

**DETERMINING OPTIMAL CHEMOTHERAPY SCHEDULES USING  
ODE MODELING AND CONTROL**

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Electrical and Computer Engineering

Spring 2013

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## ACKNOWLEDGMENTS

I would like to thank Prof. Ryan Zurakowski for all of his help and guidance during my undergraduate and graduate career here at The University of Delaware. My family, my brother, and sister for raising me right and giving me many opportunities, supportive and tough love. My professors and fellow students for their knowledge and assistance when it was needed. My significant other for her support and motivation.

## TABLE OF CONTENTS

<b>LIST OF TABLES</b> . . . . .	<b>vi</b>
<b>LIST OF FIGURES</b> . . . . .	<b>vii</b>
<b>ABSTRACT</b> . . . . .	<b>ix</b>
 <b>Chapter</b>	
<b>1 INTRODUCTION</b> . . . . .	<b>1</b>
<b>2 BIOLOGICAL BACKGROUND</b> . . . . .	<b>4</b>
2.1 Cancer . . . . .	4
2.2 Chemotherapy . . . . .	4
2.3 Chemoresistance . . . . .	7
2.4 Transfected Cells . . . . .	9
2.5 Bystander Effect . . . . .	9
<b>3 MODEL</b> . . . . .	<b>11</b>
3.1 Mathematical Model . . . . .	11
3.2 MATLAB Model . . . . .	14
<b>4 RESULTS</b> . . . . .	<b>16</b>
4.1 High Virulence Delivery Virus . . . . .	16
4.2 Low Virulence Delivery Virus . . . . .	19
4.3 Minimum Tumor Size . . . . .	21
4.4 Time to Achieve . . . . .	25
4.5 Simulations with Zero Initial Induced Chemoresistant Cells . . . . .	27
4.5.1 High Virulence Delivery Virus . . . . .	28
4.5.2 Low Virulence Delivery Virus . . . . .	30
4.5.3 Minimum Tumor Size . . . . .	32

4.5.4	Time to Achieve . . . . .	33
4.6	Sensitivity Analysis . . . . .	34
4.6.1	Low Virulence, Interval = 20, Original ICs [99 1 1 99] . . . . .	35
4.6.2	Low Virulence, Interval = 100, Original ICs [99 1 0 99] . . . . .	41
<b>5</b>	<b>CONCLUSION</b> . . . . .	<b>45</b>
	<b>REFERENCES</b> . . . . .	<b>49</b>
<b>Appendix</b>		
	<b>MATLAB CODE</b> . . . . .	<b>52</b>

## LIST OF TABLES

1.1	Parameter Values . . . . .	3
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## LIST OF FIGURES

2.1	Normal Differentiation versus Cancer Differentiation . . . . .	6
2.2	Methods of Chemoresistance . . . . .	8
3.1	Diagram of ODE model. Variables corresponding to the 4 equations are shown in blue circles. The value in the red rectangle inhibits, as shown with the dashed lines. Blue arrows show $g(t)$ decay terms. .	12
4.1	Optimal Control, High Virulence ( $a = 0.06$ ) IC(99 1 1 99) Interval = 20 . . . . .	17
4.2	Optimal Control, High Virulence ( $a = 0.06$ ) IC(99 1 1 99) Interval = 100 . . . . .	18
4.3	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(99 1 1 99) Interval = 20 . . . . .	19
4.4	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(99 1 1 99) Interval = 100 . . . . .	20
4.5	Minimum Tumor Size, IC(99 1 1 99) Interval = 20, Threshold $1e-5$	22
4.6	Minimum Tumor Size, IC(99 1 1 99) Interval = 100, Threshold $1e-5$	23
4.7	Minimum Tumor Size, IC(99 1 1 99) Interval = 1, Threshold $1e2$ .	24
4.8	Achieve Time, IC(99 1 1 99) Interval = 20, Threshold $1e-5$ . . . . .	25
4.9	Achieve Time, IC(99 1 1 99) Interval = 100, Threshold $1e-5$ . . . . .	26
4.10	Achieve Time, IC(99 1 1 99) Interval = 1, Threshold $1e2$ . . . . .	27
4.11	Optimal Control, High Virulence ( $a = 0.06$ ) IC(99 1 0 99) Interval = 20 . . . . .	28

4.12	Optimal Control, High Virulence ( $a = 0.06$ ) IC(99 1 0 99) Interval = 100 . . . . .	29
4.13	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(99 1 0 99) Interval = 20 . . . . .	30
4.14	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(99 1 0 99) Interval = 100 . . . . .	31
4.15	Minimum Tumor Size, IC(99 1 0 99) Interval = 100, Threshold $1e-1$ . . . . .	32
4.16	Achieve Time, IC(99 1 0 99) Interval = 100, Threshold $1e-1$ . . . . .	33
4.17	Achieve Time, IC(99 1 0 99) Interval = 1, Threshold $1e2$ . . . . .	34
4.18	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(198 2 2 198) Interval = 20 . . . . .	35
4.19	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(148.5 1.5 1.5 148.5) Interval = 20 . . . . .	37
4.20	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(123.75 1.25 1.25 123.75) Interval = 20 . . . . .	38
4.21	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(94.05 0.95 0.95 94.05) Interval = 20 . . . . .	39
4.22	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(89.1 0.9 0.9 89.1) Interval = 20 . . . . .	40
4.23	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(123.75 1.25 0 123.75) Interval = 100 . . . . .	42
4.24	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(108.9 1.1 0 108.9) Interval = 100 . . . . .	43
4.25	Optimal Control, Low Virulence ( $a = 0.02$ ) IC(94.05 0.95 0 94.05) Interval = 100 . . . . .	44



## ABSTRACT

Chemotherapy is mostly effective in helping cancer patients but invariably some cells will mutate to become more fit against chemotherapeutic agents. We build upon previous work that focuses on transfecting tumor cells via a delivery virus. The transfected cells then become both chemoresistant and sensitive to ganciclovir, which is an acyclic nucleotide antiviral agent. A positive selection phase of chemotherapy administration and negative selection phase of ganciclovir injection enables a chemoresistant tumor to be eradicated by a bystander effect. The bystander effect occurs when ganciclovir is applied and is strong enough to eradicate the tumor if cell populations and parameters are favorable. An ordinary differential equation model is used to represent the biological phenomena. A control system is developed in computer simulation to find the optimal treatment strategy. We improve upon previous research by modifying the cost function to measure for absolute minimum tumor size and also by incorporating realistic physiological parameters into our models.

## Chapter 1

### INTRODUCTION

Pancreatic cancer is the 4th most common form of cancer in the USA, 8th worldwide and has a poor prognosis (7). The one and five year post-diagnosis survival rates are 25% and 6% respectively. The development of cancer may involve the over-expression of oncogenes, inactivation of tumor suppressor genes or the deregulation of various signaling proteins (8). In order to determine an effective chemotherapeutic treatment schedule to cure pancreatic cancer, we build an ODE model based on previous work (14). Chemotherapy causes apoptosis in rapidly-dividing cells. This prevents cancer cells from successfully multiplying (9). However, cancerous cells can mutate to become chemoresistant. If these chemoresistant cells have a greater fitness than other cell types then they could grow to become the dominant cell type. If this happens, the cancerous tumor becomes extremely difficult to cure. To avoid this problem our model has a positive selection phase for transfected cells where chemotherapy kills the chemosensitive cells ( $X$  population). The ideas of transfection and a positive selection process were introduced by Martinez-Quintanilla et al. (10,11). In their paper, pancreatic carcinoma cell lines are transfected via a plasmid containing one of two separate genes encoding either multidrug resistance gene1 (MDR1) or Dihydrofolate reductase (DHFR), combined with a gene encoding herpes simplex virus thymidine kinase (HSV-TK). This is captured in our model with the three types of cell populations. As the proportion of the tumor that is composed of chemosensitive cells decreases, the  $Z$  cells (induced chemoresistant cells) are able to outcompete the  $Y$  type cells (natural chemoresistant cells) and thus comprise a larger portion of the tumor than before the positive selection phase.

Ganciclovir is an acyclic nucleotide antiviral agent. This transfection uses a hybrid gene that encodes an enzyme that confers ganciclovir sensitivity, and a second gene that confers resistance to another chemotherapy. After this positive selection phase the tumor is mostly comprised of cells that fall within two population types. One type,  $Y$  type cells, are chemo and ganciclovir (GCV) resistant and the other type,  $Z$ , are chemoresistant and GCV susceptible. If there are enough  $Z$ -type cells relative to the entire tumor, bystander effects due to apoptosis of  $Z$  when GCV is applied will reduce the number of  $Y$ -cells to extinction. The bystander effect originates when tyrosine kinase diffuses out of the  $Z$  cell into the neighboring cells, making them also GCV-sensitive. This will effectively eradicate any cancer because  $Z$ -type cells will also go extinct when treated with GCV. If however, the bystander effect is not strong enough to completely wipe out the  $Y$  population, then the remaining  $Y$ -cells will repopulate and become an incurable tumor.

The HSV-TK mono-phosphorylates GCV that is subsequently converted by mammalian cellular kinases to GCV-triphosphate, a potent inhibitor of DNA polymerase that blocks DNA replication and causes cell death (2, 22). DHFR and MDR1 are well known and widely utilized chemoresistance genes that can induce chemoresistance in tumor cells via transfection (5). This transfection and positive selection process are also used and accounted for in the original ODE model (14).

One way we improve this model is by updating the model parameters with biologically relevant values found from previous literature. In unpublished work by Stephany Rojas-Garcia, the author used data fitting to obtain values for several model parameters including cell growth rates and chemotherapeutic effectiveness on different cell types. The untreated tumor growth curves of 18 orthotopic implanted pancreatic tumor-bearing mice (16) were required for the elaborate data fitting approach and parameter estimation. By utilizing GFPs, Bouvet (16) reported the whole body optical image in real time of genetically fluorescent pancreatic tumors growing and metastasizing in live mice. The experimental data was fitted to a logistic growth model using a simulated annealing optimization method. Other necessary parameters were taken

from a paper by Wein et al. (15) that developed a mathematical model to analyze the infection process of a replication-competent virus to a three-dimensional tumor. The values of cell death rates for  $X$ ,  $Y$ , and  $Z$  are taken from the paper by Stark et al. (17).

The remainder of this thesis is organized in the following way: section 2 provides a biological background, section 3 presents our model, section 4 shows our results, and section 5 is our conclusion.

**Table 1.1:** Parameter Values

Parameter	Value	Meaning	Source
$d_X(t)$	0.009464	Chemotherapy Sensitive Cell Death Rate $\frac{mm^3}{day}$	M. J. Stark, et al., 1995
$d_Y(t)$	0.009464	Naturally Chemo-Resistant Cell Death Rate $\frac{mm^3}{day}$	M. J. Stark, et al., 1995
$d_Z(t)$	0.009464	Induced Chemo-Resistant Cell Death Rate $\frac{mm^3}{day}$	M. J. Stark, et al., 1995
$r$	0.0676	Chemotherapy Sensitive Cell Growth Rate $\frac{mm^3}{day}$	Data Fitting
$\lambda$	0.0676	Naturally Chemo-Resistant Cell Growth Rate $\frac{mm^3}{day}$	Data Fitting
$s$	0.0676	Induced Chemo-Resistant Cell Growth Rate $\frac{mm^3}{day}$	Data Fitting
$K$	302.3935	Tumor Carrying Capacity $mm^3$	Data Fitting
$k$	$1.0 \cdot 10^4$	Burst Size $\frac{PFU}{cell}$	Wu, 2004
$p$	24	Virus Death Rate $\frac{1}{day}$	Wu, 2001
$\Theta$	$1.0 \cdot 10^6$	Tumor Cell Density $\frac{cells}{mm^3}$	Wein et al., 2003
$R_0$	3.73	Basic Reproductive Ratio	Wein et al., 2003
$C_X(t)$	0.95	Chemotherapy Efficacy - Chemotherapy Sensitive Cell $\frac{1}{day}$	Data Fitting
$C_Y(t)$	0.0	Chemotherapy Efficacy - Naturally Chemo-Resistant Cell $\frac{1}{day}$	Data Fitting
$C_Z(t)$	0.0	Chemotherapy Efficacy - Induced Chemo-Resistant Cell $\frac{1}{day}$	Data Fitting
$g_X(t)$	0.9	Death Rate Due to GCV Sensitivity $\frac{1}{day}$	Data Fitting

