CONTROLLING RESISTANCE EVOLUTION IN CANCER GENE THERAPY

by

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My fellow students and friends, for their continued support over the past several years.

This manuscript is dedicated to:

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TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

We have developed a multi-state ordinary differential equation model representing the dynamics of cancerous cell populations under conditions consistent with the gene therapy technique proposed by Martinez-Quintanilla *et al*. The model is based on a variation of the Lotka-Volterra equations, a method to illustrate multiple species' competition to utilize a scarce resource. This system allows for investigation of the effectiveness of proposed anti-cancer gene therapy methods, including the response to selective pressures resulting from drug treatments. In this thesis, we observe the stability of the system via bifurcation analysis; highlight the importance of delivery method of drug-susceptibility genes; finally, propose a control method to optimize treatment effectiveness.

Chapter 1

INTRODUCTION

Gene therapy in the broadest sense has existed for several decades, initially proposed to replace or repair damaged human DNA [1]. The goal of therapy was to modify DNA to alleviate the effects of genetically determined diseases that although rare, respond poorly to conventional treatment methodologies. Gene therapy proposed that exogenous DNA be transduced into recipient cells. This transduction can occur as a simple uptake of proteins or nucleic acids, or through transfection by either a replication- or non-replication competent virus. Upon gaining entry to the cell, foreign transfected DNA undergoes intercellular travel to the nucleus via transport vesicles, while potentially experiencing partial degradation due to cell lysosome activity. At the nucleus, portions of foreign DNA that have arrived intact may become stabilized within the intrinsic DNA of the cell. At the conclusion of this process, the cell expresses the transduced gene through mRNA and synthesis of corresponding proteins. In this manner, health disorders caused by single gene deficiencies could be eliminated. The field of opthamology in particular has seen numerous studies conducted utilizing gene replacement therapy. Treatment to repair degenerative vision due to loss-of-function mutations may be forthcoming [2].

However, gene therapy applications are not limited to simple single gene repair. Including the ability to ameliorate the debilitating effects of genetic disorders, gene therapy was also viewed as a potentially beneficial oncologic treatment. For example, chemoprotective gene therapy admits a measure of resistance to

chemotherapy drugs. In this treatment regimen, genes immune to the effects of various drugs are introduced by transfection, conferring antidrug resistance to those cells containing the exogenous gene within their nucleus. Certain vital tissues, including bone marrow, respond particularly poorly to the toxicity of chemotherapeutic agents. Ultimately, continued exposure to the drugs leads to death of cells not specifically targeted by chemotherapy. The negative effects can be avoided through the addition of a dosage limit; however, a shorter duration of treatment also reduces the amount of time that cancer cells have to absorb and respond to the drug, potentially lessening efficacy. Chemoprotection seeks to convert tissues such as marrow to a drug resistant state, meaning less overall trauma to the body. Simultaneously, vital tissues gain the ability to withstand higher dosage limits, permitting both increased duration of treatment and the resulting ability of drugs to inhibit tumor growth.

Of course, transfection is not the only means by which cells acquire drug resistance. The natural evolution of cancer as it progresses is to lose vulnerability to chemotherapeutic agents. In fact, the very exposure to a selective stress such as chemotherapy could stimulate greater mutation rates of cancer cells from the wild type to drug resistant strains. To counteract the presence of natural mutations, gene therapy has been proposed as a means to reintroduce susceptibility to chemotherapy through gene insertion [3]; however, the spread of transfected genes has never been sufficient enough to affect the post-treatment outcome [4]. The distribution of transfected genes throughout the cancer cell (mutant and non-mutant) population is outpaced by the ability of mutated cells to proliferate and confer resistance to their progeny. It is

obvious that injecting susceptibility genes into the cancer cell population is an incomplete method on its own.

An alternative approach to this immunity mutation problem combines gene therapy techniques with concepts of both positive and negative selection. These methods exploit the dynamic evolutionary processes within the disease system that are stimulated by competition between cancer cells that occurs when a selective pressure, such as chemotherapy, is introduced. The method proposed by Martinez-Quintanilla *et al*. is as follows: Prior to chemotherapy, transfected cells are developed with the addition of a hybrid gene encoding both resistance to a specific chemotherapeutic agent, as well as susceptibility to another drug. At the onset of treatment, chemosensitive cells – those vulnerable to drug effects - are eliminated from the disease system. Meanwhile, cells having either artificial (transfected) chemoresistance or natural mutations survive due to their selective advantage. As a result, these cells exhibit greater fitness than chemosensitive cells during this first stage of treatment, are positively selected for, and continue to propagate throughout the tumor undeterred.

By itself, this stage of the proposed treatment has insignificant therapeutic effects. After chemotherapy treatment, we may actually observe greater populations of cancerous cells since the only alteration to the system has been the addition of chemoresistance. Furthermore, cells gaining resistance through naturally occurring mutations would not necessarily express the same vulnerability to the drug encoded by the exogenous gene expressed by transfected cells. Therefore, introduction of this drug to the system would be inconsequential to mutants, while negatively selecting and eradicating transfected cells. However, a significant observed bystander effect could theoretically eliminate all cells, including mutants, within the tumor. The

bystander effect is the result of transfected cells constituting a sizeable proportion of the total cancer cell population. Under such conditions, transfected cells would be more likely to interact with a greater number of neighboring cells. This interaction between transfected cells and their neighbors (or bystanders) could lead to a transfer of susceptibility. For example, the method of Martinez-Quintanilla *et al.* relies on the diffusion of a selected drug, enzymatically-converted by sensitive cells, that is not normally metabolized by resistant mutants. In this manner, the drug is able to act on all cells. Subsequent exposure activates a gene-encoded suicide mechanism that will encompass the tumor, causing negative selection and complete removal of both transfected and natural mutant cells from the system.

In this thesis, we examine a three state ordinary differential equation model to illustrate the effects of the selection processes on the cell population. As one may note, cancer cells form solid tumors and thus, spatial factors *should* be important. While a partial differential equation model would be ideal, an ordinary differential equation model should be sufficient. A logistic model of population growth is utilized to mitigate the absence of a PDE model. This constrains the growth of the model to a finite area, representing a tumor. The growth of each species of cancer cells that we have mentioned – chemosusceptible, transfected, and naturally occurring mutants – cannot exceed the carrying capacity the tumor.

The model aims to show that the role of chemotherapy in this method is to amplify the proportion of cells prone to death via a second drug, by first positively selecting for transfected cells. Through increasing this proportion, there is a subsequent expansion of the bystander effect. Perfusion of the transfected genes is promoted post-chemotherapy, yielding a more efficient stage of negative selection

upon introduction of an additional selected drug. However, as we will see, an increased bystander effect is critical in the negative selection phase. Therefore optimization of the bystander effect would enhance the viability of gene therapy as an oncologic treatment option.

Chapter 2

BIOLOGICAL BACKGROUND

Thymidine Kinase Mediated Suicide Gene Therapy [3] [4] is an anti-cancer treatment that is applied to tumors consisting of three subpopulations: chemotherapy sensitive cells, natural chemotherapy resistant cells, and hybrid transfected cells expressing both chemoresistance and susceptibility to a second, chosen drug.

Chemotherapy Sensitive Cells

In traditional chemotherapy, tumor reduction is achieved through use of numerous drugs, including methotrexate (MTX) and docetaxel. Through varying modes of action these drugs interfere with metabolic processes of the cell, halting the ability to reproduce. Chemotherapy sensitive cells, restricted from multiplying, eventually die until extinction.

Methotrexate is an antifolate compound that binds to the enzyme dihydrofolate reductase (DHFR). DHFR is normally encoded by the DNA of cells throughout the body. The typical functional role of DHFR is to act as a reducing agent on dihydrofolic acid, catalyzing the reaction that produces tetrahydrofolic acid. This compound serves as a precursor to synthesis of amino and nucleic acids such as glycine, purines, and thymidine. However, the affinity of MTX to DHFR exceeds that of dihydrofolic acid. During treatment DHFR binds to MTX, thus interfering with normal function and subsequent DNA production [5].

Docetaxel is an anti-mitotic compound that disrupts the cell cycle. The drug targets microtubules, the constituents of the cell cytoskeleton. This organelle provides the cell with structure and facilitates intercellular transport of molecules through vesicles. Microtubules are inherently unstable structures, allowing for rearrangement during the g2 m transition of the cell cycle, when a cell must grow and divide. Docetaxel alters microtubule assembly, converting them to a more stable state. As a result, microtubules lose their dynamic nature in favor of a more rigid structure. New microtubules cannot be formed and rearranged, preventing mitotic division.

The main effect of either drug is to impede tumor angiogenesis by preventing reproduction on the cellular level. Within a tumor, there is a constant competition for expansion between the cells. Chemotherapy sensitive cells exhibit a much lower fitness than mutant cells that gain immunity to drug effects. Thus, introduction of an agent like MTX or docetaxel places sensitive cells at a selective disadvantage. Susceptible cells, if not removed entirely from the tumor post-chemotherapy, will at most constitute an insignificant (~0%) proportion of the total cell population.

Natural Chemotherapy Resistant Cells

However, there typically exists a small subpopulation of cells in a tumor subpopulation of cells that do not respond to chemotherapy. Natural mutations occur due to the inherently unstable DNA structure of cancer cells. Malignant cells constantly alter their phenotype; this ability allows them to escape the programmed cell death that normal cells undergo during conditions of damaged DNA. Thus, cancer cell DNA is unstable because it is the very characteristic that allows for their existence [6]. Furthermore, the presence of a selective pressure such as chemotherapy has been

observed to cause exponential growth through clonal expansion of cells having advantageous resistance mutations [7].

