t \to +\infty} P(t) = \frac{\eta\bar{B}}{\mu_1} = \frac{b(1 - u)\bar{B}}{\mu_1} = \bar{P}.
\]

Now, using the definition of limit superior and limit inferior of a function, we have, for any \( \epsilon_2 > 0 \), \( \exists \) a large \( T_{\epsilon_2} \) such that

\[
r_1(\bar{B} - \epsilon_2) + r_2(\bar{p} - \epsilon_2) \leq A(t) \leq r_1(\bar{B} + \epsilon_2) + r_2(\bar{p} + \epsilon_2), \quad \forall t \geq T_{\epsilon_2}, \tag{29}
\]

which can be written as

\[
r_1(\bar{B} - \epsilon_2) + r_2(\bar{p} - \epsilon_2) e^{\mu_2 t} \leq (e^{\mu_2 t} A(t))' \leq (r_1(\bar{B} + \epsilon_2) + r_2(\bar{p} + \epsilon_2)) e^{\mu_2 t} \tag{30}
\]
Integrating the last inequality in the interval \([T_\varepsilon, t]\), we get
\[
\frac{r_1(\bar{B} - \varepsilon) + r_2(\bar{p} - \varepsilon)}{\mu_2} (e^{\mu_2 t} - e^{\mu_2 T_\varepsilon}) \leq e^{\mu_2 t} A(t) - e^{\mu_2 T_\varepsilon} A(T_\varepsilon)
\]
\[
\leq \frac{r_1(\bar{B} + \varepsilon) + r_2(\bar{p} + \varepsilon)}{\mu_2} (e^{\mu_2 t} - e^{\mu_2 T_\varepsilon}) \quad (31)
\]
or, after division by \(e^{\mu_2 t}\)
\[
\frac{r_1(\bar{B} - \varepsilon) + r_2(\bar{p} - \varepsilon)}{\mu_2} (1 - e^{\mu_2(T_\varepsilon - t)}) \leq A(t) - e^{\mu_2(T_\varepsilon - t)} A(T_\varepsilon)
\]
\[
\leq \frac{r_1(\bar{B} + \varepsilon) + r_2(\bar{p} + \varepsilon)}{\mu_2} (1 - e^{\mu_2(T_\varepsilon - t)}), \quad (32)
\]
If \(t \to +\infty\), according to the last inequality and since \(\varepsilon > 0\) is arbitrary, we obtain
\[
\lim_{t \to +\infty} A(t) = \frac{r_1 \bar{B} + r_2 \bar{p}}{\mu_2} = \bar{A}.
\]

Now we consider the last two equations of our system
\[
\frac{dT_c}{dt} = s + pT_c - \beta_1 T_c T_u - \mu_3 T_c
\]
\[
\frac{dT_u}{dt} = rT_u \left(1 - \frac{T_u}{k_2}\right) - \beta_2 T_c T_u - \beta_3 A T_u
\]
In order to study the global stability of the above system, we have to study the asymptotic behaviour of its solutions for \(t \to +\infty\) and so one can replace the variable \(A\) by \(\bar{A}\) in the last equation.

So, one obtains the autonomous system of two differential equations
\[
\frac{dT_c}{dt} = s + pT_c - \beta_1 T_c T_u - \mu_3 T_c
\]
\[
\frac{dT_u}{dt} = rT_u \left(1 - \frac{T_u}{k_2}\right) - \beta_2 T_c T_u - \beta_3 \bar{A} T_u
\]
which we write in the vector form
\[
\dot{Y} = F(Y(t))
\]
where, \(Y(t) = \begin{pmatrix} T_c(t) \\ T_u(t) \end{pmatrix} \)
and \(F(Y) = \begin{pmatrix} s + pT_c - \beta_1 T_c T_u - \mu_3 T_c \\ rT_u \left(1 - \frac{T_u}{k_2}\right) - \beta_2 T_c T_u - \beta_3 \bar{A} T_u \end{pmatrix} \)
Let $\alpha = \frac{1}{rT_u}$, then the simple calculation shows
\[
\forall \ (T_c, T_u) \in \mathcal{D} : \ div(\alpha F) = -\frac{s}{T_c^2 T_u} - \frac{r}{k_2 T_c} < -\frac{s}{M_1^2 M_2} - \frac{r}{k_2 M_1} = -\delta
\]

where $\mathcal{D} = \{ (T_c, T_u) \in \mathbb{R}_+^2 / 0 < T_c < \frac{s}{\mu_3 - \rho k_2} = M_1, 0 < T_u < k_2 = M_2 \}$