## Chapter 2

### BIOLOGICAL BACKGROUND

#### 2.1 Cancer

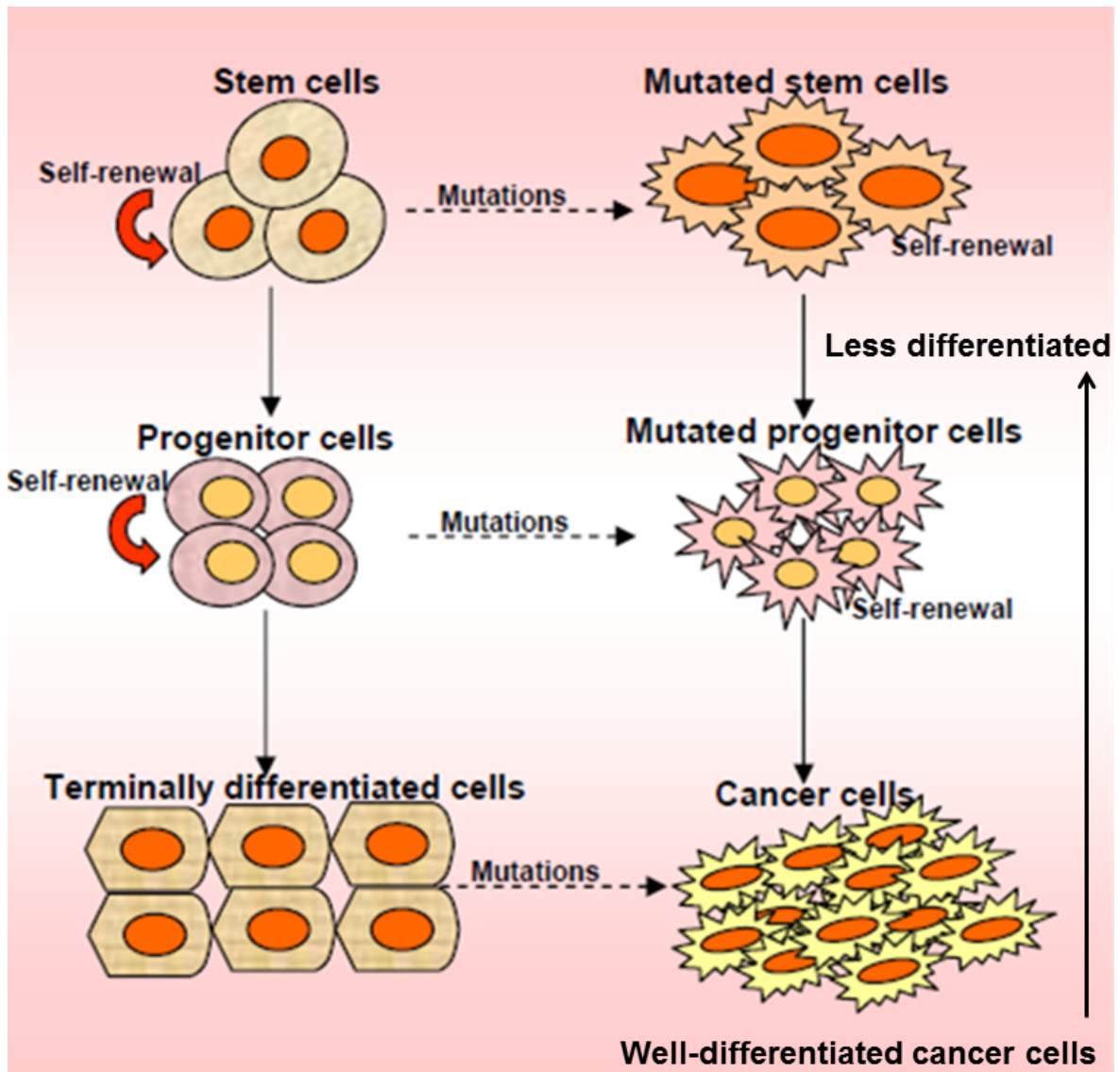
A simple definition of cancer is a disease that causes a tissue to fail to regulate its growth correctly. The genes that regulate cell growth are altered and the normal cell is transformed into a cancer cell. These genes are grouped into oncogenes, which promote cell growth and reproduction, and tumor suppressor genes, which suppress cell division. When these genes act incorrectly, i.e. by over-expressing normal oncogenes or under-expressing tumor suppressor genes, then malignant transformations are possible (1).

Smaller mutations such as point mutations, deletions, and insertions sometimes negatively affect functionality. Additionally, DNA replication handles a large amount of data and will therefore likely result in errors occasionally. Hence, intricate methods of fixing and preventing errors are innate to the process of DNA replication. Apoptosis, programmed cell death through self-destruction, happens when a severe error occurs and serves as a safety from cells against cancer. However, if a process meant to mediate errors is not successful then the mutations will remain and be passed to any daughter cells (4).

#### 2.2 Chemotherapy

Chemotherapy is the treatment of cancer with one or more cytotoxic drugs as part of a treatment schedule. Chemotherapeutic agents work by targeting cells that replicate quickly by impairing mitosis or causing apoptosis. Consequently, it not only causes harm to cancerous cells but also normal cells that characteristically divide quickly, such as cells in bone marrow and the digestive tract, which causes

adverse side effects. Chemotherapeutic drugs have a more significant affect on more differentiated tumors due to the fact that mechanisms regulating cell growth are still kept intact. As the generations of tumor cells increases, differentiation is generally lost and chemotherapeutic agents are less effective against tumors. In the area near the center of a solid tumor cell division has effectively ceased, making them insensitive to chemotherapy. Naturally and as time goes on, cancer cells gain chemoresistance.



**Figure 2.1:** In biology, differentiation describes the processes by which immature cells become more mature cells with specific functions. However when talking about cancer, differentiation describes how much or how little the tumor tissue resembles the normal tissue it came from. Well-differentiated cancer cells look more similar to normal cells and tend to grow and spread slower than poorly differentiated or undifferentiated cancer cells (23).

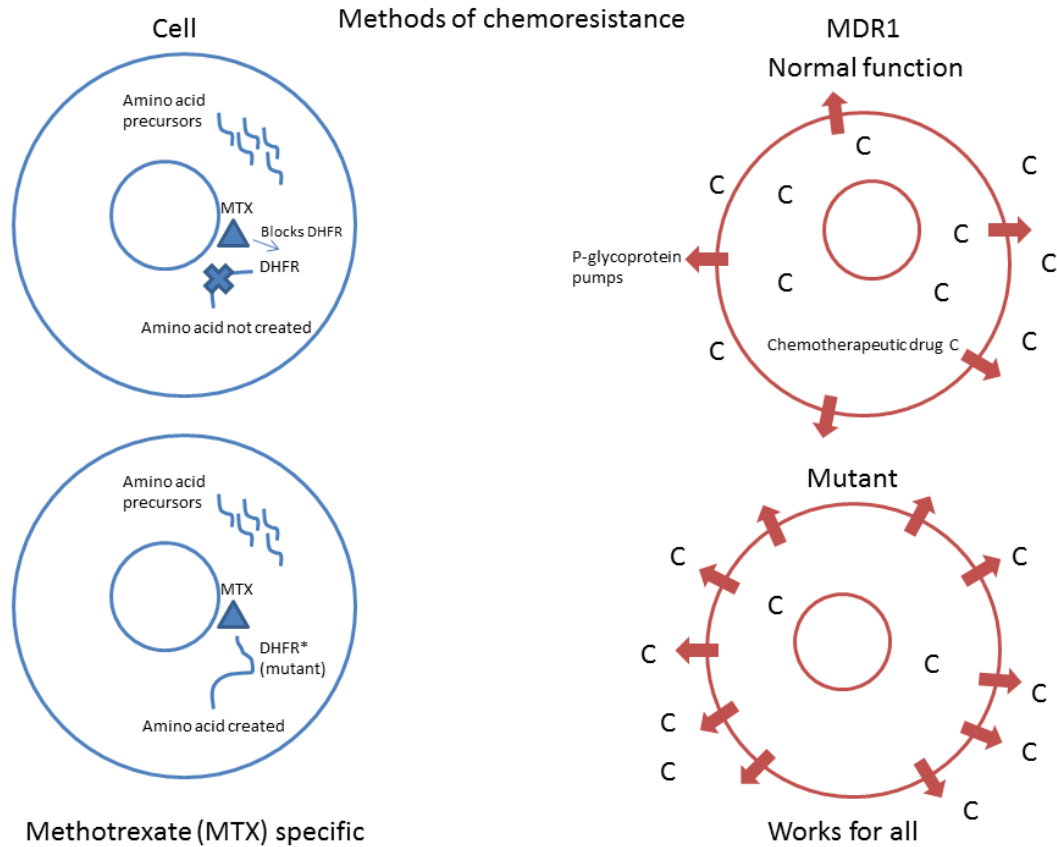
### 2.3 Chemoresistance

Cancerous cells become chemoresistant through natural mutation or transfection of engineered genes (see figure 2.2). Commonly found chemo-resistance genes are variants of dihydrofolate reductase (DHFR) and multidrug resistance gene1 (MDR1) (5) . In our model we assume that a small percentage of the initial tumor is made of chemo-resistant cells (14).

DHFR is used as a catalyst for the reduction of folate to tetrahydrofolate (3). This enzyme then acts as a cofactor in the creation of nucleic and amino acids. Methotrexate (MTX) is a chemotherapeutic agent that has a greater affinity to DHFR than folate. Therefore, DHFR more readily binds to MTX, preventing potential production of tetrahydrofolate and subsequent nucleotides. Ideally, methotrexate would show this behavior for all tumor cells. Unfortunately, mutants of DHFR show a lower affinity for the drug, which allows them to continue preferential binding to folate.

P-glycoprotein (PGP) is a facilitator of molecule movement, dealing with both extracellular and intercellular transport. PGP can act as chemotherapy efflux pump that decreases intercellular concentrations of drugs, therefore reducing their effectiveness. MDR1 up-regulates the expression of PGP, resulting in increased chemoresistance to many types of chemotherapy.





**Figure 2.2:** Left side: MTX binds with DHFR and blocks it such that no amino acids are created. With mutant DHFR (DHFR\*), the DHFR\* is not blocked by MTX because DHFR\* preferentially binds with folate instead to produces nucleotides. Right side: Small pumps on the surface of cancer cells actively move chemotherapeutic drugs from inside the cell to the outside. These pumps are present in normal cells, but mutants have more of them and therefore more chemotherapeutic drugs are pumped out.

## 2.4 Transfected Cells

In previous work by Martinez-Quintanilla et al. (10,11), the authors transfect colon and pancreatic carcinoma cells lines using a plasmid containing one of two separate genes encoding either DHFR or MDR1, together with a gene encoding herpes simplex virus thymidine kinase (HSV-TK). These transfected genes guarantee the survival from chemotherapy and consequently create a third type of cell population ( $Z$  cells).

The HSV-TK encodes an enzyme in the transfected cell which causes it to be susceptible to ganciclovir, an antiviral drug, which is popular in numerous anti-cancer gene therapy approaches due to its ease in which it can be incorporated into the DNA of cells. The cell transmission of the tyrosine kinase enzyme transforms the initially inert GCV and causes the formation of double-strand breaks in cell DNA which in turn triggers apoptosis. Intercellular transfer of the GCV to neighboring cells then occurs through gap junctions, which are 2-4 nm apart, and causes a bystander effect when transfected cells come into contact with chemo-resistant cells at gap junctions. The result leads to an apoptosis trigger in cells that are not ordinarily targeted by the drug (14).

Thus our strategy to eradicate tumor populations has two steps. In the first step, chemotherapy causes a positive selection for chemo-resistant cells by killing off all chemo-susceptible cells, leading to an increased proportion of transfected cells. Ideally, the amount of transfected cells surpasses the amount of the chemo-resistant mutant population because this results in a greater bystander effect. The second step is a negative selection stage where ganciclovir is injected and the remaining cells are eradicated through triggered apoptosis and a bystander effect.

## 2.5 Bystander Effect

Since the bystander effect is what ultimately kills the chemo and ganciclovir resistant cells if it is large enough, it is paramount that we maximize its benefits. If the bystander effect is too small though, possibly because the number of transfected

cells is too small compared to the total tumor population, then those mutant cells would not be completely destroyed after the injection of GCV. This is partly because, the low proportion of transfected cells to other cell types in the tumor translates to a low probability of exchange of metabolized ganciclovir through contact. In this unfavorable scenario, the remaining cells would proliferate leading to a tumor comprised solely of chemo and ganciclovir-resistant cells. Our research strives to find the chemotherapy schedule that grants the most favorable positive and negative selection phases and yields the greatest chance of complete tumor eradication.

## Chapter 3

### MODEL

#### 3.1 Mathematical Model

In our model we consider transfection via oncospecific viral vectors, specifically a replication-competent delivery virus. The replication-competent virus differs from the nonreplication-competent one because it has the gene necessary to evolve the lytic cycle. In the replication-competent approach, hybrid genes not only spread through mitosis of transfected cells but also by virus lysis. In our ODE model, the virus is not allowed to infect the  $Y$ -type cells because we want to focus on the dynamics that result when the virus can only infect  $X$ -type cells. Our model draws upon previous research of modeling solid tumor growth (18, 19, 20, 21).

$$\dot{\mathbf{X}} = r\mathbf{X}C_X(t) \left(1 - \frac{(\mathbf{X}+\mathbf{Y}+\mathbf{Z})}{K}\right) \quad (3.1)$$

$$-\mathbf{X} \left[ d_X + \beta V + g(t) b \left( \frac{\mathbf{Z}}{\mathbf{X}+\mathbf{Y}+\mathbf{Z}} \right) \right]$$

