ABSTRACT

Mathematical models of tumor-immune interactions provide an analytical framework by which to address specific questions about tumor-immune dynamics. We present an extension of a mathematical model developed by De Pillis et al. that accounts for the immune surveillance of tumors by natural killer (NK) cells and cytotoxic CD8+ T lymphocytes (CTL’s). NK cells mediate an innate immune response against cancer because NK cells can respond to tumor cells without the need for prior antigen-specific stimulation, while CTL’s mediate an adaptive immune response because they require antigen-specific stimulation before responding effectively. Our extended model incorporates state transitions between naive and primed CTL’s and cytokine signaling between NK cells and CTL’s. In addition, the model describes tumor-immune interactions focusing on the roles of the innate and adaptive immune responses in tumor immune surveillance. The mathematical model describes tumor-immune interactions by utilizing a system of non-linear differential equations with respect to tumor cells, NK cells, CTL’s, and antigen presenting cells. The functions describing tumor-immune growth, antigen presentation, immune response, and interaction rates are numerically simulated with a differential equation solver (GNU Octave). Parameter estimates and model validations are obtained from prior mathematical papers and medical studies related to this topic. We expect that both the innate and adaptive immune systems play independent and synergistic roles to defend the body from cancer.
I dedicate this thesis to my parents and my mentor for their support in my work. I thank Sigma Xi, the Scientific Research Society, for their support through their Grant-In-Aid of research program.
INTRODUCTION

Immunology is a broad field that seeks to understand the physiological mechanisms necessary to defend an organism against pathological invaders during states of both health and disease (Abbas et al. 2010). Before the advent of immunology, early physicians characterized various human organs that would later be deemed necessary for immunological defense; for example, the human immune system relies upon the cardiovascular system (heart, arteries, and veins), the thymus, the skin, as well as lymph vessels. Immunology can trace its origins back to ancient times where many civilizations utilized various techniques to prevent disease; for example, the Chinese (1300 BC) became resistant to smallpox by inhaling powders generated from skin lesions of the infected (Abbas et al. 2010). As immunology developed into an experimental science, it has discovered the nature of pathogenic invaders (viruses, bacteria, etc); pathogens have evolved mechanisms to subvert some aspect of a host immune system or acquired resistance to a variety of antibiotics or medicines, for example, penicillin.

Due to the complexity of foreign invaders, the human innate immune system has to present a variety of defenses to prevent and combat infection. The first defense that prevents pathogenic entry is an epithelial barrier, which is a physical barrier that prevents pathogenic movement from the external environment to host tissue (Linton 2008). Depending upon the site of entry, pathogens will encounter extra defenses in addition to the physical barrier; for example, if a pathogen tries to invade through the vaginal tract, it will encounter an acidic pH of 4.5 due to the high proton concentration contained in vaginal fluid (Abbas et al. 2010). Other barriers may have other defenses, for example secretion of lysozymes or defensins (cysteine-rich peptides), that disrupt the cell membranes of fungi and bacteria and kill them.
Pathogenic invaders will overcome the defenses epithelial barriers present and will gain entry; however, the next defense that a pathogen will encounter is circulating effector cells, such as macrophages, natural killer cells, or neutrophils. Before a circulating effector cell can digest or lyse a foreign invader, recognition must first be established in order to discriminate self from non-self (Abbas et al 2010). Circulating effector cells contain a variety of surface receptors that recognize pathogen associated molecular patterns, which are cell surface or soluble molecules that can initiate a signal transduction cascade (Abbas et al, 2010). Examples of surface receptors can include (Abbas et al. 2010):

- Toll-like receptors - these receptors recognize a variety of pathogenic molecules.
- C-type lectins - these receptors recognize bacterial surface carbohydrates with a terminal sugar
- N-formyl-Met-Leu-Phe - these receptors recognize surface peptides with N-formylmethionyl residues

Signaling cascades can result in activation of host defense, cell recruitment, and inflammation (Abbas et al. 2010).

Once recognition has been established between the pathogen and effector cell, various courses of action may be taken to eliminate the pathogen. Leukocytes and macrophages can phagocytize and kill the pathogen by fusing the newly created phagosome with a lysosome containing reactive oxygen species (ROS) (Abbas et al 2010). Other actions that macrophages can perform include the secretion of cytokines (small peptides) that stimulate inflammation at the infection site or cell recruitment to overwhelm the pathogen. Natural killer (NK) cells can recruit macrophages to the
infection site through the secretion of cytokines or kill viral infected cells through a signal transduction process that results in apoptosis (Abbas et al. 2010).

Another defense that pathogens can encounter is the complement system, which is a system of circulating effector proteins that is activated in the presence of pathogens. Complement contains a series of inactivated proteins that circulate in plasma; however, in the presence of a pathogen, it results in the rapid, sequential activation of complement proteins that can assist effector cells by recruiting macrophages, promote inflammation, as well as promote lysis of microbes through the formation of the membrane attack complex, a protein assembly that forms holes in the membrane of a pathogen, inducing free diffusion of molecules outward into the plasma (Abbas et al. 2010). If enough membrane attack complexes assemble on the surface of a pathogen, then the resulting water imbalance will kill the pathogen. Three pathways can activate the complement system as follows (figure 1):

- Alternative – complement protein is cleaved and activated on the surface of the pathogen
- Classical – antibody binding, via the adaptive immune system, activates complement cleavage
- Lectin – sugar proteins bind on surface residues on the pathogen’s surface to activate complement cleavage
Figure #1: Overview of the complement system (Adapted from Abbas et al. 2010). Activation of the complement system by pathogens results in a variety of effector function mediated by cleaved complement proteins. C3 convertase cleaves C3 into two fragments, which act to promote inflammation, to deter pathogenic movement, and opsonization, a process in which pathogens are targeted for phagocytosis. During the latter stages of the complement response, another complement protein C5 is cleaved by C5 convertase and the fragments result in inflammation and the formation of the membrane attack complex, which promotes pathogenic death via osmosis.