Immunity gained by double mutant dihydrofolate reductase (dmDHFR), for example, expresses a lower than normal binding affinity to MTX. Despite MTX presence, cellular DHFR will preferentially bind to folate and function normally. Another example is resistance conferred by multidrug resistance gene1, which encodes for expression of the P-glycoprotein (PGP). This membrane-bound protein mediates both extra- and intra-cellular movement of molecules. Essentially, PGP acts as a drug pump, mediating the active transport of chemotherapy drugs to the extracellular space, decreasing intercellular concentration in resistant cells. The ability of MDR-mutants to restrict drugs from entering their membrane allows them to continue to reproduce completely undeterred.

If we consider a two state model of population dynamics, with chemotherapy sensitive and natural chemotherapy resistant cells as the individual species, a sizeable portion of mutants will remain post chemotherapy treatment. In fact, this species will no longer compete with chemotherapy sensitive cells for resources, and will pervade the tumor. Mutants have a significant fitness advantage during this treatment, and an alternative method is required to facilitate their complete removal from the tumor.

Induced Chemotherapy Resistant Cells

A proposed solution is the addition of engineered genes to chemotherapy sensitive cells, creating a second species that would survive periods of drug treatment. Their presence would initiate competition with natural chemotherapy resistant mutants for expansion throughout the tumor. This can be achieved by transfection with either

MDR1 or the double mutant dmDHFR. Additionally, these transfected genes should introduce susceptibility to a different drug for the new cell line. One possibility commonly seen in anti-cancer gene therapy is expression of herpes simplex virus thymidine kinase (HSV-TK). While MDR1 or dmDHFR presence confers a selective advantage during chemotherapy, HSV-TK sensitizes these hybrid cells to the antiviral drug ganciclovir [3] [4].

Thymidine kinase catalyses the monophosphorylation of ganciclovir. Studies have shown that metabolism of the drug leads to incorporation of the phosphorylated product into cell DNA [8]. However, this combination also causes DNA double strand breaks (DSB). Attempted cell replication under this condition results in g2 m cell cycle arrest, as deleterious chromosomal mutations arise. This triggers a cell suicide mechanism to correct for DSBs and ensure the mutations are not replicated [9].

A significant advantage or ganciclovir is the ability to affect non-sensitive cells. Monophosphorylated ganciclovir within a cell membrane may transfer to adjacent cells through gap junctions (~2-4 nm in diameter). In this manner, the system would experience a bystander effect, where natural chemotherapy resistant cells also enter states of programmed cell death. This effect is dependent on the interaction between induced and natural chemotherapy resistant cells, requiring transfer of phosphorylated ganciclovir. Fortunately, the positive selection for resistant cells that occurs during chemotherapy will yield a favorable induced to natural resistant ratio, given a sufficient transfection at the onset of treatment.

Selection Process and Bystander Effect

The three species - chemotherapy sensitive cells, natural chemotherapy resistant cells, and hybrid transfected cells - compete and expand until selection removes cells susceptible to chemotherapy induced death. The remaining states, both induced and naturally resistant, continue to propagate, with their progeny expressing the same resistance. Treatment with antiviral drugs eliminates the induced species due to their HSV-TK transfection. However, the naturally resistant mutants are prone to the bystander effect. A significant bystander effect, we will show, can contribute to extinction of naturally occurring mutants, effectively stunting tumor angiogenesis.

Prior experimental results conducted on mice have been promising; both *in vitro* and *in vivo* attempts have supported this method. However, a number of dynamic issues related to this approach, including stability and delivery method considerations, must be explored before clinical trials in humans. We will illustrate these concerns using dynamical models in the following sections.

Chapter 3

IN VITRO MODELING

Preliminary Model

A basic approach to modeling the dynamics of anti-cancer gene therapy can be accomplished with a three state system of ordinary differential equations. We begin by investigating the *in vitro* case, where cell growth is not necessarily limited to the set amount of resources in a confined space (i.e. a tumor). Under these circumstances, transfection is assumed to occur as an injection to individual cancer cells, followed by their reinsertion to the cell community. This leads to a model of the form

$$\begin{aligned} \dot{x} &= (\lambda - d - \mu) \cdot x - C(t) \cdot x \\ \dot{y} &= (\lambda - d) \cdot y - g(t) \cdot y \\ \dot{z} &= (\lambda - d) \cdot z + \mu \cdot x - b \cdot g(t) \cdot z \end{aligned} \tag{1}$$

where **x** represents chemotherapy sensitive cells, **y** represents induced chemotherapy resistant cells, and **z** represents natural chemotherapy resistant cells. Cells of each subtype multiply at the identical exponential rate, λ; they each die at a rate, *d*. Cells of subtype **x** acquire immunity to chemotherapeutic agents through mutations and convert to subtype **z** at a nominal rate, μ; this is reflected as a growth parameter for subtype **z** cells.

Drug treatments are time controlled parameters *C(t)* and *g(t)*. *C(t)* represents the effects of chemotherapy on subtype **x**, with selectable start time and

duration of treatment. Natural and induced chemotherapy resistant cells are assumed immune to drug effects, and thus *C(t)* is absent from the equations representing subtype **y** and **z** growth. These subtypes however, are removed from the system by the effects of antiviral treatment with the drug ganciclovir, *g(t)*. Induced chemotherapy resistant cells experience the full efficacy of the drug because of the inserted susceptibility gene. Because natural chemotherapy resistant cells are not inherently prone to the drug, the dimensionless parameter *b* represents the bystander effect and diminished efficacy of ganciclovir.

Bifurcation Analysis

This model serves primarily to illustrate the significance of the bystander effect upon system stability. The model is a first order system of linear ODE's with non-constant coefficients *C(t)* and *g(t)*.

$$\frac{\partial \vec{v}}{\partial t} = \begin{bmatrix} \lambda - d - \mu - C(t) & 0 & 0 \\ 0 & \lambda - d - g(t) & 0 \\ \mu & 0 & \lambda - d - b \cdot g(t) \end{bmatrix} \begin{bmatrix} x \\ y \\ z \end{bmatrix}$$

$$\vec{v} = \begin{pmatrix} x & y & z \end{pmatrix}^T$$

Assuming chemotherapy has been successfully administered (sensitive cells are extinct), and treatment with ganciclovir is ongoing, evaluating stability is as simple as solving for the eigenvalues for the simplified matrix

$$\mathrm{A} = \begin{bmatrix} \lambda - d - g & 0 \\ 0 & \lambda - d - b \cdot g \end{bmatrix}$$

System stability is achieved for eigenvalues having negative valued real parts. There are several possible stationary points for this system; however, the only one with which we are concerned coincides with stochastic extinction of all subtypes (**x** = **y** = **z**

= 0). Holding λ, *d*, and *g(t)* constant, we were able to demonstrate the effect of a subcritical bystander effect, as well as appropriate behavior required for complete cell eradication. The results are presented in Figure 1.

This model is highly simplified - with the exception of μ, the growth of each subtype is entirely decoupled. However, the linear model allows us to illustrate several phenomenon required for successful treatment in this method. First, we observe chemotherapy establishing a selective pressure resulting in the positive selection for cells having resistance. While sensitive cells quickly reach extinction due to the effects of chemotherapy drugs, the ratio of both induced and natural chemotherapy resistant cells were greatly amplified. The dominance of either cell subtype in the post-selection environment is dependent on initial conditions at this point, as subtypes as subtypes **y** and **z** would grow at similar rates.

Next, we see that treatment with ganciclovir results in a negative selection phase for antidrug-susceptible cells. Cells of subtype **z** are not naturally sensitive and thus have a significant fitness advantage over subtype **y**. An insufficient bystander effect may temporarily suspend growth in subtype **z**; however, a rebound in growth is inevitable and will occur either at the end of ganciclovir treatment or extinction of subtype **y**. Natural chemotherapy resistant cells would continue to grow without bound, and ensuing rounds of treatment with either chemotherapy or ganciclovir would be trivial.

Finally, we observe the results of a sufficient bystander effect. Under this condition, the fitness advantage of natural chemotherapy resistant cells is negated by the ability of subtype **y** cells to transfer the metabolized ganciclovir to their neighbors. Ganciclovir triggers apoptosis in both cell lines, completely removing the tumor.

