

DYNAMIC SUICIDE GENE THERAPY CONTROL

by

LaMont Cannon

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LaMont Cannon

Approved: _____
Ryan M. Zurakowski, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
Kenneth E. Barner, Ph.D.
Chair of the Department of Electrical & Computer Engineering

Approved: _____
Michael J. Chajes, Ph.D.
Dean of the College of Engineering

Approved: _____
Charles G. Riordan, Ph.D.
Vice Provost for Graduate and Professional Education

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ABSTRACT

Based on the recent dynamic gene therapy proposed by Martinez-Quintanilla et al., we have developed a set of ordinary differential equations to model the dynamics of various cancerous cells during tumor growth and treatment. We then exploited the dependence of certain parameters within the system during therapy to devise an optimal control method for treatment. Employing the use of an optimal controller allows us to analyze the best possible outcome of the treatment under several conditions. In this thesis we will apply an optimal controller to the system under various conditions taking specific note of the total tumor burden as a result of the treatment and total treatment time. We will then prove that an optimal control approach is very helpful and may even be necessary for successful implementation of this novel cancer therapy.

Chapter 1

INTRODUCTION

The field of mathematical biology is quickly gaining popularity among researchers in effort to find novel treatments, cures, and solutions to an assortment of medical concerns. The basic procedure of mathematical biology is to first devise a mathematical model of the observable organic events based on their biological properties. The models often take the form of a partial differential equation or an ordinary differential equation used to represent the dynamical system. Next the investigator would attempt to predict the outcome of their model under certain biological pressures. Such pressures could range from a variety of different features such as treatment schedule, a certain drug efficacy, mass action rate, etc.

Once a practical model is formulated it can then be manipulated for use in several diverse applications. One popular course of action is to apply some sort of control theory to the model to achieve a desired outcome. The effort to maximize or minimize a certain aspect a therapy using the mathematical model is known as optimal control.

Optimal control is very popular among mathematical biologist. It is especially useful when trying to optimize some sort of treatment. The work of Zurakowski and Wodarz, seeks to optimize treatment by using optimal control based schedules of specific inhibitor application to increase the efficacy of an innovative new treatment approach towards cancer [13],[14].

Castiglione and Piccoli [3] hope to apply the theories of optimal control to their model to determine when and to which extent to stimulate the immune

system. Using an optimal control method they can determine when would be the best time to administer certain immunotherapeutic agents to elicit a managed immune response in the patient [3].

Mathematical biology and optimal control is not just limited to the medical field and medical applications. An example is food production, specifically food products that are produced by modern process called bacteria fermentation. Yogurt is a popular food produced via bacteria fermentation of lactose to lactic acid in milk.

Bacteriophages are type virus that infect and subsequently cause the death of bacteria. Employing mathematical biology methods and optimal control theory, scientist can use this knowledge as a manipulated variable to control the amount and bacteria used during fermentation [8]. Anthrax is used by terrorist all around the world to threaten lives in different countries. Knowledge of bacteriophages and application of control can be used to optimally minimize the effect of an anthrax breakout.

In this thesis we intend to apply mathematical biology concepts to a novel therapy approach proposed by Martinez-Qunitanilla et al [6][7]. The goal of the therapy is to overcome the current challenges faced by scientist and doctors when treating cancer, most importantly cancerous cell's resistance to chemotherapeutic drugs.

Chapter 2

BIOLOGICAL BACKGROUND

In the most basic sense, many chemotherapeutic agents work by inducing a programmed cellular suicide of the cancerous cells. This process is called apoptosis and is the primary mechanism of cell death. Regulation of the cell population is of fundamental importance to multicellular organisms. Not only must redundant cells be removed during development, as the organism is created, but controlled cellular deletions are needed in order to prevent the proliferation of cells that have acquired mutations [4],[5].

Through various processes many cancerous cells lose their apoptosis triggers as the tumor matures. For this reason many late stage cancers are highly resistant to chemotherapeutic agents. Often times after chemotherapy treatment several genomically instable, chemotherapy resistant cells remain.

2.1 Chemotherapy Resistance

Tumor cells are able to achieve states of chemo-resistance via two mechanisms: mutation (natural evolution) or transfection of engineered genes. Natural mutations occur because cancer cell DNA is in a constant state of flux, continually altering the cells phenotype. Widely employed and well characterized chemo-resistance genes are variants of dihydrofolate reductase (DHFR) and multidrug resistance gene1 (MDR1) [2].

DHFR serves as a catalyst for the reduction of folate to tetrahydrofolate [1]. This enzyme, in turn, serves as a cofactor in the production of amino and nucleic acids such as purines and thymidine. The chemotherapeutic agent methotrexate (MTX) is a compound having a greater affinity to DHFR than folate. Thus, DHFR more readily binds to MTX, preventing potential production of tetrahydrofolate and subsequent nucleotides. Ideally, methotrexate would display this characteristic for all tumor cells; however, DHFR mutants display a lower affinity for the drug, enabling them to continue preferential binding to folate.

Another instance of chemo-resistance is illustrated by MDR1. This gene encodes for the membrane bound protein P-glycoprotein (PGP). The role of PGP is a facilitator of molecule movement, mediating both extra- and inter-cellular transport. PGP effectively acts as a drug pump, decreasing intercellular concentrations of drugs, thereby limiting their effectiveness.

Either of these mutations are considered highly likely to have an established presence in cases of advanced colon or pancreatic cancer. For the purposes of our model, we assume chemo-resistant mutants constitute a portion of the tumor population. The selective pressure initiated by a chemotherapeutic agent favors these mutants, as they exhibit greater fitness than their non-mutant counterparts. Positive selection for mutants results in a post-treatment population that is devoid of chemo-susceptible cells.

2.2 Transfected Genes

In the work of Martinez-Quintanilla et al. [6][7] instances of colon and pancreatic carcinoma cell lines are transfected via a plasmid containing one of two separate genes encoding either MDR1 or DHFR, combined with a gene encoding herpes simplex virus thymidine kinase (HSV-TK).

We have discussed the implications of MDR1 or DHFR presence in the cell. Transfected genes ensure survival of the toxic effects of chemotherapy, resulting in the presence of a second subpopulation of cells after treatment.

The second gene in the plasmid, HSV-TK, encodes an enzyme that converts the host to a state of susceptibility to the antiviral drug ganciclovir. Ganciclovir is prominent in many anti-cancer gene therapy approaches because it is readily incorporated into the DNA of susceptible cells. The compound is directly responsible for the formation of double-strand breaks in cell DNA, ultimately triggering apoptosis [9]. Furthermore, it diffuses into neighboring cells - intercellular transfer occurs through gap junctions (2-4nm apart). This exchange of metabolized ganciclovir creates a bystander effect when transfected cells meet chemo-resistant mutants at gap junctions, triggering apoptosis in cells not ordinarily targeted by the drug.

The method of anti-cancer gene therapy under consideration occurs in a two-stage process. The first phase is positive selection for chemo-resistant cells during chemotherapy, resulting in an amplified proportion of transfected cells. The

population of transfected cells must outnumber that of mutant cells in order to ensure an optimal interaction between the species, required for the bystander effect. The second phase consists of negative selection phase achieved by injection of ganciclovir. Those cells prone to the antiviral drug treatment via transfection or bystander effect would undergo triggered apoptosis.

Experimental anti-cancer gene therapy has been conducted on mice cancer cells, both in vitro and in vivo [6][7]. In both instances, cell populations were measured post-therapy, indicating a significant decline corresponding to successful treatment. However, the complicated dynamics of this process make it difficult to guarantee similar success in humans, especially when delivery mechanisms are considered. The remainder of this paper serves to demonstrate the necessity of dynamic modeling and optimal control techniques in designing a robust treatment implementation for human trials.

2.3 The Bystander effect

The aforementioned bystander effect is the most important part of the biological process and is the tool that we wish to manipulate in order optimize treatment [6][7]. In the absence of a large enough bystander effect the cells that are naturally chemo-resistant would not die out after treatment; however, with an efficient bystander effect, the enrichment of the modified cells could completely eradicate the tumor after the ganciclovir treatment.

The bystander effect has the largest impact when there is a high proportion of the induced chemo-resistant cells in the tumor at the onset of the ganciclovir treatment. In other words if there is a large proportion of induced chemo-resistant

cells vs. other cells in the tumor then there is a high probability that an induced chemo-resistant cell can come into contact with another type of cell and exchange the metabolized ganciclovir.

It is known that the process governing the genesis and progression of cancers are evolutionary ones in which natural selection acts upon the inherent or acquired diversity of various somatic clones [5]. It is this very natural selection process that we wish to exploit and optimize to achieve a successful treatment.

By pulsing the chemotherapy treatment we can encourage competition between the three cell types at various times. Under the right pulse sequence the induced chemo-resistant cell ratio can reach a maximum that will allow for successful treatment. The ultimate goal of this thesis is to find that optimal pulse sequence and subsequently analyze how treatment outcomes vary subject to this optimal control when the system parameters are changed.

Chapter 3

THE MODEL

We used a logistic model to convey the dynamics of the system. In a logistic model the curve of the tumor size follows a sigmoid pattern with three key phases. The first phase is the initial exponential growth of the tumor. During this phase there are abundant resources and thus not much competition between cell types for each resource. As a result the tumor can propagate exponentially.

A linear growth section characterizes the second phase of logistic tumor growth. In this section resources are beginning to deplete as more cells are formed. As a result the growth starts to become less exponential and becomes more linear for a period.

The third and final phase of tumor growth is the plateau phase. During this phase the tumor cells have propagated so much that the available resources are running out. At this point the tumor reaches a size, called the carrying capacity at which point there is no more increase in volume, and the curve levels off into a plateau [10][11][12].

As stated previously, a delivery virus must be used in order to transfect the cancer cells. The virus would need to be at least somewhat oncospecific and could either be replication competent or non-replication competent.

3.1 Non-Replication Competent Delivery Virus

3.1.1 Non-Susceptible Naturally Resistant Cells

The basic model that we created represented the non-replication competent situation. In this case, after the initial virus bolus no more virus are produced. This is done through the use of a replication-defective viral vector. These viral vectors are able to penetrate and integrate into host DNA; however, the coding regions responsible for reproduction have been deleted. This means that this type of virus, a non-replication competent delivery virus, can infect a host cell but cannot enter into the reproductive cycle or lytic cycle to produce any progeny. Thus, after all of the viruses have transfected a cell, there will be no more viral transfection during the treatment unless another bolus is administered. In order to model such dynamics we developed a simple set of non-linear differential equations

$$\begin{aligned}\dot{x} &= r\mathbf{x}C_x(t)\left[1 - \frac{(\mathbf{x} + \mathbf{y} + \mathbf{z})}{K}\right] - \mathbf{x}[d_x + \beta\mathbf{v} + g(t)b\left(\frac{\mathbf{z}}{\mathbf{x} + \mathbf{y} + \mathbf{z}}\right)] \\ \dot{y} &= \lambda\mathbf{y}C_y(t)\left[1 - \frac{(\mathbf{x} + \mathbf{y} + \mathbf{z})}{K}\right] - \mathbf{y}[d_y + g(t)b\left(\frac{\mathbf{z}}{\mathbf{x} + \mathbf{y} + \mathbf{z}}\right)] \\ \dot{z} &= \beta\mathbf{x}\mathbf{v} + s\mathbf{z}C_z(t)\left[1 - \frac{(\mathbf{x} + \mathbf{y} + \mathbf{z})}{K}\right] - \mathbf{z}[d_z + g(t)] \\ \dot{v} &= -\mathbf{v}(u + \beta\mathbf{x})\end{aligned}\tag{1}$$

In this model x , y , and z represent the chemotherapy sensitive, the naturally chemotherapy resistant, and the induced chemotherapy resistant cell populations respectively. The individual exponential growth rates of each cell type are

represented by r , λ , and s respectively and their death are represented by d_x , d_y , and d_z . The free virus population is represented by v , which decays exponentially at a rate $(u+\beta x)$. Because the virus is non-replication competent there is no growth term in the virus equation.

The previous terms are the general expressions associated with most models that reproduce population dynamics. There are several key terms in our model that are necessary to show the interaction and dynamics associated with this competition-suicide gene therapy. First there are the chemotherapy treatment efficacy terms $C_x(t)$, $C_y(t)$ and $C_z(t)$, which are time-dependent terms that affect the growth rate of each cell type. They are modeled as time-dependant terms because the chemotherapy can be on or off at various times during the treatment.

The variable K represents the carrying capacity of the tumor. It is a fundamental element of a logistic tumor growth equation and summarizes the cell population that the tumor can sustain given the surrounding conditions. The variable β is the infection rate or efficacy of the virus. In this set of equations it describes a rate of change from state x to state z .

Perhaps the most important part of the model for this type of therapy is the aforementioned ganciclovir and bystander effects. Similar to the chemotherapy treatment, the ganciclovir treatment is modeled as a time-dependent term denoted by the variable $g(t)$. The bystander effect is represented by the variable $b(z/(x+y+z))$ in the model. It is a function of the ratio of the amount of induced or transfected chemotherapy resistant over the total amount of cells in the tumor. It affects the chemotherapy sensitive cells and the naturally chemotherapy resistant

cells as the induced chemotherapy resistant cells share its metabolized ganciclovir when it comes into contact with the other cell types. The probability that such an interaction will take places is embedded in the function $(z/(x+y+z))$.

Symbol	Definition
x	Chemotherapy Sensitive (Uninfected) Cell Population
y	Naturally Chemo-Resistant Cell Population
z	Induced Chemo-Resistant (Infected) Cell Population
v	Viral Load
r	Chemotherapy Sensitive Cell Growth Rate
λ	Natrually Chemo-Resistant Cell Growth Rate
s	Induced Chemo-Resistant Cell Growth Rate
β	Infection Rate (Efficacy) Constant
C_x	Time-Dependant Chemotherapy Treatment Effect on Chemotherapy Sensitive Cells
C_y	Time-Dependant Chemotherapy Treatment Effect on Naturally Chemo-Resistant Cells
C_z	Time-Dependant Chemotherapy Treatment Effect on Induced Chemo-Resistant Cells
d_x	Chemotherapy Sensitive Cell Death Rate
d_y	Natrually Chemo-Resistant Cell Death Rate
d_z	Induced Chemo-Resistant Cell Death Rate
K	Tumor Carrying Capacity
b	Induced Chemo-Resistant Population Ratio Dependant Bystander Effect
g	Time Dependant Ganciclovir Effect
u	Virus Death Rate

Table 1 ODE Symbol Definitions.

3.1.2 Susceptible Naturally Resistant Cells

The previous model showed the case where only the chemotherapy sensitive cells were able to be infected by the virus and become induced chemotherapy

resistant cells. The following model shows the case where both the chemotherapy sensitive and the naturally chemotherapy resistant cells are susceptible to infection by the virus.

$$\begin{aligned}
\dot{x} &= rxC_x(t)\left[1 - \frac{(x+y+z)}{K}\right] - x[d_x + \beta v + g(t)b\left(\frac{z}{x+y+z}\right)] \\
\dot{y} &= \lambda yC_y(t)\left[1 - \frac{(x+y+z)}{K}\right] - y[d_y + \beta v + g(t)b\left(\frac{z}{x+y+z}\right)] \\
\dot{z} &= \beta xv + \beta yv + szC_z(t)\left[1 - \frac{(x+y+z)}{K}\right] - z[d_z + g(t)] \\
\dot{v} &= -v(u + \beta x + \beta y)
\end{aligned} \tag{2}$$

The model is very similar to the model for the non-susceptible naturally resistant cells case, with a few key exceptions. There is now a βyv term in the second and third states that signify the infection is now infecting the naturally resistant cells and turning them into induced chemotherapy resistant cells at a mass action rate proportional to β . The free virus now decays from the system a little faster as a result of the additional mass action term βyv from the virus infecting the naturally resistant cells.

3.2 Replication Competent Delivery Virus

The previous models illustrated the case where the delivery virus was administered and could not replicate, therefore the virus load decayed naturally due to death and as a result of infecting a cell. Next we observe the case where the virus is able to replicate and produce progeny via the lytic cycle. During viral infection in the lytic cycle the free virus attaches on to a susceptible cell and injects its genetic information into the cell. The viral genes then integrate themselves into the genetic

information of the cell and assemble into new viruses. After enough viruses have been assembled the host cell undergoes a virus induced cell death, the cell lyses and all of the free viruses are released.

3.2.1 Non-Susceptible Naturally Resistant Cells

The following model depicts the dynamics of the therapy given a replication competent delivery virus is used during inoculation and only chemotherapy susceptible cells are vulnerable to infection.

$$\begin{aligned}
 \dot{x} &= rxC_x(t)\left[1 - \frac{(x + y + z)}{K}\right] - x[d_x + \beta v + g(t)b\left(\frac{z}{x + y + z}\right)] \\
 \dot{y} &= \lambda yC_y(t)\left[1 - \frac{(x + y + z)}{K}\right] - y[d_y + g(t)b\left(\frac{z}{x + y + z}\right)] \\
 \dot{z} &= \beta xv + szC_z(t)\left[1 - \frac{(x + y + z)}{K}\right] - z[d_z + a + g(t)] \\
 \dot{v} &= kaz - v(u + \beta x)
 \end{aligned} \tag{3}$$

This model is similar to the previous non-susceptible naturally resistant cell model. The difference though, is that the Induced chemotherapy resistant cells now die out faster. They not only die of natural cell death d_z they also die out because of lysing do to the viral infection, or the lytic rate a . Also the virus now has a growth term, kaz , where k is the burst size, which reflects the amount of free virus that is released when the cell lyses.