Each pathway activates C3 Convertase, which is a protein dimer composed of two complement proteins that acts to promote successive cleavage of complement proteins. All pathways lead to the same result of inflammation, cell recruitment, as well as the formation of the membrane attack complex.

If the innate system cannot deter pathogenic invasion in a host, then the adaptive immune system must be primed via antigen presentation in order to take over immune functions. Unlike the innate immune system, the adaptive immune system deters pathogenic attack through activation of lymphocytes of B and T cell lineages (Abbas et al. 2010). Activation of B and T cells requires a cell (dendritic, macrophage, B cell, etc.) that has the capacity to convert an antigenic substance (protein, sugar, or cell residue) into a polypeptide signaling molecule and present via a major histocompatibility complex molecule (MHC). MHC molecules can be classified as the following:
- Class I - Receptor generated from degraded viral proteins in the cytosol, via proteosomes, and activates CD8$^+$ Cytotoxic T lymphocytes (CTL)
- Class II - Receptor generated in response to extra cellular bacteria and activates CD4$^+$ Helper T lymphocytes (HTL)
- Class III - Encodes for other immune components such as complement components, heat shock proteins, and cytokines to promote cell recruitment as well as inflammation

All nucleated cells in the immune system encode MHC class I receptors, while B-cells, dendritic cells, and macrophages encode MHC class II receptors.

CD8$^+$ Cytotoxic T lymphocytes perform the dirty work of cell-mediated immunity by activating apoptosis in an infected, damaged, or dysfunctional cell. Once MHC class I molecules from an infected cell interacts with a naïve CTL, the CTL becomes primed and releases perforin and granulysin proteins, which create a osmotic gradient capable of lysis, as well as serine proteases to induce apoptosis. To prevent extensive damage during an infection, CTL activation is heavily regulated and requires a strong MHC/antigen affinity as well as HTL surface proteins (Abbas et al. 2010). Upon remission of a pathogenic attack, most of a CTL population is destroyed except for a few remnants that are retained as memory cells, which can quickly regenerate and eliminate a pathogenic attack.

Unlike CTLs, HTLs mediate immunity by maximizing the adaptive immune response of CTL activation and cell recruitment via cytokine secretion. Once a MHC class II molecule from an infected cell interacts with a naïve HTL, the HTL becomes primed and differentiates into one of three forms:
- **Effector** – These cells secrete cytokines to stimulate circulating effector CTL, HTL, B cells, and macrophages.

- **Memory** – These cells retain the affinity of the MHC/antigen of the original cell and can be reactivated during a secondary immune response.

- **Regulatory** – These cells regulate the cell-mediated response by suppressing CD8 and CD4 functionality.

Depending upon the partner cell that attaches to the naïve HTL, the primed effector cell will release various chemokines and cytokines. For example, a macrophage will induce interleukin-2 and interleukin-10 (IL) secretion that stimulates the recruitment of circulating CTLs and macrophages, while a B cell will induce IL-4, IL-5, IL-6, IL-10, and IL-13 secretion to promote antibody class switching as well as increasing antibody production (Abbas et al. 2010). Unlike effector HTLs, memory HTLs remain in dormancy after being primed in order to retain the affinity of MHC/antigen binding; however, during a secondary immune response, memory HTLs can differentiate into other subpopulations to respond to a pathogenic attack much faster than the first response.

Regulatory HTLs, unlike the other two classes, can be beneficiary and detrimental to the immune process depending upon the ailment encountered. Regulatory HTLs act in bystander suppression by hindering MHC/antigen attachment as well as suppressing IL-10 and tumor necrosis factor secretion to stop cell recruitment (Abbas et al. 2010). Inhibition of certain cell-mediated functions is necessary to maintain homeostasis in an individual; however, inhibition can create a “slippery-slope” since too much inhibition leads to chronic disease, while too little inhibition leads to autoimmune disorders.

The cancer immunosurveillance hypothesis has had a long history of development
since the early 20th century. In 1909, Paul Ehrlich, a German scientist, proposed the idea that the immune system could suppress an overwhelming number of carcinomas (Dunn et al. 2004). This idea was not well tested until the early 1950’s when the field of immunology had matured. Early research in mouse-bred lines discovered that Ehrlich’s hypothesis did not refer to tumor immunology and instead proved the theory of allograft rejection; however, research in the latter 20th century supported Ehrlich’s hypothesis as many laboratories demonstrated that mice could become immunized against cancer through allograft transplantation of tumors with chemical carcinogens. This discovery provided the cornerstone of the cancer immunosurveillance hypothesis since tumor antigens present in an individual’s immune system are required to elicit an immune response; if no distinctive structures associated with tumor cells exist, then no recognition would be established (Dunn et al. 2004). F. Macfarlane Burnet and Lewis Thomas, well-known immunologists during the late 20th century, speculated that for cancer immunosurveillance to work, lymphocytes would need to act aggressively in order to recognize and eliminate a cancer threat. Cancer immunosurveillance theory revolves around the three “E’s” (Figure 2), which are (Dunn et al. 2004):

- **Elimination** – The establishment of a strong cancer surveillance network by both the innate and adaptive immune system which seeks to eliminate cancer populations
- **Equilibrium** – The long term process of combat between a cancer population and the cancer surveillance network
- **Escape** – The overpowering of the cancer surveillance network by strong tumor variants, which results in host death
Figure #2: Overview of the cancer immunosurveillance hypothesis (Adapted from Dunn et al. 2004). Cancer immunosurveillance arises from deleterious mutations in the regulatory system of the cell cycle mainly caused due to many factors such as carcinogens or radiation. Transformed cells have their cell cycle put in “overdrive” and result in a cell growth explosion resulting in the formation of cancer. Innate and adaptive immunity then activates in response to this process and both seek to destroy newly formed cancer populations. Cancer immunosurveillance starts in the “elimination” phase in which components of innate and cell mediated immunity seek to hinder cancer propagation; however, if cancer populations can evade immune surveillance by producing different antigen epitopes that the immune system cannot recognize, then “elimination” transitions to “equilibrium.” “Equilibrium” is the second phase in which innate and cell mediated immunity are in check with cancer populations. The cancer immunosurveillance response does not have a profound impact on cancer propagation since the immune system is overwhelmed with processing different antigens cancer cells produce as a result of mutation in the antigen conversion process. Thus, the cancer immunosurveillance response has to keep up at a fast pace due to the diversity in cancer antigen production and processing; however, if a host’s immune system cannot process antigens anymore, then cancer can overwhelm the host and “equilibrium” transitions to “escape.” “Escape” is the final phase in which a host’s immune system cannot contain a cancer population. Cancer escapes, due to immune inhibition by T regulatory cells, and the result is host death.