Hence the system (33) satisfies the Dulac-Bendixson condition, which ensures that the system (33) has no non-constant periodic solutions and this condition obviously is robust under small $C^1 –$ perturbations of $F$. Then, it follows from Michael and James (1995) that every semi trajectory either tends to an equilibrium point inside $\mathcal{D}$ or the boundary of $\mathcal{D}$. Now if we choose the domain $\mathcal{D}'$ instead of $\mathcal{D}$ as in Figure.6 $\mathcal{D}' = \mathcal{D} \cup K, K = \{ (T_c, T_u) / (T_c - T_c)^2 + T_u^2 < \rho, T_c < 0, \rho > 0 \}$, then in the interior of $\mathcal{D}'$, the only equilibrium is the disease free one, which is locally asymptotically stable. It is easy to show, that the boundary of $\mathcal{D}'$ can not be a limit of any solutions with initial conditions in $\mathcal{D}'$. Indeed, a solution can not approach asymptotically the boundary $\partial K$ of the half disk $K$ since its center is the locally stable equilibrium point. At the same time, no solution can converge to the boundary part $\partial \mathcal{D} \setminus \partial K$ since $\partial \mathcal{D}$ does not contain any equilibrium points. Thus, all solutions starting in $\mathcal{D}'$ converge as $t \to +\infty$ to the disease free equilibrium (see Figure 7).

4.5 Bifurcation Analysis

We now perform bifurcation analysis of the proposed model to obtain threshold values of the parameters where the qualitative behavior of the system changes. To verify this, we use estimated parameter values given in table 1. Though the system has four equilibrium points, from clinical point of view, the cancer free equilibrium point is more important to study because it gives us under what situation the patient will be tumor free. Therefore it is necessary to study the bifurcation analysis of the equilibrium point $\hat{E}(B, P, A, T_c, 0)$.

The cancer free equilibrium point $\hat{E}$ will be stable if $\rho < \frac{\beta}{\mu_3} + \beta A = r^*$, otherwise unstable. When $r = \frac{\beta}{\mu_3} + \beta A = r^*$, it shows one zero eigen value of the system (1 - 5), other eigen values are real and negative. Therefore, the stability of the cancer free equilibrium point $\hat{E}$ changes as $r$ crosses the threshold value $r = \frac{\beta}{\mu_3} + \beta A = r^*$ and the system experiences a transcritical bifurcation around $\hat{E}$, with the tumor growth parameter $r$ as the bifurcation parameter. To verify this analytically, we apply Sotomayor’s theorem Perko
(1991) to obtain the existence of transcritical bifurcation for the system (1−5) around the tumor free equilibrium $\hat{E}$ when $r = r^*$. The dynamical system (1−5) can be represented in vector form as

$$f(B, P, A, T_c, T_u) = \begin{pmatrix}
auB \left(1 - \frac{B}{k_1}\right) - b(1 - u)B \\
b(1 - u)B - \mu_1P \\
r_1B + r_2P - \mu_2A \\
\frac{s + pT_cT_u}{r} - \beta_1T_cT_u - \mu_3T_c \\
\frac{rT_u}{k_2} - \beta_2T_cT_u - \beta_3AT_u
\end{pmatrix}$$

The Jacobian matrix $J$ at $\hat{E}$ and its transpose have a single eigen value $\lambda = 0$ with corresponding eigen vector $v^T = \begin{bmatrix} 0 & 0 & \frac{(p - \beta_1)s}{\mu_3} & 1 \end{bmatrix}$ and $u^T = [0 \ 0 \ 0 \ 0 \ 1]$. Differentiating the vector expression with respect to the bifurcation parameter $r$ about the equilibrium point $\hat{E}$, we obtain

$$f_r(\hat{E}, r) = \begin{pmatrix} 0 & 0 & 0 & 0 & T_u \left(1 - \frac{T_u}{k_2}\right) \end{pmatrix}^T |_{\hat{E}} = [0 \ 0 \ 0 \ 0 \ 0]^T$$