3.2.2 Susceptible Naturally Resistant Cells

The next model also shows the dynamics of therapy given a replication competent delivery virus. However, not only are the chemotherapy sensitive cells

susceptible, but the naturally chemotherapy resistant cells are also susceptible to infection by the virus.

$$\begin{aligned}
\dot{x} &= rx C_x(t) \left[1 - \frac{(x+y+z)}{K} \right] - x \left[d_x + \beta v + g(t) b \left(\frac{z}{x+y+z} \right) \right] \\
\dot{y} &= \lambda y C_y(t) \left[1 - \frac{(x+y+z)}{K} \right] - y \left[d_y + \beta v + g(t) b \left(\frac{z}{x+y+z} \right) \right] \\
\dot{z} &= \beta x v + \beta y v + sz C_z(t) \left[1 - \frac{(x+y+z)}{K} \right] - z [d_z + a + g(t)] \\
\dot{v} &= kaz - v(u + \beta x + \beta y)
\end{aligned} \tag{4}$$

Once again the model is essentially the same as the previous susceptible naturally resistant cell model with a few differences. Again the induced chemotherapy resistant cells die out faster as a result of the extra death term, a , which represents the lytic rate.

These four models are all very similar however their differences have a large effect on the effectiveness of the therapy. In the remainder of this thesis we will analyze and try to optimize the therapy with respect to the models that we have created.

Chapter 4

ANALYSIS

Before beginning any kind of optimal control investigation we must break down the different dynamics of the model. It is important to know how the dynamics change when certain parameters within the system are altered. Various changes in the parameters will demonstrate the variety of situations that are biologically feasible in the system. Once we have a good feel for how the dynamics will change we will have a good idea of how to approach the problem from a control standpoint.

4.1 Chemotherapy Effect

4.1.1 No Chemotherapy Administered

The following plot in figure 1 shows the output of the model when no chemotherapy treatment is given.

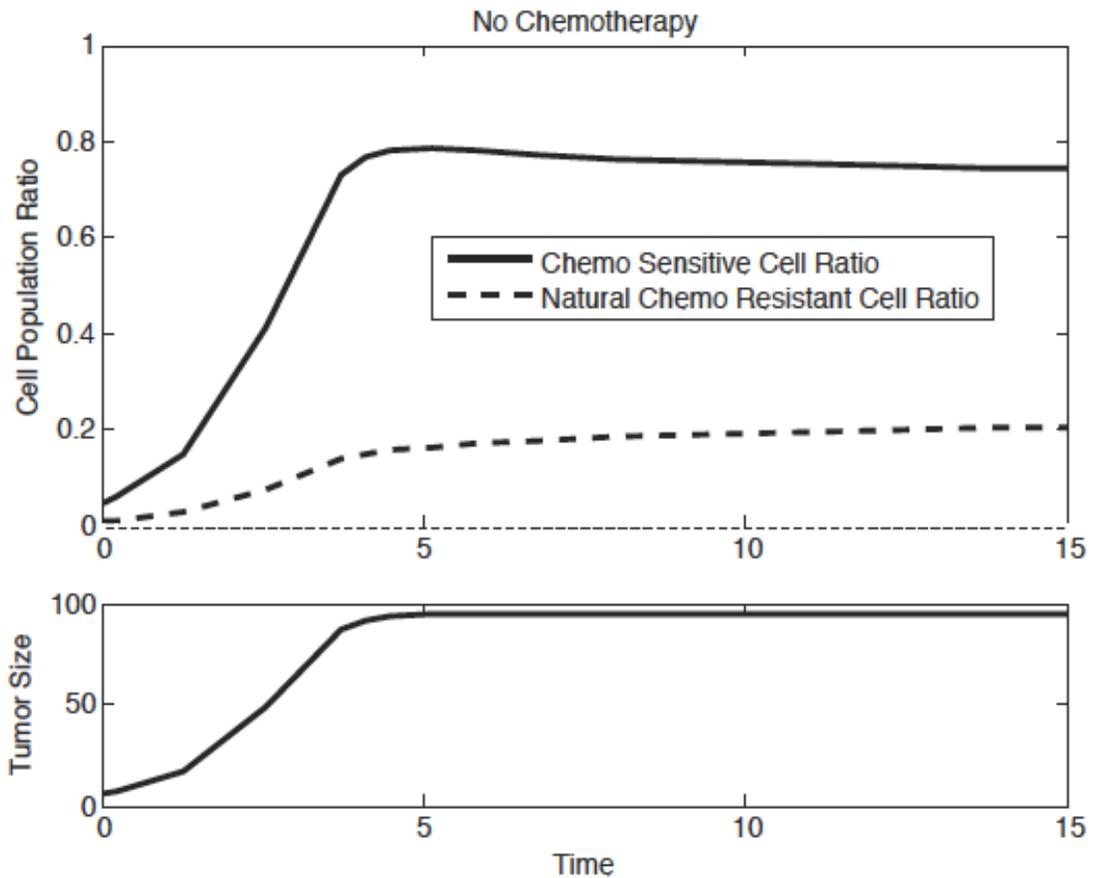


Figure 1 No Chemotherapy. With no chemotherapy treatment administered, $(C(t))$, the model produces a normal tumor growth plot. The y-axis of the upper part of the plot is cell population ratio characterized as the amount of each type of cell over the carrying capacity of the tumor. The y-axis of the lower portion of the plot is the tumor size characterized as the tumor size as a percent of the total carrying capacity. Parameters values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, and $C_x = C_y = C_z = 0$.

The plot shows the expected sigmoid growth curve as the tumor size increases towards 100 percent of its carrying capacity. The key aspect of this plot that we should note is the particular growth patterns of each type of cell, chemotherapy sensitive cells and natural chemotherapy resistant cells. The

chemotherapy sensitive cell ratio quickly increases as the tumor propagates while the natural chemotherapy resistant cell ratio rises much more slowly.

After around day five, the tumor has approached a significant portion of its carrying capacity and thus cannot increase much in total size anymore. At this point the chemotherapy sensitive cell ratio begins to decline slightly while the natural chemotherapy resistant cell ratio increases slowly. This is due to the fact that since the tumor can no longer grow due to lack of resources, the existing chemotherapy sensitive cells lose more and more of their apoptosis triggers and become chemotherapy resistant.

4.1.2 Chemotherapy Administered

After considering the situation where there is no chemotherapy applied in the model we show how the model reacts to a chemotherapy treatment.

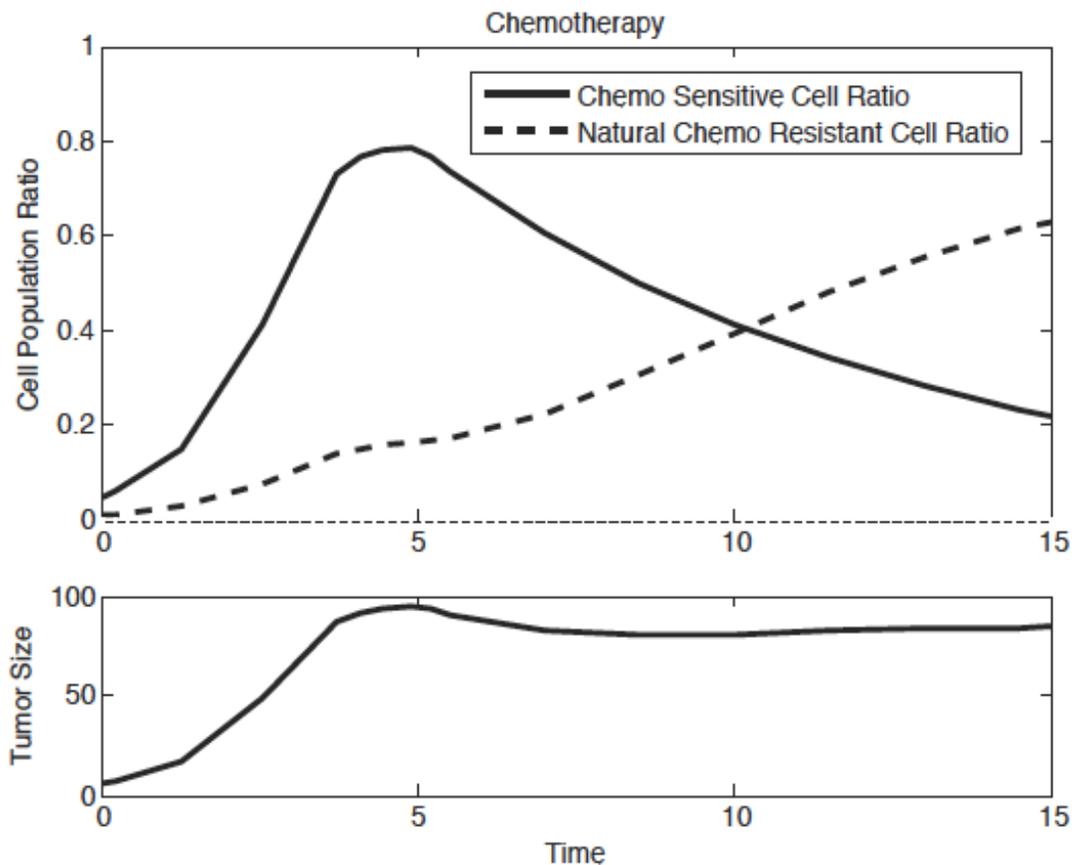


Figure 2 Chemotherapy. Chemotherapy is now administered at day five. All axes are the same as the previous figure. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, and $C_x = 0.05$ $C_y = C_z = 0.95$.

Similar to the figure 1, the total tumor size plot is shows the sigmoid growth pattern; however, there are some distinct differences after the initial growth period when the chemotherapy is delivered.

After the chemotherapy is delivered at day five the total tumor size dips a bit and then begins to recover. What happens here is that the chemotherapy has begun to kill off the chemotherapy sensitive cells and as a result the chemotherapy resistant cells now begin to dominate the cell population. This can be seen in the top portion of the plot.

This situation is the very reasons that many late stage cancers cannot be treated with chemotherapy and eradicated. Eventually after enough time the majority of the cells in the tumor will become chemotherapy resistant and then must be removed surgically. Usually, surgery is impossible because at late stage, the cancer has metastasized.

This plot demonstrates the need of some sort of intervention to restrict an event like this from happening during cancer treatment. Throughout the rest of this thesis we intend to introduce a dynamic therapy that can overcome this problem and show how using a robust optimal control approach can be applied to totally eradicate each tumor.

4.2 Fitness: Non-Replication Competent Delivery Virus

We have verified that our model behaves as we expect with respect to the chemotherapy sensitive cells and the natural chemotherapy resistant cells when chemotherapy is either administered or not administered. We must now extend the analysis of our model to include the delivery virus and in consequence the induced chemotherapy resistant cells. We will start by analyzing the different fitnesses of each type of cell after the tumor is inoculated with a non-replication competent delivery virus.

4.2.1 Natural Chemotherapy Resistant Cells More Fit

First, we will look at the case where the natural chemotherapy resistant cells are able to outcompete the induced chemotherapy resistant cells for resources and as a result should thrive after the chemotherapy sensitive cells have been wiped out by treatment.

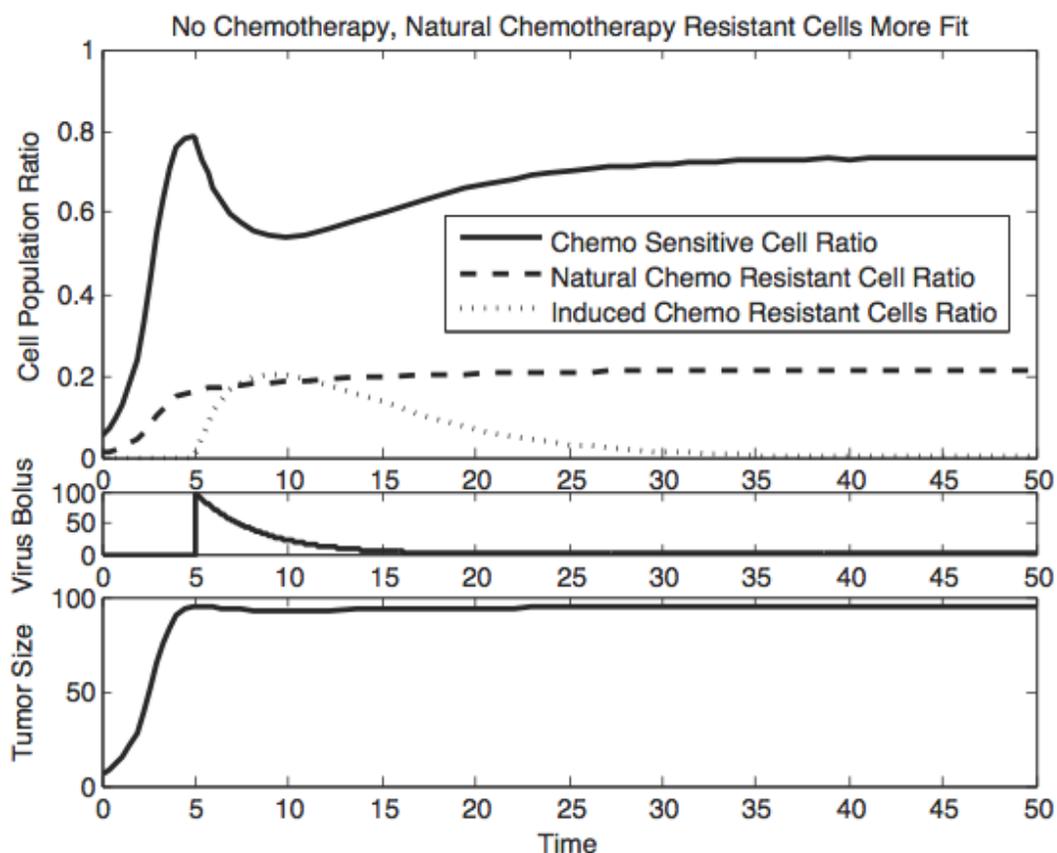


Figure 3 No Chemotherapy and Natural Chemotherapy Resistant Cells More Fit. No chemotherapy treatment and now a non-replication competent delivery virus is added at day five. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = C_y = C_z = 0$, $\lambda = 0.9$, and $s = 0.7$.

The plots in Figure 3 show a tumor allowed to grow almost until it reaches carrying capacity just before day five. At this point the tumor is approximately 80 percent chemotherapy sensitive cells and 20 percent natural chemotherapy resistant cells. At day five a virus bolus is given and the chemotherapy sensitive cell ratio begins to drop as the cells become infected and turn into induced chemotherapy sensitive cells. The natural chemotherapy resistant cell ratio slightly increases as a result and in time the chemotherapy sensitive cell ratio recovers

because they are significantly more fit without the introduction of chemotherapy treatment. The Induced chemotherapy resistant cells then die out.

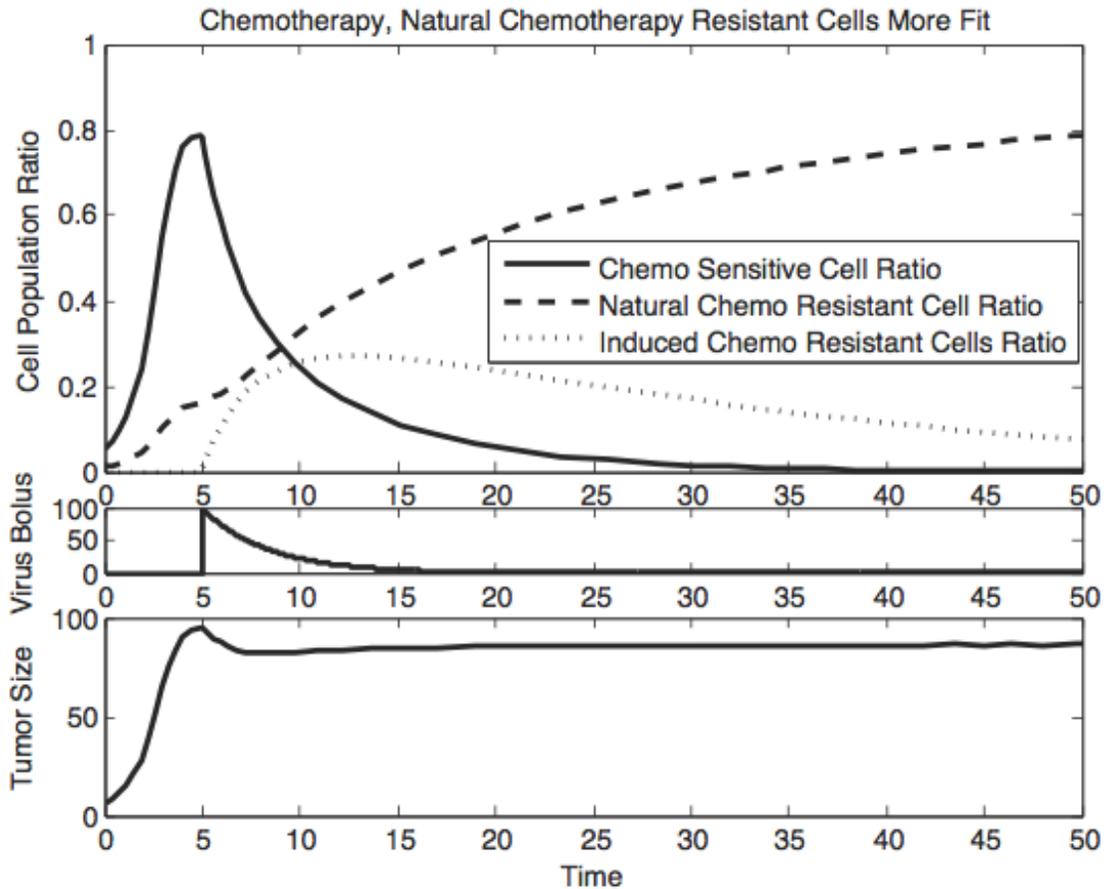


Figure 4 Chemotherapy and Natural Chemotherapy Resistant Cells More Fit. Chemotherapy treatment and non-replication competent delivery virus are both delivered at day five. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = 0.05$ $C_y = C_z = 0.95$, $\lambda = 0.9$, and $s = 0.7$.