In this thesis, I present a mathematical model that describes the conditions in which the three “E’s” exist in the cancer immunosurveillance hypothesis between cancer populations as well as the innate and adaptive immune systems. We expect that both the
innate and adaptive immune systems play independent and synergistic roles to defend the body from cancer.
METHODS

The biological assumptions taken into consideration during the development of this model are based on accepted knowledge of immune function, which include:

1. Tumor cells grow exponentially in absence of an immune response. This assumption is based on prior work. Other forms of population growth such as logarithmic growth have been considered during the development of this model; however, exponential growth was chosen since it is the simplest form to go along with the cancer immunosurveillance hypothesis (Edelstein-keshet 2005).

2. Both NK and CTL cells can kill tumor cells.

3. Tumor cells can have the potential to elicit endogenous defenses in primed cells. Both NK and CTL cells respond to tumor cells by secreting various cytokines, interferon gamma as well as perforin proteins. The “effectiveness” of how well the innate and adaptive immune systems eliminate cancer depends upon the number of cells present as well as the cytotoxic potential of individual cells. This model does not evaluate the cytotoxic potential of each cell as a response to increases in cell number, but combines the two and measures the combined increase as a response to tumor cells.

4. As with innate immunity, NK cells are abundant and constantly circulating in the immune system (de Pillis et al. 2005).

5. With cell-mediated immunity, T cells are abundant in their naïve stage and differentiate into CD8⁺ Cytotoxic T lymphocytes, CD4⁺ Helper T lymphocytes, and CD4⁺ FoxP3 T regulatory lymphocytes after encountering tumor antigen by APC. Naïve T cells derive from hematopoietic stem cells in bone marrow and
migrate to the thymus in which they both express CD4 and CD8 surface proteins. The thymus undergoes positive and negative selection to discriminate "self" from "non-self" reacting T cells in order to prevent an autoimmune disorder. "Self" reacting cells are deactivated while "non-self" cells continue to develop and differentiate (Abbas et al. 2010). Overtime, one of the surface proteins, CD4 and CD8, are deactivated and naïve T cells transition into a CTL, HTL, or a regulatory HTL. This model simplifies the thymus maturation process by allowing each specialized cell type to have their own naïve populations and immediately transition into mature cells in presence of tumor antigen.

6. The activation of cell-mediated immunity is regulated by tumor antigen acquisition by antigen presenting cells, such as Langerhan’s, B cells, and macrophages.

7. CTLs, HTLs, and regulatory HTLs secrete IL-2 to activate and recruit circulating effector cells.

8. Regulatory HTLs decrease CTL and HTL populations by inactivating cell activation or cytokine secretion. Current trends in immunology assume that inhibiting regulatory HTL function decreases the time in which immune cells interact with cancer. This model assumes that regulatory HTLs make up no more than five percent of cell-mediated immune populations, but still impact CTL and HTL function (Ahmadzadeh et al 2006).

Utilizing the eight assumptions from above, the system can be described as ten coupled differential equations where each equation gives the rate of change of a
particular cell population in terms of growth and death, cell-cell kill, cell recruitment, and cell inactivation. In general:

\[ \frac{\Delta \text{population}}{\Delta \text{time}} = (\text{growth} - \text{death}) + (\text{recruitment} - \text{inactivation} - \text{cell kill}) \]

The eight above assumptions are, in essence, an improvement to a model proposed by Lisette G. de Pillis et al. in 2005 in which the primary investigator considered cancer immunosurveillance based on interactions between cancer, CTL, and NK cell populations; however, their model did not consider deeper details of the hypothesis, such as HTL assistance, cytokine secretion, as well as T regulatory cell inhibition of CTLs and HTLs. The new model designated for this thesis is as follows:

For Cancer Cells:

\[ \frac{dC}{dt} = r_c C - k_1 NC - k_2 T_e C \] (1)

Equation #1 describes the change of cancer populations in which the state variable of this equation is \( C \). Cancer populations propagate \( r_c \) at a fixed rate and die off due to cell-cell interactions between NK cells and CTL’s \( k_1 \) and \( k_2 \).

For NK Cells:

\[ \frac{dN}{dt} = b_N - d_N N + \frac{r_n NC}{m_n + NC} - l_n NC \] (2)

Equation #2 describes the change of NK cell populations in which the state variable of this equation is \( N \). NK cells are born at a fixed rate \( b_N \) and die off \( d_N \) in proportion to population levels. In addition, NK cells are recruited in response to cancer antigen presentation at a fixed rate \( r_n \) and \( m_n \) as well as die off due to cell-cell interactions with cancer \( l_n \).