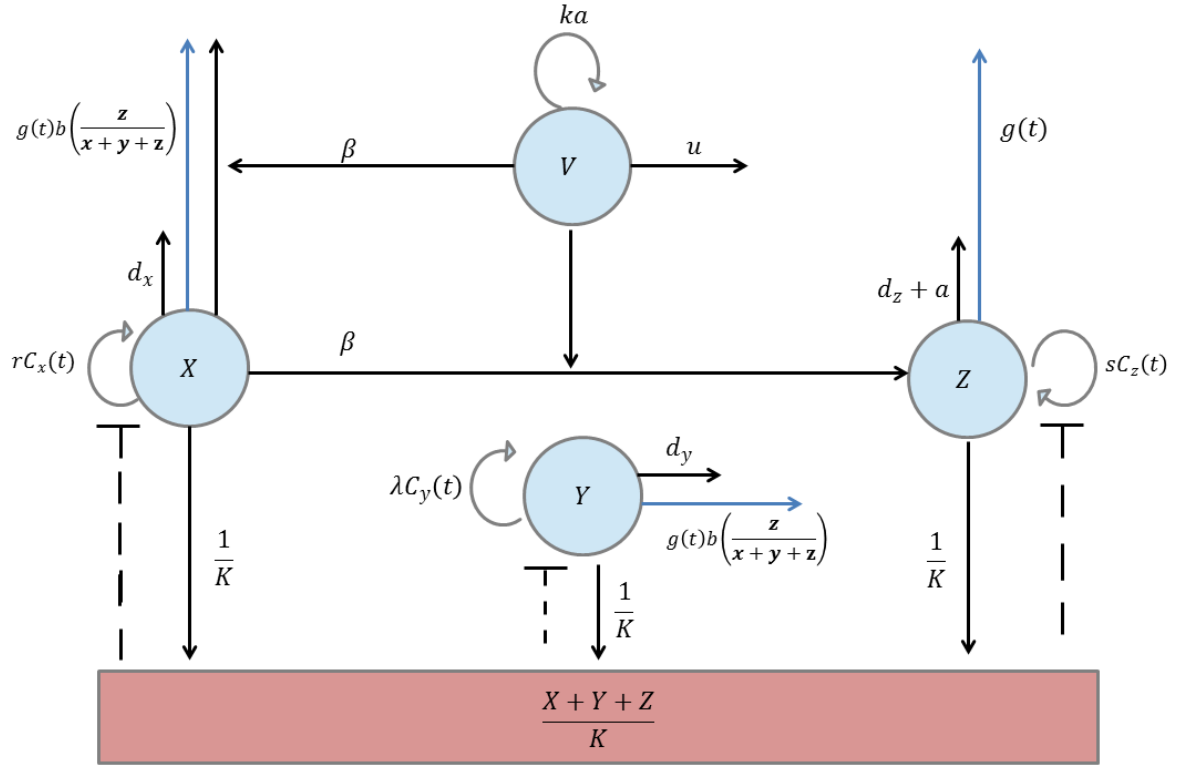
$$\dot{\mathbf{Y}} = \lambda\mathbf{Y}C_Y(t) \left(1 - \frac{(\mathbf{X}+\mathbf{Y}+\mathbf{Z})}{K}\right) \quad (3.2)$$

$$-\mathbf{Y} \left[ d_Y + g(t) b \left( \frac{\mathbf{Z}}{\mathbf{X}+\mathbf{Y}+\mathbf{Z}} \right) \right]$$

$$\dot{\mathbf{Z}} = \beta\mathbf{X}\mathbf{V} + s\mathbf{Z}C_Z(t) \left(1 - \frac{(\mathbf{X}+\mathbf{Y}+\mathbf{Z})}{K}\right) \quad (3.3)$$

$$-\mathbf{Z} [d_Z + a + g(t)]$$

$$\dot{\mathbf{V}} = ka\mathbf{Z} - u\mathbf{V} \quad (3.4)$$



**Figure 3.1:** Diagram of ODE model. Variables corresponding to the 4 equations are shown in blue circles. The value in the red rectangle inhibits, as shown with the dashed lines. Blue arrows show  $g(t)$  decay terms.

$X$  are chemosensitive / ganciclovir insensitive cells,  $Y$  represent chemoresistant / ganciclovir insensitive cells,  $Z$  are chemoresistant / ganciclovir sensitive cells, and  $V$  are the viruses that contain the gene that confers chemoresistance and GCV susceptibility.  $r$ ,  $\lambda$ , and  $s$  are exponential growth rates while  $d_X$ ,  $d_Y$ , and  $d_Z$  are natural decay rates of each cell type.  $K$  is the carrying capacity of the tumor.  $C_X$ ,  $C_Y$ , and  $C_Z$  are multiplicative constants that represent the efficacy of chemotherapeutic treatments on the respective species.  $g(t)$  represents the injection of ganciclovir, scaled for efficacy, that mainly affects the transfected cells. The bystander effect is modeled by

$$b \left( \frac{Z(t)}{X(t) + Y(t) + Z(t)} \right)$$

where its effect increases as the ratio of  $Z$  over the total population increases. A sigmoid function is used to model the bystander effect. It is a dimensionless approximation of the probability that type  $X$  and  $Y$  cells are located close to type  $Z$  cells. This assumption is reasonable because as the amount of transfected cells increases toward the tumor carrying capacity, the likelihood of interaction between cells of type  $X$ ,  $Y$ , and  $Z$  increases. In our model we use a linear approximation of the generic sigmoid function  $b$ .

$\beta$  is a mass action rate that corresponds to infection related dynamics. A larger value of mass action rate leads to increased growth of  $Z$ -type cells and decreased growth of  $X$  and  $Y$  populations. Virulence is represented by parameter  $a$ . Burst size  $k$  is the number of free virus released when a cell is lysed.

The model parameters in our replication-competent delivery virus model can be changed to make a low virulence scenario as well as a high virulence scenario. A high virulence virus spreads through the tumor quicker, however the higher infection rate leads to an increase in burst size and thus a higher virus-induced death rate. A low virulence virus spreads slowly and has a lower burst size. We try to find the treatment switch time from chemotherapy to ganciclovir that achieves the smallest minimum tumor size.

$$\min (X(t) + Y(t) + Z(t))$$

Minimum tumor size better captures tumor extinction than the previous cost function of

$$\max \left( \frac{Z(t)}{X(t) + Y(t) + Z(t)} \right)$$

used by Cannon et al (14). Maximizing bystander effect is a tool that leads to tumor extinction but there are non-favorable cases for low virulence where the ratio of

$$\left( \frac{Z(t)}{X(t) + Y(t) + Z(t)} \right)$$

could be increasing while the tumor size increases.

Because a low virulence delivery virus spreads slowly throughout the tumor, it is able to spread in an optimal way. As chemotherapy kills the  $X$ -type cells, the more prevalent  $Z$ -type cells outcompete the  $Y$ -type and the tumor can be completely eradicated with the application of GCV and the resulting bystander effect. The tradeoff of a low virulence delivery virus is that the slow proliferation means that the patient must be exposed to the toxic chemotherapy for longer times.

On the other hand, a high virulence delivery virus spreads quickly but also has a higher burst size. Consequently the cells have an increased viral death rate due to infection. The transfected cells increase to a high peak quickly then begin to decrease. If the tumor is left untreated the natural chemotherapy resistant cells are able to outcompete the transfected cells and the tumor becomes untreatable making the window of opportunity smaller. In both cases by introducing ganciclovir at the optimal time, the greatest reduction in tumor size is achieved.

### 3.2 MATLAB Model

We use initial conditions of  $X = 99, Y = 1, Z = 1, V = 99$  or  $X = 99, Y = 1, Z = 0, V = 99$  and define  $C = 1$  if chemotherapy is being applied and  $C = 0$  if chemotherapy is not being applied. The schedule of chemotherapy treatment is not allowed to switch faster than a feasible interval which we define. We decimate our total treatment duration into 10 intervals of equal length. Our model is capable of finding optimal schedules that include timed pulses of chemotherapy, however due to the fact that turning on and off chemotherapy rapidly can be biologically unrealistic, we only test continuous-dose schedules of different durations. In each interval chemotherapy

is either administered or not administered and when the cost function is optimal we switch from chemotherapy to GCV. For example, a treatment schedule of 1111100000 would mean that chemotherapy was applied for 5 intervals, then chemotherapy turned off while GCV turned on for the remaining duration.

The model's optimization method according to the cost function is beneficial because it finds the most favorable treatment schedule out of all of the different possibilities (on the order of  $2^{10}$  including timed pulses). This is especially necessary when using the high virulence, fast spreading delivery virus because the tumor will reach a minimum size quickly but will also regrow quickly.

In some cases, the tumor size continues to decrease as long as chemotherapy is applied. While this behavior seems perfect at first, further investigation shows that because the cost function is optimal at the end of the 10 intervals of chemotherapy, the model never switches from chemotherapy to ganciclovir and no bystander effect takes place. To ensure that the *Y*-type cells will be killed via the bystander effect, we define a number where GCV will be applied if the tumor size decreases below this threshold i.e. ( $10^{-5}$ ,  $1e-5$ ). Consequently our 99% effectiveness approach may cut off the optimal cost function early.

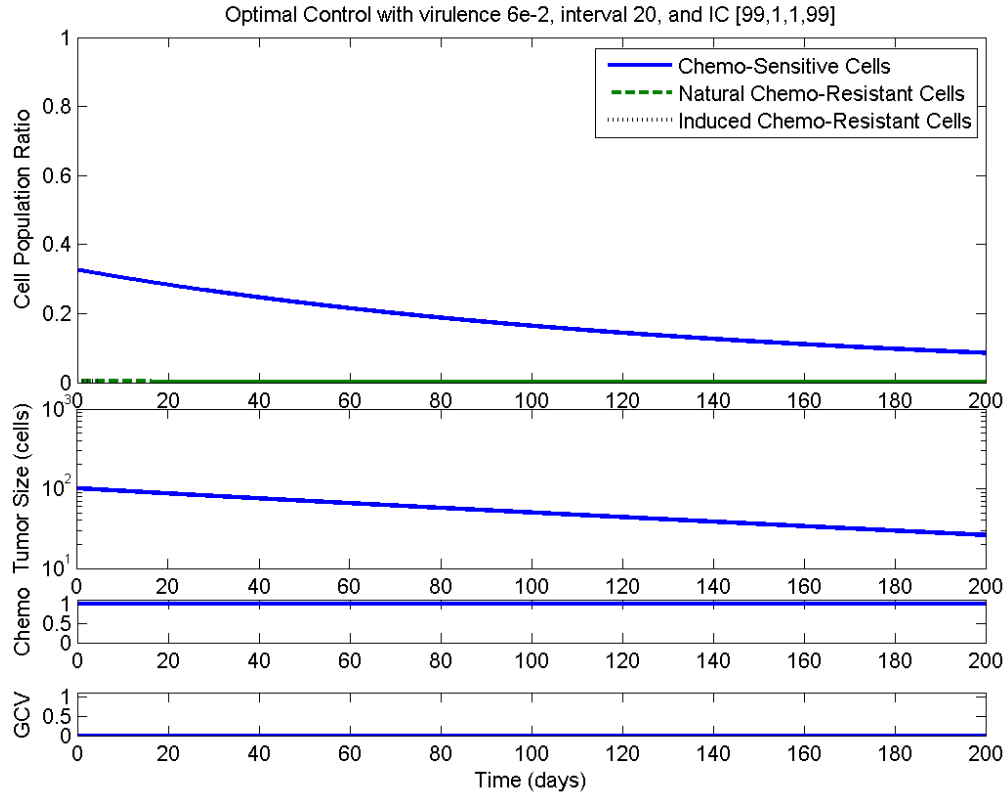


## Chapter 4

### RESULTS

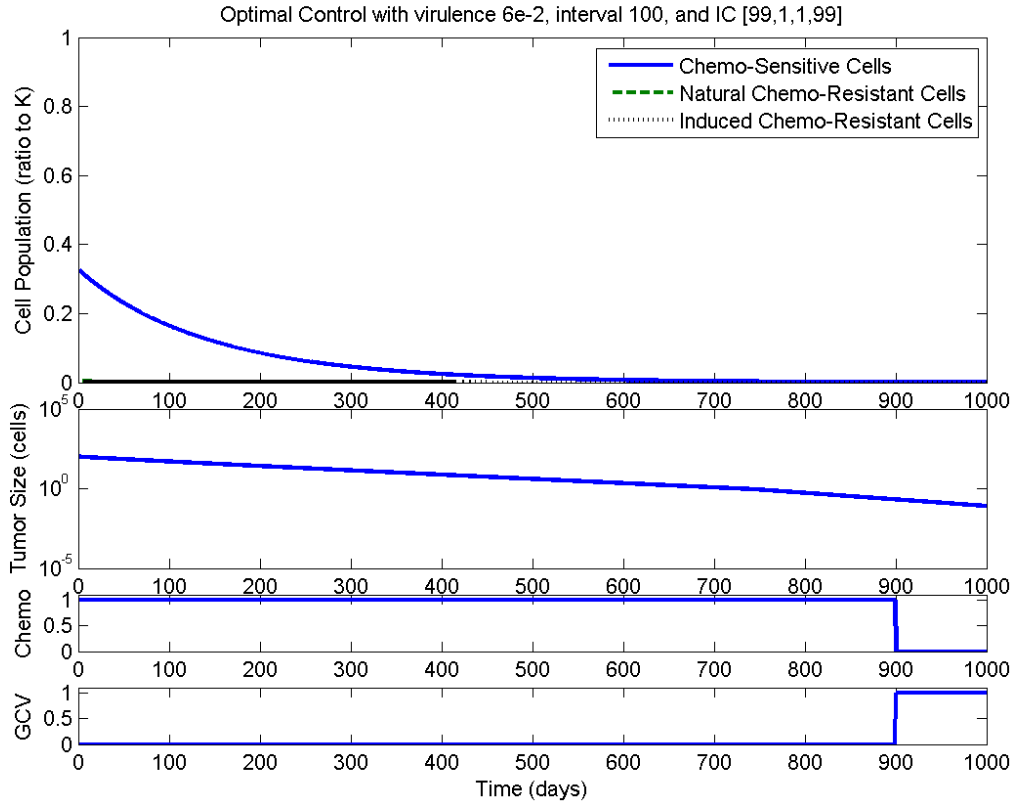
#### 4.1 High Virulence Delivery Virus

We first examine the behavior of a delivery virus with high virulence. This means the virus has a high infection rate and high lytic rate and therefore it spreads and causes damage quickly to cells. However, this also means it's more likely to kill off all the *X*-type cells that it can infect and so induced chemotherapy-resistant cells will not adequately spread throughout the tumor.



**Figure 4.1:** Treatment fails because GCV is never applied because the cost function is never satisfied. Tumor size doesn't decrease significantly enough.