The plots in figure 4 show a similar situation as figure 3 however, now chemotherapy treatment is given at the same time as the virus bolus. Consequently, the chemotherapy sensitive cells totally die out. Because the natural chemotherapy resistant cells are more fit than the induced chemotherapy resistant cells, their cell

ratio slowly increases toward carrying capacity. The induced chemotherapy resistant cell ratio slowly decreases.

This result is what we wish to prevent because it will lead to total therapy failure as shown later. This happens because we have no way of eliminating the natural chemotherapy resistant cells without the presence of a significant amount of induced chemotherapy cells, via the bystander effect.

4.2.2 Induced Chemotherapy Resistant Cells More Fit

Next we will consider the condition where the induced chemotherapy cells are more fit after chemotherapy is introduced. In this case we would expect to see that after chemotherapy treatment the induced chemotherapy resistant cells would thrive and be the prevalent cell type in the tumor.

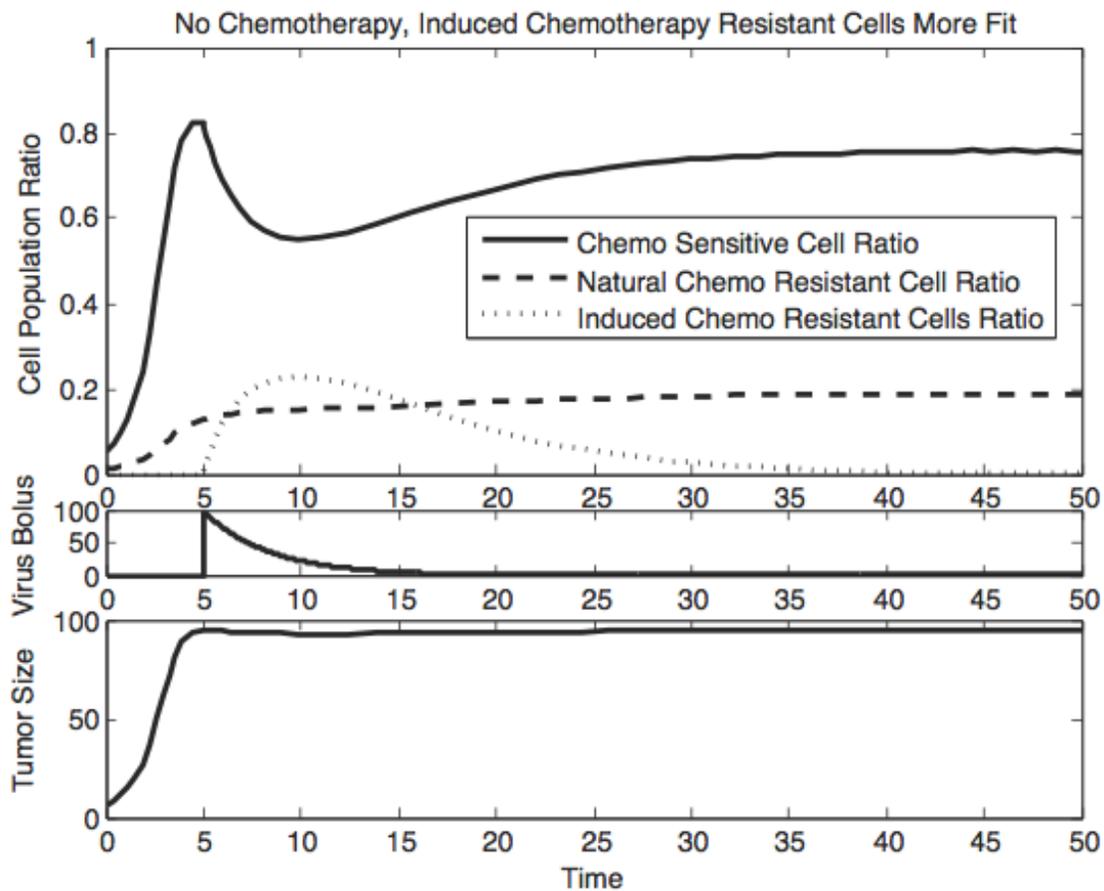


Figure 5 No Chemotherapy and Induced Chemotherapy Resistant Cells More Fit. No chemotherapy treatment and now a non-replication competent delivery virus is added at day five. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = C_y = C_z = 0$, $\lambda = 0.7$, and $s = 0.9$.

The plots in figure 5 are similar to the plots in figure 3, the tumor is allowed to grow approaching carrying capacity then a virus bolus is introduced at day five, only this time because the induced chemo therapy resistant cells are more fit, they have a higher peak and they die out slower as the chemotherapy sensitive cells recover.

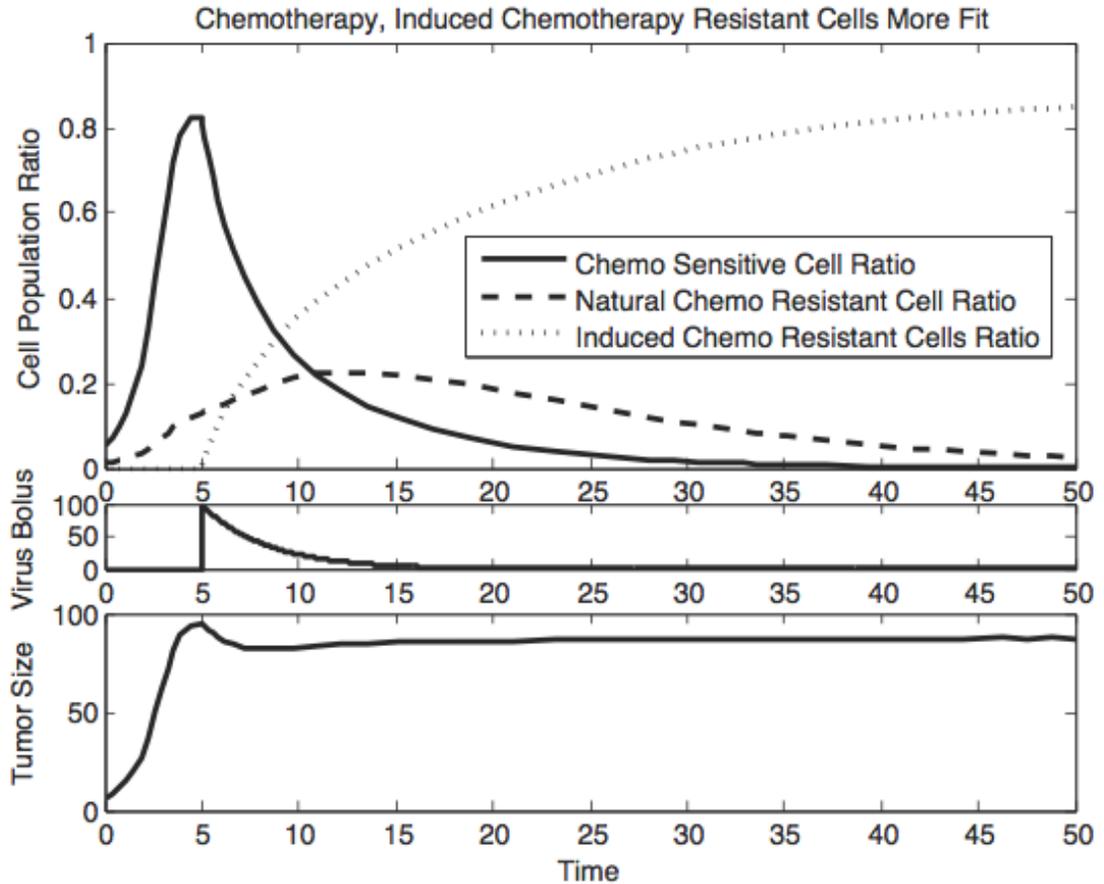


Figure 6 Chemotherapy and Induced Chemotherapy Resistant Cells More Fit. Chemotherapy treatment and non-replication competent delivery virus are both delivered at day five. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = 0.05$ $C_y = C_z = 0.95$, $\lambda = 0.7$, and $s = 0.9$.

Figure 6 illustrates the effect if the chemotherapy is given at the same time as the virus bolus. In these plots, as the chemotherapy sensitive cells die out as a result of the chemotherapy treatment, the induced chemotherapy resistant cell ratio grows towards carrying capacity and natural chemotherapy resistant cell ratio slowly declines. This is the favorable result of therapy after the chemotherapy is administered. Since we are left with just induced chemotherapy resistant cells, we should be able to totally eradicate the tumor after ganciclovir treatment.

Note that in the case where the naturally chemotherapy resistant cells were more fit, the outcome would lead to treatment failure if there were no intervention. A way that this situation could be modified to lead to successful therapy would be through the use of multiple virus boluses. In figure 3, the induced chemotherapy resistant cell ratio increased rapidly at first. This is due to the many free viruses around to infect the chemotherapy resistant cells. After the free virus population declined the induced chemotherapy cell ratio peaked and then declined as well.

We intend to implement an optimal controller to see what would be the optimal virus bolus pattern so that we would be able achieve a quick successful therapy even in the event that the induced chemotherapy resistant cells are not as fit.

4.3 Fitness: Replication Competent Delivery Virus

In the previous section we examined the dynamics of our model after the introduction of a non-replication competent delivery virus. In this section we will observe what happens if the delivery virus is able to produce progeny and hence create more virus and infect more cells.

In that we are now dealing with a replication competent virus, a virus that has different properties than the non-replication competent form, we elected to categorize them into one of two categories, high and low virulence. A virus with high virulence has a high infection rate and a high lytic rate. It will spread quickly throughout the tumor and create a lot of damage to the cells that it infects. The downside is that because it spreads so quickly and kills off all the cells that it can

infect, it does not allow the induced chemotherapy resistant cells to become adequately spread in the tumor.

A virus with low virulence has a lower infection rate and a lower lytic rate. This type of virus spreads slowly and does not do as much damage to the host cell. As a result this type of virus enables the induced chemotherapy resistant cells to spread throughout, and become the dominant cell type in the tumor.

	Infection Rate (β)	Lytic Rate (a)
High Virulence (Figure 5)	0.0025	0.35
Very High Virulence (Figure 6)	0.004	0.56
Low Virulence (Figure7)	0.0018	0.252
Very Low Virulence (Figure 8)	0.0006	0.084

Table 2 Infection and Lytic Rates Vs Virulence.

The table above shows the different types of viruses and their infection and lytic rates that are used in the following figures that show how viruses with different virus virulence act on the tumor.

4.3.1 High Virulence Delivery Virus

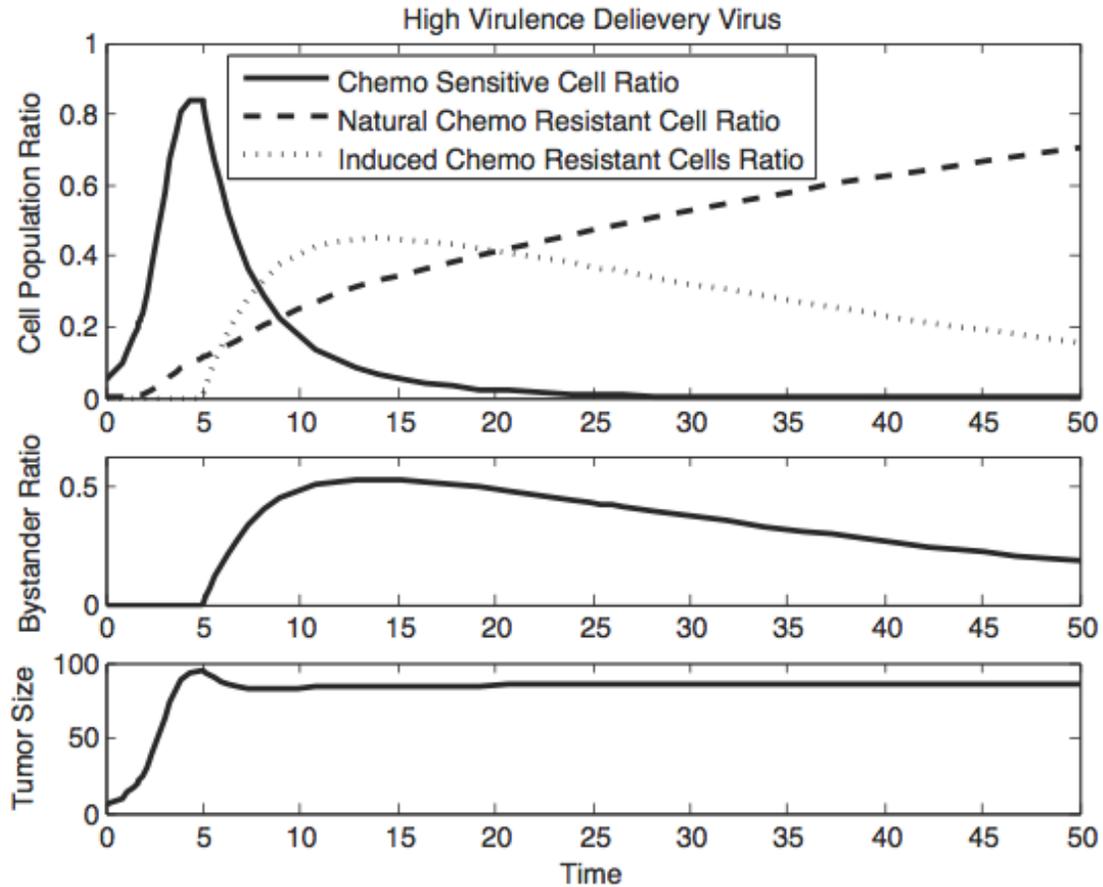


Figure 7 High Virulence Delivery Virus. A high virulence delivery virus is given in bolus form at day five. Parameter values: $\beta = .0025$ and $a = 0.35$

The plots in figure 7 show the dynamics of each cell type when a high virulence virus is used. As expected the virus spread quickly and the induced chemotherapy resistant cell ratio increases rapidly and then begins to decline slowly while the natural chemotherapy resistant cell ratio steady increases. This outcome would lead to treatment failure, as the natural chemotherapy resistant cells cannot be removed.

Another key thing to note about these figures is the bystander ratio. This is the plot of the ratio of induced chemotherapy resistant cells over the total cell population as it changes through time. This is important because in order for our ganciclovir treatment to be successful during the negative selection portion of treatment the bystander ratio must be sufficiently high. A declining bystander ratio like the one in figure 7 will indicate that the ganciclovir treatment will be less effective as time goes on.

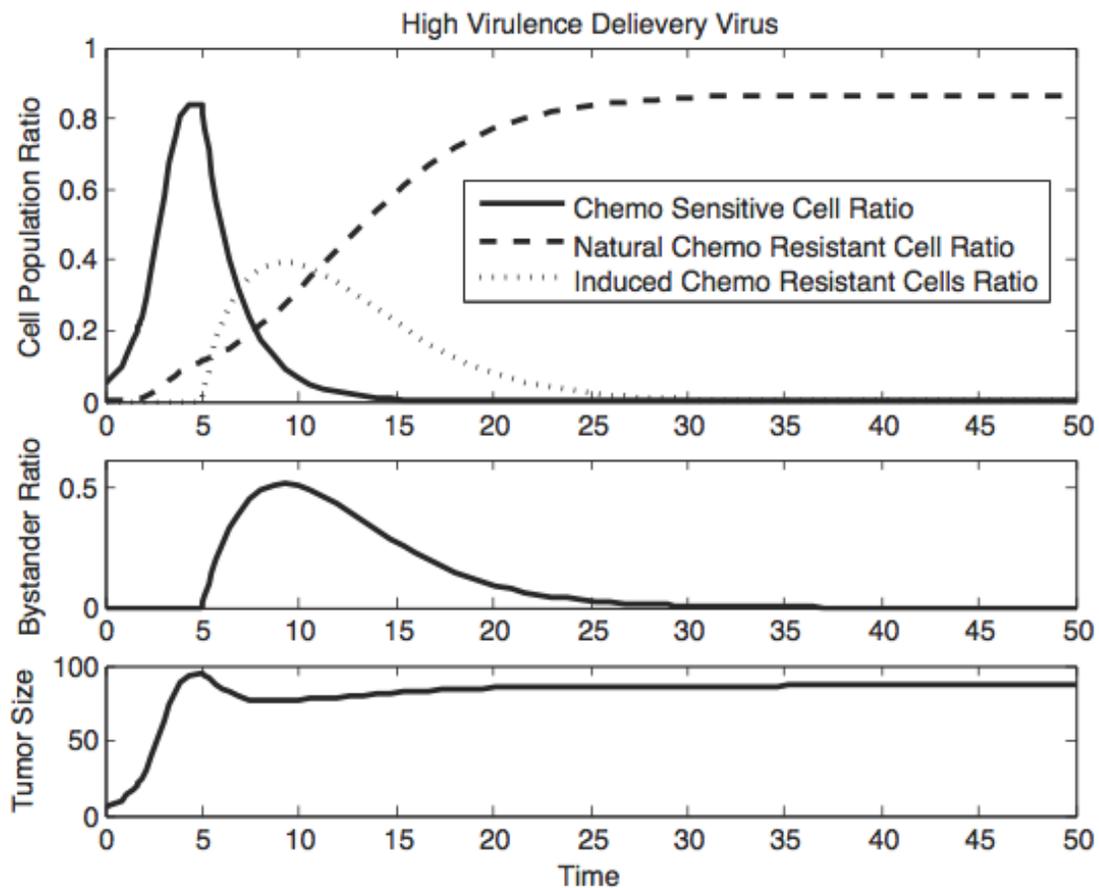


Figure 8 Very High Virulence Delivery Virus. A higher virulence delivery virus is given in bolus form at day five. Parameter values: $\beta = .004$ and $a = 0.56$

The plots in figure 8 show the dynamics of another high virulence delivery virus; however, the delivery virus is even more virulent this time. The virus quickly infects again and the induced chemotherapy cell ratio increases rapidly, but this time it declines much faster and the natural resistant chemotherapy cell ratio grows faster. In this figure the bystander ratio rises and falls very quickly indicating that there is an even smaller window for successful therapy.