Now,

(i) $u^T f_r(\hat{E}, r) = [0 \ 0 \ 0 \ 1][0 \ 0 \ 0 \ 0]^T = 0$

(ii) $u^T [df_r(B, P, A, T_c, T_u : r) v] = \begin{pmatrix} 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} 0 \\ 0 \\ 0 \\ v_5 \end{pmatrix} = v_5 = 1 \neq 0.$

where

$$Df_r(B, P, A, T_c, T_u : r) v = \frac{\partial Df_r(B, P, A, T_c, T_u : r) v}{\partial B} v_1 + \frac{\partial Df_r(B, P, A, T_c, T_u : r) v}{\partial P} v_2 + \frac{\partial Df_r(B, P, A, T_c, T_u : r) v}{\partial A} v_3 + \frac{\partial Df_r(B, P, A, T_c, T_u : r) v}{\partial T_c} v_4 + \frac{\partial Df_r(B, P, A, T_c, T_u : r) v}{\partial T_u} v_5.$$
at the steady state $\hat{E}(\mathcal{B}, \mathcal{P}, \mathcal{A}, T_c, 0)$

$$(iii) \ u^T [D^2 f(\hat{E}; r)](v, v) = (0 \ 0 \ 0 \ 1) \begin{pmatrix} 0 \\ 0 \\ 0 \\ (p - \beta_1)v_4v_5 \\ -\frac{\beta_2}{k_2}v_5^2 - 2\beta_2v_4v_5 \end{pmatrix}$$

$$= -2 \left[ \frac{r}{k_2} + \frac{(p - \beta_1)s\beta_2}{\mu_3^2} \right]$$

$$= -2 \left[ \frac{\beta_2s + \beta_3\mu_3A}{k_2} + \frac{(p - \beta_1)s\beta_3}{\mu_3^2} \right] \neq 0$$

where

$$D^2 f(\hat{E}, r)(v, v) = \frac{\partial^2 f(\hat{E}, r)}{\partial B^2}v_1v_1 + \frac{\partial^2 f(\hat{E}, r)}{\partial B\partial P}v_1v_2 + \frac{\partial^2 f(\hat{E}, r)}{\partial P\partial B}v_2v_1$$

$$+ \frac{\partial^2 f(\hat{E}, r)}{\partial P^2}v_2v_3 + \frac{\partial^2 f(\hat{E}, r)}{\partial B\partial A}v_1v_3 + \frac{\partial^2 f(\hat{E}, r)}{\partial A\partial B}v_3v_1$$

$$+ \frac{\partial^2 f(\hat{E}, r)}{\partial A^2}v_3v_3 + \frac{\partial^2 f(\hat{E}, r)}{\partial B\partial T_c}v_1v_4 + \frac{\partial^2 f(\hat{E}, r)}{\partial T_c\partial B}v_4v_1$$

$$+ \frac{\partial^2 f(\hat{E}, r)}{\partial T_c^2}v_4v_4 + \frac{\partial^2 f(\hat{E}, r)}{\partial B\partial T_u}v_1v_5 + \frac{\partial^2 f(\hat{E}, r)}{\partial T_u\partial B}v_5v_1$$

$$+ \frac{\partial^2 f(\hat{E}, r)}{\partial T_u^2}v_5v_5 + \frac{\partial^2 f(\hat{E}, r)}{\partial P\partial T_c}v_2v_3 + \frac{\partial^2 f(\hat{E}, r)}{\partial A\partial P}v_3v_2$$

$$+ \frac{\partial^2 f(\hat{E}, r)}{\partial P\partial T_u}v_2v_4 + \frac{\partial^2 f(\hat{E}, r)}{\partial T_c\partial P}v_4v_2 + \frac{\partial^2 f(\hat{E}, r)}{\partial P\partial T_u}v_2v_5$$

$$+ \frac{\partial^2 f(\hat{E}, r)}{\partial T_u\partial P}v_5v_2 + \frac{\partial^2 f(\hat{E}, r)}{\partial A\partial T_c}v_3v_4 + \frac{\partial^2 f(\hat{E}, r)}{\partial T_c\partial A}v_4v_3$$

$$+ \frac{\partial^2 f(\hat{E}, r)}{\partial A\partial T_u}v_3v_5 + \frac{\partial^2 f(\hat{E}, r)}{\partial T_u\partial A}v_5v_3 + \frac{\partial^2 f(\hat{E}, r)}{\partial T_c\partial T_u}v_4v_5$$

$$+ \frac{\partial^2 f(\hat{E}, r)}{\partial T_u\partial T_c}v_5v_4$$

Thus all three properties (see appendix) for the existence of transcritical bifurcation hold and we conclude by Sotomayar’s theorem, that the system undergoes transcritical bifurcation as the tumor intrinsic growth rate $r$, crosses the threshold value $r^*$.  

5 Numerical Results

We perform the numerical simulation of system (1-5) using the parameter values for Table 1. We like to see how the dynamics of the system changes with
the change of some parameter value, keeping the other parameters fixed.