For Naïve CD8:

\[ \frac{dT_n}{dt} = b_t - d_t T_n - m_a A_p T_n \] (3)

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Equation #3 describes the change of naïve CD8 populations in which the state variable of this equation is \((T_n)\). Naïve CD8 populations are born at a fixed rate \((b_t)\) and die off \((d_t)\) in proportion to population levels. Naïve CD8 populations then transition from the naïve to primed states due to cancer antigen acquisition \((m_a)\) by antigen presenting cells, which then present the processed cancer antigen to naïve populations.

\[
\frac{dT_e}{dt} = m_a A_p T_n - d_T T_e + r_T T_e I_2 - i_T R T_e \quad (#4) - \text{Effector CD8}
\]

Equation #4 describes the change of primed CD8 populations in which the state variable of this equation is \((T_e)\). Naïve CD8 populations primed with cancer antigen transition from their naïve to primed states to combat cancer (first term) and die off \((d_T)\) in proportion to population levels. Primed CTL populations are then influenced due to memory cell recruitment by interleukin-2 \((r_T)\) and are inhibited \((i_T)\) by T regulatory cells.

\[
\frac{dH_n}{dt} = b_n - d_n H_n - m_a A_p H_n \quad (#5) - \text{Naïve CD4}
\]

Equation #5 describes the change of naïve CD4 populations in which the state variable of this equation is \((H_n)\). Naïve CD4 populations are born at a fixed rate \((b_n)\) and die off \((d_n)\) in proportion to population levels. Naïve CD4 populations then transition from the naïve to primed states due to cancer antigen acquisition \((m_a)\) by antigen presenting cells, which then present the processed cancer antigen to naïve populations.

\[
\frac{dH_e}{dt} = m_a A_p H_n - d_H H_e + r_H H_e I_2 - i_H R H_e \quad (#6) - \text{Effector CD4}
\]

Equation #6 describes the change of primed CD4 populations in which the state variable of this equation is \((H_e)\). Naïve CD4 populations primed with cancer antigen transition from their naïve to primed states to combat cancer (first term) and die off \((d_T)\).
in proportion to population levels. Primed HTL populations are then influenced due to memory cell recruitment by interlukin-2 ($r_H$) and are inhibited ($i_H$) by T regulatory cells.

$$\frac{dI_2}{dt} = C_I I_2 - d_I I_2 - r_{H} H_e I_2 - r_{T} T_e I_2 - r_{R} R I_2 \quad (#7) - \text{Interlukin-2 production}$$

Equation #7 describes the change of interleukin-2 concentration in which the state variable of this equation is ($I_2$). IL-2 is produced at a constant rate ($C_I$) by primed immune cells, mainly of HTL lineage, and is consumed in varying proportions ($r_{H}$, $r_{T}$, and $r_{R}$) to recruit circulating memory cells to combat cancer populations. In addition, IL-2 denatures ($d_I$) in proportion to population levels.

$$\frac{dA_p}{dt} = r_a C - d_a A_p \quad (#8) - \text{Antigen Presenting Cells}$$

Equation #8 describes the change of antigen presenting cells in which the state variable of this equation is ($A_p$). APC populations are primed ($r_a$) in a direct proportion to cancer antigen secretion and die off ($d_a$) in proportion to population levels.

$$\frac{dR_n}{dt} = b_R - d_R R_n - m_a A_p R_n \quad (#9) - \text{Naïve CD4 FoxP3}$$

Equation #9 describes the change of naïve T regulatory populations in which the state variable of this equation is ($R_n$). Naïve T regulatory populations are born at a fixed rate ($b_R$) and die off ($d_R$) in proportion to population levels. Naïve T regulatory populations then transition from the naïve to primed states due to cancer antigen acquisition ($m_a$) by antigen presenting cells, which then present the processed cancer antigen to naïve populations.
\[
\frac{dR_e}{dt} = m_a A_p R_n - d_R R_e + r_R R_e l_2 \quad (#10) - \text{Effector CD4 FoxP3}
\]

Equation #10 describes the change of primed T regulatory populations in which the state variable of this equation is \( R_e \). Naïve CD4 populations primed with cancer antigen transition from their naïve to primed states to inhibit host immune function (first term) and die off (\( d_R \)) in proportion to population levels. Primed T regulatory populations are then influenced due to memory cell recruitment by interleukin-2 (\( r_R \)), which aid in regulating the CTL and HTL response.

GNU Octave, an open source math-modeling program, subjected the above model to numerical integration to determine the conditions in which cancer elimination, equilibrium, or escape might persist as well as determine “biologically” relevant parameter estimates for cancer immunosurveillance via bifurcation analysis, which will be addressed later. A list of all parameters and estimated values are listed on the next page:
<table>
<thead>
<tr>
<th>Parameter and Units</th>
<th>Parameter Description</th>
<th>Parameter Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_c$ (1/day)</td>
<td>cancer propagation</td>
<td>$5.41 \times 10^{-7}$</td>
<td>(de Pillis et al. 2005).</td>
</tr>
</tbody>
</table>
| $k_1$, $k_2$, and $l_n$ (cell/day*nL) | interaction between cancer and NK/CD8 cells | 3.50 $\times 10^{-12}$  
4.60 $\times 10^{-9}$  
1.00 $\times 10^{-3}$ | (de Pillis et al. 2005). |
| $b_n$ and $d_n$ (cell/day*nL) (1/day) | birth (fixed) and death of NK cells | $1.30 \times 10^{-2}$  
4.12 $\times 10^{-4}$ | (Pillis et al. 2005). |
| $r_n$ and $m_n$ (1/day) (cell$^2$/nL) | recruitment of circulating NK cells | 2.50 $\times 10^{-8}$  
20.2 | (de Pillis et al. 2005). |
| $m_a$ (1/day) | antigen presentation | $1.00 \times 10^{-4}$ | (Kim et. al 2006) |
| $b_c$ and $d_c$ (cell/nL*day) (1/day) | birth and death rates of naïve CD4 | 8.55  
3.00 $\times 10^{-9}$ | (Kim et. al 2006) |
| $d_f$, $d_i$, and $d_R$ (1/day) | death of effector CD4, CD8, and CD4 FoxP3 cells | 2.00 $\times 10^{-5}$  
4.00 $\times 10^{-6}$  
1.00 $\times 10^{-9}$ | (Kim et. al 2006) |
| $r_f$, $r_i$, and $r_R$ (cell/nL*day) | recruitment of circulating effector cells | 3.75 $\times 10^{-9}$  
1.88 $\times 10^{-9}$ | (de Pillis et al. 2005). |
| $i_T$ and $i_H$ (1/day) | inhibition of CD4 and CD8 activity by CD4 FoxP3 | 5.00 $\times 10^{-7}$ | (de Pillis et al. 2005). |
| $b_s$ and $d_s$ (cell/nL*day) (1/day) | birth and death rates of naïve CD8 | 6.00  
3.00 $\times 10^{-8}$ | (Kim et. al 2006) |
| $b_s$ and $d_s$ (cell/nL*day) (1/day) | birth and death rates of naïve CD4 FoxP3 | 4.50 $\times 10^{-7}$  
3.00 $\times 10^{-8}$ | (Kim et. al 2006) |
| $C_f$ and $d_f$ (1/day*nL) (1/day) | production and degradation of IL-2 | 1.00 $\times 10^{-5}$  
1.00 $\times 10^{-7}$ | (Kim et. al 2006) |
| $r_a$ and $d_a$ (1/day) | antigen secretion and death of antigen presenting cells | 1.00 $\times 10^{-4}$  
3.00 $\times 10^{-8}$ | (Kim et. al 2006) |