4.3.2 Low Virulence Delivery Virus

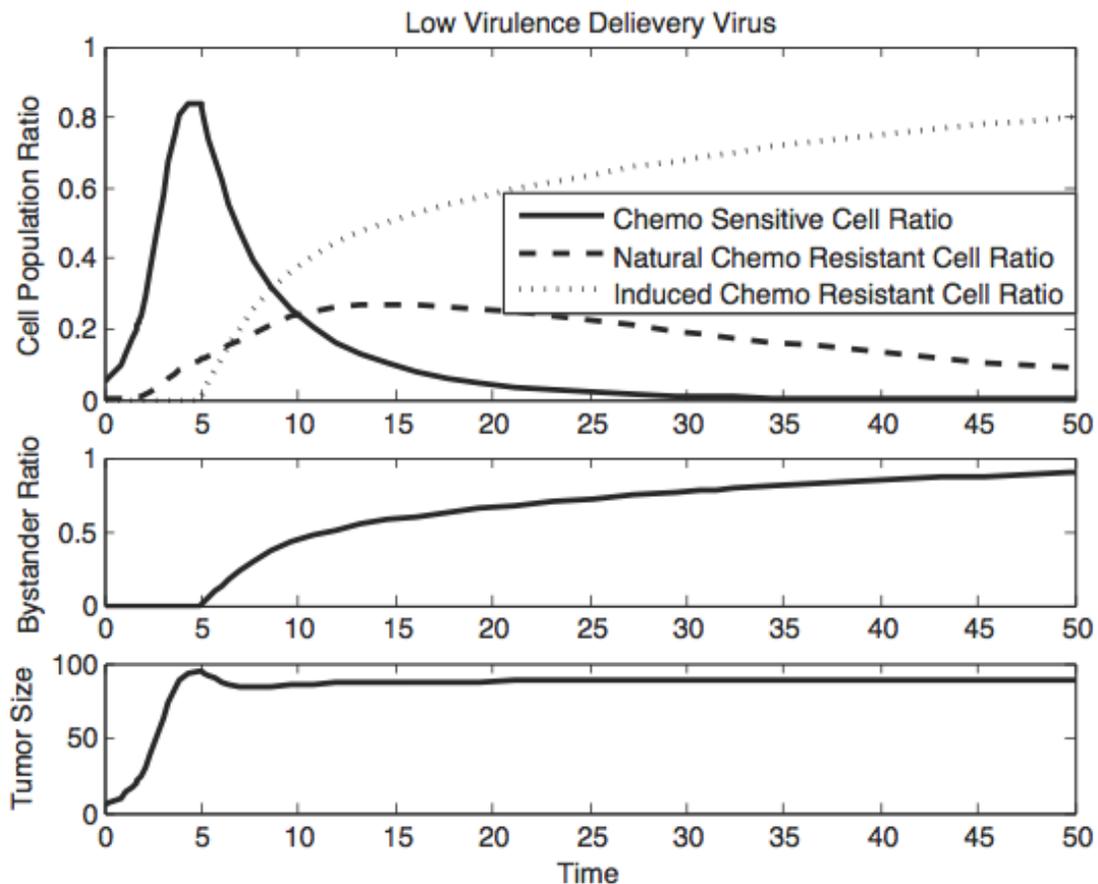


Figure 9 Low Virulence Delivery Virus. A low virulence delivery virus is given in bolus form at day five. Parameter values: $\beta = .0018$ and $a = 0.252$.

The dynamics of the system using a low virulence virus is shown in figure 9. The most noticeable difference is that this time the virus does not spread as quick and the induced chemotherapy resistant cell ratio slowly grows throughout the whole tumor. The natural resistant cell ratio rises initially then slowly declines.

The bystander ratio plot indicates that as time goes on successful treatment with the ganciclovir becomes more and more likely as the natural chemo resistant cells take over and become the most prevailing cells in the tumor.

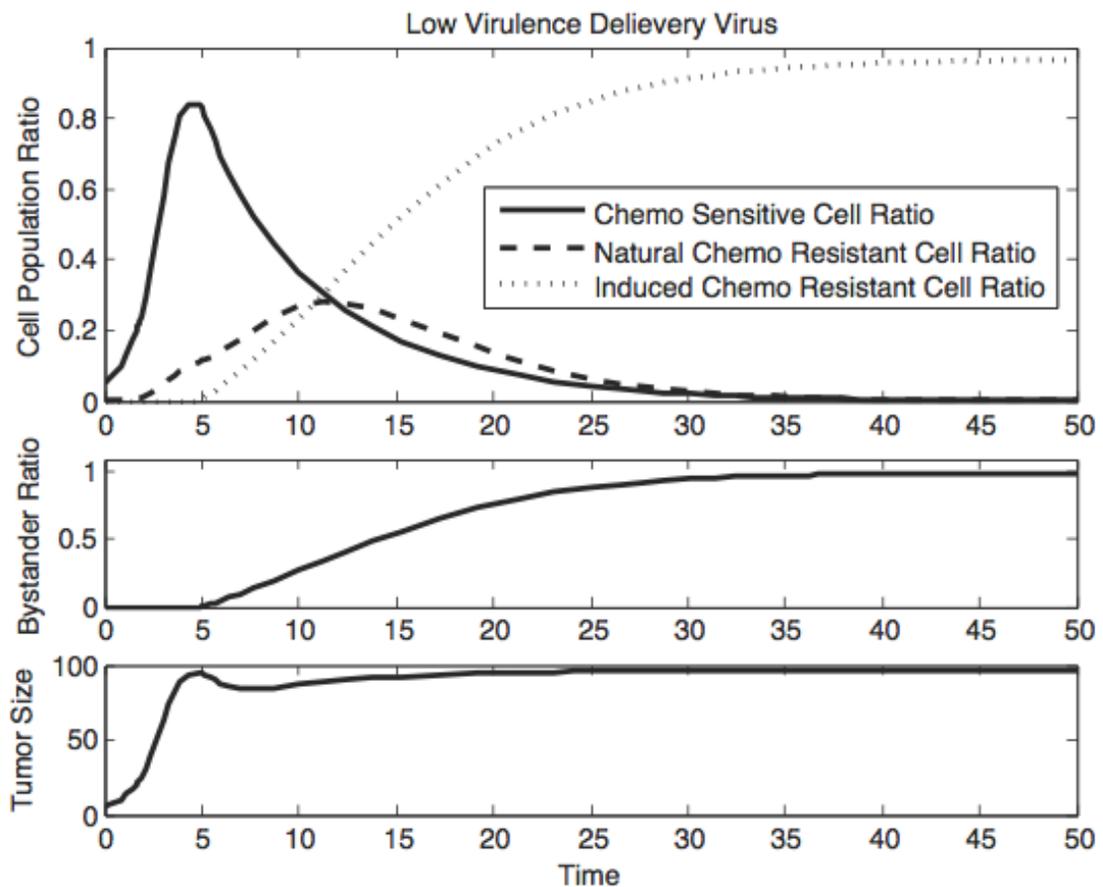


Figure 10 Very Low Virulence Delivery Virus. A lower virulence delivery virus is given in bolus form at day five. Parameter values: $\beta = .0006$ and $a = 0.084$.

Figure 10, shows the dynamics of the system when an even less virulence virus is used. The virus spreads even slower and as a result the induced chemotherapy cell ratio grows at a slow rate showing the familiar sigmoid growth curve eventually becoming even more prevalent in the tumor. The bystander ratio again shows that as time goes on successful ganciclovir treatment becomes more and more likely to be successful.

4.4 Ganciclovir Timing: Treatment Failure vs. Treatment Success

As we have seen in the previous sections the tumor does not propagate in any sort of predetermined manner. In fact, there are many factors that determine what cell type becomes dominant in the tumor. There are several ways in which the tumor could grow that would lead to treatment failure; however, the aim of the treatment is to force the tumor to grow, through the period of positive selection followed by negative selection, to achieve treatment success.

Even if we manage to achieve the type of growth patterns that we desire through the positive selection phase of the treatment, total treatment success is still not guaranteed. Proper timing of the negative selection phase, the ganciclovir delivery, is vital. Too early or too late and the treatment may fail.

4.4.1 Treatment Failure

The following figures show the outcome of treatment for three different scenarios when the ganciclovir is administered after a high virulence delivery virus has been given, similar to the virus in figure 7.

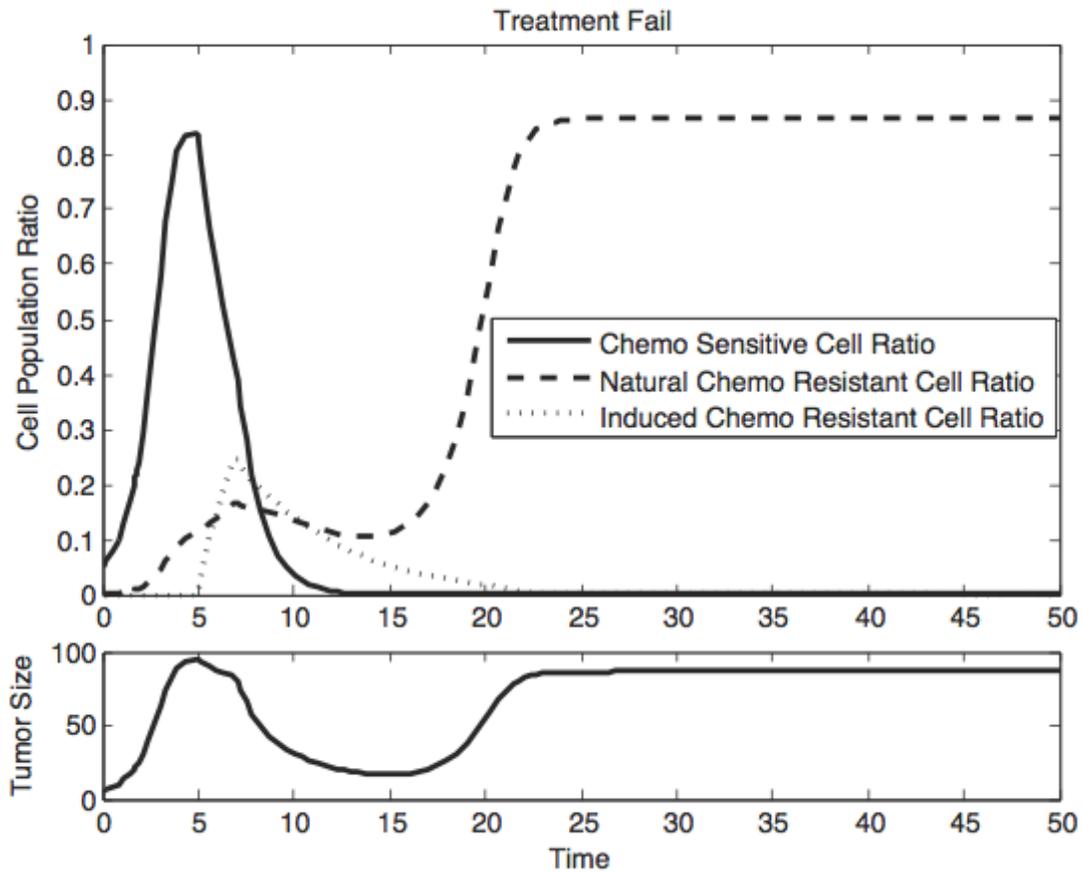


Figure 11 Early Treatment Failure. Treatment failed because ganciclovir was administered too early at day seven. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = 0.05$ $C_y = C_z = 0.95$, $\beta = .0025$ and $a = 0.35$, $g = 0.7$

The first scenario as conveyed in figure 11 is the situation where the negative selection process what started too early, the ganciclovir was delivered prematurely. Here the ganciclovir was administered at day 7 leading to overall treatment failure as natural chemotherapy resistant cells grow to become the dominate cell type as a result.

Treatment has failed in this scenario because the induced chemotherapy resistant cells have not been given enough time to spread; therefore, the bystander

ratio is not sufficient to produce a bystander effect that would eliminate the natural chemotherapy resistant cells.

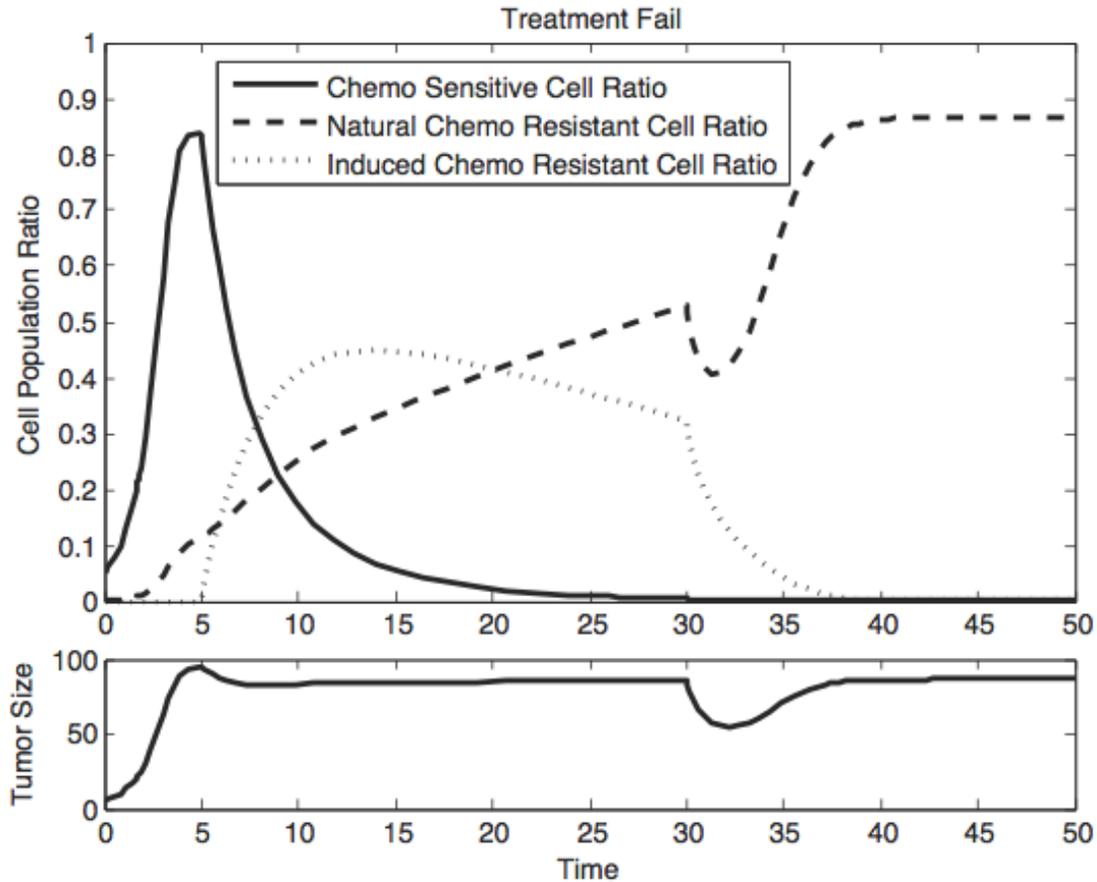


Figure 12 Late Treatment Failure. Treatment failed because ganciclovir was administered too late at day 30. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = 0.05$ $C_y = C_z = 0.95$, $\beta = .0025$ and $a = 0.35$, $g = 0.7$

The scenario expressed in figure 12, shows the circumstance where the negative selection process was begun too late. Here the ganciclovir was delivered too late which once again led to overall treatment failure as the natural chemotherapy resistant cells grow dominant in the tumor.

Treatment has failed in this scenario because the induced chemotherapy resistant cells have been allowed to peak and then begin to decline while the natural chemotherapy resistant cells have been allowed to continue growing before the negative selection process is begun.

4.4.2 Treatment Success

The last scenario, articulated in figure 13, is treatment success. Here the ganciclovir was delivered at a correct time for treatment success.