**Equilibrium and Stability:**

- The boundary equilibrium point \( E(0, 0, 0, \frac{s}{\mu_3}, 0) = E(0, 0, 0, 80996.9, 0) \) exists for the parameter values in Table 1. The eigen value of the variational matrix implies that it is a saddle point and hence unstable.
- The antibody free equilibrium point is \( \tilde{E}(0, 0, 0, 4.9936 \times 10^9, 4.2799 \times 10^7) \) for the parameter values in Table 1, it is unstable.
- Since \( au - b(1 - u) = .011 > 0 \), the cancer free equilibrium point exists and is given by \( \hat{E} = (6.875 \times 10^6, 3.0938 \times 10^6, 2.7669 \times 10^6, 80996.9, 0) \). It is locally asymptotically stable if \( r < \frac{s\beta_1 k_1 (1 - u)}{\alpha a \mu_1 \mu_2} = 0.0457 (= r^*, \text{ say}) \). The locally asymptotically stable cancer free equilibrium point is also globally stable, which has been shown numerically in figure 7.
- For the set of parameter value in Table 1, the interior equilibrium point is \( E^*(6.875 \times 10^6, 3.0938 \times 10^6, 2.7669 \times 10^6, 4.7549 \times 10^9, 4.2799 \times 10^7) \) and the eigen values of the variational matrix are \((-0.011, -0.02, -1.3666, -0.0158 + 0.3821 i, -0.0158 - 0.3821 i)\). Hence, it is locally asymptotically stable.

We now use the model to predict responses to the treatment at the population model. The model is simulated for response to treatment for patients with a range of immune strength. For a given set of immune systems parameters the effectiveness of immune system against cancer depends on four control parameters namely, \( p \) (rate of recruitment of cytotoxic T-lymphocytes), \( \beta_1 \) (death rate of cytotoxic T-lymphocytes due to interaction with tumor cells), \( \beta_2 \) (kill rate of cancer cells by cytotoxic T-lymphocytes) and \( \beta_3 \) (rate of cancer cell death by antibodies). These values are varied in a biologically reasonable range to predict the changes in the dynamics of the system and to ascertain how a cancer free state can be reached.

**Varying the rate of recruitment of cytotoxic T-lymphocytes (p):**

The long term behavior of all the state variables, that is B-Cells, plasma Cells, antibodies, cytotoxic T-lymphocytes and cancer cells are shown in Figure 8 for \( p = 6.8 \times 10^{-8} \), with other parameter values remaining same (Table 1). B cells grow and reaches its steady state (fig 8A) but the plasma cells show a sharp decline and then increase to reach the steady state (fig 8B). This may be due to the fact that the initial plasma cells present in the body is used to secrete antibodies, till replaced by the new plasma cells, obtained from B-cell differentiation. The antibodies also reach the steady state after some transient dynamics (fig 8C). With this recruitment value of cytotoxic T-lymphocytes, the cancer free equilibrium is unstable, hence the CTL’s as well as antibodies fail to control the cancer cells, which attain large cancer cell steady state (fig 8E). As \( p \) is increased, both CTLs and cancer cells show
oscillations(Figure9(A,B,C,D,E,F)), which increase with the increment of $p$ and ultimately becomes a table limit cycle (see Figure9(G,H)). Antibodies show similar dynamics as seen in (fig 8C).

**Varying $\beta_1$, the death rate of CTL’s by cancer cells:**

Figure 10 shows the dynamic of B-Cells, plasma cells, antibodies, CTL’s and cancer cells for $\beta_1 = 9.5 \times 10^{-9}$, other parameter values as in Table 1. With this set of parameter values, the cancer free state is unstable. There is no change of dynamics for B cells, plasma cells and antibody but the dynamics of the CTL’s and cancer cells are oscillatory as the large cancer cells steady state (interior equilibrium point) is a stable focus. As we increase the value of the parameter $\beta_1$, the changes in the dynamics of antibodies, CTL, and cancer cells is shown in Figure 11. Figure 11(A, D, G) shows that there is no change in the dynamics of antibodies due to change in the parameter $\beta_1$. This is because of the fact that there is no interaction between antibodies and CTLs. But as we increase the value of $\beta_1$, the oscillation in the cancer cells vanishes and reaches a new internal equilibrium(large cancer cells steady state). This change of dynamics is due to the fact that the interior equilibrium changes from stable focus(fig.11(C)) to a stable node(Fig.11(I)) as the death rate of CTLs by the cancer cells increases 11(I).