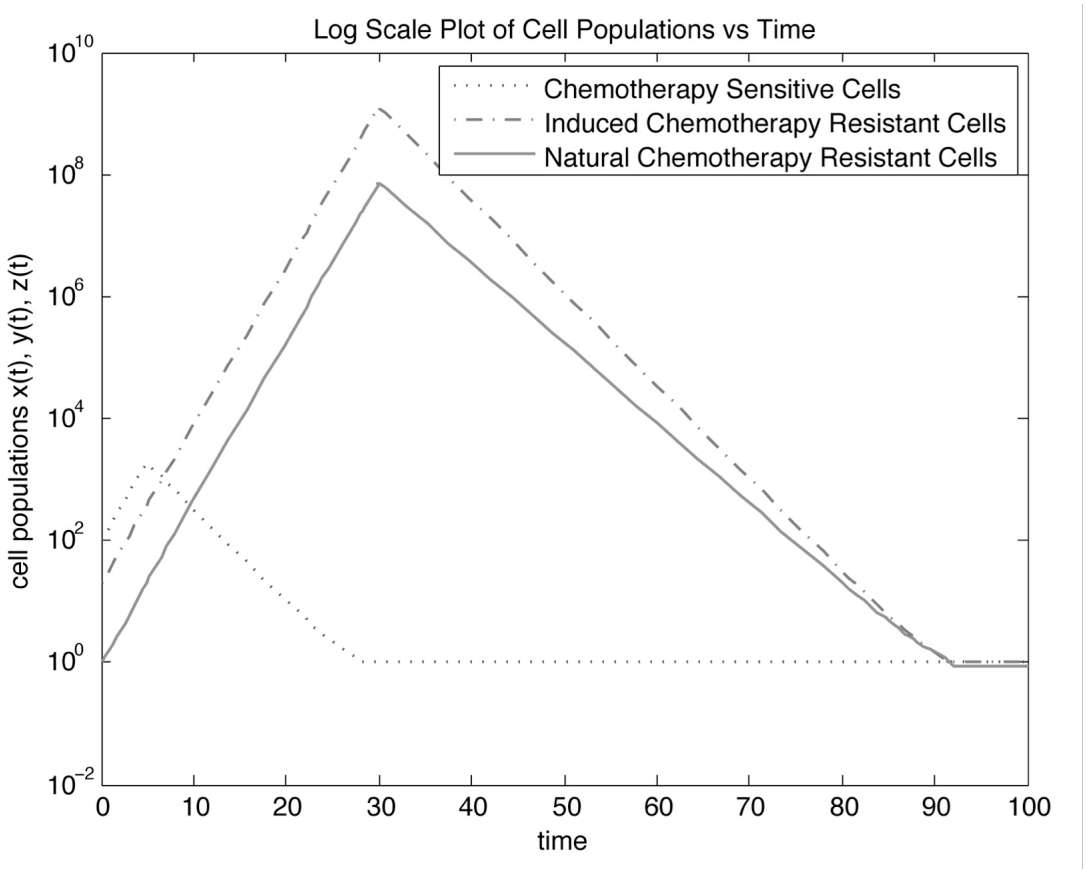

Figure 1 **Successful *In Vitro* Treatment.** Parameter values: C(t) = 0.95, g(t) = 0.95, b = 0.95. Chemotherapy begins at t = 5 for a duration of 24 units, resulting in extinction of chemotherapy sensitive cells. Subsequent ganciclovir input to the system removes all remaining cells. Note: although plots approach a value of 10^0, in actuality they reach zero. The value shown is done arbitrarily due to the log plot.

Chapter 4

IN VIVO MODELING

Prior studies of anti-cancer gene therapy assume transfection is achieved by isolating natural chemosensitive cells from other cell lines and injecting a hybrid plasmid individually. These transfected cells are then reinserted to the tumor cell community to interact with natural chemotherapy resistant and natural chemotherapy sensitive cells. This is a labor intensive process and transfection can only occur as quickly as plasmids can physically be inserted to each cell.

This method of transfection is certainly a viable option for in vitro studies conducted on simple cell cultures where initial population conditions can be kept manageable. However, anti-cancer gene therapy necessitates a much greater number of transfected cells to achieve sufficient bystander effect killing and therefore, successful treatment in humans. Furthermore, the spread of natural chemotherapy resistant cells *in vivo* introduces time constraints to avoid dominance by this species and the ensuing diminished bystander effect. Thus, individual transfection is rendered an impractical methodology.

We will instead consider transfection achieved via oncospecific viral vectors. Viruses can be utilized to infect tumor cells with engineered hybrid plasmids, without altering other tissue types. We evaluate the feasibility of two infection methods: replication competent and replication defective delivery viruses. The dynamics of each method can be very different, and thus multi-state models are developed for each case. For the replication competent case, we must also investigate

the effects of a virus with varying degrees of virulence, as well as the corresponding changes to drug treatments that are required.

Non-Replication Competent Delivery Virus

Replication-defective viral vectors are capable of integrating with host cell DNA; however, the coding regions associated with reproduction have been deleted from their genes. Thus, a replication defective virus cannot accomplish full reproductive functionality as can a virus that is capable of entering the lytic cycle.

Replication-defective viruses can adsorb to cell membranes to infect a host. Other functions associated with the lytic cycle such as copying of genes or lysing of the host membrane, are unachievable. Non-replication competent viruses operate in a manner similar to the lysogenic cycle [10]. This method of reproduction is common in certain temperate bacterial viruses, and results in creation of a prophage, the integration of viral nucleic acids with that of the host. Prophages are passed to daughter cells as a result of mitotic division; therefore we assume that the delivery virus is capable of reproducing and infecting further cells in this manner.

Inoculation of transfected genes by replication-defective viruses results in a fixed maximum number of free delivery virus. Without the ability to make copies of their genetic material or lyse a host, growth of free virus does not occur. The initial number of free delivery virus goes to zero upon infection, thus we can assume that after this point the free viral population does not change over time, and we do not require an additional state for the model. Such an instance would yield the system of differential equations

$$\dot{\mathbf{x}} = r\mathbf{x}C_x(t)\left(1-\frac{(\mathbf{x}+\mathbf{y}+\mathbf{z})}{K}\right)-\mathbf{x}\left[d_x+g(t)b\left(\frac{\mathbf{z}}{\mathbf{x}+\mathbf{y}+\mathbf{z}}\right)\right]$$

$$\dot{\mathbf{y}} = \lambda\mathbf{y}C_y(t)\left(1-\frac{(\mathbf{x}+\mathbf{y}+\mathbf{z})}{K}\right)-\mathbf{y}\left[d_y+g(t)b\left(\frac{\mathbf{z}}{\mathbf{x}+\mathbf{y}+\mathbf{z}}\right)\right] \qquad (2)$$

$$\dot{\mathbf{z}} = s\mathbf{z}C_z(t)\left(1-\frac{(\mathbf{x}+\mathbf{y}+\mathbf{z})}{K}\right)-\mathbf{z}\left[d_z+g(t)\right]$$

where **x** represents chemotherapy sensitive/ganciclovir insensitive cells; **y** are chemotherapy resistant/ganciclovir insensitive cells; and **z** are the chemotherapy sensitive/ganciclovir sensitive cells. The exponential growth rates for each species are r, λ, and s, respectively, while d_x, d_y, and d_z represent their natural death rates.

The overall tumor growth, consisting of the sum of all cell species, is restricted by the carrying capacity, *K*. This is an element of the logistic model of population growth that has been incorporated into the model. In an actual system, cells will compete for limited resources such as nutrients or space. It is more realistic for a tumor to have a finite carrying capacity due to finite resources when compared to the unlikely case of unrestricted growth for the linear *in vitro* model. Because of this amendment to our model in equation (1), the set of ordinary differential equations becomes nonlinear. The parameter *K* not only limits the maximum tumor size, but encourages competition for growth between the species. Those cells exhibiting greater fitness, either through mutations or favorable selective pressures, will occupy a larger portion of the tumor. A decrease in population size in one subtype will yield an increase in the others.

$C_x(t)$, $C_y(t)$, and $C_z(t)$ are inputs representing the timed application of chemotherapy, chosen according to its relative effect on individual species' growth. Natural chemotherapy sensitive cells will obviously experience a greater effect under

this treatment. The application of ganciclovir is also an input, *g(t)*, again chosen according to its relative efficacy, that acts primarily on those cells that have been infected by the delivery virus. The bystander effect discussed in Section II is modeled as

$$b\left(\frac{\mathbf{z}}{\mathbf{x}+\mathbf{y}+\mathbf{z}}\right) \tag{3}$$

indicating that the effect on proximal cells increases as transfected cells achieve tumor dominance.

In this model, we have elected to represent bystander killing as a monotonic function, having properties similar to that of the sigmoid function. This is a dimensionless approximation of the likelihood that both natural chemotherapy resistant and sensitive cells are neighbored by cells that have been infected by the delivery virus. This is a reasonable assumption; as the transfected cell population multiplies towards the bounds of the carrying capacity, *K*, non-transfected cells are almost certain to have interaction with ganciclovir sensitivity genes. It is impossible for the likelihood to decrease with each additional cell of subtype **z**; therefore, the bystander effect must be at least a positive semi-definite monotonic function (though we have simplified by assuming it to be strictly increasing).

For the purposes of this thesis and our ODE model, we have chosen to further simplify our system equations with a linear approximation of the generically sigmoid function, *b*. This ensures that the bystander effect is a function having a first derivative that is strictly positive. It should be notes that a partial differential model in this instance would likely represents a more appropriate choice in modeling the system; nonetheless, the dynamics of anti-cancer gene therapy have not previously

been investigated with mathematical models of any sort. This ODE model should be sufficient for a preliminary investigation.

Chemotherapy System Effects

Initial conditions for our model can be changed to demonstrate system dynamics under different starting points. Realistically, they should reflect a tumor population that is largely chemotherapy sensitive, with few cells having the natural resistance mutation. This is because, prior to the initial chemotherapy treatment, resistant cells have never enjoyed a selective advantage associated with a selective pressure. Mutant cells may grow in number, but the absence of a chemotherapy input, *C(t)*, does not enable them to outcompete other cells. They remain a subset of the tumor population, occupying a smaller ratio than sensitive cells whose growth rate is much larger than the mutation rate.

Upon administering a chemotherapeutic agent however, the input causes a dramatic decrease in the number of sensitive cells. Subtype **x** is the population especially vulnerable to decline due to the high chemotherapy efficacy for the species (C_x). This sudden drop causes a temporary reduction in overall tumor size since chemotherapy sensitive cells were the dominant population prior to treatment. Yet, cells of subtypes **y** and **z** have a significant selective advantage because the drug efficacy for these cells, C_y and C_z, are negligible. Because of their chemoresistance, their growth rates outpace that rate of death due to drug inputs. Their continued growth yields a rebound in tumor size, approaching pre-chemotherapy levels. The extinction of sensitive cells allows chemotherapy resistant cells to compete for and occupy the space vacated by those removed.