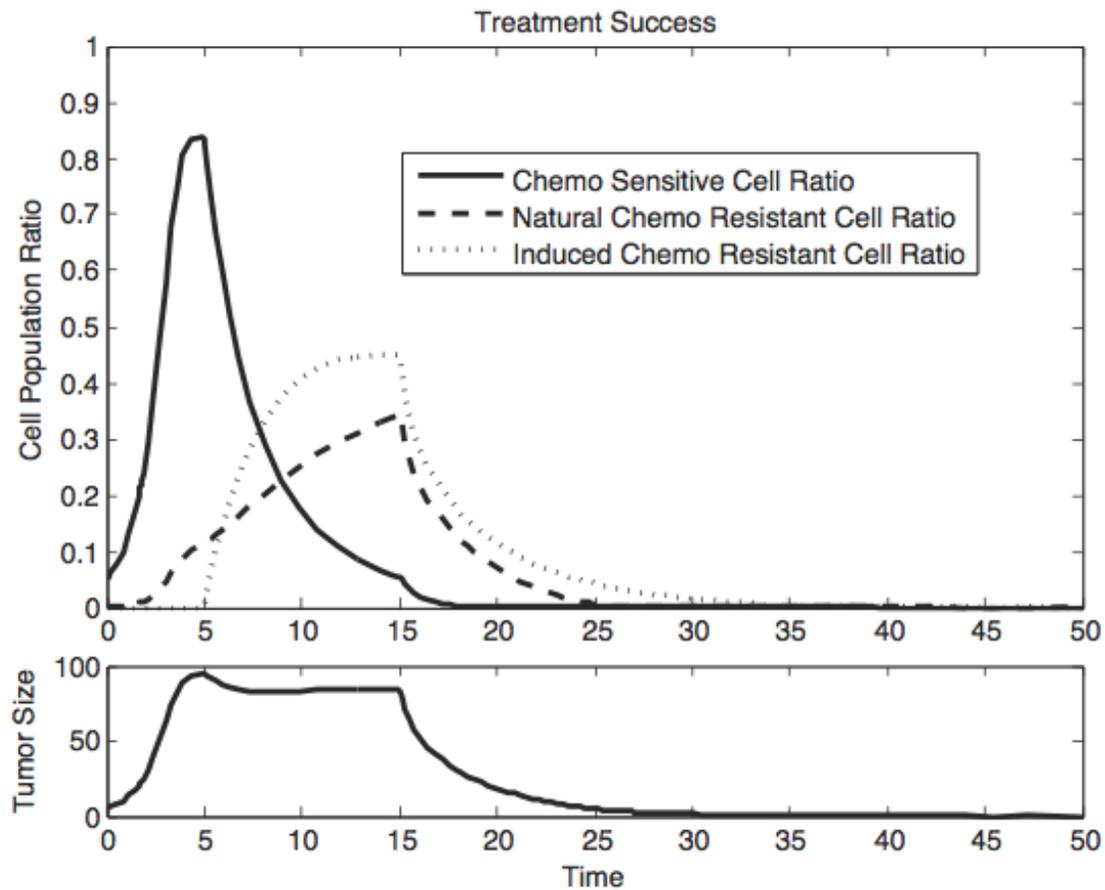


Figure 13 Treatment Success. Dynamic therapy treatment was successful due to effective ganciclovir delivery timing. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = 0.05$, $C_y = C_z = 0.95$, $\beta = .0025$ and $a = 0.35$, $g = 0.7$

Treatment has been achieved in this scenario because the negative selection process was begun during the window of opportunity that ensures successful treatment. This window exists around the peak of the bystander ratio plot shown in

figure 7. For this particular situation the ganciclovir was delivered at day 15 that corresponds to a point just after the peak on the bystander ratio plot from figure 7.

Chapter 5

CONTROL ANALYSIS

The proposed dynamic cancer treatment of having a period of positive selection followed by a period of negative selection seems straightforward. In fact for certain parameters treatment success is often achieved without any problems; however, there are many situations where the parameters are such that a solution that would yield successful treatment is not trivial.

In this case we must consider another approach in attempt to accomplish our goal of totally eradicating the tumor. Most of the parameters that make up the system are set by nature and thus out of our control; nevertheless, there are a few parameters that we can vary to our benefit.

From the small number of parameters that we can control we will select two in particular to utilize for our research and analysis in this thesis: 1, when and how often the chemotherapy is administered, and for what time duration and 2, when and how often the virus boluses are provided. We will exploit these parameters to design an optimal controller that will give the best outcome of therapy under any given circumstance.

5.1 Optimal Chemotherapy Control

In our previous analysis of the dynamics during treatment we assumed that the chemotherapy was given at the same time that the virus bolus was introduced, once the tumor reached carrying capacity. The chemotherapy then continued throughout the duration of dynamic therapy, in the course of both the positive and

the negative selection periods. Because the distribution of the chemotherapy is something that we can control over time, we begin to wonder if there is another method that will yield a superior result.

As stated before, one of the key features to the success of the therapy is the bystander ratio.

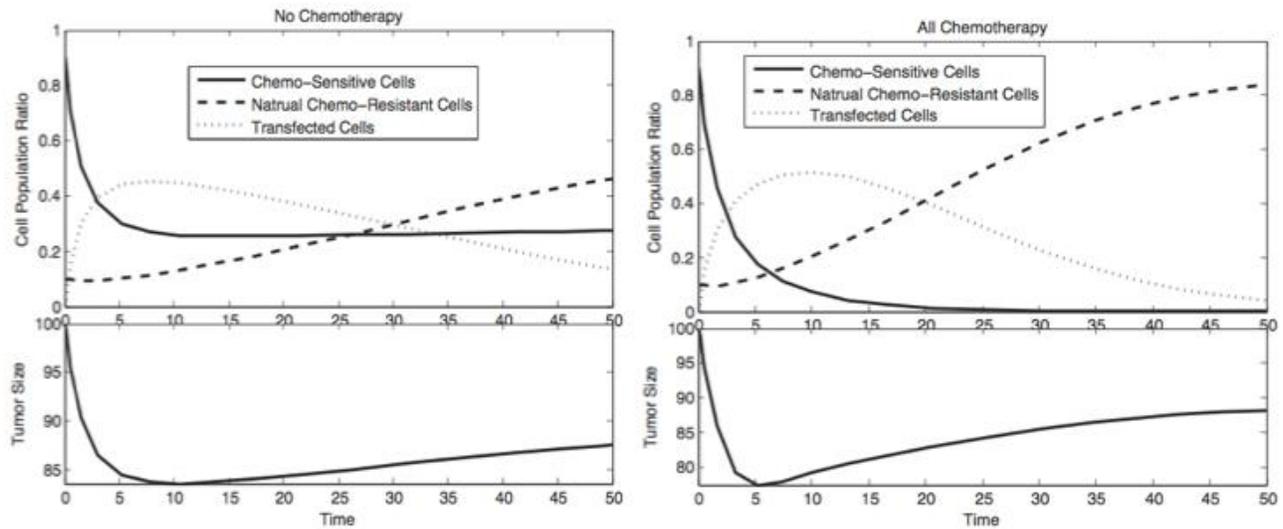
$$\frac{z(t)}{x(t) + y(t) + z(t)}$$

We intend to take advantage of our ability to change the chemotherapy timing as a way to design an optimal controller that will maximize our cost function, the bystander ratio. By choosing the bystander ratio as our cost function that we wish to maximize we will be able to ensure that we have the best chance of success during the negative selection, ganciclovir delivery, phase. This process is denoted by

$$\max_{t \in [t, T_{Final}], C_x \in \mathcal{C}} \frac{z(t)}{x(t) + y(t) + z(t)}$$

Where C is the chemotherapy that is applied to all of the cells ($C_x(t)$ $C_y(t)$ $C_z(t)$), which we will alter in time to maximize our cost function. In order to create the open loop controller to find the optimal chemotherapy pulse schedule we first limited the search space by discretizing the possible switching times. We set up 10

discrete regions where the chemotherapy could be either “on” or “off.” This allowed the open loop controller to cycle through 1024 possibilities to find the optimal schedule.



(A)

(B)

Figure 14 No Control. The plots show the situation where the virus is administered and no controller applied for the chemotherapy. Parameter values: $x_0 = 90$, $y_0 = 10$, $z_0 = 0$, $v_0 = 100$, $C_x = 0.05$, $C_y = C_z = 0.95$, $\beta = .004$ and $a = 0.2$

The plots above, in figure 14, assume that the tumor has been allowed to grow sufficiently such that it has reached its maximum carrying capacity when the virus is given. The dynamics of figure 14A show the simple case where the virus is provided and allowed to so spread throughout the tumor with no chemotherapy treatment present. The dynamics of figure 14B show the complete opposite case where the virus is given at the same time that the treatment is began.

It is clear that the dynamics in figure 14B, where the chemotherapy was applied the whole time, offers a better chance for success than the dynamics of figure 14A where no chemotherapy treatment was given. The transfected cells were able to gain a slightly higher prevalence, with the chemotherapy treatment present, before their population began to die out and the natural resistant cells took over the tumor. This means that the bystander ratio would slightly higher for that case, which would give a higher probability for successful therapy when the ganciclovir was distributed.

The next question is if there is a better option that will give a greater chance for successful therapy. We believe that some combination of these two will yield the best dynamics for successful treatment by means of some form of pulsed chemotherapy treatment. By employing a pulsed technique we should be able to take advantage of the natural competitive nature of the different type of cells to optimize the bystander ratio.

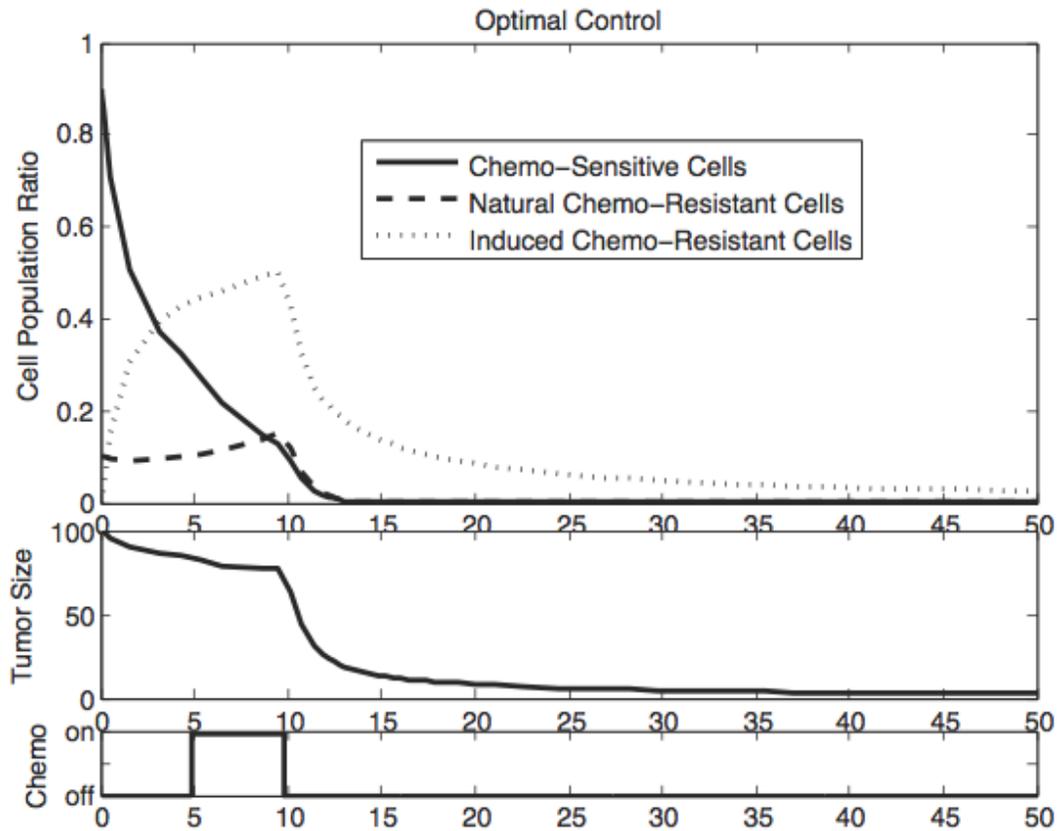


Figure 15 Optimal Chemotherapy Control. Parameter values: $x_0 = 90$, $y_0 = 10$, $z_0 = 0$, $v_0 = 100$, $C_x = 0.05$, $C_y = C_z = 0.95$, $g = 0.7$, $\beta = .004$ and $a = 0.2$

The plots in figure 15 show the result of running our optimal chemotherapy control on the system. The system has the same parameters and initial conditions as the plots in figure 14. The only difference is that here the optimal control has found that a single pulse from day 5 to 10 would maximize the bystander ratio for this particular parameter set and initial conditions. Also, another function of the optimal controller is to apply the ganciclovir treatment at the point when the bystander ratio is maximized.

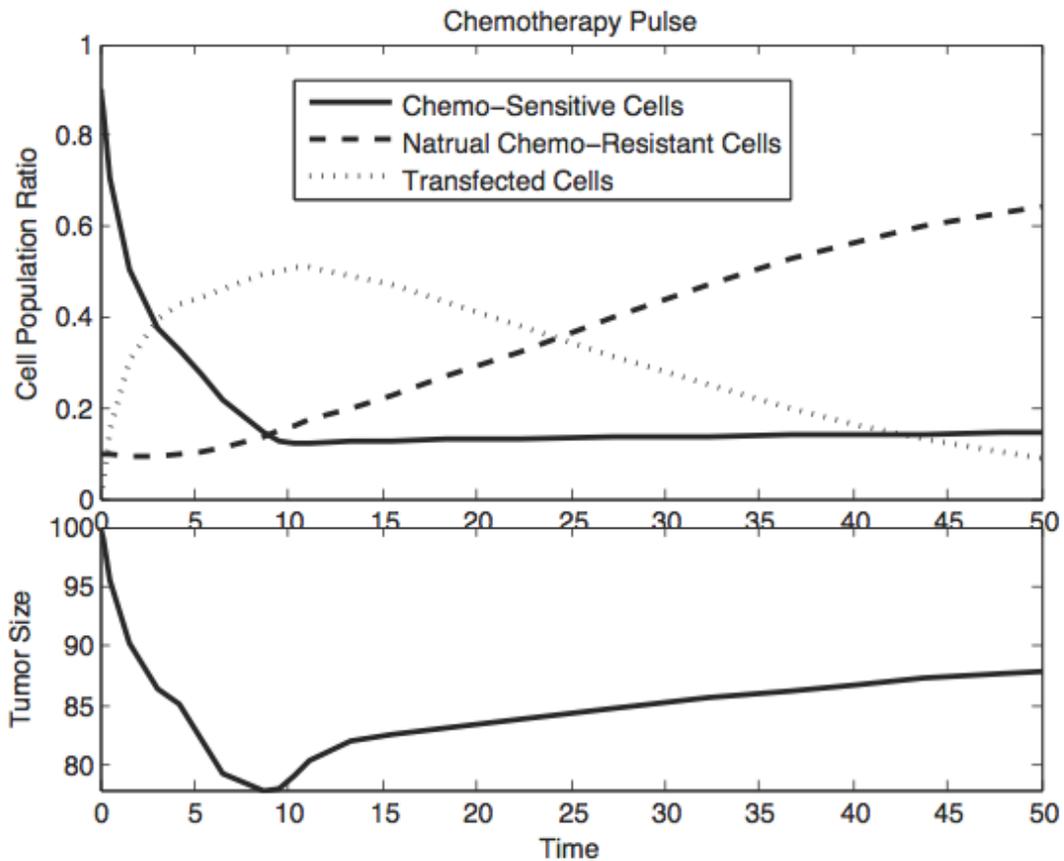


Figure 16 Chemotherapy Pulse With No Ganciclovir Treatment.
 Parameter values: $x_0 = 90$, $y_0 = 10$, $z_0 = 0$, $v_0 = 100$, $C_x = 0.05$, $C_y = C_z = 0.95$, $\beta = .004$ and $a = 0.2$

In figure 16 there is a chemotherapy pulse from day 5 to day 10 as in the optimal control case, except there is ganciclovir treatment. Comparing the plots in figure 16 with the plots from figure 14, a somewhat linear increase can be noticed in the transfected cells during the chemotherapy pulse. This behavior is exactly what allows the bystander ratio to be maximized.

During the period of pulsed treatment the dynamics are switched. As a result the transfected cells become more fit than the other cells and begin to grow at a

faster rate than in the case when there was no chemotherapy given or when chemotherapy was administered for the whole time.

The dynamic cancer therapy seeks to take advantage of the natural competition of the different cell types and with the use of the optimal controller it is possible to make a specific cell type more fit during certain periods of time to use as an advantage. It is evident from figure 15 that the optimal controller was able to achieve successful therapy with the total tumor size decreasing to zero after the ganciclovir treatment; however, another important detail of the optimal therapy is that the chemotherapy dose is kept to a minimum. Since treatment with chemotherapy often has negative side effects, the fact that this treatment can significantly decrease the dose shows great promise in the field of oncology.