**Varying $\beta_2$, the kill rate of cancer cells by CTL’s:**

By local stability analysis we observed that if $\beta_2 > \frac{\mu_1}{r\mu_3}(r - \beta_3 k_1[a_u - b(1 - u)])(= 1.16 \times 10^{-5} Ab^{-1}hr^{-1})$, then the cancer free equilibrium is stable otherwise unstable. Therefore, for any value of $\beta_2 < 1.16 \times 10^{-5} Ab^{-1}hr^{-1}$, the CTL’s and cancer cells reach the interior steady states through damped oscillation (see Figure 12), as it is a stable focus. As the value of $\beta_2$ crosses 1.16 × 10⁻⁵ and increases, the cancer cells are eradicated very fast(see Figure 13)(I) since the cancer free equilibrium is stable now and the large cancer cells steady state does not exist. The cell count of CTL’s also decreases at a faster rate with the increment of $\beta_2$ (see Figure 13)(H).

**Varying $\beta_3$, the kill rate of cancer cells by antibodies:**

Figure 14 shows the dynamics of antibodies, CTL’s and cancer cells for varying $\beta_3$. If $\beta_3 > \frac{\mu_1}{r}[\mu_1 r_1 + b r_2(1 - u)][a_u - b(1 - u)](= 3.57 \times 10^{-10} Ab^{-1}hr^{-1})$, cancer free equilibrium point is stable(see 14(I)) otherwise unstable. For $\beta_3 < 3.57 \times 10^{-10}$, the interior equilibrium is a stable focus (see 14(B,C,E,F)) , implying high cancer cells interior point. However for $\beta_3 > 3.57 \times 10^{-10}$, cancer free state is obtained, no matter what the initial size is. Thus, in order to realistically effect a cure when the cancer free equilibrium point is unstable, a therapy or
treatment must ensure that not only the cancer burden must be reduced but
the therapy itself is capable of changing the parameter values of the system.
In this context, monoclonal antibody therapy of cancer is suggested as treat-
ment, which may be capable of changing the system parameters. Monoclonal
antibodies can be used to target a number of cancer associated targets, includ-
ing tumor associated blood vessels, vascular growth factors, diffuse malignant
cells like leukemia, cancerous cells within a solid tumor and tumor associated
stroma like fibroblasts.

6 Conclusion

We have formulated a mathematical model representing interaction between
cancerous cells and immune system considering both humoral and cell-mediated
immune system. Using sensitivity analysis we have identified the parameters
that are sensitive and by subset selection, we have identified the one that
are estimable from given experimental data Tutt et al. (2002)(figure 4A, pg
No.2723). We then estimate those parameters using least square method.
Though there are four equilibria, we concentrate only on two equilibrium
points, namely, cancer free equilibrium and high cancer equilibrium. Cancer
can be driven to either of these states in simulations, depending on the relative
strength of the patient’s immune systems, considering the role of antibodies
and CTL’s. By simulation, it is revealed that Cytotoxic T lymphocytes and
antibodies play an important role in bringing down the cell count of cancer
cells. This suggest that proper activation of the system parameters in the con-
tion obtained, namely \( \beta_2 \) and \( \beta_3 \)(see Theorem 3), the effectiveness of the
cytotoxic T lymphocyte and antibodies to kill the cancerous cells respectively.
We observe that for certain values of \( \beta_2 \) and \( \beta_3 \), one can control the unlimited
growth of the cancerous cell population. As the values of \( \beta_2 \) and \( \beta_3 \) crosse
certain threshold value, the cancerous cells population always decays to zero,
no matter what the initial size is.

The parameter sensitivity analysis yield results that are intuitively rea-
sonable, which also highlight which parameter(s) to target so that the cancer
burden is reduced. For example, if we get a better idea of how to influence
the parameters \( \beta_2 \) and \( \beta_3 \) biologically (parameters, which effect the kill rates
of cancer by CTL’s and antibody respectively), a large increase in both of
them will result in our immune system more efficient to eradicate cancer cells
than the immune system resulting from a change in the other immune system
parameters.

There are several notable clinical observations that are important for the
next stage of model development. Though the antibodies have the ability to kill
cancerous cells through direct, the process is not instantaneous and followed
by some time lag. This can be captured by introducing interaction delay into
the model, which may mimic some notable clinical observations missing in this
model.
Acknowledgements This study was supported by the Ministry of Human Resource Development (MHRD) (Grant No.).

Appendix: Sotomayor Theorem (Perko (1991))

Consider the system of ordinary differential equations

$$\frac{dx}{dt} = f(x, \eta), \; x \in \mathbb{R}^n,$$

where $\eta \in \mathbb{R}$ is a system parameter. It is assumed that the function $f$ is sufficiently differentiable so that all the derivatives appearing in that theorem are continuous on $\mathbb{R}^n \times \mathbb{R}$. We denote the matrix of partial derivatives of the components of the vector field $f$ with respect to the components of $x$ by $Df$ and the vector of partial derivatives of the components of $f$ with respect to parameter $\eta$ denoted by $f_\eta$.