Under the conditions of our model, subtypes **y** and **z** grow at an identical rate ($r = \lambda$, $d_y = d_z$). Therefore, the cell line that is able to achieve tumor dominance post-selection is dependent on the system initial conditions. Whichever subtype existed in greater numbers prior to chemotherapy will outcompete the other. This is critical when considering the required level of delivery virus infection. Successful ganciclovir treatment at this phase is entirely dependent on the concentration of ganciclovir sensitive cells, **z**. Initial conditions may mandate explosive growth of subtype **y** cells if the natural mutation is sufficiently high, or the inoculation with transfected genes has not sufficiently spread. Under this scenario, a weaker bystander effect will inevitably lead to ganciclovir failure. This sensitivity to initial conditions may be seen in Figure 2, where natural chemotherapy resistant cells constitute a larger portion of the initial tumor population.

Conversely, if the delivery virus can more efficiently infect chemotherapy sensitive cells, we can assume a number of subtype **z** cells greater than those of subtype **y**. Under these conditions, infected cells are amplified most by chemotherapy, making them the most populous tumor cell species and enhancing bystander effect killing. Obviously, the ratio between these species will depend on the difference between their initial conditions. As the initial disparity increases, the steady state values for each species will grow further apart until the ideal bystander effect (when all **y** cells are indirectly killed by ganciclovir) is achieved.

Ganciclovir treatment affects sensitive and bystander effect-sensitive cells, causing a reduction in tumor size. Ideally, this reduction results in a tumor size nadir that would coincide with stochastic extinction of all cells. However, an insufficient bystander effect will permit the tumor size to rebound following ganciclovir treatment.

This represents the worst-case scenario, as cells of subtype **y** will be the only subtype to survive the treatment. As the lone tumor cell type, treatment with either drug becomes useless. Further treatments using this method are no longer an option.

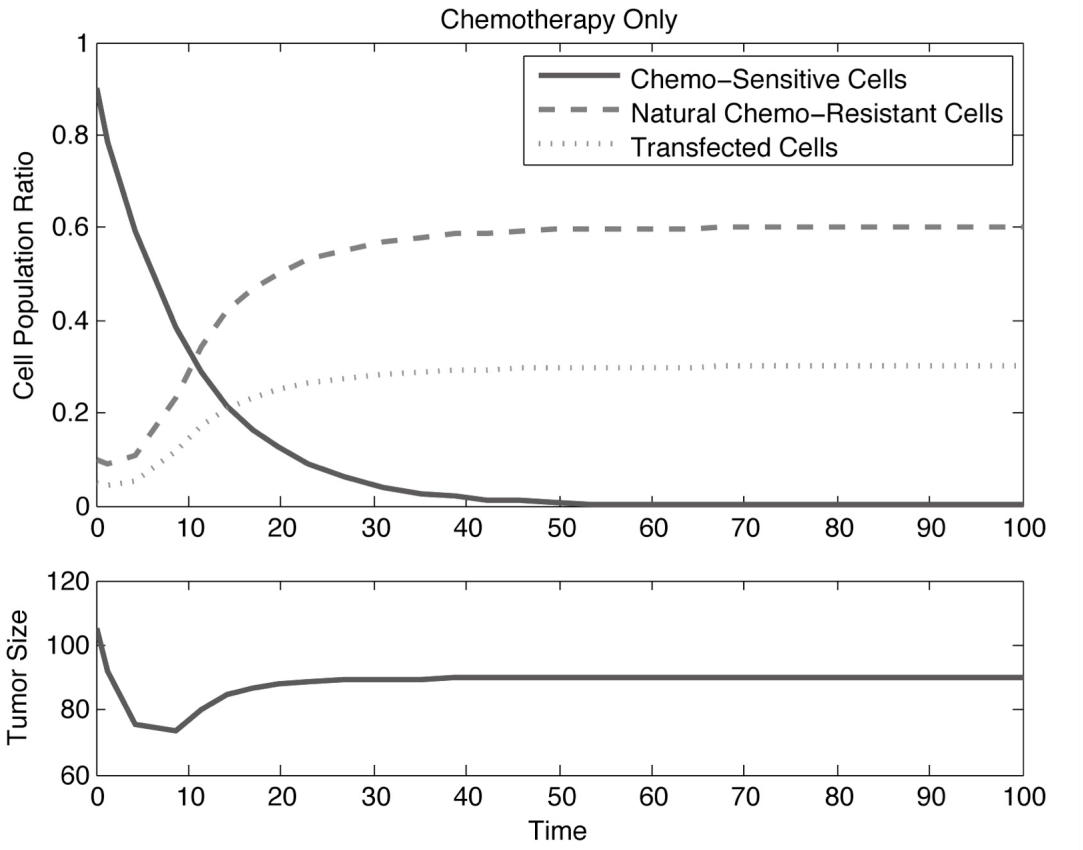

Figure 2 **Initial Condition Effects.** Parameter Values: $\mathbf{x}_0 = 90$, $\mathbf{y}_0 = 10$, $\mathbf{z}_0 = 5$, $C_x = 1$, $C_y = C_z = 0$, $g = 0$. Application of chemotherapy results in a steep decline in sensitive cells and overall tumor size. The greater initial number of natural resistant cells allows them to outcompete the cells with transfected genes. Application with ganciclovir would only eliminate subtype **z**.

Ganciclovir Timing

To avoid the aforementioned instance where the entire tumor is resistant to further treatment, we seek to find the appropriate timing for the ganciclovir input. Because our ultimate goal is to induce stochastic extinction of all cell types in the system, ganciclovir input should be chosen to maximize the bystander effect. Given equal initial conditions for both natural and transfected chemotherapy resistant cells, we restrict our investigation to possible differences in cell fitness under periods of extended chemotherapy. Appropriate ganciclovir input can change if either species of resistant cells is less responsive to chemotherapy than the other.

Until now, our model has only considered the possibility that subtype **y** and **z** cells enjoy the same selective advantage under chemotherapy. We now introduce the condition that their fitness levels are less similar. It is reasonable to assume that one subtype may exhibit a greater degree of fitness than the other. For example, natural mutations may confer dmDHFR resistance while infected cells may carry the gene expressing MDR1. Mutants with dmDHFR resistance would be immune to the effects of MTX, while infected cells could only regulate the intracellular drug concentration to limit its effect. To model this, we can manipulate the system parameters $C_y(t)$ and $C_z(t)$. The observed dynamics may provide incentive to prolong chemotherapy, administering ganciclovir simultaneously.

If natural chemotherapy resistant cells have even a marginally greater fitness over transfected cells ($C_y(t) < C_z(t)$), subtype **y** will be preferably selected for by chemotherapy treatment. This will be observed as a continuous rise in the **y** population that is able to outgrow any possible reduction in number due to chemotherapy. Cell of subtype **z** however, can only increase to a maximum point

before decaying. The initial increase is due to the ability of transfected cells to outcompete chemotherapy sensitive cells; however, transfected cells are outcompeted themselves by natural mutations. Figure 2 illustrates this effect, where we assume a chemotherapy efficacy of 0.1 for transfected cells, while natural resistance confers complete immunity. The greatest bystander effect achievable by the system under these circumstances is created when ganciclovir is administered at this maximum point.

Alternatively, transfected genes may be engineered to grant a more significant degree of chemoresistance to subtype **z**. If transfected cells enjoy a selective advantage over natural chemotherapy resistant cells ($C_y(t) > C_z(t)$), we would observe dynamics similar to the previous case; however, subtypes **y** and **z** exchange roles. In this favorable scenario, ganciclovir treatment would be postponed to allow for further transfected cell growth and thereby, the bystander effect.

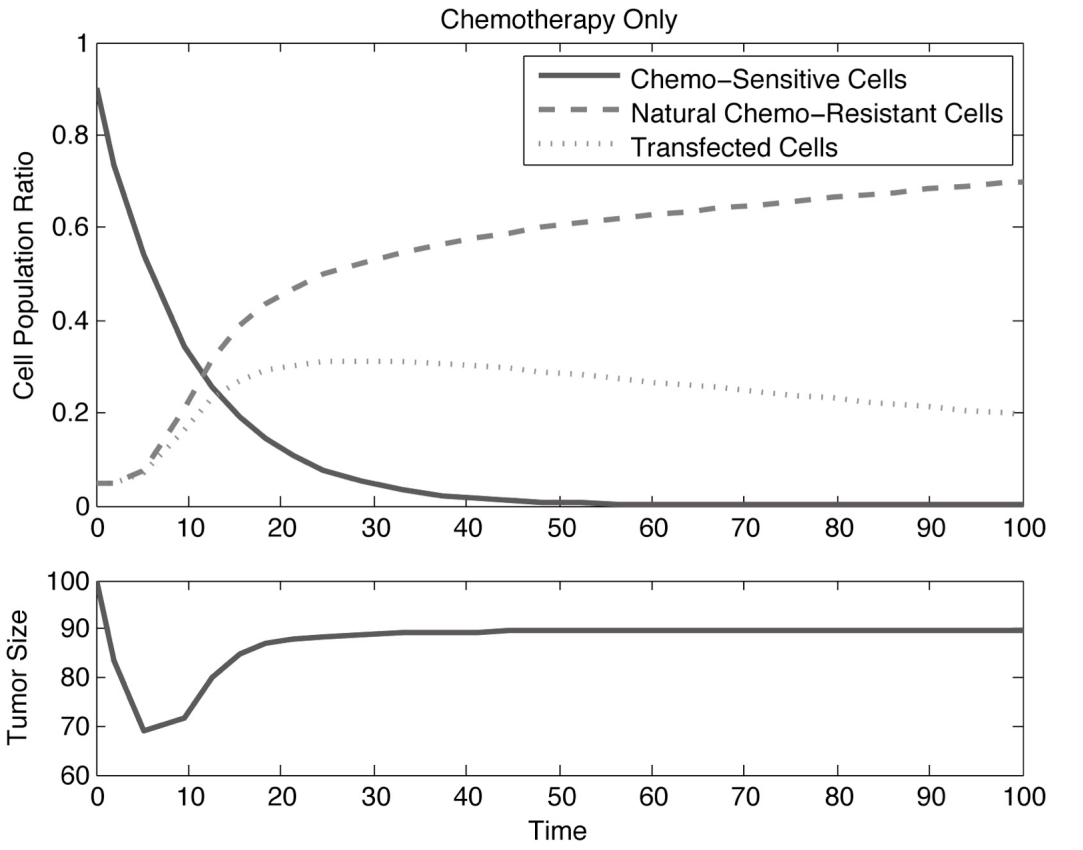

Figure 3 **Natural Chemotherapy Resistant Cell Advantage.** Parameter values: $\mathbf{x}_0 = 90$, $\mathbf{y}_0 = \mathbf{z}_0 = 5$, $C_x = 1$, $C_y = 0.1$, $C_z = 0$, $g = 0$. Under chemotherapy treatment, the cell that is most resistant to drug effects will become most dominant. Here, transfected cells reach a maximum point before decaying. The greatest bystander effect possible would be at this point ($t = 28.6$).