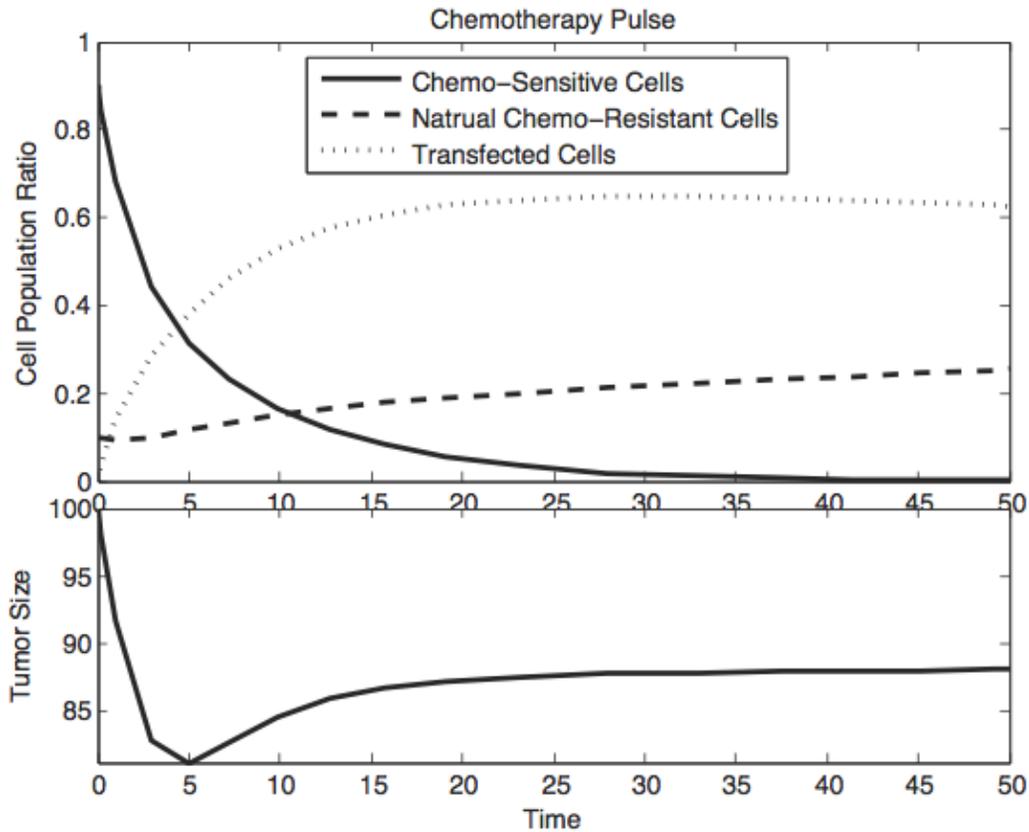


Figure 17 Chemotherapy Applied and Natural Chemotherapy Resistant Cells More Fit. Parameter values: $x_0 = 90$, $y_0 = 10$, $z_0 = 0$, $v_0 = 100$, $C_x = 0.05$ $C_y = C_z = 0.95$, $\beta = .0022$ and $a = 0.11$

The plots in figure 17 show a different case from the previous figures. Here, once again chemotherapy is being applied for the whole time starting when the virus is given. In this case however, the natural chemotherapy resistant cells are slightly less fit. None the less, they will still become dominant in the long run, as the transfected cells peak and then slowly start to decrease while the natural chemotherapy resistant cells continually increase. There is a much bigger window for success in this case, although if treatment is given too early there will be a total failure because all of the chemotherapy sensitive cells die out early. It turns out that

the optimal control solution to this problem is an especially interesting and nontrivial one.

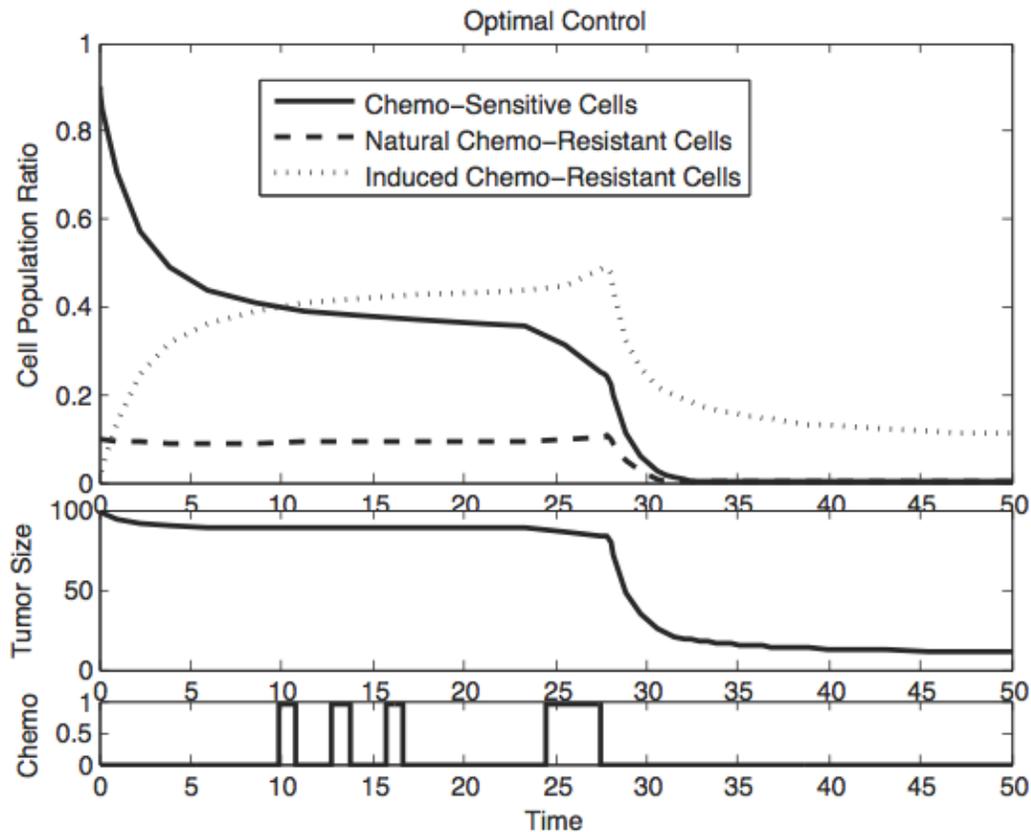


Figure 18 Optimal Control Solution. Parameter values: $x_0 = 90$, $y_0 = 10$, $z_0 = 0$, $v_0 = 100$, $C_x = 0.05$, $C_y = C_z = 0.95$, $g = 0.7$, $\beta = .0022$ and $a = 0.11$

The plots in figure 18 show the optimal chemotherapy control solution the case from figure 17. The solution is unique in that it requires three small chemotherapy pulses followed by a longer chemotherapy pulse and then treatment with the ganciclovir. Administering the chemotherapy in this sequence has some

interesting characteristics. Initially, as in figure 17, natural chemotherapy resistant cells gained a slight fitness advantage as time continues but this treatment schedule prevents them from achieving dominance. This happens because the chemotherapy sensitive cells are not killed right away by a large chemotherapy dose. Instead the chemotherapy is given in small increments, which suppresses the ability for the natural chemotherapy resistant cells to expand. The virus then has more target cells available to infect in the long run and is able to spread until the final chemotherapy pulse causes the chemotherapy sensitive cell population to crash.

5.2 Virus Bolus Control

The second method that we wish to exploit in order to control the outcome of the therapy is the use of the virus bolus. In the instances where a non-replication competent virus is used the dynamics are altered slightly. Since the virus cannot replicate in this situation, the transfected cells often do not become prevalent enough just from the initial bolus injection.

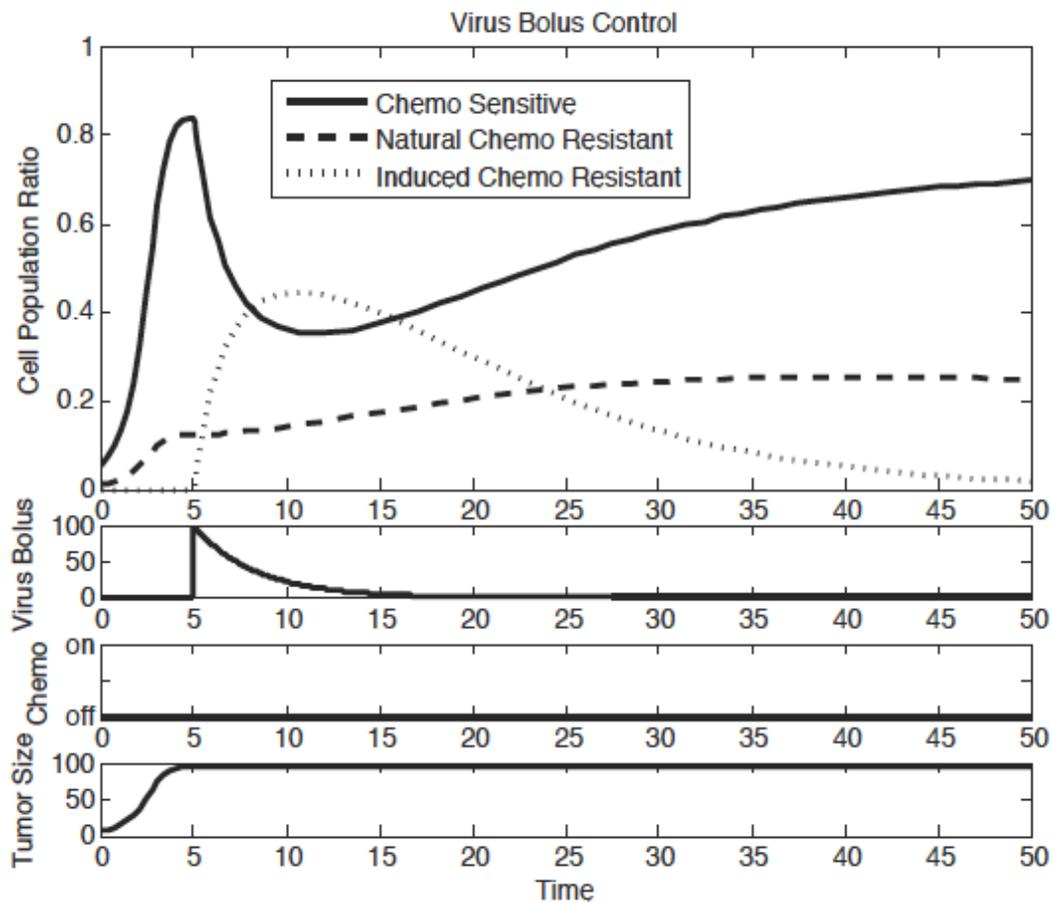


Figure 19 Virus Bolus Control: Single Virus Bolus. Single virus bolus when tumor reaches carrying capacity at day 5 Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $\beta = .004$ and $a = 0.2$

In figure 19 we see the general case, similar to the previous plots of the dynamics for a non-replication competent virus. Here the tumor is allowed to grow to carrying capacity and is then subjected to the virus bolus. After the virus bolus is given the induced cell population rises and chemo-sensitive cell population falls as a result of infection. The virus population declines exponentially to zero and the induced chemotherapy resistant cells soon follow since there are no more virus around to infect the chemotherapy sensitive cells.

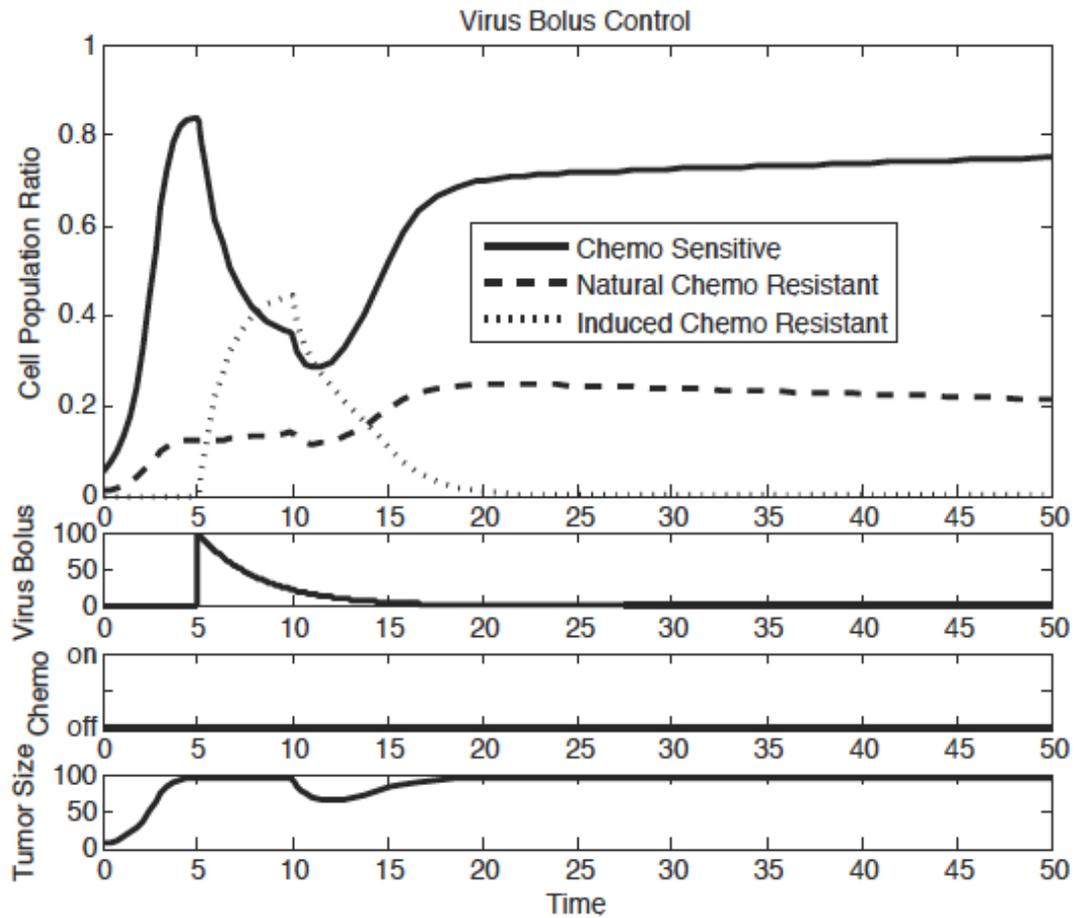


Figure 20 Virus Bolus Control: Early Ganciclovir Treatment. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $g = 0.7$, $\beta = .004$ and $a = 0.2$

Upon treatment of the previously discussed tumor with the ganciclovir, we can see that the therapy is not successful in figure 20. It is not complete treatment failure since the chemotherapy sensitive cells are able to rebound after the ganciclovir treatment.

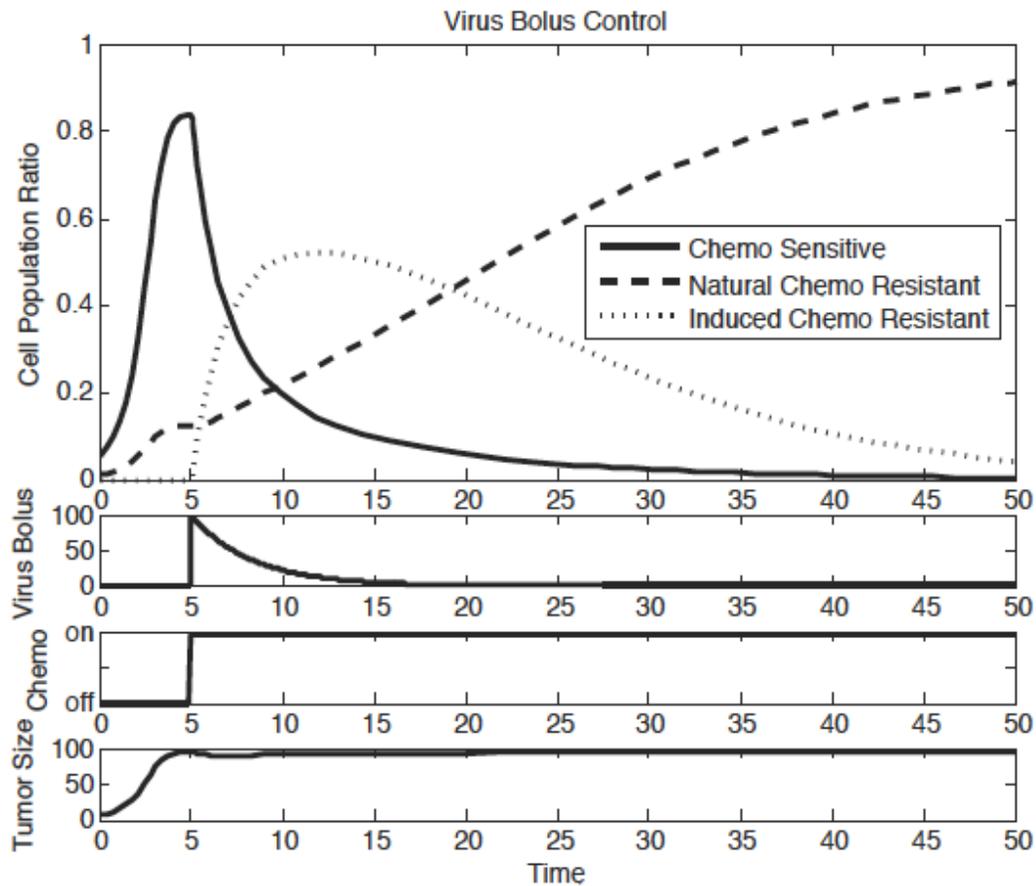


Figure 21 Virus Bolus Control. Chemotherapy is Administered. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 100$, $C_x = 0.05$ $C_y = C_z = 0.95$, $\beta = .004$ and $a = 0.2$

If instead of treating with the ganciclovir, chemotherapy is provided at the same time as the virus the dynamics change significantly. In figure 21, it can be seen that because the chemotherapy is given, the chemotherapy sensitive cells die out and as a result the natural chemotherapy resistant cells begin to dominate. There is a very small window around day 10 where treatment with ganciclovir might yield successful therapy but if that opportunity is missed then treatment will be a failure because the induced chemo therapy resistant cells will die out.