Table #1: Estimated parameter values incorporated into the above cancer immunosurveillance model.
RESULTS

The above model can be used to simulate the conditions in which cancer immunosurveillance falls under one of the three “E’s” of the cancer immunosurveillance hypothesis. Elimination, the first “E” of the immunosurveillance hypothesis, requires antigen capture and presentation to activate this process. Once antigen recognition is established, cell-mediated immunity can intervene in cancer propagation, which is illustrated in figure 3:

Figure #3: A hypothetical cancer immunosurveillance response with the following initial conditions C = 20,000 cells, N=2,500 cells, T_n=H_n= 1000 cells and R_n=50 cells with Table #1 parameter values.

In the stage of elimination, cancer propagation is assumed weak due to a strong host immune response (Dunn et al. 2004). Here, cancer populations (bolded blue) around
20,000 cells within a nanoliter of body fluid are rapidly eliminated due to a strong HTL and CTL proliferation and differentiation. Although the NK cell population (green) remains constant, intervention by NK cells of the innate immune system is only present at the beginning of the response. Cell mediated immunity takes over cancer immunosurveillance after 100 days due to rapid antigen acquisition through NK cells secreting interferon-gamma as well as various chemokines that inhibit blood vessel formation (Dunn et al. 2004). Dead tumor debris is then acquired by APC cells (light blue line) that travel to the lymph nodes and prime naïve T cells. There is rapid proliferation and recruitment after antigen presentation in which HTLs and CTLs migrate to the site of infection and deplete cancer populations. T regulatory cells see little action as they only make up five percent of lymphocyte populations. Although there is function, as shown by the model, T regulatory cells cannot inhibit the rapid expansion of mature CTL and HTL populations due to constant IL-2 secretion.

Equilibrium, the second “E” of the cancer immunosurveillance hypothesis, occurs immediately right after the elimination stage. In equilibrium, host immunity is at “even odds” with cancer populations in that cancer propagation is equal to host immune intervention. Cancer variants that survive the initial elimination stage rapidly proliferate until cancer populations match the host’s immune response such that there is an equilibrium established between cancer division and death; equilibrium may also arise during the initial immune response if cancer cells mutate antigen variants that cannot be recognized by CTLs or HTLs, which is shown in figure 4:
Figure #4: A hypothetical cancer immunosurveillance response with the following initial conditions $C = 20,000$ cells, $N=2,500$ cells, $T_n=H_n=1000$ cells and $R_n=50$ cells with Table #1 parameter values except for $k_1 = 3.50 \times 10^{-18}$ and $k_2 = 4.60 \times 10^{-11}$. The changes in $k_1$ and $k_2$ values result from a strong cancer immunosurveillance response decaying overtime, reflecting the transition from “elimination” to “equilibrium,” primarily due to cancer populations producing mutated antigen variants at a fast rate. Hence, cell-mediated immunity must produce CTLs and HTLs at a fast rate in order to keep up with the diversity of cancer antigens being produced. Because of antigen diversity, interactions between cancer, NK cells, and CTL’s are weakened since primed cells with one specific type of cancer antigen can kill off cancer cells expressing the recognized antigen; however, cancer cells expressing a different type of antigen can avoid the cancer immunosurveillance response. Hence, the immunosurveillance process must process another type of antigen in order to kill off cancer cells that avoided the first immunosurveillance response. The transition from “elimination” to “equilibrium” results in cancer populations remaining constant during the immunosurveillance response. Please refer to Table #1 for parameter definitions and equation #1 to see how $k_1$ and $k_2$ influences cancer populations.

During equilibrium, tumor populations (blue) will spontaneously mutate in order to avoid APC antigen acquisition; a mutation, whether it will affect a protein or one amino acid, can influence cancer immunosurveillance. T cell populations, both HTLs and CTLs, (green and tan) will differentiate and proliferate to survey host tissue that contains
cancerous cells that possess the recognized antigen; however, due to antigen mutation, T cells can no longer influence cancer dynamics. As tumor cells with recognized antigens are destroyed, those with mutated antigen can proliferate and maintain the population at equilibrium such that it undergoes a state of dormancy, which can last for about 20 years (Dunn et al. 2004). Parameter changes in figure 4 reflect a change in a host’s defense or cancer propagation. As a host’s defense weakens or cancer propagation ($r_c$ – not shown in the above graph) strengthens, a host’s immune system has to work twice as hard in order to maintain cancer populations in equilibrium (figure 4). A host’s immune system will need to have the capability of producing T lymphocytes at a high rate in order to maintain cancer populations in check such that cancer remains in a benign state. In this case, a high cell proliferation and differentiation rate may not be feasible for a host’s immune system.