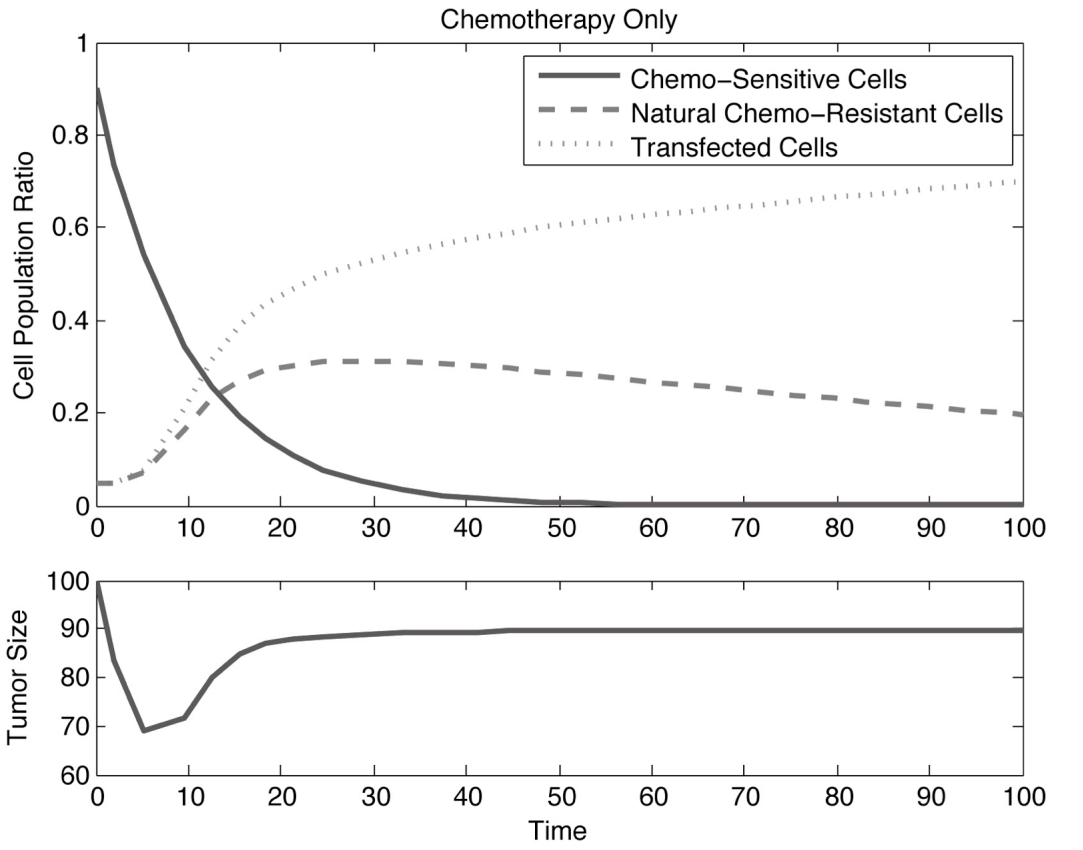

Figure 4 **Transfected Chemotherapy Resistant Cell Advantage.** Parameter values: $\mathbf{x}_0 = 90$, $\mathbf{y}_0 = \mathbf{z}_0 = 5$, $C_x = 1$, $C_y = 0$, $C_z = 0.1$, $g = 0$. Under chemotherapy treatment, the cell that is most resistant to drug effects will become most dominant. Here, natural chemotherapy resistant cells reach a maximum, followed by extended decay. Bystander effect only increases over time.

Replication Competent Delivery Virus

Replication competent viruses reproduce primarily via the lytic cycle. In addition to the spread of ganciclovir susceptibility through host mitosis, the free virus population grows exponentially with the release of multiple viruses for each cell lysis. Free virus particles are then capable of further infecting potential hosts, which in turn leads to demise in host cell population.

Plasmid delivery by replication-competent viruses has certain advantages over the replication defective case. A delivery virus capable of infecting all cells would rather quickly cause infected cells to become the most populous tumor cell species. These infected cells would all express the susceptibility gene, and would consequently be eliminated during ganciclovir treatment. This would avoid the aforementioned possibility in replication defective systems of ganciclovir treatment failure, leading to unrestrained growth of natural resistant cells. A nonspecific delivery virus would always be in the presence of viable hosts (unless each subtype has reached extinction), continually spreading ganciclovir susceptibility.

Of course, there are tradeoffs and this choice of delivery virus might not always produce desired results. Because viral replication requires the destruction of host cells, this method is beneficial only if the infection can spread through all cell lines, equally inhibiting growth. If the virus is specific to one cell type, such as the natural chemotherapy sensitive cells, the virus itself will have effectively established a selective advantage for the species that is not infected. We will show that in this case, infected cell populations will reach a peak before declining once susceptible hosts have been depleted. As is the case when ganciclovir treatment fails with a non-replication competent delivery virus, the remaining subtype is allowed to propagate

through the tumor completely free of competition and any future bystander effect killing.

Virus particles can be more than one thousand times smaller in size than host cells. As a result, they are not in direct competition with the remaining subtypes for the carrying capacity of the tumor. We will not consider free viruses subject to the same spatial constraints dictated by the logistic model of population growth.

We model transfection of ganciclovir susceptibility genes by replication competent viruses by implementing a fourth state and including additional parameters to our original model of equation 1.

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

In this model, **v** is introduced as the free virus population having the gene that will confer both chemoresistance and ganciclovir susceptibility. If we assume all subtypes are prone to infection, free virus particles are able to infect hosts proportional to the mass action rates β_x and β_y. These rates depend on the interaction between the virus and non-infected cells. An infection in subtype **x** or **y** is reflected in the model by a decline in population and an increase in that of subtype **z**.

Once infected, hosts cells are lysed at the lytic rate, *a*. This rate is a reflection of the relative virulence of the infecting virus and can have significant consequences for system dynamics. Virulence refers to the ability of the virus to

directly cause host cell death. A virus displaying low virulence, for example, will have longer latent periods, during which time free virus particles are produced within the cell. A highly virulent virus, however, will induce cell lysis after only a short post-infection delay. High virulence can be represented with larger values for the parameter *a*. For every instance of cell lysis, multiple free virus particles are released. The amount per cell is referred to as the burst constant, *k*. These viruses decay at the rate *u*, similar to the natural death parameters for each cell subtype.

The results of our model simulations were fairly predictable. As Figure 5 shows, infected cells will outcompete other subtypes if the delivery virus is able to infect subtype **x** and **y** cells. As the most populous cell species, infected cells will undergo apoptosis and cause a great degree of bystander effect killing under ganciclovir.

The characteristic of naturally occurring mutants to have a degree of genetic instability may cause them to be less prone to viral infection. Constant changes in their DNA could possibly make successful integration and transcription of the transfected gene problematic. Additionally, natural mutations might result in the ejection of ganciclovir sensitivity genes, similar to the manner in which subtype **y** cells are considered to have initially lost chemotherapy sensitivity. Susceptibility of all cells to infection by the delivery virus should not be assumed; in fact, a delivery virus specific to subtype **x** may be more realistic. We will see that depending on the virulence of the virus, this may necessitate a more dynamic treatment approach.Figure 6, however, demonstrates the problem consistent with a species specific delivery virus. Subtype **x** cells are most populous when the virus is introduced to the system. This abundance of hosts coincides with a rise in infected cells that produce further free

virus particles, but also causes a rapid decline in the population **x**. Subtype **z** can only maintain its level of growth in the presence of **x**, and begins to decline once the virus has caused complete transfer from natural chemotherapy sensitivity to resistance.