**Theorem 4** (Perko (1991))(Sotomayor): Suppose that $f(x_0, \eta_0)$ and that the $n \times n$ matrix $B \equiv Df(x_0, \eta_0)$ has a simple eigenvalue $\lambda = 0$ with eigen vector $v$ and that $B^T$ has an eigen vector $u$ corresponding to the eigen value $\lambda = 0$. Furthermore, suppose that $B$ has $k$ eigenvalues with negative real parts and $(n - k - 1)$ eigenvalues with positive real parts.

(i) If the following conditions are satisfied

$$u^T f_\eta(x_0, \eta_0) \neq 0, \quad u^T[D^2 f(x_0, \eta_0)(v, v)] \neq 0 \quad (34)$$

Then there is a smooth curve of equilibrium point of (34) in $\mathbb{R}^n \times \mathbb{R}$ passing through $(x_0, \eta_0)$ and tangent to the hyperplane $\mathbb{R}^n \times \eta_0$. Depending on the signs of the expressions in (35), there are no equilibrium points of (34) near $x_0$ when $\eta < \eta_0$ (or $\eta > \eta_0$) and there are two equilibrium points of (34) near $x_0$ when $\eta > \eta_0$ (or $\eta < \eta_0$). The two equilibrium points of (34) near $x_0$ are hyperbolic and have stable manifolds of dimension $k$ and $k + 1$ respectively, that is, the system (34) experiences a saddle-node bifurcation at the equilibrium point $x_0$ as the parameter $\eta$ passes through the bifurcation value $\eta = \eta_0$. The set of $C^\infty$-vector fields satisfying the above conditions is an open, dense subset in the Banach space of all $C^\infty$, one parameter vector fields with an equilibrium point at $x_0$ having a simple zero eigenvalue.

(ii) If the following conditions are satisfied

$$u^T f_\eta(x_0, \eta_0) = 0, \quad u^T[D f(x_0, \eta_0)v] \neq 0 \quad u^T[D^2 f(x_0, \eta_0)(v, v)] \neq 0 \quad (35)$$

the system (34) experiences a transcritical bifurcation at the equilibrium point $(x_0, \eta_0)$ as the parameter $\eta$ varies through the bifurcation value $\eta = \eta_0$. 

References


cells, The Journal of Clinical Investigation, 119(8), 2143-2159.


### Table 1 Parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter values and Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a ) (growth rate of large B-cells)</td>
<td>( 0.1 \ hr^{-1} ) (Perelson et al. (1976))</td>
</tr>
<tr>
<td>( b ) (conversion rate of large B-cells into plasma cells)</td>
<td>( 0.01 \ hr^{-1} ) (Perelson et al. (1976))</td>
</tr>
<tr>
<td>( \mu_1 ) (natural death rate of plasma cells)</td>
<td>( 0.01 \ hr^{-1} ) (Perelson et al. (1976))</td>
</tr>
<tr>
<td>( k_1 ) (carrying capacity of large B-cells)</td>
<td>( 10^9 \text{cells} ) (Perelson et al. (1976))</td>
</tr>
<tr>
<td>( u ) (the fraction of daughter cells which remain as large B-cells)</td>
<td>( 0.1 ) (Perelson et al. (1976))</td>
</tr>
<tr>
<td>( r_1 ) (rate at which large B-cells secrete antibodies)</td>
<td>( 100 \text{Ab cell}^{-1} \text{sec}^{-1} ) (Nossal and Makela (1962))</td>
</tr>
<tr>
<td>( r_2 ) (rate at which plasma cells secrete antibodies)</td>
<td>( 1000 \text{Ab cell}^{-1} \text{sec}^{-1} ) (Nossal and Makela (1962))</td>
</tr>
<tr>
<td>( \mu_2 ) (natural death rate of antibodies)</td>
<td>( 1.3666 \text{sec}^{-1} ) (estimated)</td>
</tr>
<tr>
<td>( s ) (natural source term of Cytotoxic T-lymphocyte)</td>
<td>( 1.3 \times 10^4 \text{cells/day} ) (Kuznetsov et al. (1994))</td>
</tr>
<tr>
<td>( p ) (increasing rate of Cytotoxic T-lymphocyte due to interaction with cancerous cells)</td>
<td>( 7.2641 \times 10^{-8} \text{Ab}^{-1} \text{sec}^{-1} ) (estimated)</td>
</tr>
<tr>
<td>( \beta_1 ) (death rate of Cytotoxic T-lymphocyte due to interaction with cancerous cells)</td>
<td>( 6.8891 \times 10^{-8} \text{Ab}^{-1} \text{sec}^{-1} ) (estimated)</td>
</tr>
<tr>
<td>( \mu_3 ) (natural death rate of Cytotoxic T-lymphocyte)</td>
<td>( 0.1665 \text{sec}^{-1} ) (estimated)</td>
</tr>
<tr>
<td>( r ) (the intrinsic growth rate of cancerous cells)</td>
<td>( 0.9889 \text{day}^{-1} ) (estimated)</td>
</tr>
<tr>
<td>( k_2 ) (carrying capacity of cancerous cells)</td>
<td>( 1.336 \times 10^9 \text{cells} ) (estimated)</td>
</tr>
<tr>
<td>( \beta_2 ) (the death rate of cancerous cells due to interaction with Cytotoxic T-lymphocyte)</td>
<td>( 1.9169 \times 10^{-10} \text{Ab}^{-1} \text{sec}^{-1} ) (estimated)</td>
</tr>
<tr>
<td>( \beta_3 ) (death rate of cancerous cells due to interaction with antibodies)</td>
<td>( 1.6530 \times 10^{-11} \text{Ab}^{-1} \text{sec}^{-1} ) (estimated)</td>
</tr>
</tbody>
</table>
Fig. 1 The schematic diagram illustrate the interaction between B cells, plasma cells, antibody, Cytotoxic T lymphocyte (CTL) and cancer cells.
Fig. 2 The figure shows the sensitivity graph for different parameter of the system.