For a high virulence of 0.06 we see that the GCV is never applied within 200 days. Tumor size continues to slowly decrease as the chemotherapy kills the  $X$  type cells. At the end of the 200 days the  $X$  type cells constitute 0.1 of the carrying capacity of the tumor while the populations of  $Y$  and  $Z$  type cells are much smaller. Therefore the interval is set to 100 for illustrative purposes.



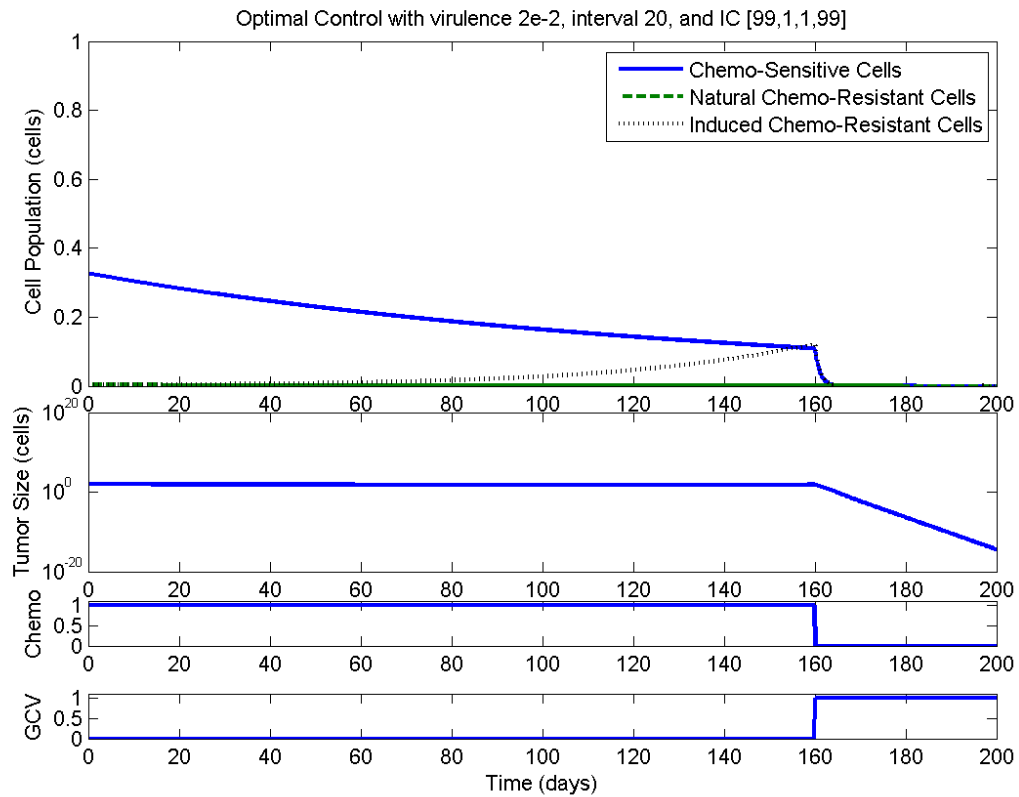
**Figure 4.2:** Using a high virulence delivery virus, a successful treatment needs an interval of 100 days. With these parameters, the optimal treatment schedule is to apply chemotherapy for 900 days then switch to GCV.

While this treatment schedule does decrease the tumor size to less than one cell, it also requires the patient to receive chemotherapy doses for 900 days which may or may not be biological possibly since chemotherapeutic agents are cytotoxic. At time = 900 when the GCV is applied, we see from the plot of tumor size that it is approximately equal to one. So our control algorithm did not require a 99 percent cutoff because it did not reach  $1e-5$  cells for tumor size. It is probable that this treatment eradicates

the tumor completely so that it does not rebound.

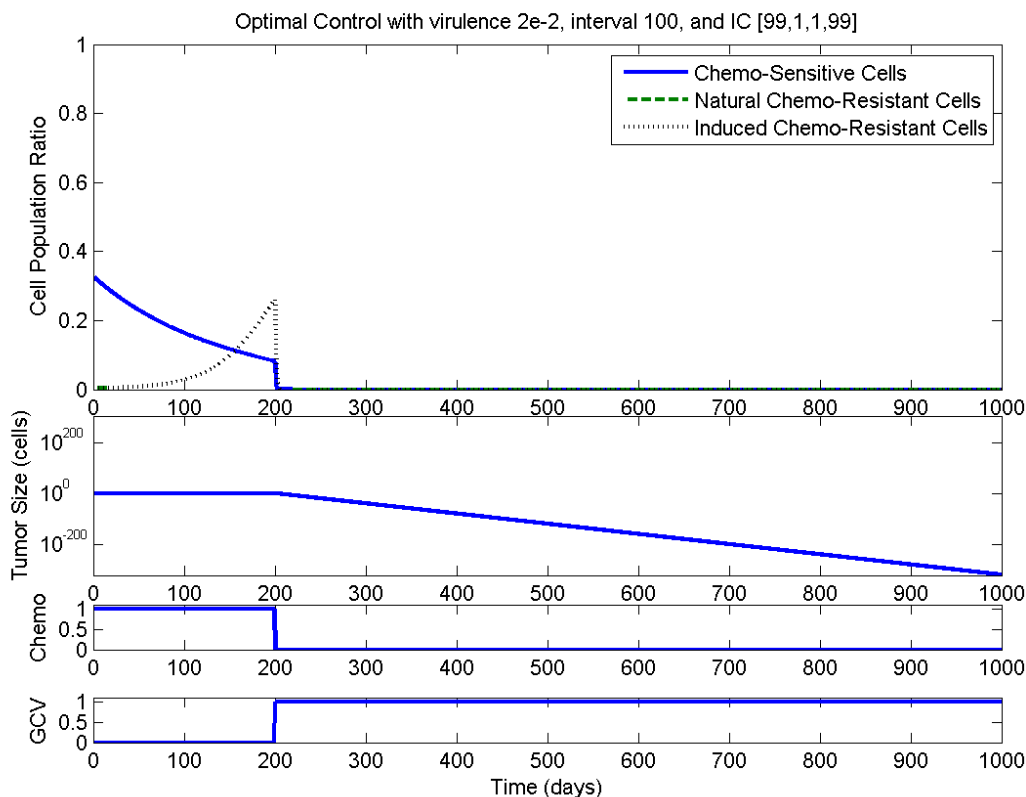
## 4.2 Low Virulence Delivery Virus

Next we look at the simulations when using a low virulence delivery virus. The low infection and lytic rate means this type of virus has a higher probability of enabling the induced chemotherapy-resistant cells to become the dominant cell type within the tumor.



**Figure 4.3:** Our system finds a successful treatment schedule with a GCV application time of 160 days. The bystander effect causes enough apoptosis to completely kill off the tumor.

From the figure above we see that indeed the  $Z$  type cells were the dominant population when GCV was applied at 160 days. As the number of  $X$  type cells decreases, the  $Z$  type cells outcompete the  $Y$  type species until the cost function is optimal. Here the bystander effect is enough to decrease the tumor size to approximately  $10^{-15}$ ; we consider this treatment schedule a success in reaching complete tumor eradication.



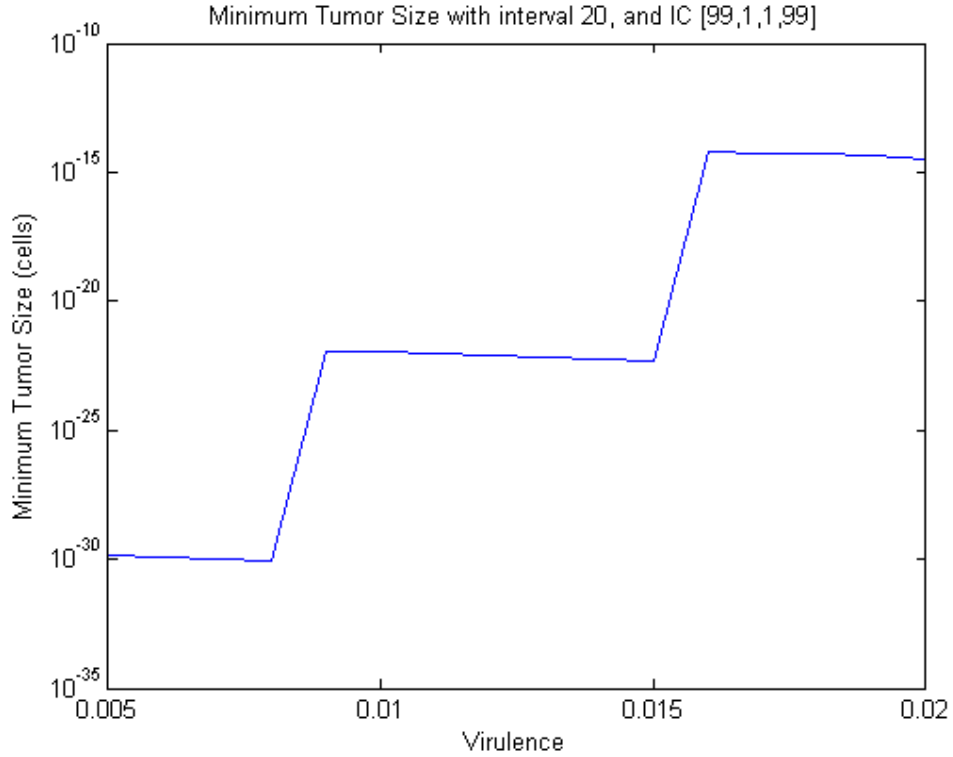
**Figure 4.4:** The algorithm is able to determine a treatment success and the GCV is applied at 200 days. Though it is a treatment success, the longer required chemotherapy time is unfavorable for the health of the patient.

When the interval parameter is equal to 100 days we still have a successful

treatment. However, because the chemotherapy can only switch on and off based on our interval we see that chemotherapy is turned off at time 200 days whereas from the previous figure we saw that switching at 160 days also led to a successful treatment. With an interval of 100 days, the patient would have to stay on chemotherapy for 40 days more. Hence it is valuable to have shorter intervals when possible.

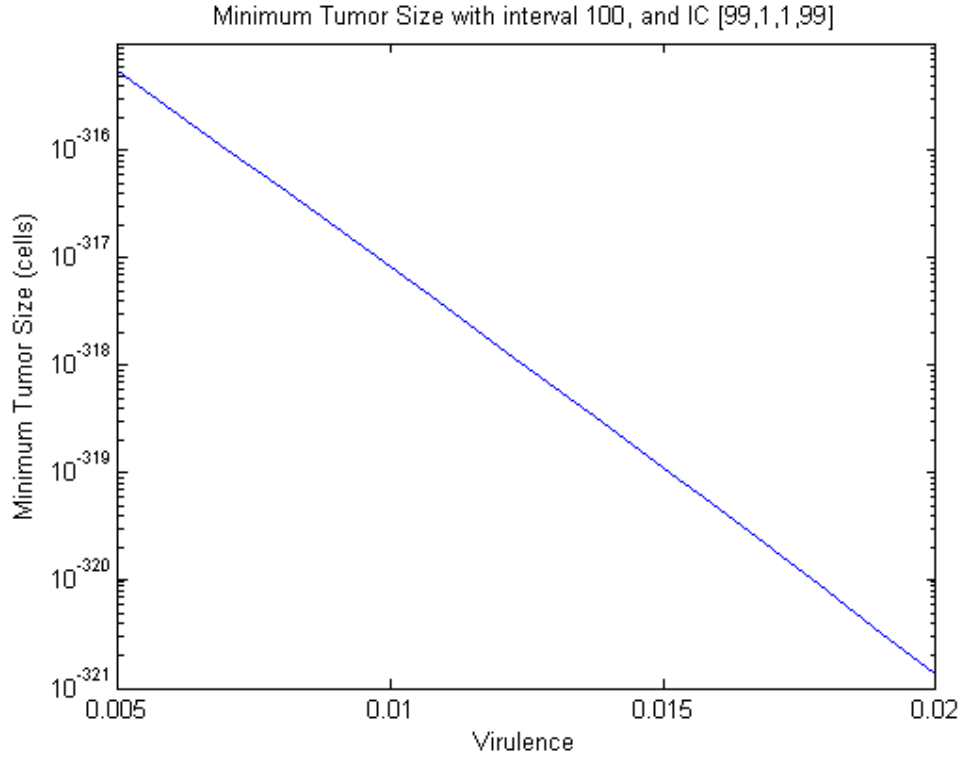
### **4.3 Minimum Tumor Size**

By looping through our code with different values for viral virulence and storing important results of each iteration, we are able to plot the minimum tumor size achieved vs. the virulence of the virus for varying parameter values. We choose virulence values from 0.005 to 0.02 with 0.001 increments because as seen from previous figures, the low virulence delivery virus has more favorable results as compared to a high virulence delivery virus.