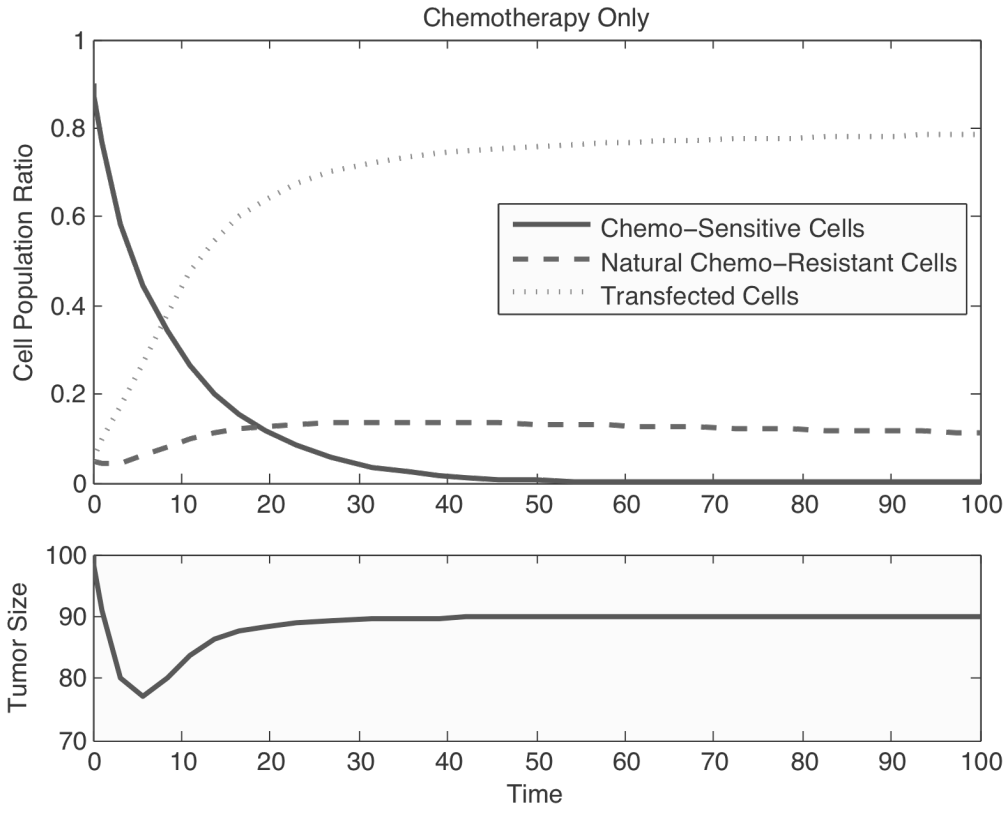

Figure 5 **All Cells Infection-Susceptible.** Parameter values: $C_x(t) = 0.95$, $C_y(t) = C_z(t) = 0$, $g_x(t) = g_y(t) = g_z(t) = 0$, $\beta_x = \beta_y = 0.0015$, k =1. If all cells can become infected, subtype **z** will gain prevalence while subtype will gain prevalence while subtype **y** peaks and begins gradual decline. Ganciclovir has yet to be administered in this figure.

Chapter 5

OPTIMAL CONTROL

Use of a replication competent delivery virus specific to natural chemotherapy sensitive cells requires a single alteration to the model in equation 4. Removing the possibility of subtype **y** infection translates to a removal of the mass action term signifying interaction with free virus, β_y, and yields the set

$$\begin{aligned}
\dot{\mathbf{x}} &= r\mathbf{x}C_x(t)\left(1-\frac{(\mathbf{x}+\mathbf{y}+\mathbf{z})}{K}\right)-\mathbf{x}\left[d_x+\beta\mathbf{v}+g(t)b\left(\frac{\mathbf{z}}{\mathbf{x}+\mathbf{y}+\mathbf{z}}\right)\right] \\
\dot{\mathbf{y}} &= \lambda\mathbf{y}C_y(t)\left(1-\frac{(\mathbf{x}+\mathbf{y}+\mathbf{z})}{K}\right)-\mathbf{y}\left[d_y+g(t)b\left(\frac{\mathbf{z}}{\mathbf{x}+\mathbf{y}+\mathbf{z}}\right)\right] \\
\dot{\mathbf{z}} &= \beta\mathbf{x}\mathbf{v}+s\mathbf{z}C_z(t)\left(1-\frac{(\mathbf{x}+\mathbf{y}+\mathbf{z})}{K}\right)-\mathbf{z}\left[d_z+a+g(t)\right] \\
\dot{\mathbf{v}} &= ka\mathbf{z}-u\mathbf{v}
\end{aligned} \tag{5}$$

Intuitively, we might consider a delivery virus that quickly spreads as the best choice to accelerate the treatment schedule, or shorten the duration. Yet, it is not really this simple; a fast-spreading virus would be one that rapidly infects hosts. Such a characteristic could be represented in the model by increasing the interaction term, β_x. The rate of interaction between free virus and natural chemotherapy sensitive cells, however, can only increase if they are more likely to meet – i.e. there are more free viruses present in the system.

A fast spreading virus will attempt to saturate the tumor with free virus particles by increasing the lytic rate, *a*, to quickly replicate and release new virions

into the system. An increased lytic rate physically means that the period between infection and lysis is shortened, thus reducing the time the virus has to produce genetic copies. This in turn, results in a smaller burst size, k, for the fast-spreading virus. This tradeoff between virus particles per lysis (larger k) and virus particles per time (larger a) favors the latter.

For this reason, the infection rate and virus-induced cell death rate are coupled in nature. In this section, we investigate the both the low and high virulence cases for a replication competent delivery virus. Through our model, we simulate the actions of a fast spreading, high virulence and slow spreading, low virulence delivery virus. Each instance represents different dynamics, which will have implications for the overall drug treatment method.

Low Virulence

Perhaps contrary to our intuition, simulation revealed that the system dynamics associated with a slow spreading delivery virus would coincide with the simplest treatment scheduling. As in previous cases, introduction of chemotherapy caused only sensitive cells to reach extinction levels. This decline is further accelerated by interaction with the virus and transfer to a chemotherapy resistant state. However, a relatively small lytic rate will not cause subtype **x** to decrease before infected cells attain a certain critical population level. At this point, the sheer number of infected cells allows them to out-compete natural mutants simply by their natural growth rate. Once this critical level is reached and the virus has few remaining targets, infected cells are the most prevalent in the tumor.

Simultaneous to the initial growth of infected cells, natural chemotherapy resistant cells begin to increase in number – a drug induced consequence of the

declining numbers of their sensitive counterparts. However, their growth is far outpaced by the infected cell population that grows by both natural replication (mitosis) as well as newly infected cells. At the previously mentioned critical level, the natural mutant population will reach a maximum before beginning a gradual decrease. Their decline is exponential in nature, but with a very long half-life, as it is driven only by the competition for resources, rather than the efficacy of drug treatment. Theoretically, natural mutations could be removed from the system in this manner; however, time to completely decay is prohibitively long to make this a viable treatment option. Of course, ganciclovir treatment can be administered and will cause infected cells, sensitive to its effects, to decay. As a result of the significantly large bystander effect, the natural chemotherapy resistant cells also decay to zero.

In Figure 6, we see the initial decline in the natural sensitive cell population that occurs during periods of chemotherapy. The selective advantage of both infected cells and natural mutants causes subtypes **y** and **z** to increase; the number of infected cells is shown to increase more rapidly due largely to new infections and smaller lytic rate. Finally, Figure 6 shows significant bystander effect killing once natural chemotherapy cells have been driven to extinction and infected cells have become prevalent.

While this treatment option is simple and is nearly guaranteed to be effective, a slow spreading, low virulence delivery virus requires a longer time to operate than would a high virulence virus. Ganciclovir treatment must be delayed until natural chemotherapy resistant cells can be driven to extinction by bystander effect. Furthermore, the system is within a human cancer patient, and treatment time should be minimized as much as possible.

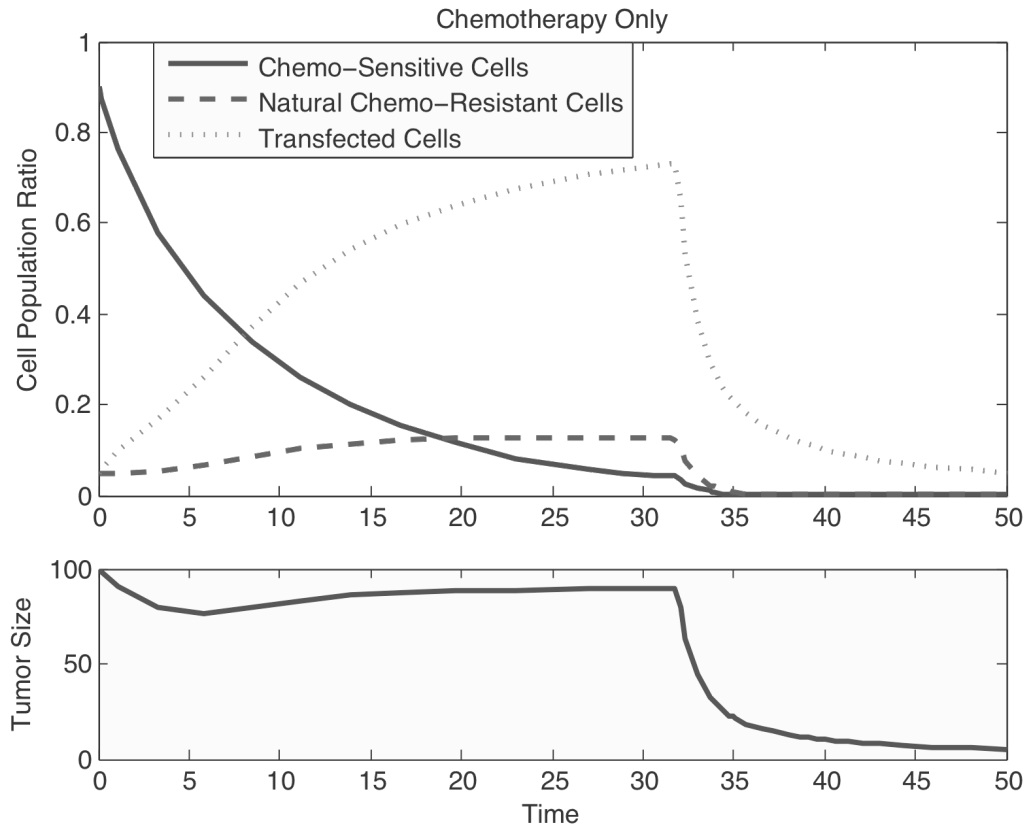

Figure 6 **Low Virulence Delivery Virus.** Parameter Values: $C_x(t) = 0.95$ for $t\epsilon[t_0,30]$, $g_z(t) = 0.9$ for $t\epsilon[32,t_{final}]$, $b = 1.5$, $\beta_x = \beta_y = 0.0015$, $k = 1$. Chemotherapy successfully amplifies the ratio of transfected cells. Ganciclovir is only effective if treatment is delayed until ratio of transfected cells has reach critical level. Entire tumor is reduced with a sufficient bystander effect.