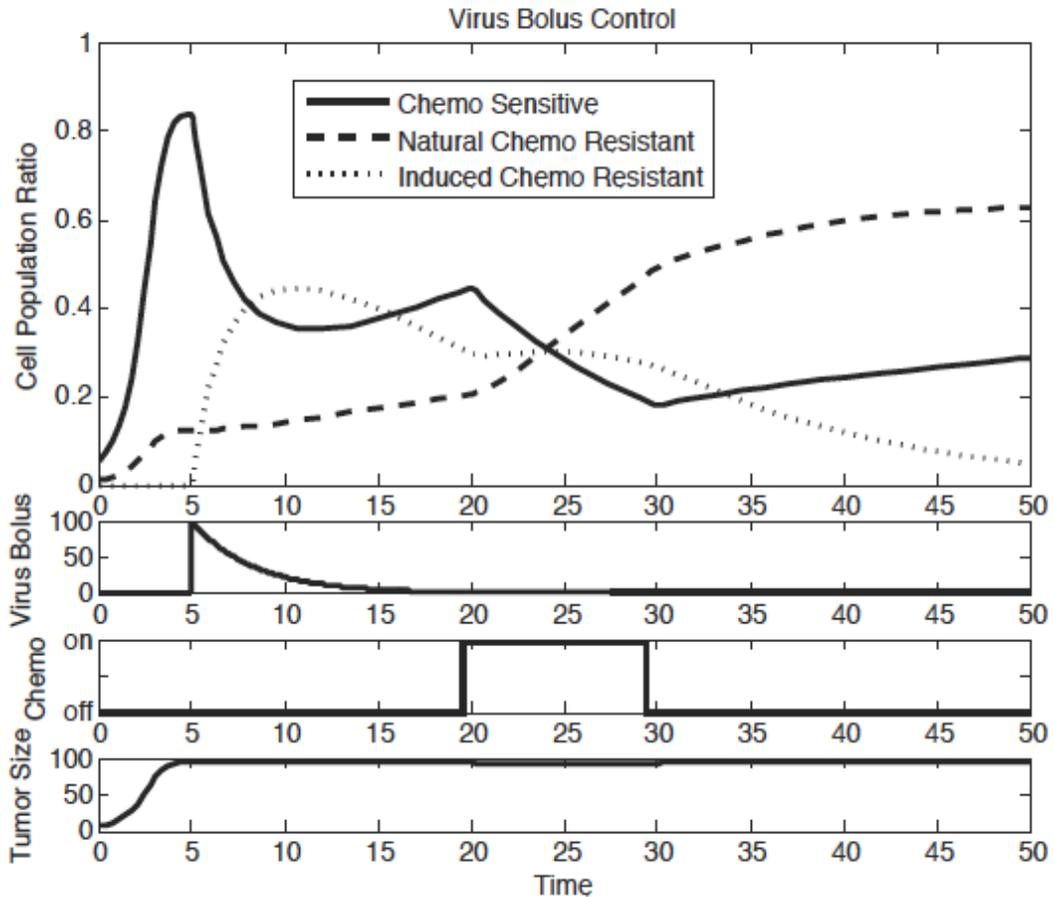


Figure 22 Virus Bolus Control: Single Pulse of Chemotherapy. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = 0.05$, $C_y = C_z = 0.95$, $\beta = .004$ and $a = 0.2$

As we have learned from the optimal chemotherapy control section, continuous application of the chemotherapy is not the most favorable option when it comes to controlling the dynamics of the therapy. In figure 22 a single pulse of chemotherapy is administered from around day 20 to day 30. This alone does not lead to successful therapy since the natural chemotherapy resistant cells are still the

prevalent cell type in the tumor It does set up a good place to give another virus bolus injection, since the previous virus load has depleted and the induced chemotherapy resistant cells start to slightly increase again around day 20.

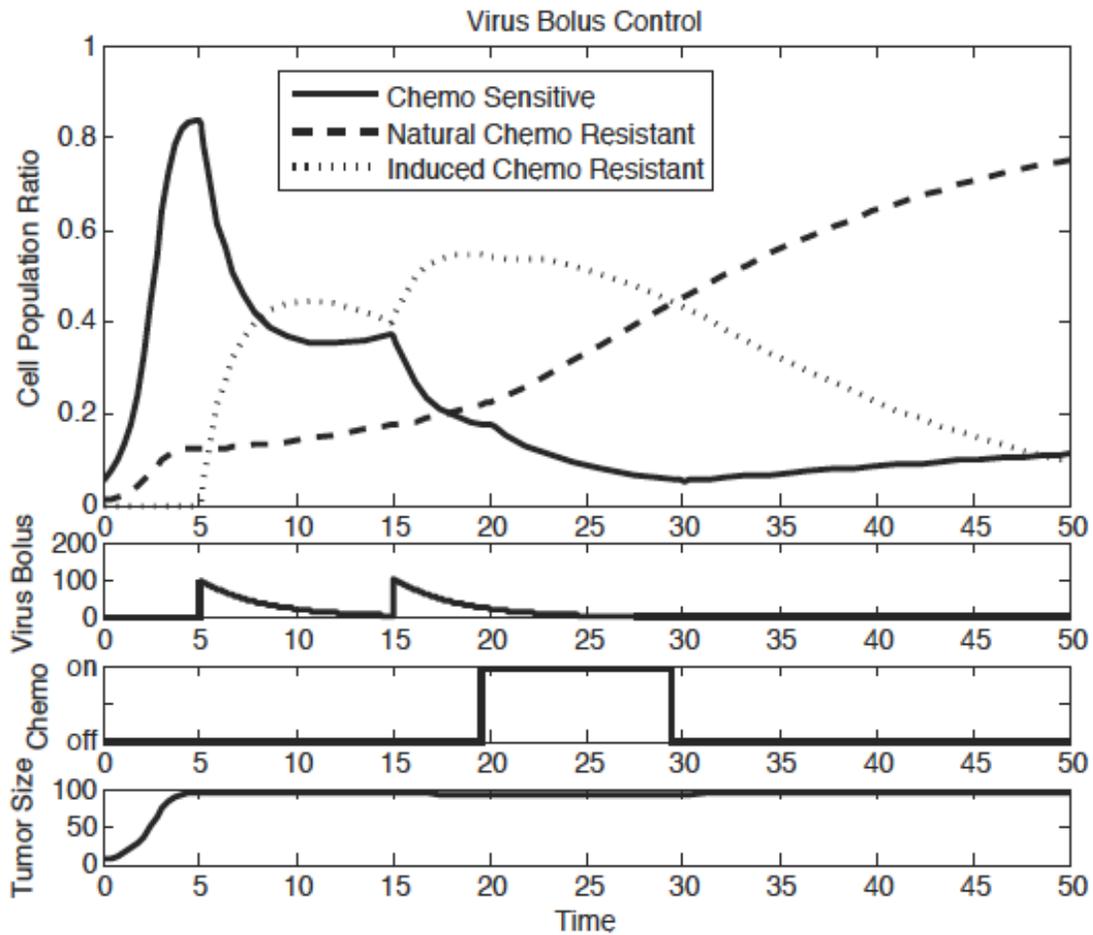


Figure 23 Virus Bolus Control: Two Boluses are Administered. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = 0.05$ $C_y = C_z = 0.95$, $\beta = .004$ and $a = 0.2$

After a second virus bolus is administered at day 15, the chances for successful therapy are significantly increased as can be seen in figure 23. As a result of the second virus bolus injection, the induced chemotherapy resistant cells are able to spread quickly through the tumor for a short period of time giving rise to a period with a high bystander ratio. This situation should be ideal for successful therapy. One thing to note is that the chemotherapy sensitive cell population has drastically decreased as a result of the chemotherapy and the two bolus injections. This means the amount of virus bolus injections that can be provided and give some useful result is severely limited because of the decreased prevalence of the chemotherapy sensitive cells.

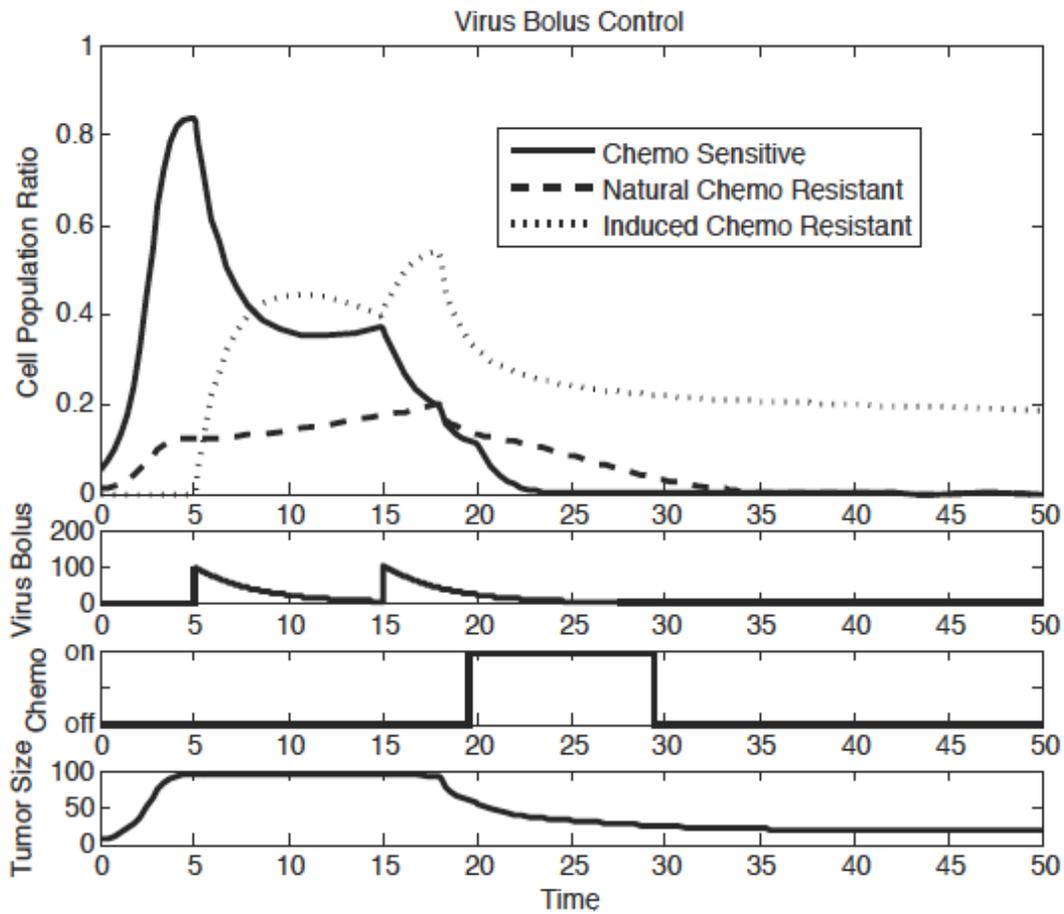


Figure 24 Virus Bolus Control: Successful Therapy. Parameter values: $x_0 = 5$, $y_0 = 1$, $z_0 = 0$, $v_0 = 0$, $C_x = 0.05$, $C_y = C_z = 0.95$, $g = 0.7$, $\beta = .004$ and $a = 0.2$

After treatment with ganciclovir at day 18 the total tumor size begins to decline rapidly as can be seen in figure 24. Even though the total tumor burden has not decreased by the end of the time window, the only cells that remain are the induced chemotherapy resistant cells. This means that the tumor can easily be eradicated with more ganciclovir treatment. Figure 24 was shows a particular schedule that works to achieve successful therapy. It demonstrates how better

therapies can be achieved using less intuitive solutions. Through the application of an optimal controller, even better solutions can be achieved.

Not only chemotherapy pulsing but also bolus injections can be used as a very effective method for controlling the dynamics of the various tumor cell growth. Using these inputs that we can control to our advantage we were able to show the importance of a control approach to the problem of dynamic cancer therapy.

Chapter 6

CONCLUSION

In this thesis we developed a set of ordinary differential equations to model the dynamics of various cancer cell types during the course of a novel dynamic cancer therapy. We first introduced the new cancer treatment concept, which consisted of a period of positive selection from transfection of some of the cancer cells via a virus delivery vector and then treatment with the chemotherapy. This was followed by a period of negative selection implemented by exposing the remaining cells to ganciclovir. Provided there was a sufficient bystander effect between the transfected cells and the remaining cells the tumor would be totally eradicated.

First we modeled a non-replication competent delivery virus. These viruses when introduced into the system are not able to replicate. As a result, the growth and spread of the transfected cells was severely hindered. The competition of the cell types and their individual fitnesses were also studied. We modeled both cases: 1 where the virus could infect only the chemotherapy sensitive cells and 2 where the virus could infect both the chemotherapy sensitive cells and the naturally resistant cells. We found that when the natural chemotherapy resistant cells were susceptible to virus infection, there was a much greater chance for successful therapy.

We next studied the case where a replication competent delivery virus was used. Using a virus that can replicate and produce more viruses in the system brings about new conditions in the system. If the virus is highly virulent it will spread quickly but as a result will bring about more damage and cause greater virus

induced cell death. The low virulent virus would proliferate slowly and cause less cell damage allowing for significant spread which would yield successful therapy at the price of time. It spreads slowly and would take a long time to infect sufficient levels of cells to be effected during the negative selection period. For all virulence levels, the timing of the negative selection process is crucial. If the ganciclovir is administered too early for a slow spreading virus the treatment could fail. For a quick spreading virus there is a only a small window of opportunity where the bystander effect is large enough to achieve successful therapy after the negative selection process has begun.

The most important analysis of thesis is the control study. We studied how an open loop optimal control analysis can be applied to the replication competent delivery virus situation in order to achieve the optimal therapy. By pulsing the chemotherapy treatment we were able to maximize the bystander effect and in turn achieve the best chance for successful therapy for various parameters. We also studied the effect of using multiple bolus injections to control the outcome or therapy. More work needs to be done to develop an optimal control analysis for the bolus injections. Nevertheless, in both cases therapy proved successful with minimal chemotherapy dosage.

This type of study shows the importance of using mathematical models to study the complex dynamics of biological systems. We have also shown that a control analysis can also be used to optimize therapy techniques to ensure the highest probability of success.

APPENDIX

MATLAB CODE

Optimal Control

```
%function [seqoptim,costoptim] = findoptimalsequence(N);
N = 10;
NN = 2^N;
cost1 = 0;
costold = cost1;
Tmax = 0;
for ii = 0:NN-1
    seq1 = dec2bin(ii,N);
    [cost1, Tmax1] = PancOptimTest(seq1);
    if cost1 > costold
        seqoptim = seq1;
        costold = cost1;
        Tmax = Tmax1;
    end
end
costoptim = costold;
PancPlotOptim(seqoptim, Tmax);
```

```
function [Cost,Tmax] = Pancreaswvcfinal(seq1)
clc;
close all;
tttt=cputime;
length = max(size(seq1));
```

```
% -----
-----
```

%Model Parameter Definitions

```
global Tmax seq r e K d n C I lambda delta b s a G t0 t1 t2
t3 q j Beta p L k f u w Cn Ci Gr XGr YGr ZGr I t4 t5 ti
interval
```

```
seq = seq1;
q = 1;      % uninfected tumor growth rate
e = 1;      % B-R exponent
K = 100;    % B-R carrying capacity [((1-
d/r)^(1/e))*Carrying capacity]
d = .1;     % natural death rate uninfected tumor
```

```

n = .0007;      % rate of change to chemoresistant tumor
C = .95;       % Chemotherapy treatment efficacy
Cn = 0;        % Chemotherapy treatment efficacy (natural
chemo resistant)
Ci = 0;        % Chemotherapy treatment efficacy (induced
chemo resistant)
I = 1.1;       % Beta-a ratio
f = .9;        % growth rate of natural chemo resistant tumor
cells
delta =.1;     % death rate of natural chemo resistant tumor
cells
b = 1.75;      % death rate due to bystander effect
j = .9;        % growth rate of induced chemo resisntant tumor
cells
a = I*.1;      % deat rate of induced chemo resistant tumor
cells
G = .7;        % death rate of due to ganciclovir (GCV)
sensitivity
Beta = I*.002; % virus mass action rate
p = .3;        % virus death rate
L = .00;       % Lytic rate
k = .5;        % Burst size
u = .1;        % Chemotherapy efficacy on chemoresistant
w = .0;        % Chemotherapy efficacy on Induced Resistant
                % resistant back to sensitive term?
% -----
% -----
for ti = 0:0
% Treatment Times
%ti = 15; % Pulse time increment
t0 = 0; % Chemo treatment beginning time (day)
t1 = 0; % Chemo treatment end time (day)
t2 = 0; % GCV treatment begin time (day)
t3 = 0; % GCV treatment end time (day)
interval = 5; %Fastest switch time in days

% -----
% -----

% ODE
%Tr = 100;
Tr = interval*length;
sol = ode45(@go, [0.01, Tr], [90 10 0 100]);

ymax=min(sol.y(2,:));
zmax=max(sol.y(3,:));
[Cost,Tmax] =

```

```

max(sol.y(3, :)./(sol.y(1, :)+sol.y(2, :)+sol.y(3, :)));
Tmax = sol.x(Tmax);
% -----
-----

end
% Plots
lw = 2;

% -----
-----

% Differential Equation Functions
function ddt = go(t,y)
global seq r e K d n C I lambda delta b s a G t0 t1 t2 t3 q
j bb Beta p L k f u w GG CCi CCn Cn Ci t4 t5 ti interval
X = (y(1));
Y = (y(2));
Z = (y(3));
V = (y(4));

% Floor Functions
if X < .9
    r = 0;
else if X > .9
    r = q;
end
end

if Y < 1.5
    lambda = 0;
else if Y > 1.5
    lambda = f;
end
end

if Z < .9
    s = 0;
else if Z > .9
    s = j;
end
end
rn = round(rand);

```

```

if str2num(seq(ceil(t/interval))) == 1

    CC = C;
    CCn = Cn;
    CCi = Ci;
    GG = 0;
    bb = 0;
end
if str2num(seq(ceil(t/interval))) == 0

    CC = 0;
    CCn = 0;
    CCi = 0;
    GG = 0;
    bb = 0;
end
%R0 = (Beta*r*k*L)/((a+L+GG)*(d+n+CC)*p);

% Differential Equations
ddt = [r*X*(1-CC)*(1 - ((X + Y + Z)^e)/(K^e)) - X*(d + n +
Beta*V + bb*(Z/(X+Y+Z)))
      n*X + Y*(1-Cn)*lambda*(1 - ((X + Y + Z)^e)/(K^e)) -
Y*(delta + bb*(Z/(X+Y+Z)))
      Beta*X*V + Z*(1-Ci)*s*(1 - ((X + Y + Z)^e)/(K^e)) -
Z*(a + GG + L)
      k*a*Z - p*V];