**Figure 4.5:** We see a general positive correlation with three distinct tiers and a large range in y-axis values.

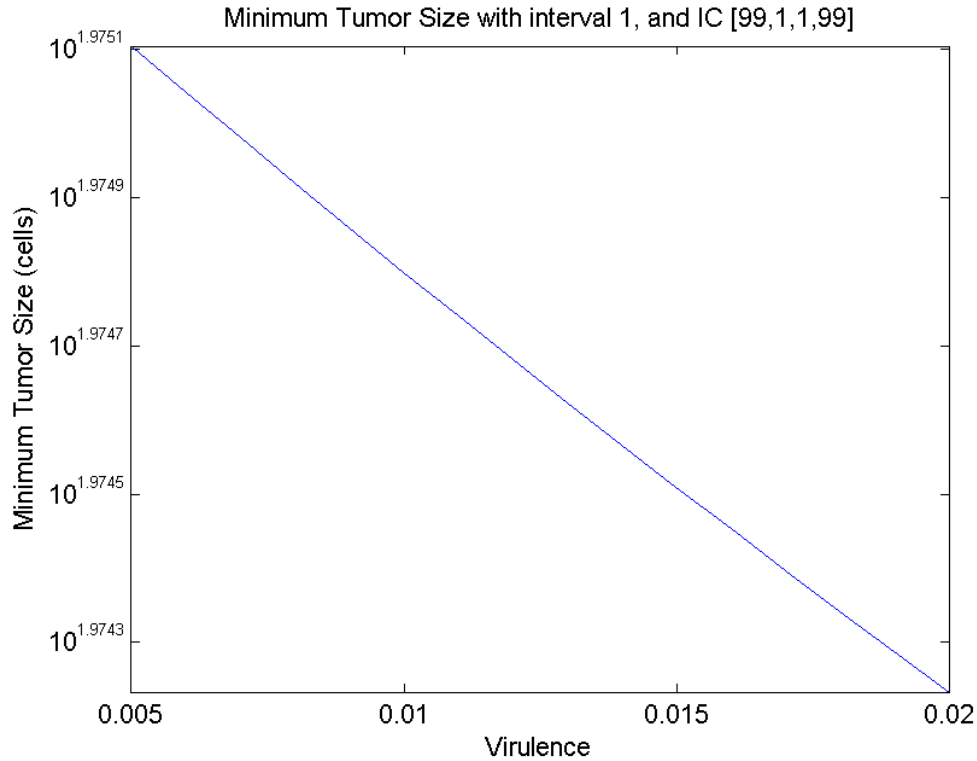
The figure above allows us to further analyze the dynamics of our system. When  $\text{interval} = 20$  there is a general positive correlation between the minimum tumor size reached and virulence. There appears to be three levels of cell population and when the virulence passes a certain value, the minimum tumor size reached jumps to the next grouping. A change of 0.015 in virulence corresponds to a  $10^{-15}$  change in minimum tumor size.



**Figure 4.6:** We see that the data is now negatively correlated with a constant slope. The range of y-axis values is smaller than in the previous case where interval = 20.

Changing the interval to 100 changes the minimum tumor size versus virulence to have a negative correlation. Unlike before, the slope of the line here is relatively constant. Here the same 0.015 difference in virulence only causes a change in number of cells of approximately  $10^{-5}$ .



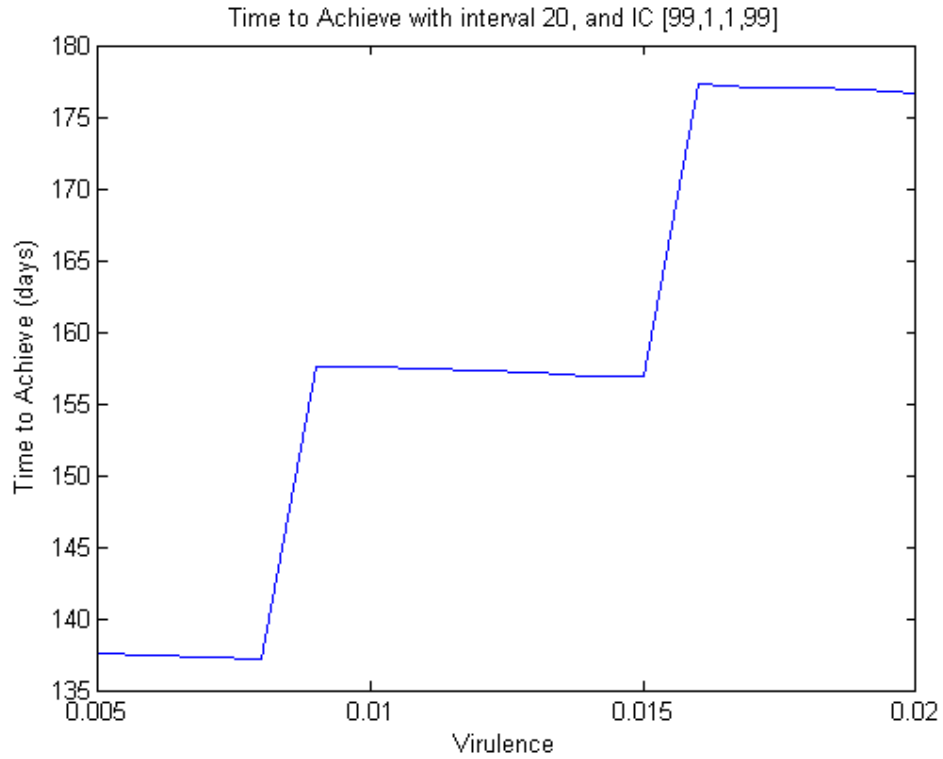


**Figure 4.7:** With a smaller interval we have to increase the value of the tumor size threshold to  $1e2$ . The figure appears to be negatively correlated with a constant slope but noticing the small range of the y values we determine that the minimum tumor size isn't affected by the virulence much.

With an interval of 1 day we see a very slight negative correlation given the y-axis values. However it should be noted that the threshold had to be set to  $1e2$  because the tumor size was never reduced below this number via chemotherapy. The optimal control plot for these parameters would have shown a treatment failure. As seen from the figures of minimum tumor size vs. virulence, the shape and behavior of the plot can change depending on what the interval is set to.

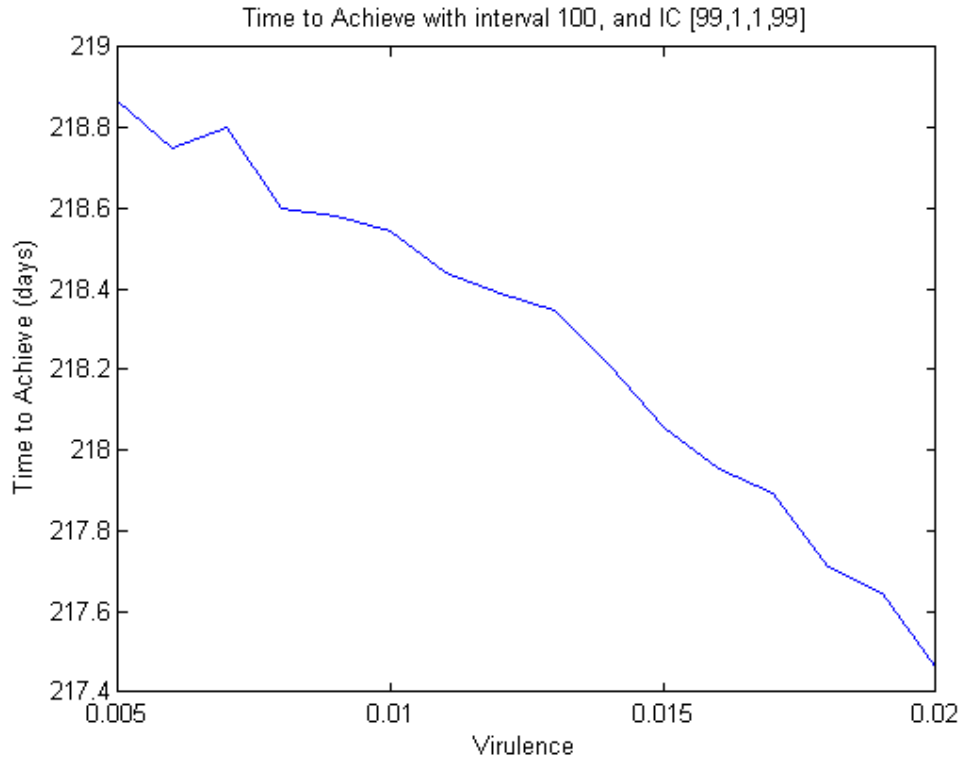
#### 4.4 Time to Achieve

In order to further analyze the behavior of the model we also plot the time to achieve minimum tumor size vs. virulence.



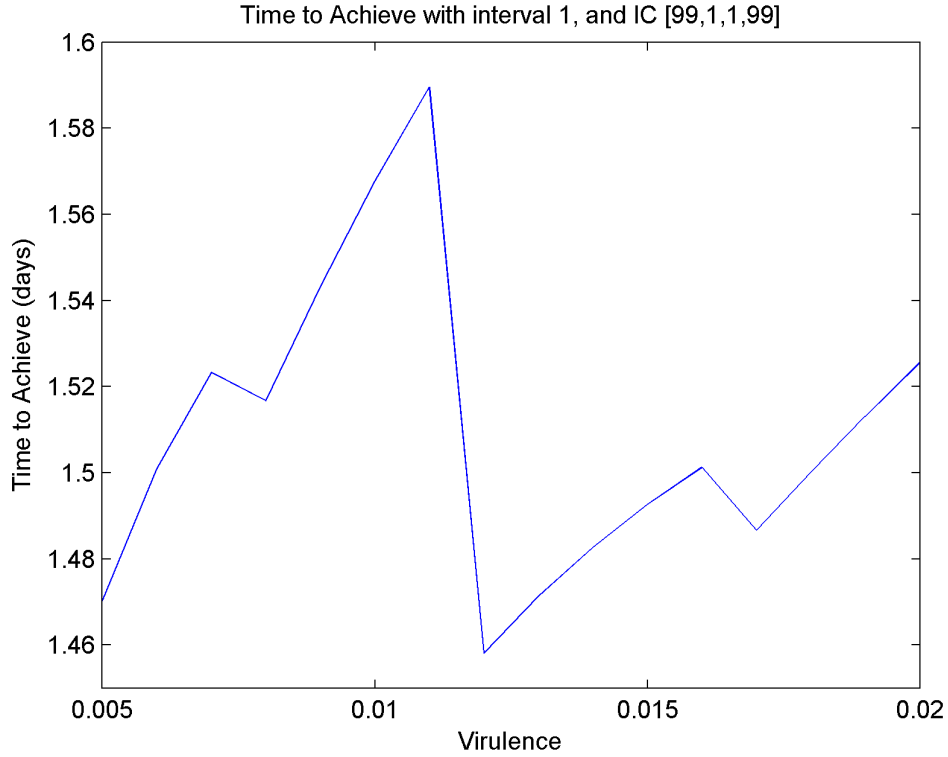
**Figure 4.8:** We see an overall positive correlation with three groupings of y values and a y-axis range of 138 to 178 days.

The two data sets are mostly positively correlated but again with three distinct tiers when interval = 20, same as minimum tumor size vs. virulence. A range of about 138 to 178 days corresponds to a virulence of 0.005 and 0.02 respectively.



**Figure 4.9:** We again see a negatively correlated series with relatively constant slope and a small range in y values. This behavior is consistent with the minimum tumor size vs. virulence plot with corresponding parameter values.

Note on this figure the scale of the y-axis. There is a negative correlation with relatively constant slope but the range of time to achieve is only approximately 1.4 days.



**Figure 4.10:** There is an overall increase in time to achieve until a certain level of virulence is reached. After this value the time to achieve drops back down. There doesn't appear to be any general trend for this scenario.

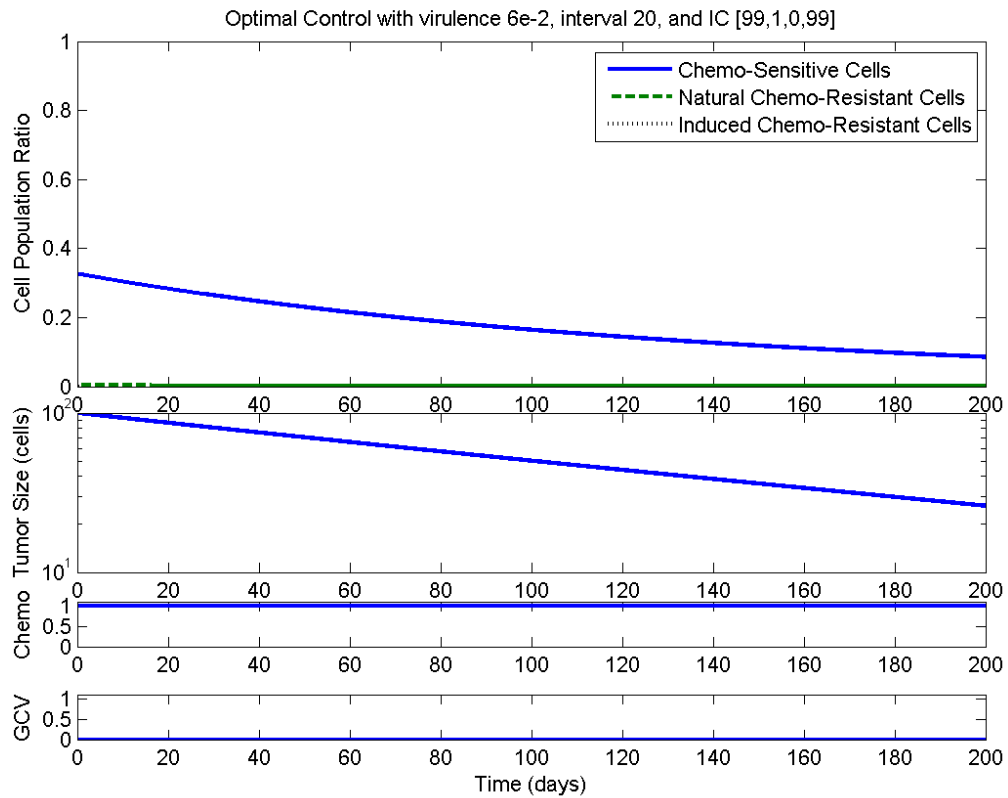
For the extreme case of an interval of one day, we again had to set the threshold level to  $1e2$ . The time to achieve increases until virulence reaches 0.011, at which point it plummets and regains slowly. The range of time to achieve is only 0.14 days and there doesn't appear to be any overall trend.