High Virulence

In order to reduce treatment time, a highly virulent delivery virus should be utilized. Theoretically, this would allow for the infected cell population to grow at the quickest rate possible. However, the coupling of the infection rate, β_x, with the virus-induced host cell death rate, *a*, causes unanticipated system behavior. Additional infected cells are gained when free viruses interact with susceptible hosts. However, the spread of infection is negated because frequent interaction requires numerous free viruses, and therefore frequent host cell lysis. The two actions of the delivery virus are offset by each other as newly infected cells increase the population at a rate similar to the decline due to lysis. As a result, the net growth of the infected cell population remains mostly unchanged. While they cannot be outcompeted by the natural chemotherapy resistant cells, they cannot achieve tumor prevalence either without some favorable selective pressure. Without the additional growth due new infections, ganciclovir sensitive cells will never replicate quick enough to induce efficient bystander effect killing.

The use of a highly virulent delivery virus renders the simple treatment seen in the low virulence case useless. We still intend to maximize the bystander effect, therefore ganciclovir should only by introduced at the peak value for

$$\frac{\mathbf{z}(t)}{\mathbf{x}(t) + \mathbf{y}(t) + \mathbf{z}(t)} \tag{6}$$

A more dynamic approach is required to find the optimal timing for chemotherapy. This lead to the optimal control formulation to find the appropriate treatment schedule, C_x, satisfying

$$\max_{t_0 \in [t, T_{Final}], C_x \in \mathscr{C}} \left[\frac{\mathbf{z}(t)}{\mathbf{x}(t) + \mathbf{y}(t) + \mathbf{z}(t)} \right] \tag{7}$$

subject to equation 5 and the given initial conditions. $\mathscr{C}$ defines the set of all clinically acceptable application schedules.

Here, t_0, describes the point at which cells of subtype **y** obtain prevalence in the tumor. As in all previous iterations of this model, successful ganciclovir treatment is most likely to occur at this point; however, that likelihood is dependent on the ability of the chemotherapy schedule to effectively amplify the infected cell ratio. Certain chemotherapy application schedules will induce a greater maximum than others. Therefore, an optimal control method is the ideal approach to find the schedule coinciding with the maximum peak.

Maximizing Cost Function

For simplicity of implementation, chemotherapy treatment is considered to be either applied at full efficacy, or not applied at any given time ($C_x(t)=1$ or $C_x(t)=0$). Application of chemotherapeutic agents is decimated into ten intervals over a length of fifty units of time. For each of these five unit long intervals, application of chemotherapy or non-application can alternate; however, the transition time is finite. Cycling through all possible combinations of chemotherapy treatment - of which there are 2^{10} - allows us to identify the switching method that maximizes the cost function.

As seen in figure 7, the optimal chemotherapy application for a highly virulent delivery virus is not likely to be the simple, single-dose schedule. Instead, a sort of pulsed chemotherapy delivery is preferred, with multiple timed pulses. A single, continuous application of chemotherapy rapidly drives the sensitive cells to extinction. By pulsing chemotherapy, chemotherapy sensitive cells are allowed to

recover for a period of time, allowing for further infection. This has previously been shown in other cases of combination chemotherapies involving gene therapies, illustrating the necessity of a model-based approach to sequence design [12] [13].

This pulsing established by the optimal controller in turn maximizes the bystander effect because the greatest possible percentage of infected cells in the tumor coincides with the optimal chemotherapy treatment schedule. Subsequent introduction of ganciclovir achieves the greatest possible reduction of tumor size, completely eliminated all cell species. While complete tumor removal was also achieved in the low virulence case, optimal control with a highly virulent delivery virus significantly decreases the duration of treatment, exposing a patient to drugs for the shortest length of time possible.

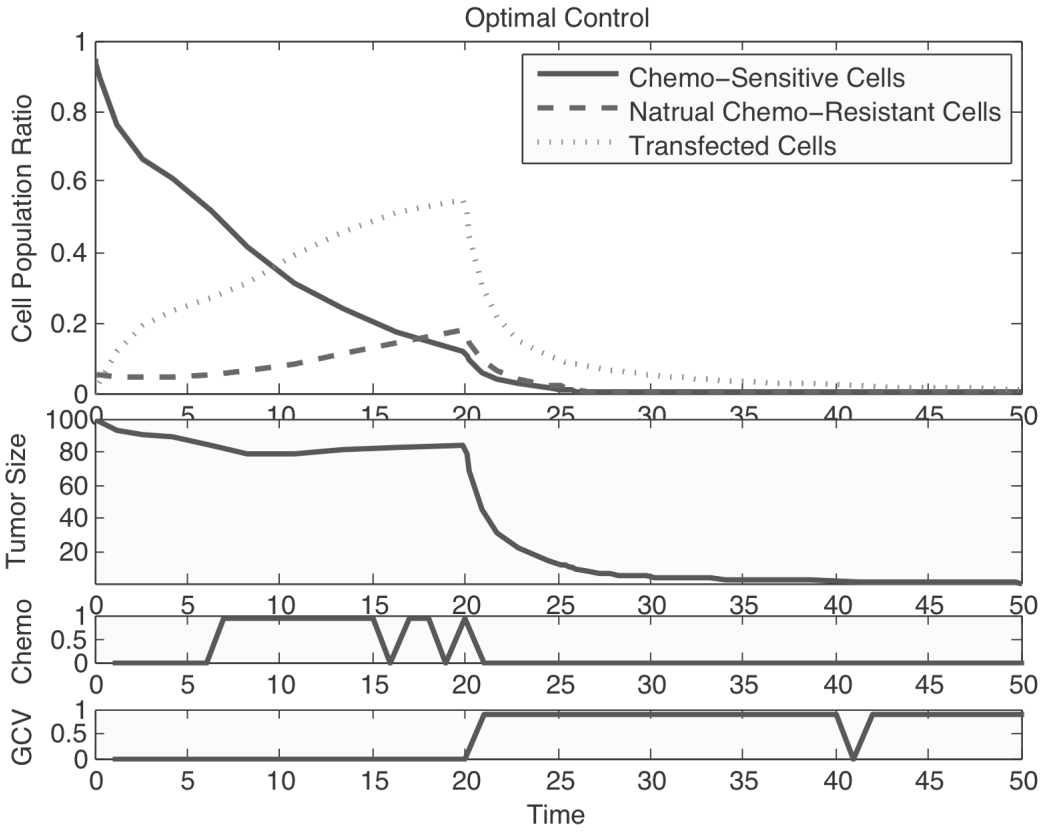

Figure 7 **High Virulence Delivery Virus.** Parameter Values: $C_x = 1$, $g_z(t) = 0.9$, $b = 1.5$, $\beta_x = \beta_y = 0.0015$, $k = 1$. Pulsed chemotherapy successfully amplifies the ratio of transfected cells. Ganciclovir is effective if applied when the cost function is maximized. This point is reached quicker than in the low virulence case, shortening treatment duration. Entire tumor is reduced with a sufficient bystander effect.

CONCLUSIONS

In this thesis, we utilized mathematical models to gain insights to the dynamics of a novel anti-cancer gene therapy technique. By first modeling an *in vitro* system with limitless possible population growth, we displayed the ability of chemotherapy to act as a favorable selective advantage for resistant cells, and ganciclovir to establish negative selection for sensitive cells. The model also demonstrated the result of a sufficient bystander effect in killing cells not directly sensitive to ganciclovir.

The *in vivo* model of a non-replication competent delivery virus pointed out the phenomena associated with chemotherapy as a selective pressure under different initial conditions. The dynamics reveal the need for an initial population of chemotherapy resistant/ganciclovir sensitive cells to be greater than the initial population of natural chemotherapy resistant mutants. Furthermore, the model illustrated the dynamics of a system where cells of one species were relatively more fit to survive chemotherapy. Even a slight competitive advantage allows the cells of greater fitness to become prevalent in the tumor.

The *in vivo* model of a replication competent delivery virus introduced the concept of virulence. A delivery virus of low virulence allowed for infected cells to outcompete natural chemotherapy resistant cells purely due to the nature of their growth. However, we saw the problem inherent with this application – unrealistic treatment duration. The spread of infection was simply too slow, necessitating the use of a more virulent delivery virus.

Finally, the *in vivo* model of a fast-spreading delivery virus demonstrated the need for optimal control. This model allowed for an optimal chemotherapy treatment schedule that would provide the system with the benefits of a highly virulent virus (quick spread), without the drawbacks (prohibitive lytic rate). Optimal treatment illustrated the desired result of complete tumor cell clearance in a feasible length of time.

From the analysis, it is evident that mathematical modeling of systems is vital to understanding the complex dynamics involved. Combination therapy strategies show promise to provide an alternative option to traditional anti-cancer treatment methods, specifically when such regimes would fail. Before this technique can be implemented on human subjects, however, a more thorough understanding of the system must be gained. We have created several models in this thesis but acknowledge that many of the parameters have been considered in ideal or simplified states. Future investigations should better investigate the spatial effects, and utilize estimation of parameters from existing tumor and viral vector models.