Escape, the third “E” of the cancer immunosurveillance hypothesis, means death for the host immune response as cancer propagation overcomes host defenses and spreads beyond the area of infection, essentially hindering all host function. Cancer populations in this stage exponentially proliferate as according to figure 5:
Figure #5: Cancer immunosurveillance response with the following initial conditions C = 20,000 cells, N=2,500 cells, T_T=H_T= 1000 cells and R_T=50 cells with Table #1 parameter values except for \( r_c = 5.41 \times 10^{-3} \), \( k_1 = 3.50 \times 10^{-18} \) and \( k_2 = 4.60 \times 10^{-17} \). The changes in \( r_c \), \( k_1 \), and \( k_2 \) values result from cancer overwhelming a host's immunosurveillance response, reflecting the transition from "equilibrium" to "escape," primarily due to a strong cancer propagation rate and a weakening immune response due to T cell inhibition and antigen processing. Because of antigen diversity and a strong cancer propagation rate, interactions between cancer, NK cells, and CTL's are weakened since primed cells with a specific type of cancer antigen can kill off cancer cells expressing the recognized antigen; however, cancer cells expressing a different type of antigen can avoid the cancer immunosurveillance response. In addition, T regulatory cells (not shown) inactivate CTLs and HTLs in order to maintain a host's homeostasis. Cancer populations can take these processes to their advantage and propagate at a fast rate, which results in host death. Please refer to Table #1 for parameter definitions and equation #1 to see how \( k_1 \) and \( k_2 \) influences cancer populations.

In the escape phase, cancer variants that have survived the equilibrium phase have successfully evolved escape mechanisms to avoid the host immune response and can now proliferate without regard to the host environment. Host immune response has severely weakened due to a variety of factors, such as FoxP3 regulatory inhibition and antigen switching, such that T cell populations (green and tan) grow at a slow rate and are eradicated at a faster rate than during the elimination and equilibrium stages. Tumor
variants have other mechanisms by which escape is possible, such as suppressive
cytokines, to inactivate IL-2 secretion and stop host immune response (Dunn et al. 2004).

Parameter changes in figure 5 reflect a change in a host’s immune system or
cancer propagation. As cancer propagation increases within a host, dormant cancer
populations transition from a benign to an active state and the host undergoes a great deal
of damage whether it be pancreatic, breast, or ovarian cancer. A host’s immune system
cannot catch up with a fast cancer propagation rate and in order to do so would need to
have an overactive immune system, which is not physically possible due to T regulatory
influence. As addressed above, T regulatory cells consist of only five percent of
lymphocyte populations but can have a major influence on cancer immunosurveillance.
As a host’s immune function goes into overdrive, T regulatory cells will inactivate any
cells that go beyond normal function; for example, targeting tissue cells in addition to
cancer cells. T regulatory cells deactivate overactive CTLs or HTLs in order to maintain
homeostasis and this response will result in host death.

Bifurcation analysis, or parameter estimation, requires non-dimensionalization of
a mathematical model to determine how computed solutions behave in regards to
parameter switching. In the process of bifurcation analysis, relations between parameter
and parameter “families” can be discovered and show how switching one can influence
the behavior of another. Bifurcation analysis of the above model reveals three distinct
families between cancer propagation ($r_c$) and host immune function as well as their
behavior in figures 6 to 8:

- $R_a$ and $M_a$ – Antigen production and acquisition (figure 6)
- $I_T$ and $I_h$ – Inhibition of CTLs and HTLs by CD4 FoxP3 cells (figure 7)
• C1 – Cytokine production (figure 8)

Figure #6 – A bifurcation diagram of cancer propagation against lymphocyte inhibition. Any values \((r_c, m_a/r_a)\) below the bolded blue line in the graph \((r_c, m_a/r_a)\) will result in cancer populations overcoming the host while values above the bolded blue line will result in the elimination of cancer. Note, any \(M_a\) and \(R_a\) values below \(R_c = 1.2\) are not show to complete accuracy due to the high range of \(M_a\) and \(R_a\) beyond \(R_c = 1.2\)

Antigen production by cancer cells \((r_a)\) and antigen acquisition by APCs \((m_a)\) are assumed to have linearity with cancer propagation (assumption #5) since each cancer cell produces one antigenic variant and APCs (B cells, etc.) quickly process a variant for cell-mediated immunity activation. For values of \(r_c\) between \(0 < r_c < 1.2\), \(m_a\) and \(r_a\) values are between \(0 < m_a/r_a < 30\) due to linearity. Thus, for each secreted antigen from a cancer cell, an APC must quickly process it to activate cell-mediated immunity. Thus, there are biologically relevant parameter values for \(m_a\) and \(r_a\). Beyond \(r_c > 1.2\),
bifurcation analysis reveals biologically irrelevant parameters ranging from $30 < m_a/r_a < 1 \times 10^{80}$ This is primarily due to cancer propagation overwhelming a host’s immune system by producing antigenic variants in greater numbers compared to APCs that can process them. Overwhelming of the APC process results in a compromised immune system and eventually host death.

Figure #7 — A bifurcation diagram of cancer propagation against lymphocyte inhibition. Any value ($r_c$, $r_H$) above the bolded blue line will result in cancer populations overcoming the host while values below the bolded blue line will result in the elimination of cancer.

T regulatory cell inhibition and cancer propagation are assumed to have an inverse relationship similar to the function $y = \frac{1}{x}$. As cancer propagation increases within a host, from “equilibrium” to “escape”, the host’s immune system must work twice as hard in order to prevent host death. A problem encountered during the transition from “equilibrium” to “escape” is T cell regulation, which influences cancer
immunosurveillance by deactivating CTL sentinels and HTLs. Thus, in order to maintain cancer in the “equilibrium” stage, T regulatory function must decrease in order for a host to prevent host death. For values of cancer propagation between \(1 < r_c < 2\), T regulatory function \(i_T\) and \(i_H\) takes on values between \(1 < i_T/i_H < 0.2\), which indicates that in order for a host to maintain cancer in the equilibrium stage, inactivation of HTLs and CTLs must decrease to effectively combat cancer; however, if cancer propagation goes beyond \(r_c > 2\) there is a finite limit in which T regulatory function can be suppressed. From \(2 < r_c < 6\), the area of this interval is significantly smaller compared to the interval \(1 < r_c < 2\), which indicates that a host can tolerate T regulatory suppression for so long to deter cancer propagation until cancer overcomes a host’s immune machinery.