Fig. 3 The figure indicate the $L_2$ norm of different parameter. $L_2$ norm of the parameter $p, \beta_1, r, \beta_3, r_2, \mu_2, \mu_3, \beta_2$ is large compare to the other parameter of the system. So $\mu_3, r, p, \beta_1, k_2, \beta_2, a, \beta_3, \mu_2$ are more sensitive.
Fig. 4 The figure shows that best fit line for estimation of the parameter \( r k_2 \) using real data Tutt et al. (2002) in absence of immune system. Applying least square method the estimated value of \( r \) and \( k_2 \) are \( r = 0.9889 \text{ day}^{-1} \) and \( k_2 = 1.336 \times 10^9 \text{ cells} \).

Fig. 5 The figure shows the curve of best fit for estimation of the parameter \( \mu_2, p, \beta_1, \mu_3, \beta_2, \beta_3 \) using real data Tutt et al. (2002). Applying least square method, the estimated value of the parameters are \( \mu_2 = 1.3666 \text{ sec}^{-1}, \ p = 7.2641 \times 10^{-8} \text{ Ab}^{-1} \text{ hr}^{-1}, \ \beta_1 = 6.8891 \times 10^{-8} \text{ Ab}^{-1} \text{ hr}^{-1}, \ \mu_3 = 0.1605 \text{ sec}^{-1}, \ \beta_2 = 1.9169 \times 10^{-10} \text{ Ab}^{-1} \text{ hr}^{-1}, \ \beta_3 = 1.6530 \times 10^{-11} \text{ Ab}^{-1} \text{ hr}^{-1} \).
Fig. 6 The domain $D'$ where the cancer free equilibrium point is globally asymptotically stable.

Fig. 7 The figures show the global stability of the cancer free equilibrium point. The cancer free equilibrium is globally stable for $r < 0.0457$. The values of $r = 0.0099$. 
Fig. 8 The figure shows the dynamics of all the state variables, namely B cells, Plasma cells, Antibodies, CTL and Cancer cells for $p = 6.8 \times 10^{-8}$ $Ab^{-1} hr^{-1}$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_c(0) = 9 \times 10^4$, $T_u(0) = 1 \times 10^9$, other parameter values are same as given Table 1.
Fig. 9 The figures (A, B, C) show the dynamics of Antibody, CTL and Cancer cells for different values of \( p \) keeping other parameter value fixed as in Table 1. (i) figure A is for \( p = 7 \times 10^{-8} \) \( Ab^{-1}hr^{-1} \) with initial condition \( B(0) = 6 \times 10^6, P(0) = 3 \times 10^9, A(0) = 2 \times 10^9, T_c(0) = 4 \times 10^9, T_u(0) = 1 \times 10^7 \) (ii) figure B is for \( p = 8.1 \times 10^{-8} \) \( Ab^{-1}hr^{-1} \) with initial condition \( B(0) = 6 \times 10^6, P(0) = 3 \times 10^9, A(0) = 2 \times 10^9, T_c(0) = 4 \times 10^9, T_u(0) = 1 \times 10^7 \) (iii) figure C is for different \( p = 4.5 \times 10^{-6} \) \( Ab^{-1}hr^{-1} \) with initial condition \( B(0) = 6 \times 10^6, P(0) = 3 \times 10^6, A(0) = 2 \times 10^9, T_c(0) = 4 \times 10^9, T_u(0) = 3 \times 10^4 \). Figure (D, E, F) shows the formulation of limit cycle as we increase the value of \( p \). The values of \( p \) are in figure (D) \( p = 7.2 \times 10^{-8} \), figure (E) \( p = 8.1 \times 10^{-8} \) and figure (F) \( p = 2 \times 10^{-7} \).
Fig. 10 The figure shows the dynamics of all the state variables, namely B cells, Plasma cells, Antibodies, CTL and Cancer cells for $\beta_1 = 9.5 \times 10^{-5}$ Ab$^{-1}$hr$^{-1}$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_c(0) = 4 \times 10^3$, $T_u(0) = 2 \times 10^3$, other parameter values as in Table 1.