#### 4.5 Simulations with Zero Initial Induced Chemoresistant Cells

We then run similar simulations for the case where the initial condition of  $Z$  type cells is 0 because this is more biologically realistic since there initially shouldn't be any induced chemoresistant cells in the tumor. However we find that with these initial

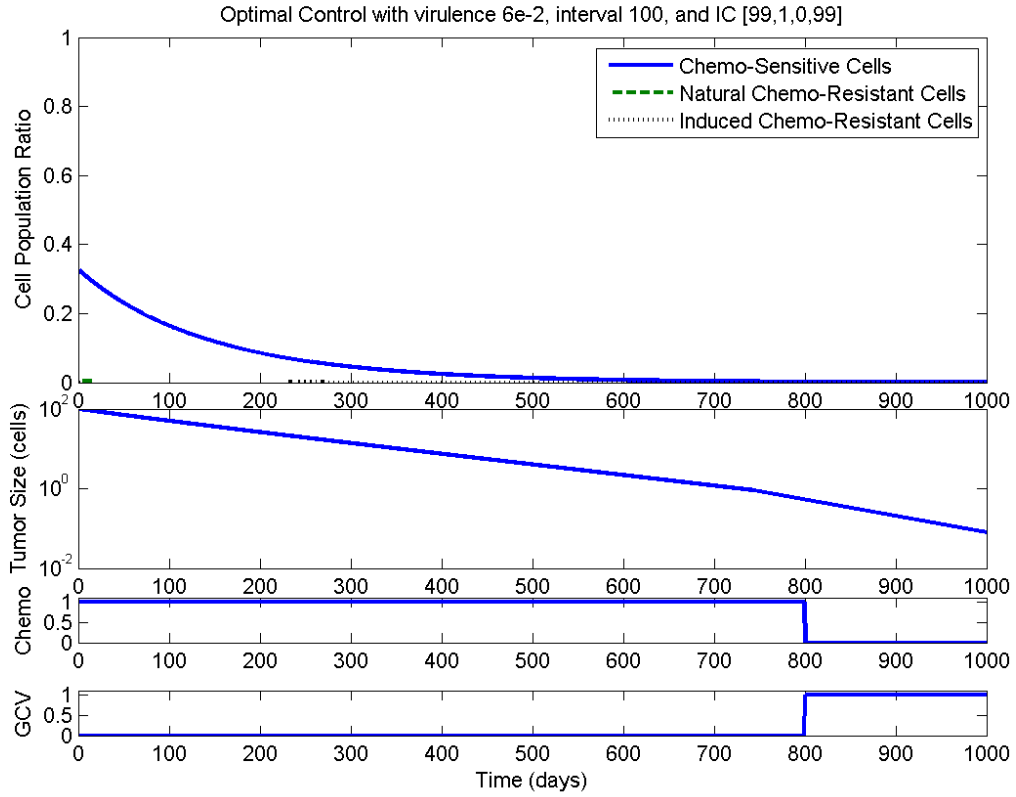
conditions of  $[99,1,0,99]$  for cell types  $(X, Y, Z, V)$  respectively, result in treatment schedules that require the patient to be on chemotherapy for significantly more time to reach a successful treatment and therefore are not always biologically feasible.

#### 4.5.1 High Virulence Delivery Virus



**Figure 4.11:** The treatment results in a failure because chemotherapy administration never switches to GCV treatment. Therefore no bystander effect occurs to completely eradicate the tumor.

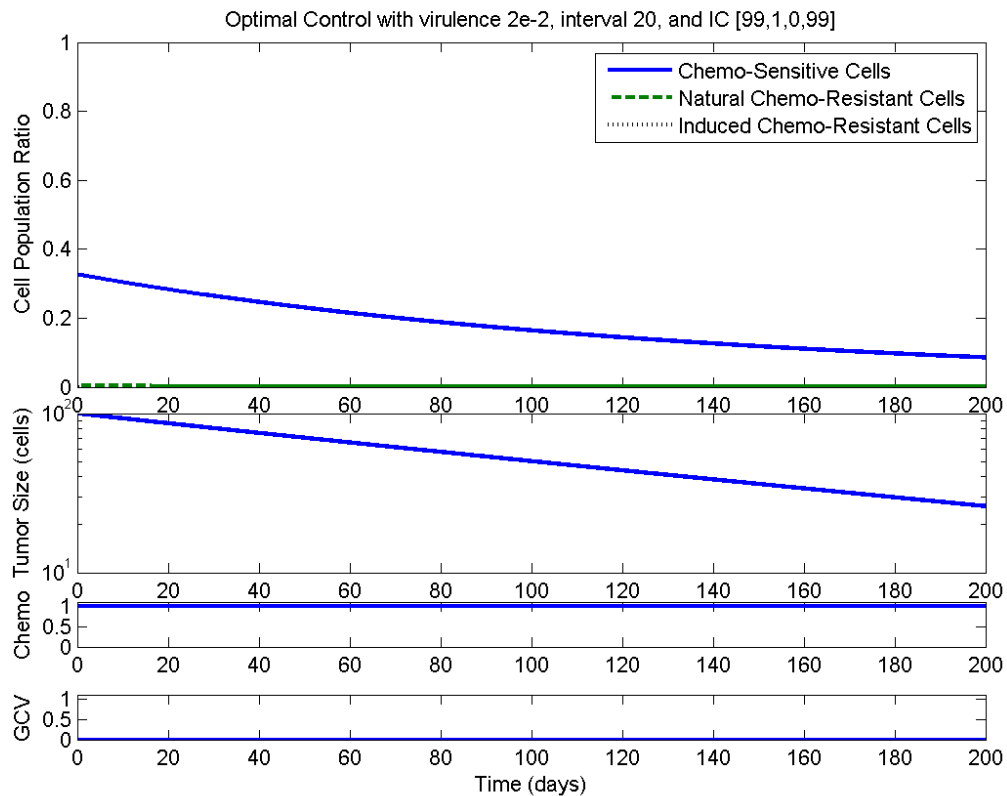
Ganciclovir is never applied within the treatment schedule.



**Figure 4.12:** We find that these initial conditions improve the treatment by requiring chemotherapy for 100 less days when using initial conditions of (99 1 1 99) and interval = 100.

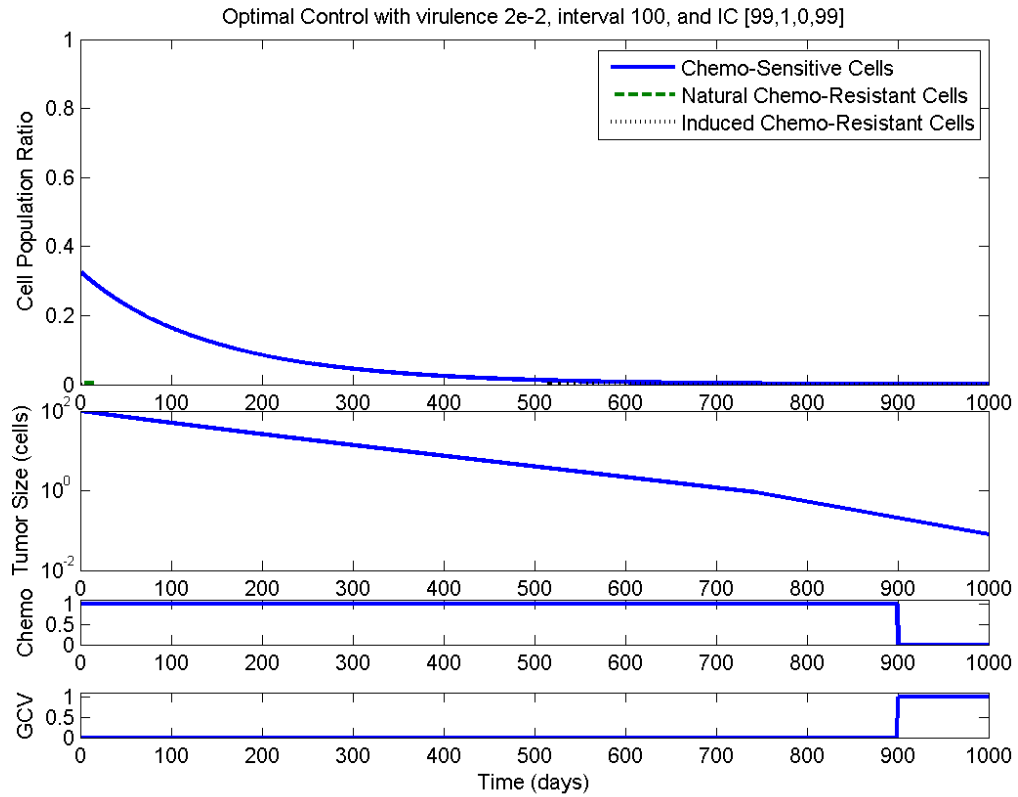
GCV is applied at time = 800 days, 100 days earlier than the corresponding case with the same parameters that use initial conditions of [99 1 1 99].

## 4.5.2 Low Virulence Delivery Virus



**Figure 4.13:** The low virulence virus results are made worse by the (99 1 0 99) initial conditions. With the interval set to 20 days, the treatment now fails because GCV is never applied within the allowed time period.

We focus on the results that utilize a low virulence delivery virus. With these initial conditions GCV is never applied. Compare to corresponding previous figure using IC [99 1 1 99]. Now the  $Z$  type cells are not produced as much and the cost function is never satisfied within the time constraint, resulting in a failed treatment schedule.



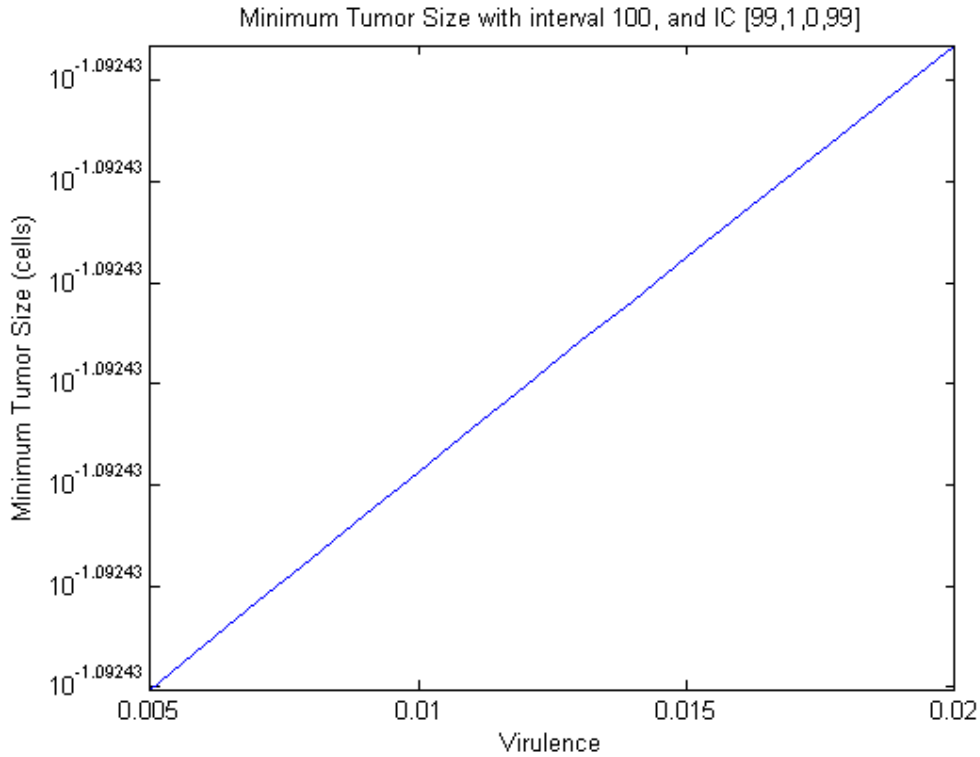
**Figure 4.14:** When the interval equals 100 days the chemotherapy schedule requires 900 days instead of only 200 days as in the previous corresponding simulation with ICs of [99 1 1 99]. With these new initial conditions the patient is subjected to the adverse effects of chemotherapy for substantially longer.

We again see that the more biologically relevant initial conditions of [99 1 0 99] give results that are worse for the patient.



### 4.5.3 Minimum Tumor Size

The minimum tumor size and time to achieve minimum tumor size vs. virulence plots have their thresholds changed to  $1e-1$  for interval = 100 because the tumor size never reaches the 99% effectiveness cutoff that we earlier defined as  $1e-5$ . The results for cases with interval = 20 and interval = 1 are omitted because they require the threshold to be set equal to 10 and 100 respectively, which is uninformative.