Figure #8 – A bifurcation diagram of cancer propagation against cytokine secretion. Any value \((r_c, C_c)\) below the bolded blue line will result in cancer populations overcoming the host while values above the bolded blue line will result in the elimination of cancer.
Cytokine secretion and cancer propagation are assumed to have a hyperbolic relationship similar to the Michaelis-Menten function $y = \frac{ax}{b+x}$. Cytokine secretion is an important process in cancer immunosurveillance as this protein family is essential for T cell recruitment of circulating HTLs, CTLs, and T regulatory cells and other immune processes. For values of $r_c$ between $0 < r_c < 10$, $C_i$ values are between $0 < C_i < 7$; however beyond $r_c > 10$, $C_i$ remains constant around seven, which indicates saturation. This means no matter what value of $r_c$ a cancer population may have, cancer propagation can be deterred if cytokine secretion remains at seven or above; however, any value less than seven for any $r_c > 10$ means that the host succumbs to death.
DISCUSSION

Numerical analysis can give insightful information to a problem simply by varying initial conditions as well as parameter values, which is versatile since a mathematical model can adapt to any given situation, such as the model above and cancer immunosurveillance. By varying only three parameters in the cancer equation ($r_c$, $k_1$, and $k_2$), cancer and host immune populations can transition from “Elimination” to “Equilibrium” and to “Escape”; varying other parameters in the other nine equations, such as birth rates on the naïve T cell populations, may give similar results that describe these transitions in cancer immunosurveillance. Variation of host immune or cancer parameters can give insight into the behaviors of all populations and hence, parameter and initial value switching can adapt, to a variety of cancer patients to describe their current cancer’s stage in cancer immunosurveillance.

Mathematical modeling, however, has its limitations in terms of model building and adaptation. Model building can only incorporate as much relevant biological information and assumptions as it can since this can affect the analytic phase of model building. Complex models, such as the one above, cannot be easily solved by hand and instead require the use of numerical solvers to see how results behave; smaller models are easier to manipulate and solve by hand, but are a simplified “story” of the issue at hand. Since accuracy is more important than simplicity, a larger model was opted for this project.

Cancer immunosurveillance is a complex immune process and more than a simplified mathematical model, such as the one above and the one proposed by Lisette G. de Pillis et al. (2005). As stated in the introduction, cancer immunosurveillance is a complex process that requires antigen recognition in order to activate cell-mediated
immunity. Innate immunity, for example, can defend a host against cancer populations up to a certain degree (Lin et al. 2007). Thus, cell-mediated immunity needs to be activated on the instant of cancer recognition in order to assist innate immunity functions (Figures 3-5); yet, innate immunity facilitates cell-mediated immunity activation through antigen/MHC class I binding. Hence, innate immunity plays an independent and synergistic role in cancer immunosurveillance. On the onset of cancer immunosurveillance, figures 3 and 4 depict no change in natural killer populations since assumption 4 states “NK cells are abundant and constantly circulating in the immune system.” Hence, the model sees little changes or activity in NK cell populations during the cancer immunosurveillance response. If the model had several more equations to describe the innate immune responses (macrophages, complement, etc.), then innate immunity would play a larger role in cancer immunosurveillance; the only contribution innate immunity gives to the model to describe the cancer immunosurveillance process is NK cell activity.

Cell-mediated immunity, once activated, assists the weak innate immune response by killing off cancer and infected host cells via CD8⁺ cytotoxic T lymphocyte interactions as well as CD4⁺ helper T lymphocyte rolling recruitment by IL-2 secretion. Some specialized T lymphocyte subpopulations, however, can provide deleterious effects on cell-mediated immunity. FoxP3 CD4⁺ T regulatory lymphocyte populations can hinder cell-mediated function in order to maintain homeostasis within a host if it perceives a lymphocyte’s response to an infection as too strong. Although not shown in figures 3 through 5, T regulatory cells consist only of five percent of a host’s lymphocyte population. To have a strong influence to deter cancer immunosurveillance, FoxP3
production would need to be greater than that of HTLs and CTLs, which is not biologically possible. Another population is CD1d natural killer T cells that can reside in both innate and adaptive immunity (Chaturvedi et al. 2011). Their role in cancer immunosurveillance theory is relatively unknown, due to its recent discovery, but this subpopulation is hypothesized to have a negative impact on cancer immunosurveillance. CD1d-NK T lymphocytes recognize various pathogens that express CD1d and act on those populations, while pathogens that do not express CD1d can invade CD1d surveillance; this may facilitate the transition of cancer immunosurveillance from “elimination” to “equilibrium” or possibly “escape” in part of CD1d cells lacking in the diversity of antigen response compared to other cell populations.

The three stages of cancer immunosurveillance are unique in that each has its own outcome:

- **Elimination** – The establishment of a strong cancer surveillance network by both the innate and adaptive immune system which seeks to eliminate cancer populations
- **Equilibrium** – The long term process of combat between a cancer population and the cancer surveillance network
- **Escape** – The overpowering of the cancer surveillance network by strong tumor variants, which results in host death

By understanding the dynamics of each process, the results can have numerous applications in cancer diagnosis and cancer prevention since this model can be “tailored” to describe a patient’s condition. The cancer immunosurveillance response is relatively not a quick process as the model depicts as cellular immune processes may take days,
weeks, months, or even years. The phases of “equilibrium” and “escape” also follow similar parallels that of “elimination” (figures 4 and 5). In figure 3, cancer populations (blue bolded line – see above) start out at 20,000 cells and at the onset of the cancer immunosurveillance response, immediately drop to zero cells around 1,500 days. In figure 4, cancer populations (blue bolded line – see above) stay constant around 20,000 cells past 1,500 days and figure 5 depicts a surge in cancer populations starting around 1,500 days. The model assumes days, rather than weeks or years, due to the time scale introduced into GNU Octave and its relative tolerance in computing the solutions for the above model; naturally, most mathematical models introduce an arbitrarily determined time scale (seconds, hours, etc.) to show that the results can be measured in any timescale.