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Appendix A

MATLAB CODE

InVitro.m

```
% Preliminary In Vitro Model
% This matlab program includes an ODE solver
% that finds the state values over a chosen
% run time.  The values are then plotted over
% time on a log-scale


function InVitro
clc;
close all;

%Model Parameter Definitions
global lambda d myu e1 e2 b t0 t1 t2 t3 C G
lambda = 1;   % Natural cell growth rate
d = 0.4;        % Natural cell death rate
myu = 0.001;     % Chemoresistance mutation rate
e1 = 0.95;      % Chemotherapy (MTX) drug efficacy
e2 = 0.95;      % Antiviral (GCV) drug efficacy
b = 0.95;        % Bystander Effect
% ------------------------------------------------------

% Treatment times
t0 = 5;    % Chemo treatment beginning time
t1 = 29;   % Chemo treatment end time
t2 = 30;     % GVC treatment begin time
t3 = 95;   % GVC treatment end time
% ------------------------------------------------------

% ODE
Tr = 100;
sol = ode45(@therapy, [0, Tr], [100 20 1]);
% ------------------------------------------------------
```

```
% Plots
lw = 2;
figure;
subplot(3,1,1)
plot(sol.x, sol.y(1,:),'-', 'LineWidth',lw);
ylabel('Chemo sensitive cell level');
xlabel('time (days)');
title('Cell Populations vs time');
hold all
subplot(3,1,2)
plot(sol.x, sol.y(2,:),':', 'LineWidth',lw);
ylabel('Induced chemo resistant cell level');
xlabel('time (days)');
hold all
subplot(3,1,3)
plot(sol.x, sol.y(3,:),'--', 'LineWidth',lw);
ylabel('Natural chemo reisistan cell level');
xlabel('time (days)');

figure;
semilogy(sol.x, sol.y(1,:),':', 'LineWidth',1);
hold all
semilogy(sol.x, sol.y(2,:),'-.', 'LineWidth',1);
hold all
semilogy(sol.x, sol.y(3,:),'-', 'LineWidth',1);
hold all
legend('Chemotherapy Sensitive Cells', 'Induced
Chemotherapy Resistant Cells', 'Natural Chemotherapy
Resistant Cells')
ylabel('cell populations x(t), y(t), z(t)');
xlabel('time');
title('Log Scale Plot of Cell Populations vs Time');
% ------------------------------------------------------

% Differntial Equation Functions
function ddt = therapy(t,y)
global lambda d myu e1 e2 b t0 t1 t2 t3 C G
X = floor(y(1));
Y = floor(y(2));
Z = floor(y(3));
C = e1 * (t>t0) * (t < t1);
G = e2 *(t>t2) * (t<t3);
ddt = [ (lambda-d-myu)*X - C*X
    (lambda-d)*Y - G*Y
    (lambda-d)*Z + myu*X - b*G*Z];
```

InVivo.m

```
% This matlab program simulates the in vivo
% model including both delivery viruses.
function InVivo
clc;
close all;
% -------------------------------------------------------
%Model Parameter Definitions
global 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
lambda = 1; % NCR growth rate
s = 1;      % ICR growth rate
r =1;       % CS growth rate
q = 1;      % uninfected tumor growth rate
e = 1;      % exponent
K = 100;    % carrying capacity
d = .1;     % natural death rate uninfected tumor
n = .00;    % rate of change to chemoresistant tumor
C = 0.95;   % Chemotherapy treatment efficacy
Cn = 1;     % Chemotherapy treatment efficacy
Ci = 1;     % Chemotherapy treatment efficacy
I = 0.90;   % rate of change to induced chemo resistant
f = 0.9;    % growth rate of natural chemo resistant
delta =.1;  % death rate of natural chemo resistant
b = 1.5;
j = 1;      % growth rate of induced chemo resistant
a = I*.1;   % deat rate of induced chemo resistant tumor
G = .9;     % death rate due to ganciclovir (GCV)
Beta = I*.0015;  % virus mass action rate
p = .3;     % virus death rate
L = .01;    % Lytic rate
k = 1;      % Burst size
u = .1;     % Chemotherapy efficacy on chemoresistant
w = .0;     % Chemotherapy efficacy on Induced Resistant
            % resistant back to sensitive term?
% -------------------------------------------------------
% Treatment Times
t0 = 0;    % Chemo treatment beginning time (day)
t1 = 5;    % Chemo treatment end time (day)
t2 = 21;   % GCV treatment begin time (day)
t3 = 23;   % GCV treatment end time (day)
```

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

% ODE
Tr = 50;
sol = ode45(@go, [0, Tr], [90 5 5 50]);
% ------------------------------------------------------

% 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;

figure;
subplot(3,1,1:2)
plot(sol.x, KXGr, '-', 'LineWidth', lw);
hold all
plot(sol.x, KYGr, '--', 'LineWidth', lw);
hold all
plot(sol.x, KZGr, ':', 'LineWidth', lw);
ylabel('Cell Population Ratio');
legend('Chemo-Sensitive Cells ', 'Natural Chemo-Resistant
Cells', 'Transfected Cells')
axis([0 Tr 0 1])
title(['Chemotherapy Only' ])
subplot(3,1,3)
plot(sol.x, Gr, '-', 'LineWidth', lw)
xlabel('Time')
ylabel('Tumor Size')
% ------------------------------------------------------

% Differntial Equation Functions
function ddt = go(t,y)
global 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
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

% Treatment Times
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);

% Differential Equations
%INFECTION SUSCEPTIBLE RESISTANT CELLS
% ddt = [r*X*(1-CC)*(1 - ((X + Y + Z)^e)/(K^e)) - X*(d +
          Beta*V + bb*(Z/(X+Y+Z)))
%     Y*Cn*lambda*(1 - ((X + Y + Z)^e)/(K^e)) - Y*(delta
          + Beta*V + bb*(Z/(X+Y+Z)))
%     Beta*X*V + Beta*Y*V + Z*Ci*s*(1 - ((X + Y +
          Z)^e)/(K^e)) - Z*(a + GG + L)
%     k*L*Z - p*V];
%INFECTION RESTRICTED TO CS AND ICR CELLS
ddt = [r*X*(1-CC)*(1 - ((X + Y + Z)^e)/(K^e)) - X*(d +
          Beta*V + bb*(Z/(X+Y+Z)))
    Y*(Cn)*lambda*(1 - ((X + Y + Z)^e)/(K^e)) - Y*(delta
          + bb*(Z/(X+Y+Z)))
    Beta*X*V + Z*(Ci)*s*(1 - ((X + Y + Z)^e)/(K^e)) –
          Z*(a + L + GG)
    k*L*Z - p*V];
```

```
%NON COMPETENT REPLICATION VIRUS
% ddt = [r*X*(1-CC)*(1 - ((X + Y + Z)^e)/(K^e)) - X*(d +
          GG*(Z/(X+Y+Z)))
%      Y*(Cn)*lambda*(1 - ((X + Y + Z)^e)/(K^e)) - Y*(d +
          GG*(Z/(X+Y+Z)))
%      Z*(Ci)*s*(1 - ((X + Y + Z)^e)/(K^e)) - Z*(d + GG)
%      0];
```

findoptimalsequence.m

```
% This matlab program runs simultaneously
% with InVivoFinal to run through all possible
% chemotherapy treatment schedules over a
% selected duration, to find the optimal
% sequence to maximize the cost function.
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);
```

InVivoOptim.m

```
% This matlab program runs simultaneously with
% findoptimalsequence.m to maximize the cost
% function to find the best chemotherapy treatment
% schedule.
function InVivoOptim(seq1, Tmax)
clc;
close all;
tttt=cputime;
length = max(size(seq1));

% ------------------------------------------------------
%Model Parameter Definitions
global 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
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
Ci = 0;     % Chemotherapy treatment efficacy
I = 1.5;    % Beta-a ratio
f = 1;      % growth rate of natural chemo resistant
delta =.1;  % death rate of natural chemo resistant
b = 1.5;    % death rate due to bystander effect
j = 1;      % growth rate of induced chemo resistant
a = I*.1;   % deat rate of induced chemo resistant
G = .9;     % death rate of  due to ganciclovir
Beta = I*.001;  % 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], [95 5 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;
figure;
subplot(8,1,1:4)
plot(sol.x, KXGr, '-', 'LineWidth', lw);
hold all
plot(sol.x, KYGr, '--', 'LineWidth', lw);
hold all
plot(sol.x, KZGr, ':', 'LineWidth', lw);
%xlabel('time');
ylabel('Cell Population Ratio');
legend('Chemo-Sensitive Cells ', 'Natrual Chemo-Resistant
Cells', 'Transfected Cells')
axis([0 Tr 0 1])
title(['Optimal Control'])
```

```
subplot(8,1,5:6)
plot(sol.x, Gr, '-', 'LineWidth', lw)
%xlabel('Time')
ylabel('Tumor Size')
axis([0 Tr min(Gr) 100])
subplot(8,1,7)
plot(CCC, 'Linewidth', lw)
axis([0 Tr  0 1])
ylabel('Chemo')
subplot(8,1,8)
plot(GGG, 'LineWidth', lw)
xlabel('Time')
ylabel('GCV')
axis([0 Tr 0 1])

% ---------------------------------------------------------
% Differntial Equation Functions
function ddt = go(t,y)
global 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
rn = round(rand);
```

```
% Treatment Times0
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

h = round(t)+1;
CCC(h) = CC;
GGG(h) = GG;

% 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];
```