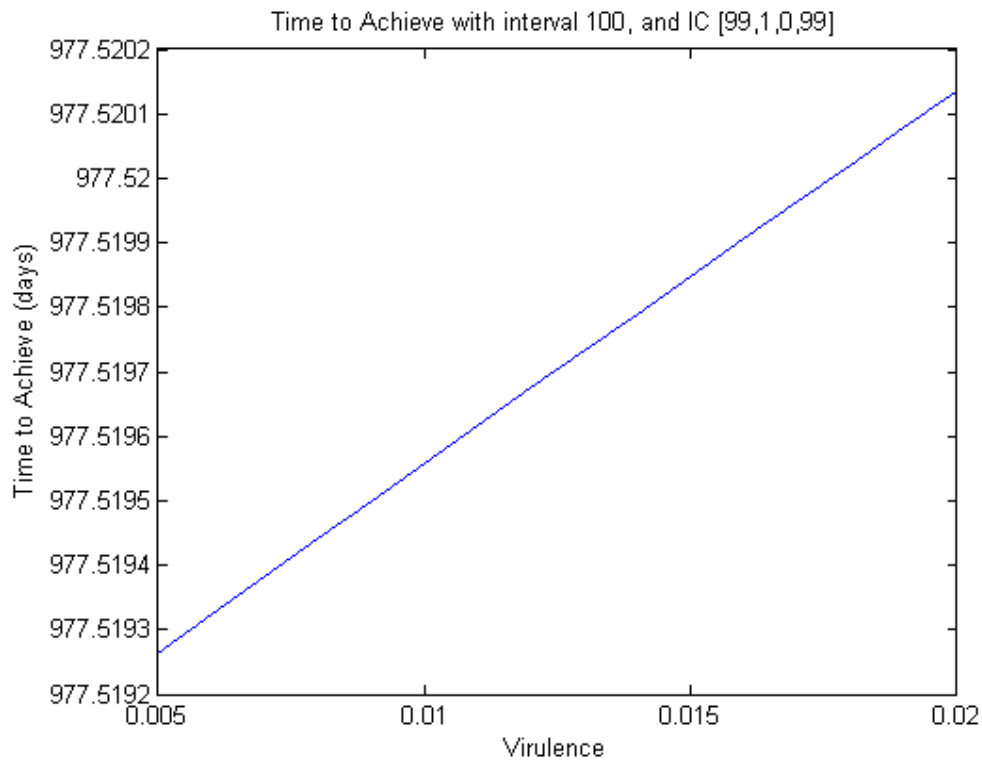


**Figure 4.15:** The y-axis values that show that minimum tumor size with the listed parameters is unaffected by virulence. The positive correlation of the graph is from the trivial increase in minimum tumor size not reflected on the plot axis.

Note the y-axis values that show that minimum tumor size with the listed parameters is unaffected by virulence. The same behavior is observed in the case

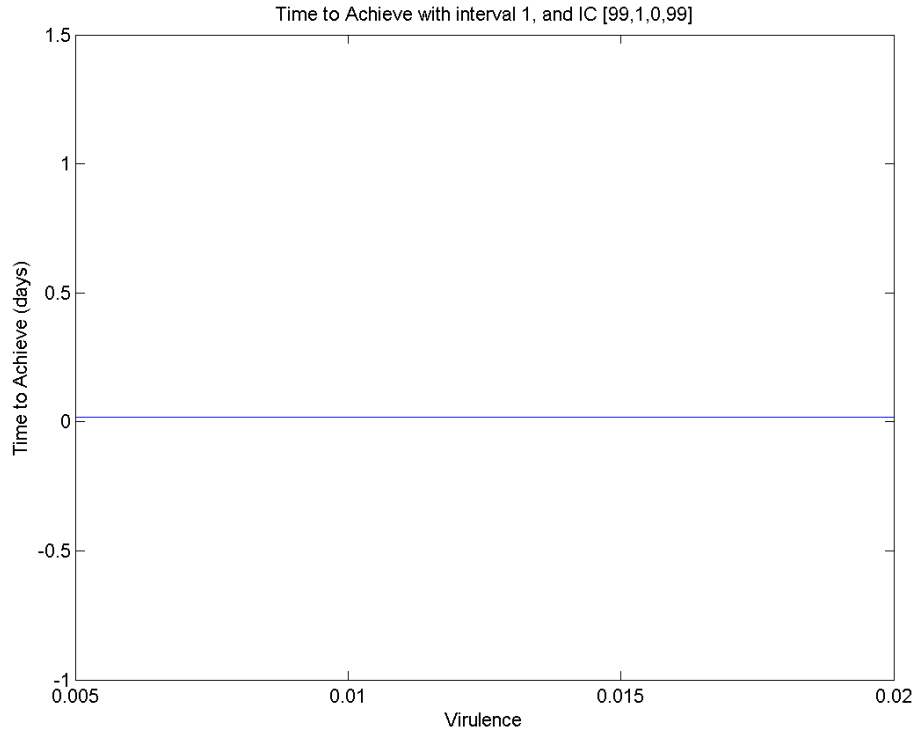
where interval = 1 and threshold  $1e2$  (not shown). The positive correlation of the graph is from the trivial increase in minimum tumor size not reflected on the plot axis.

#### 4.5.4 Time to Achieve



**Figure 4.16:** Upon careful inspection of the figure and its axes values, it is determined that time to achieve is unaffected by virulence when using the listed parameter values.

There appears to be a slight positive correlation with constant slope. However, again looking at the y-axis values, realistically the time to achieve in this case is unaffected by virulence because the range of achieve time is only 0.10 days.



**Figure 4.17:** Time to achieve is unaffected by virulence.

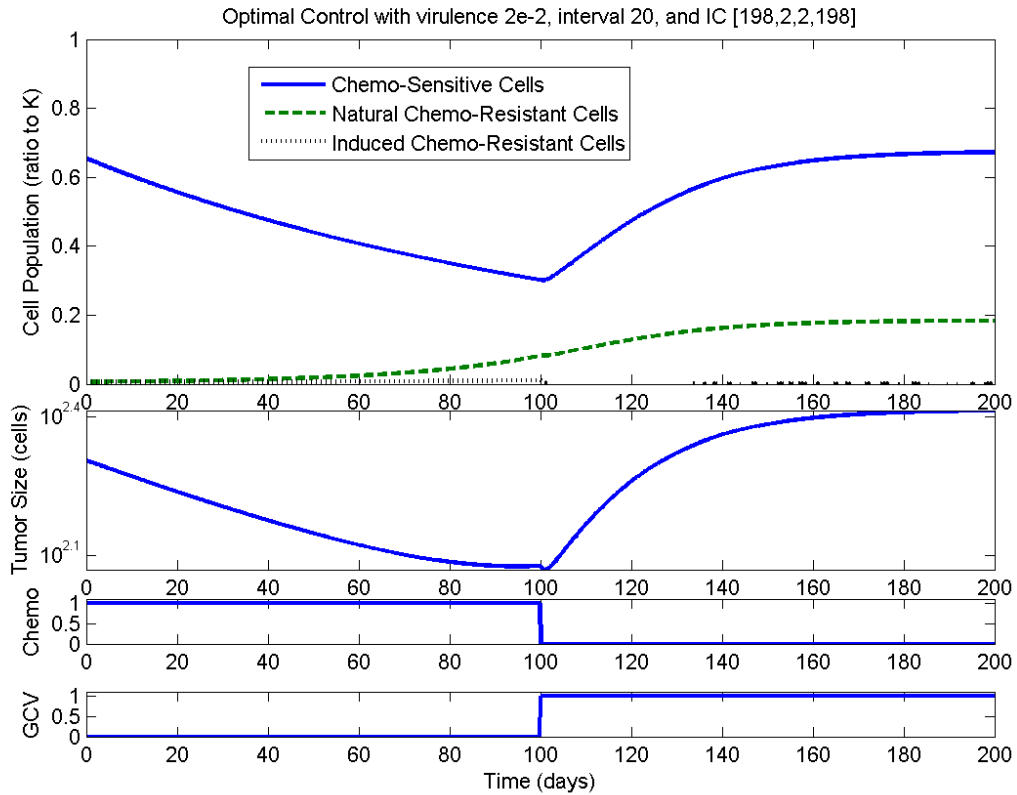
The figure above clearly reflects the previous behavior when interval = 100 and time to achieve is unaffected by virulence. It is important to remember that a threshold requiring  $1e2$  is considered a treatment failure.

#### 4.6 Sensitivity Analysis

We perform sensitivity analysis on our model by running simulations with variants of our original initial conditions and seeing how the treatment schedules and successes change. Ideally we want to observe a trend where the original treatment and success does not drastically change when the ICs are scaled by different factors.

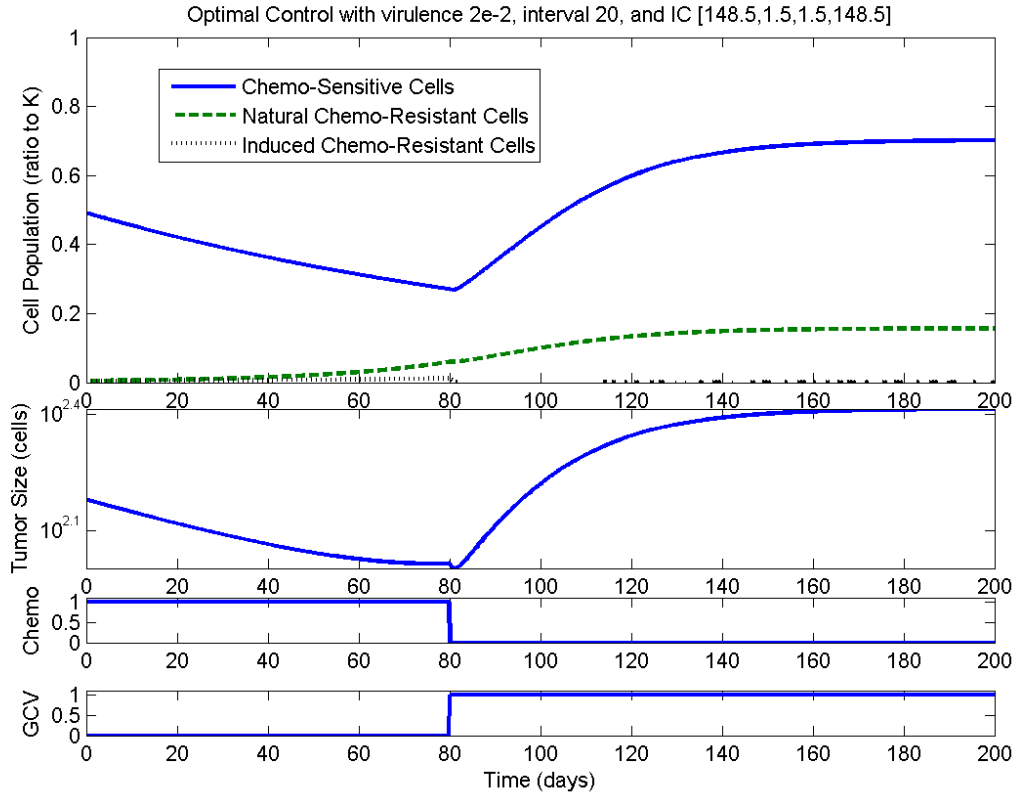
#### 4.6.1 Low Virulence, Interval = 20, Original ICs [99 1 1 99]

We first examine the case of virulence = 0.02 and interval = 20. We run several simulations using the original initial conditions of [99 1 1 99] scaled by various factors in order to obtain an approximate range in which simulations remain successful treatments.



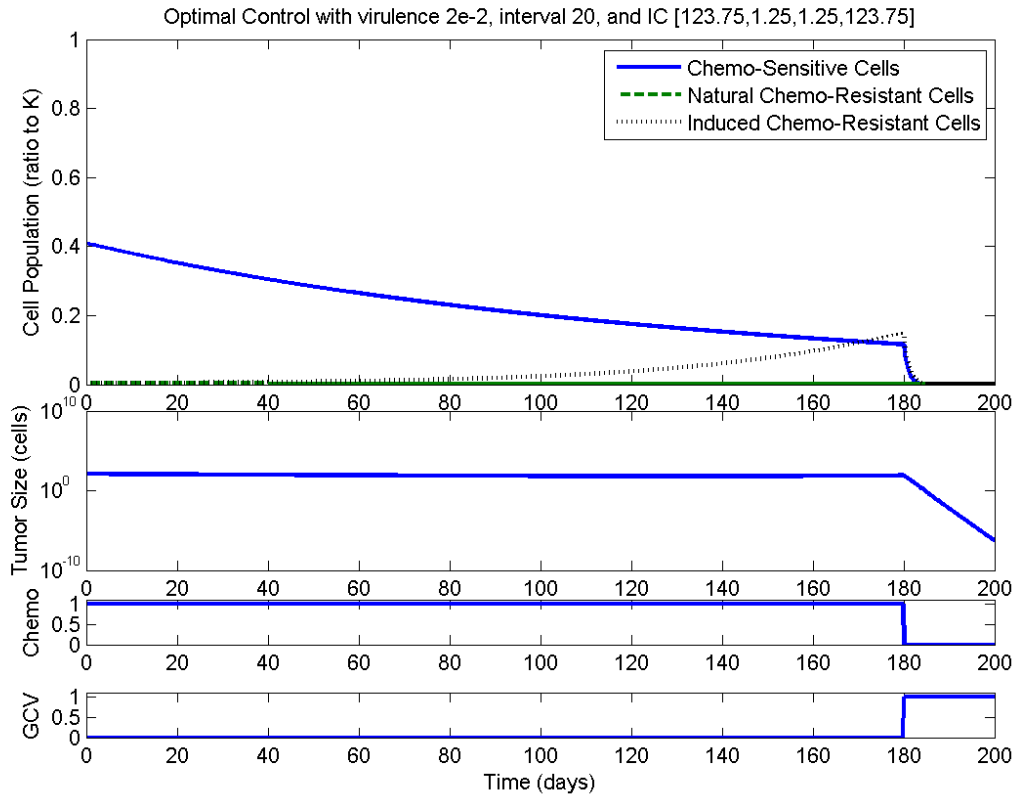
**Figure 4.18:** The output is a treatment failure where chemotherapy is switched to GCV at 100 days. Due to the fact that the ratio of the  $Z$  type cells to the total tumor size is too small at this time, the bystander effect is too small to stop the tumor size from rebounding.