Regardless of the timescale, the ultimate goal of cancer immunosurveillance is to protect the host from succumbing to many types of cancer. The main proponents of the cancer immunosurveillance response are HTLs and CTLs primed after being exposed to cancer antigens by APCs (B cells, dendritic, etc.). In figure 3, HTL and CTL populations (green and tan lines – see above) rise as a response and kill off cancer populations, which follows the phase of “elimination.” Figure 4 also sees a rise in HTL and CTL populations, but cancer populations stay at the same level around 20,000 cells, which follows the phase of “equilibrium.” Figure 5 sees hindered growth of CTL and HTL populations as cancer populations have successively evaded the cancer immunosurveillance response, which follows the “escape” phase. These three figures can be thought of as three different patients with different types of cancer (pancreatic, prostate, colon, etc.) but with similar initial conditions and their immune systems at a different stage in cancer immunosurveillance theory. Depending upon the abundance of CTLs, HTLs, T
regulatory cells, NK cells, cancer cells, and IL-2 concentrations, cancer
immunosurveillance will be in either “elimination, equilibrium, or escape.”

Cellular dynamics can also affect cancer immunosurveillance. Changes in three
parameters that specify cancer, CTLs, and NK cell interactions as well as cancer
propagation can force the cancer immunosurveillance response to switch between
“elimination, equilibrium, and escape.” In the model’s context of “elimination,” cancer
populations were eliminated due to $k_1$ and $k_2$ values being $3.50 \times 10^{-12}$ and $4.60 \times 10^{-7}$;
however, if $k_1$ and $k_2$ were decreased to reflect a weakened immune response ($k_1 =
3.50 \times 10^{-18}$ and $k_2 = 4.60 \times 10^{-11}$), then cancer immunosurveillance transitions from
“elimination” to “equilibrium,” which results in less interactions between cancer, CTLs,
and NK cells and cancer populations remaining constant. A further decrease of $k_1$ and $k_2$,
along with an increase in cancer propagation ($r_c$), ($r_c = 5.41 \times 10^{-3}$, $k_1 = 3.50 \times 10^{-18}$, and $k_2
= 4.60 \times 10^{-17}$) will result in transition from “equilibrium” to “escape” and cancer wins
resulting in host death. Therefore, the above mathematical model has established
parameter conditions for cancer immunosurveillance theory:

- **Elimination:** $k_1 > 3.50 \times 10^{-12}; k_2 > 4.60 \times 10^{-7}; r_c = 5.41 \times 10^{-7}$
- **Equilibrium:** $3.50 \times 10^{-18} < k_1 < 3.50 \times 10^{-12}; 4.60 \times 10^{-11} < k_2 < 4.60 \times 10^{-7}$
- **Escape:** $k_1 = 3.50 \times 10^{-18}; 4.60 \times 10^{-17} < k_2 < 4.60 \times 10^{-11}; 5.41 \times 10^{-7} < r_c <
5.41 \times 10^{-3}$

Bifurcation analysis can validate or provide new information to a subject that is
being modeled. Figures 7 and 8, for example, show that cancer propagation can be
hindered if cytokine production is increased (Figure 8) or T regulatory function is
decreased (Figure 7), while figure 6 depicts antigen acquisition must rise in response to
cancer propagation. Figure 6 shows that antigen acquisition has to spontaneously rise in response to a faster cancer propagation rate; this proves impossible unless a host has accumulated a variety of mutations in his or her genome that will give rise to APCs with incredible function. Thus, any values beyond \( r_c = 1.2 \) are biologically irrelevant since it is not possible for a host to produce APCs with such incredible function.

Figures 7 and 8, however, show that if host cytokine production (figure 8) or T regulatory function (figure 7) is decreased, a host may have a better chance of survival due to enhanced immune function. Figure 7 shows that there is an inverse relationship between cancer propagation and CTL/HTL inhibition; this proves only possible if cancer immunosurveillance is in “elimination” and “equilibrium” where T regulatory cells have little influence on CTL and HTL function. Thus, all values of \( r_c \) and \( i_T/I_H \) are biologically relevant since beyond \( r_c = 6 \), T regulatory cells have a significant impact upon CTL and HTL function, which correlates with the escape phase. Figure 8 shows that that for increasing values of cancer propagation, cytokine secretion \( (C_i) \) reaches saturation around a value of 7, which indicates that during the transition from “elimination” to “escape,” a host needs to produce IL-2 in great concentrations in order to prevent death. Thus, only values of \( r_c \) from \( 0 < r_c < 10 \) are only biologically relevant since a host can only produce so much IL-2 during the cancer immunosurveillance response; however, beyond \( r_c > 10 \), this is only possible in a hospital setting, during the “escape” phase, where cytokine injections are needed to aid a critically ill patient. Regardless, bifurcation analysis of this non-dimensionalized model provides insightful information to the cancer immunosurveillance response. For example medicines, such as cytokine injections (Margolin 2008) (figure 8) or T regulatory inhibitors (Ozao-choy et al. 2009) (figure 7),
may assist immunocompromised patients during the “escape” phase of cancer immunosurveillance.

The future of this model is constant improvement since refinement is necessary to describe all processes of cancer immunosurveillance. Here, the model has shown that both the innate and adaptive immune systems play independent and synergistic roles in cancer immunosurveillance as well as establish the conditions of the cancer immunosurveillance hypothesis of “Elimination,” “Equilibrium,” and “Escape” by varying three distinct parameters of the cancer equation; however, other parameter values may be varied to produce the same results. This model will most likely take part in a Ph.D. that will follow similar subject matter explored in this thesis.
REFERENCES


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