Fig. 11 The figures show the dynamics of Antibody, CTL and Cancer cells for different values of $\beta_2$ keeping other parameter value fixed as on Table 1.(i) figure A is for $\beta_1 = 6.5 \times 10^{-8}$ Ab$^{-1}$hr$^{-1}$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_c(0) = 4 \times 10^3$, $T_u(0) = 2 \times 10^3$. (ii) figure B is for $\beta_1 = 7.2 \times 10^{-8}$ Ab$^{-1}$hr$^{-1}$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_c(0) = 3 \times 10^9$, $T_u(0) = 2 \times 10^3$. (iii) figure B is for $\beta_1 = 7.26 \times 10^{-8}$ Ab$^{-1}$hr$^{-1}$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_c(0) = 1 \times 10^3$, $T_u(0) = 1 \times 10^3$. 
Fig. 12 The figure shows the dynamics of all the state variables, namely B cells, Plasma cells, Antibodies, CTL and Cancer cells for $\beta_2 = 2.5 \times 10^{-10} \text{Ab}^{-1} \text{hr}^{-1}$ with initial condition $B(0) = 6 \times 10^9$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_r(0) = 3 \times 10^6$, $T_u(0) = 4 \times 10^7$, other parameter values as in Table 1.

Fig. 13 The figures show the dynamics of Antibody, CTL and Cancer cells for different values of $\beta_2$ keeping other parameter value fixed as on Table 1. (i) figure A is for $\beta_2 = 7.5 \times 10^{-6} \text{ Ab}^{-1} \text{ hr}^{-1}$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_r(0) = 1 \times 10^5$, $T_u(0) = 1 \times 10^7$ (ii) figure B is for $\beta_2 = 1.13 \times 10^{-5} \text{ Ab}^{-1} \text{ hr}^{-1}$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_r(0) = 8 \times 10^4$, $T_u(0) = 1 \times 10^6$. (iii) figure C is for increasing $\beta_2$ where $(\beta_2 > 1.16 \times 10^{-5} \text{ Ab}^{-1} \text{ hr}^{-1})$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_r(0) = 8 \times 10^4$, $T_u(0) = 1 \times 10^6$. 
Fig. 14 The figures show the dynamics of Antibody, CTL and Cancer cells for different values of $\beta_3$ keeping other parameter value fixed as on Table 1. (i) figure A is for $\beta_3 = 1.5 \times 10^{-10} \text{Ab}^{-1} \text{hr}^{-1}$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_c(0) = 2 \times 10^9$, $T_u(0) = 4 \times 10^7$. (ii) figure B is for $\beta_3 = 3.4 \times 10^{-10} \text{Ab}^{-1} \text{hr}^{-1}$ with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_c(0) = 7 \times 10^7$, $T_u(0) = 4 \times 10^7$. (iii) figure C is for different $\beta_3$ where ($\beta_3 > 3.574 \times 10^{-10} \text{Ab}^{-1} \text{hr}^{-1}$) with initial condition $B(0) = 6 \times 10^6$, $P(0) = 3 \times 10^6$, $A(0) = 2 \times 10^9$, $T_c(0) = 8 \times 10^4$, $T_u(0) = 1 \times 10^6$. 

$\beta_3 = 5.6 \times 10^{-10}$
$\beta_3 = 9.5 \times 10^{-10}$
$\beta_3 = 1.7 \times 10^{-9}$
$\beta_3 = 6.9 \times 10^{-10}$