```

```

function Pancreaswvcfinal(seq1, Tmax)
clc;
close all;
tttt=cputime;
length = max(size(seq1));

```

```

% -----
-----

```

```

%Model Parameter Definitions
global LL ss CGGG CCCC CCC GGG h Tmax seq r e K d n C I

```

```
lambda delta b s a G t0 t1 t2 t3 q j Beta p L k f u w Cn Ci
Gr XGr YGr ZGr I t4 t5 ti interval
```

```
seq = seq1;
q = 1;      % uninfected tumor growth rate
e = 1;      % B-R exponent
K = 100;    % B-R carrying capacity [((1-
d/r)^(1/e))*Carrying capacity]
d = .1;     % natural death rate uninfected tumor
n = .0007;  % rate of change to chemoresistant tumor
C = .95;    % Chemotherapy treatment efficacy
Cn = 0;     % Chemotherapy treatment efficacy (natural
chemo resistant)
Ci = 0;     % Chemotherapy treatment efficacy (induced
chemo resistant)
I = 1.1;    % Beta-a ratio
f = .9;     % growth rate of natural chemo resistant tumor
cells
delta = .1; % death rate of natural chemo resistant tumor
cells
b = 1.75;   % death rate due to bystander effect
j = .9;     % growth rate of induced chemo resistant tumor
cells
a = I*.1;   % deat rate of induced chemo resistant tumor
cells
G = .7;     % death rate of due to ganciclovir (GCV)
sensitivity
Beta = I*.002; % virus mass action rate
p = .3;     % virus death rate
L = .00;    % Lytic rate
k = .5;     % Burst size
u = .1;     % Chemotherapy efficacy on chemoresistant
w = .0;     % Chemotherapy efficacy on Induced Resistant
% resistant back to sensitive term?
% -----
-----
for ti = 0:0
% Treatment Times
%ti = 15; % Pulse time increment
t0 = 0; % Chemo treatment beginning time (day)
t1 = 0; % Chemo treatment end time (day)
t2 = 0; % GCV treatment begin time (day)
t3 = 0; % GCV treatment end time (day)
interval = 5; %Fastest switch time in days
% -----
-----
```

```

% ODE
%Tr = 100;
Tr = interval*length;
sol = ode45(@go, [0.01, Tr], [90 10 0 100]);

ymax=min(sol.y(2,:));
zmax=max(sol.y(3,:));
Cost = max(sol.y(3,:)./(sol.y(1,:)+sol.y(2,:)+sol.y(3,:)));
% -----
-----

end
% Plots
lw = 2;

Gr = sol.y(1,:)+sol.y(2,:)+sol.y(3,:);
XGr = sol.y(1,:).*(Gr.^-1);
YGr = sol.y(2,:).*(Gr.^-1);
ZGr = sol.y(3,:).*(Gr.^-1);

KXGr = sol.y(1,+)/K;
KYGr = sol.y(2,+)/K;
KZGr = sol.y(3,+)/K;

MAXIMUM_NCR = max(KYGr);
MAXIMUM_ICR = max(KZGr);
MAXIMUM_BRATIO = max(ZGr);

ss = (Tr/LL):(Tr/LL):(Tr/LL)*LL;

figure;
subplot(8,1,1:5)
plot(sol.x, KXGr, '-', 'LineWidth', lw);
hold all
plot(sol.x, KYGr, '--', 'LineWidth', lw);
hold all
plot(sol.x, KZGr, 'k:', 'LineWidth', lw);
xlabel('time');
ylabel('Cell Population Ratio');
legend('Chemo-Sensitive Cells ', 'Natural Chemo-Resistant
Cells', 'Induced Chemo-Resistant Cells')
axis([0 Tr 0 1])
title(['Optimal Control'])
subplot(8,1,6:7)
plot(sol.x, Gr, '-', 'LineWidth', lw)
xlabel('Time')

```

```

ylabel('Tumor Size')
%axis([0 Tr min(Gr) 100])
axis([0 Tr 0 100])
subplot(8,1,8)
plot(ss, CCCC, 'Linewidth', lw)
%plot(CCC, 'Linewidth', lw)
axis([0 Tr 0 1])
ylabel('Chemo')
% subplot(8,1,8)
% plot(ss, CGGG, 'Linewidth', lw)
% %plot(GGG, 'LineWidth', lw)
xlabel('Time')
% ylabel('GCV')
% axis([0 Tr 0 G+.1])

% -----
% -----

% Differential Equation Functions
function ddt = go(t,y)
global LL ss CGGG CCCC CCC GGG h Tmax seq r e K d n C I
lambda delta b s a G t0 t1 t2 t3 q j bb Beta p L k f u w GG
CCi CCn Cn Ci t4 t5 ti interval
X = (y(1));
Y = (y(2));
Z = (y(3));
V = (y(4));

% Floor Functions
if X < .9
    r = 0;
else if X > .9
    r = q;
end
end

if Y < 1.5
    lambda = 0;
else if Y > 1.5
    lambda = f;
end
end

if Z < .9
    s = 0;

```

```

else if z > .9
    s = j;
end
end

if str2num(seq(ceil(t/interval))) == 1

    CC = C;
    CCn = Cn;
    CCI = Ci;
    GG = 0;
    bb = 0;
end
if str2num(seq(ceil(t/interval))) == 0

    CC = 0;
    CCn = 0;
    CCI = 0;
    GG = 0;
    bb = 0;
end
if t > Tmax
    CC = 0;
    CCn = 0;
    CCI = 0;
    GG = G;
    bb = b;
end
ONE = ones(1,100);
h = round(t)+1;
CCC(h) = CC;
BCCC = CCC' * ONE;
CCCC =
[BCCC(1,:), BCCC(2,:), BCCC(3,:), BCCC(4,:), BCCC(5,:), BCCC(6,
), BCCC(7,:), BCCC(8,:), BCCC(9,:), BCCC(10,:), BCCC(11,:), BCCC(
12,:), BCCC(13,:), BCCC(14,:), BCCC(15,:), BCCC(16,:), BCCC(17,
), BCCC(18,:), BCCC(19,:), BCCC(20,:), BCCC(21,:), BCCC(22,:), BC
CC(23,:), BCCC(24,:), BCCC(25,:), BCCC(26,:), BCCC(27,:), BCCC(2
8,:), BCCC(29,:), BCCC(30,:), BCCC(31,:), BCCC(32,:), BCCC(33,
), BCCC(34,:), BCCC(35,:), BCCC(36,:), BCCC(37,:), BCCC(38,:), BCC
C(39,:), BCCC(40,:), BCCC(41,:), BCCC(42,:), BCCC(43,:), BCCC(44
,:), BCCC(45,:), BCCC(46,:), BCCC(47,:), BCCC(48,:), BCCC(49,:),
BCCC(50,:), BCCC(51,:)];
LL = length(CCCC);
GGG(h) = GG;
BGGG = GGG' * ONE;
CGGG =

```

```
[BGGG(1,:),BGGG(2,:),BGGG(3,:),BGGG(4,:),BGGG(5,:),BGGG(6,
),BGGG(7,:),BGGG(8,:),BGGG(9,:),BGGG(10,:),BGGG(11,:),BGGG(
12,:),BGGG(13,:),BGGG(14,:),BGGG(15,:),BGGG(16,:),BGGG(17,
),BGGG(18,:),BGGG(19,:),BGGG(20,:),BGGG(21,:),BGGG(22,:),BG
GG(23,:),BGGG(24,:),BGGG(25,:),BGGG(26,:),BGGG(27,:),BGGG(2
8,:),BGGG(29,:),BGGG(30,:),BGGG(31,:),BGGG(32,:),BGGG(33,:)
,BGGG(34,:),BGGG(35,:),BGGG(36,:),BGGG(37,:),BGGG(38,:),BGG
G(39,:),BGGG(40,:),BGGG(41,:),BGGG(42,:),BGGG(43,:),BGGG(44
,:),BGGG(45,:),BGGG(46,:),BGGG(47,:),BGGG(48,:),BGGG(49,:),
BGGG(50,:),BGGG(51,:)];
```

```
% Differential Equations
ddt = [r*X*(1-CC)*(1 - ((X + Y + Z)^e)/(K^e)) - X*(d + n +
Beta*V + bb*(Z/(X+Y+Z)))
      n*X + Y*(1-Cn)*lambda*(1 - ((X + Y + Z)^e)/(K^e)) -
Y*(delta + bb*(Z/(X+Y+Z)))
      Beta*X*V + Z*(1-Ci)*s*(1 - ((X + Y + Z)^e)/(K^e)) -
Z*(a + GG + L)
      k*a*Z - p*V];
```

Virus Bolus Control

```
function viruscontrol
clc;
close all;
% -----
-----
```

```
%Model Parameter Definitions
global LL CCCC CGGG CCC GGG h r e K d n C I lambda delta b
s a G t0 t1 t2 t3 t4 t5 q j Beta ss CCCC p L k f u w Cn Ci
Gr XGr YGr ZGr I t V inV
```

```
q = 1; % uninfected tumor growth rate
e = 3; % B-R exponent
K = 100; % B-R carrying capacity [((1-
d/r)^(1/e))*Carrying capacity]
d = .1; % natural death rate uninfected tumor
n = .0007; % rate of change to chemoresistant tumor
C = .95; % Chemotherapy treatment efficacy
Cn = 0; % Chemotherapy treatment efficacy (natural chemo
```

```

resistant)
Ci = 0;      % Chemotherapy treatment efficacy (induced chemo
resistant)
I = 2;      % rate of change to induced chemo resistant
f = .9;      % growth rate of natural chemo resistant tumor
cells
delta = .1; % death rate of natural chemo resistant tumor
cells
b = 1.75;    % death rate due to bystander effect
L = 0;      % Lytic rate
j = .9;      % growth rate of induced chemo resisntant
tumor cells
a = I*.1;    % deat rate of induced chemo resistant tumor
cells
G = .7;      % death rate of due to ganciclovir (GCV)
sensitivity
Beta = I*.002; % virus mass action rate
p = .3;      % virus death rate

inCS = 5;    % Initial Chemo Sensitive Cell
inNCR = 1;   % Initial Natrual Chemo Resistant Cells
inICR = 0;   % Initia Induced Chemo Resistant Cells
inV = 100;   % Initial Viral load

y0 = [inCS inNCR inICR];
k = .5;      % Burst size
u = .1;      % Chemotherapy efficacy on chemoresistant
w = .0;      % Chemotherapy efficacy on Induced Resistant
              % resistant back to sensitive term?
% -----
% -----

% Treatment Times
t0 = 20;     % Chemo treatment beginning time (day)
t1 = 30;     % Chemo treatment end time (day)
t2 = 18;     % GCV treatment begin time (day)
t3 = 100;    % GCV treatment end time (day)
t4 = 5;      % Virus Bolus 1 Time
t5 = 15;     % Virus Bolus 2 Time

% -----
% -----

% ODE
Tr = 50;
tx = 0:.01:Tr;
Vx1 = inV*exp(-p*(tx-t4)).*(tx>t4);

```

```

Vx2 = inV*exp(-p*(tx-t5)).*(tx>t5);
Vx = Vx1 + Vx2;

sol = ode23(@go, [0, Tr], y0);

% -----
% -----

% Plots
lw = 2;

Gr = sol.y(1,:)+sol.y(2,:)+sol.y(3,:);
KGr = (sol.y(1,:)+sol.y(2,:)+sol.y(3,:))/K;
% XGr = sol.y(1,:).*(Gr.^-1);
% YGr = sol.y(2,:).*(Gr.^-1);
% ZGr = sol.y(3,:).*(Gr.^-1);

KXGr = sol.y(1, :)/K;
KYGr = sol.y(2, :)/K;
KZGr = sol.y(3, :)/K;

ss = (Tr/LL):(Tr/LL):(Tr/LL)*LL;

%Vi = sol.y(4,:)/inV;

figure;
subplot(7,1,1:4)
plot(sol.x, KXGr, '-', 'LineWidth', lw);
hold all
plot(sol.x, KYGr, '--', 'LineWidth', lw);
hold all
plot(sol.x, KZGr, 'k:', 'LineWidth', lw);
xlabel('time');
ylabel('Cell Population Ratio');
legend('Chemo Sensitive', 'Natural Chemo Resistant' ,
'Induced Chemo Resistant')
axis([0 Tr 0 1])
title(['Virus Bolus Control' ])
% subplot(5,1,4)
% plot(sol.x, Vi, '-', 'LineWidth', lw);
% ylabel('Virus')
subplot(7,1,5)
plot(tx,Vx, 'LineWidth', lw)
ylabel('Virus Bolus')

```

```

subplot(7,1,6)
plot(ss, CCCC, 'Linewidth', lw)
axis([0 Tr 0 1])
ylabel('Chemo')
subplot(7,1,7)
plot(sol.x, Gr, '-', 'LineWidth', lw)
xlabel('Time')
ylabel('Tumor Size')
axis([0 Tr 0 K])

% -----
% -----

% Differential Equation Functions
function ddt = go(t,y)
global LL CCCC CGGG CCC GGG h r e K d n C I lambda delta b
s a G t0 t1 t2 t3 t4 t5 q j bb Beta ss p L k f u w GG CCI
CCn Cn Ci inV
X = (y(1));
Y = (y(2));
Z = (y(3));
%V = (y(4));

% Floor Functions
if X < .9
    r = 0;
else if X > .9
    r = q;
end
end

if Y < .9
    lambda = 0;
else if Y > .9
    lambda = f;
end
end

if Z < .9
    s = 0;
else if Z > .9
    s = j;
end
end

```

```

CC = C*(t>t0)*(t<t1);
CCn = Cn*(t>t0)*(t<t1);
CCi = Ci*(t>t0)*(t<t1);
GG = G*(t>t2)*(t<t3);
bb = b*(t>t2)*(t<t3);

if t < .5;
h = round(t)+1;
else if t > .5
h = round(t)+1;
    end
end

%plot fix
ONE = ones(1,100);

CCC(h) = CC;
BCCC = CCC' * ONE;
CCCC =
[BCCC(1,:),BCCC(2,:),BCCC(3,:),BCCC(4,:),BCCC(5,:),BCCC(6,:),
BCCC(7,:),BCCC(8,:),BCCC(9,:),BCCC(10,:),BCCC(11,:),BCCC(12,:),
BCCC(13,:),BCCC(14,:),BCCC(15,:),BCCC(16,:),BCCC(17,:),
BCCC(18,:),BCCC(19,:),BCCC(20,:),BCCC(21,:),BCCC(22,:),BCCC(23,:),
BCCC(24,:),BCCC(25,:),BCCC(26,:),BCCC(27,:),BCCC(28,:),
BCCC(29,:),BCCC(30,:),BCCC(31,:),BCCC(32,:),BCCC(33,:),
BCCC(34,:),BCCC(35,:),BCCC(36,:),BCCC(37,:),BCCC(38,:),BCCC(39,:),
BCCC(40,:),BCCC(41,:),BCCC(42,:),BCCC(43,:),BCCC(44,:),
BCCC(45,:),BCCC(46,:),BCCC(47,:),BCCC(48,:),BCCC(49,:),
BCCC(50,:),BCCC(51,:)];
LL = length(CCCC);
GGG(h) = round(GG);
BGGG = GGG' * ONE;
CGGG =
[BGGG(1,:),BGGG(2,:),BGGG(3,:),BGGG(4,:),BGGG(5,:),BGGG(6,:),
BGGG(7,:),BGGG(8,:),BGGG(9,:),BGGG(10,:),BGGG(11,:),BGGG(12,:),
BGGG(13,:),BGGG(14,:),BGGG(15,:),BGGG(16,:),BGGG(17,:),
BGGG(18,:),BGGG(19,:),BGGG(20,:),BGGG(21,:),BGGG(22,:),BGGG(23,:),
BGGG(24,:),BGGG(25,:),BGGG(26,:),BGGG(27,:),BGGG(28,:),
BGGG(29,:),BGGG(30,:),BGGG(31,:),BGGG(32,:),BGGG(33,:),
BGGG(34,:),BGGG(35,:),BGGG(36,:),BGGG(37,:),BGGG(38,:),BGGG(39,:),
BGGG(40,:),BGGG(41,:),BGGG(42,:),BGGG(43,:),BGGG(44,:),
BGGG(45,:),BGGG(46,:),BGGG(47,:),BGGG(48,:),BGGG(49,:),
BGGG(50,:),BGGG(51,:)];

```

```

%V = inV*exp(-p*(t-t4))*(t>t4);
V1 = inV*exp(-p*(t-t4)).*(t>t4);
V2 = inV*exp(-p*(t-t5)).*(t>t5);
V = V1 + V2;

% Differential Equations
ddt = [r*X*(1-CC)*(1 - ((X + Y + Z)^e)/(K^e)) - X*(d + n +
Beta*V + GG*bb*(Z/(X+Y+Z)))
      n*X + Y*(1-Cn)*lambda*(1 - ((X + Y + Z)^e)/(K^e)) -
Y*(delta + GG*bb*(Z/(X+Y+Z)))
      Beta*X*V + Z*(1-Ci)*s*(1 - ((X + Y + Z)^e)/(K^e)) -
Z*(a + GG)];

```

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