In the first scenario we increase all the initial conditions by a factor of 2, resulting in [198 2 2 198]. From the plot we see that this results in a treatment failure whereas the original case of [99 1 1 99] was a success. Here the tumor size decreases to  $10^{2.1}$  as chemotherapy is applied, then rebounds after treatment is switched to GCV. During the application of chemotherapy from 0 to 100 days we see that the natural chemo-resistant cells outcompete the induced chemo-resistant cells. This unfavorable situation yields a bystander effect that is too minimal to completely eradicate the tumor. This is seen graphically in the small dip in tumor size that occurs slightly after 100 days.



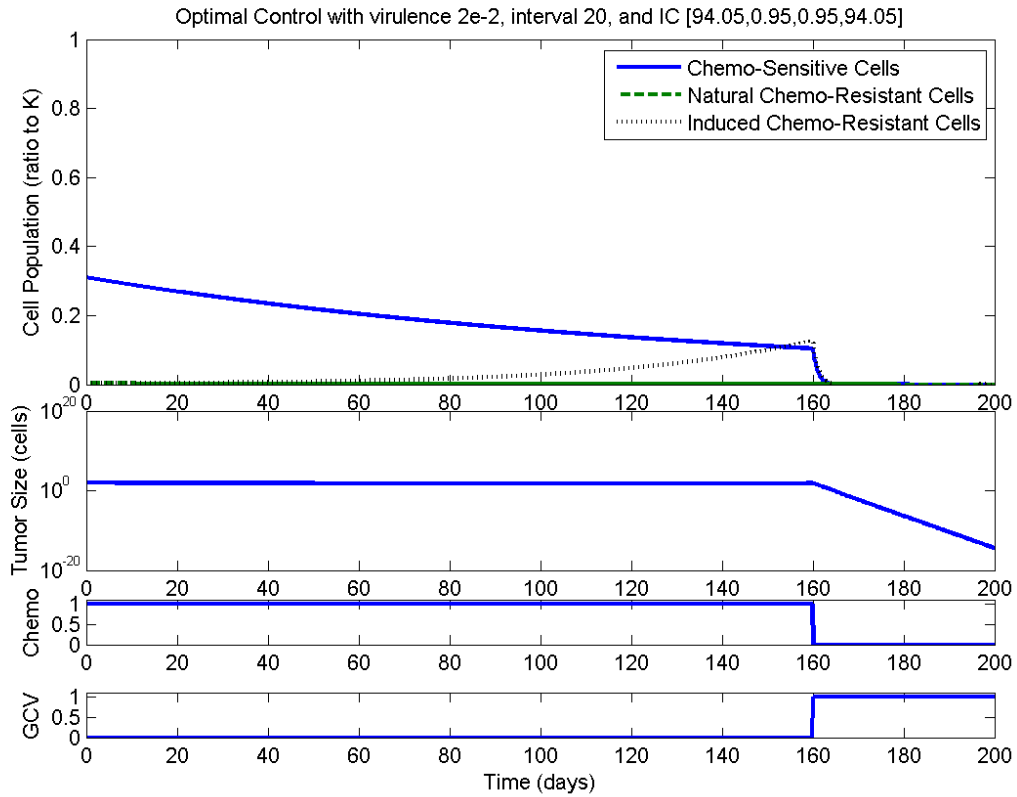
**Figure 4.19:** Now with a differently scaled set of initial conditions, the time at which GCV is applied is 80 days. Similar behavior is observed and we have a treatment failure.

In the second run we use initial conditions of  $[148.5 \ 1.5 \ 1.5 \ 148.5]$ , an increase by a factor of 1.5. Again we notice that the schedule leads to a treatment failure because the  $Z$  type cells do not increase as fast as the  $Y$  type cells and the resulting bystander effect is not strong enough to avoid tumor regrowth. When GCV is applied at 80 days, the chemotherapy has reduced the tumor size to approximately  $10^2$  cells. Though this is a treatment failure, it performs better than the case for ICs of  $[198 \ 2 \ 2 \ 198]$ .



**Figure 4.20:** We have a successful treatment and GCV is applied at 180 days when  $Z$  cells are the dominant type.

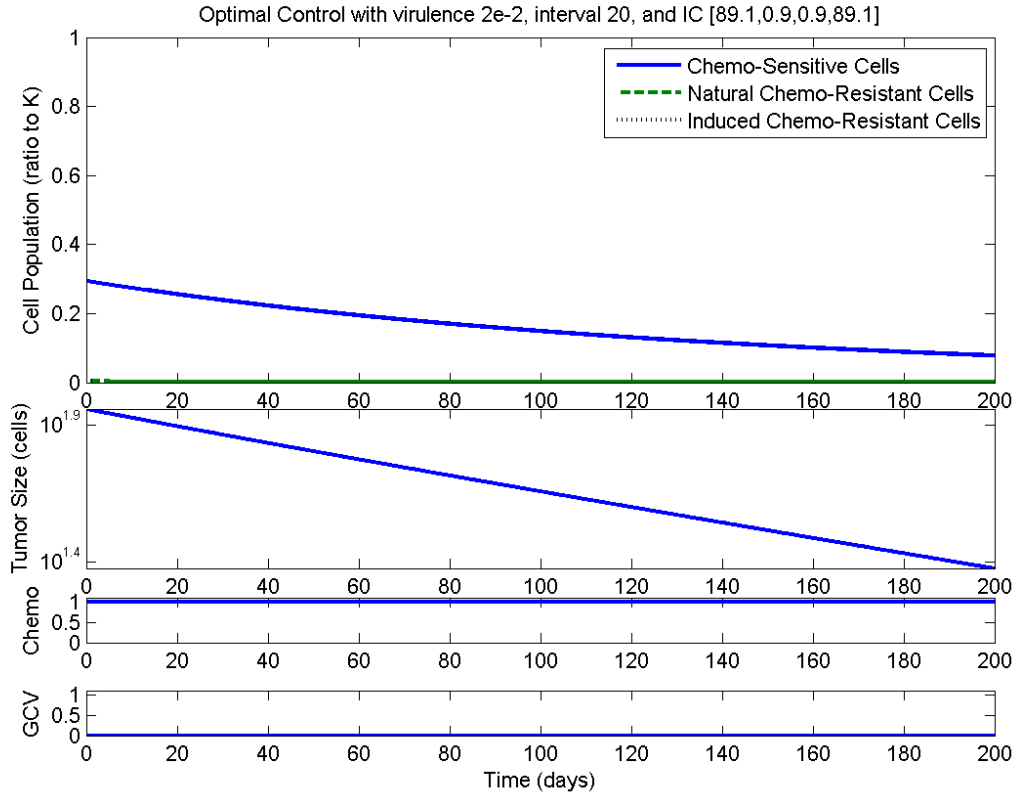
Here, the treatment completely cures the tumor for an IC increase factor of 1.25. Using  $[123.75, 1.25, 1.25, 123.75]$ , chemotherapy is administered until time = 180 days. Inspection of the cell populations shows that the  $Z$  type cells outcompete the  $Y$  type cells and become the dominant cell type. Thus all cell types plummet after GCV is applied. In the original case of  $[99 \ 1 \ 1 \ 99]$  and using the same parameters, the treatment switched from chemotherapy to GCV at 160 days.



**Figure 4.21:** The treatment is a success and the bystander effect that takes place at 160 days is large enough to completely eradicate the tumor.

This plot looks very similar to the original case. Treatment success is obtained when using 0.95 of the original ICs and both simulations apply GCV at 160 days.





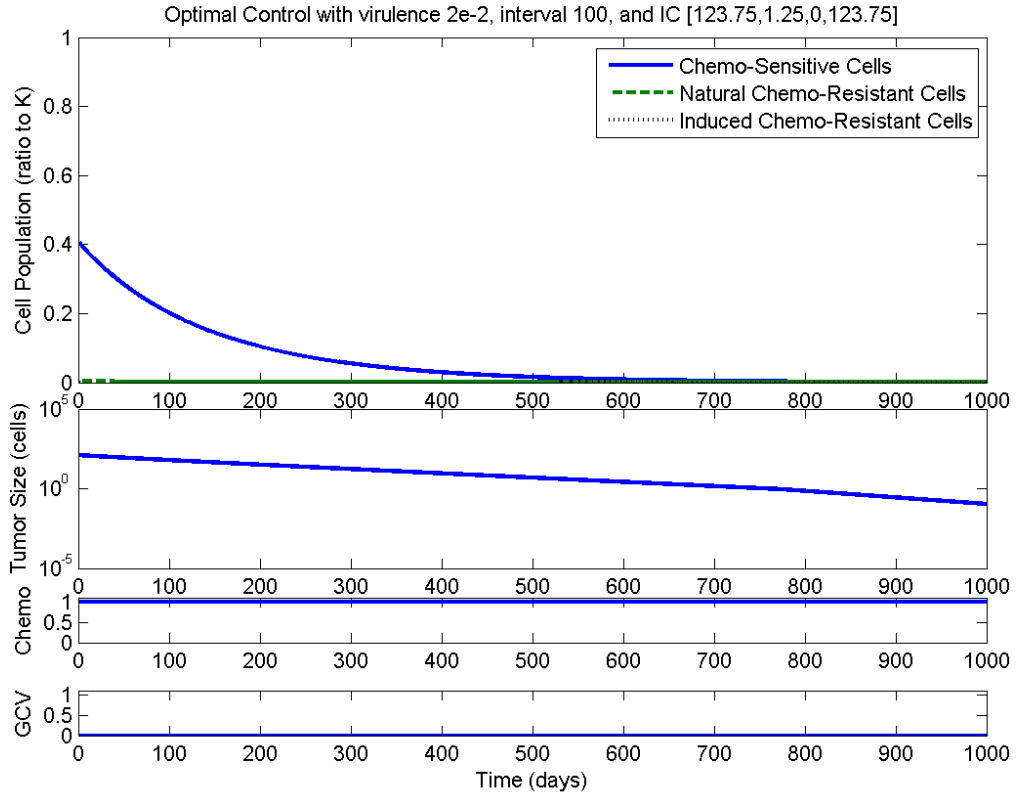
**Figure 4.22:** The treatment reverts back to a failure when we use a factor of 0.9, ICs of  $[89.1 \ 0.9 \ 0.9 \ 89.1]$  because GCV is never applied within the duration of the simulation. This is because the tumor size continues to decrease as long as chemotherapy is applied, and simultaneously the tumor size never reaches the threshold where we could logically assume that the tumor would stochastically go extinct.

Finally we look at an approximate lower bound for ICs that still result in a successful treatment. Multiplying the original ICs by 0.9 leads to a treatment failure where GCV is never applied within the allowed period. It is possible that GCV would

be applied if we specified a longer time period but that would also require the patient to remain on chemotherapy for a longer time. From analysis of the previous figures in this section, we conclude that the system is capable of finding a treatment schedule that completely kills the tumor if the factor by which the initial conditions are changed is between 0.95 and 1.25.

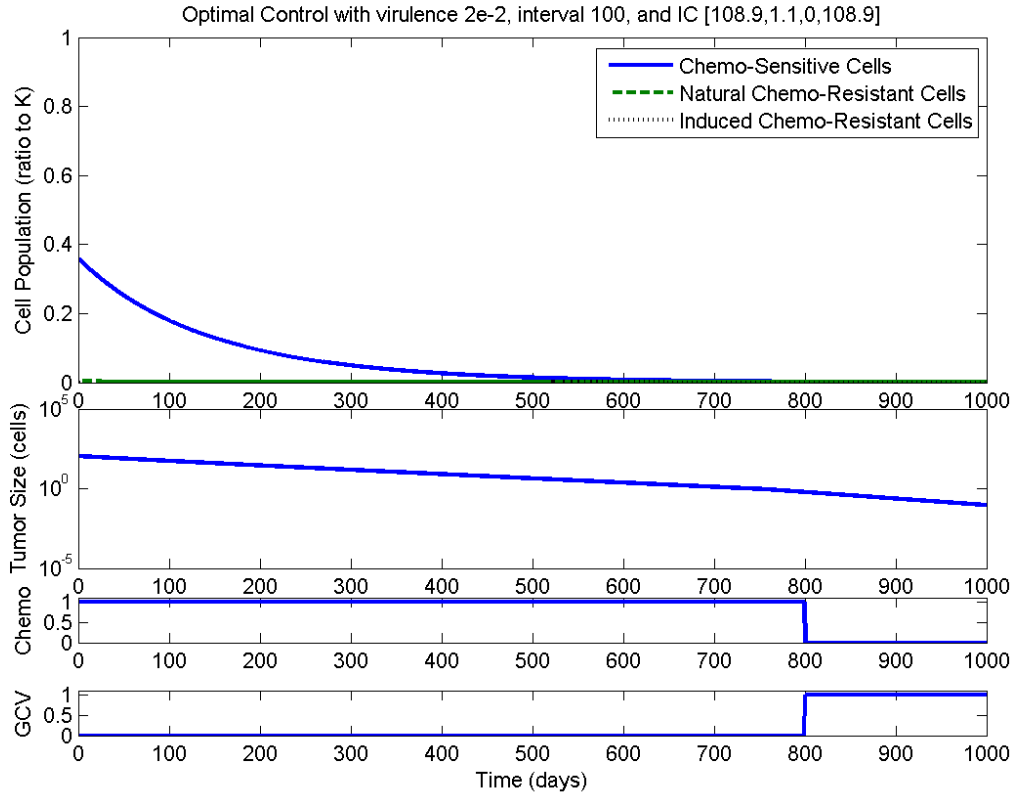
#### **4.6.2 Low Virulence, Interval = 100, Original ICs [99 1 0 99]**

Out of the many different possible parameter and IC combinations that we could use next to analyze stability analysis, we choose virulence = 0.02, interval = 100, and the original initial conditions to be scaled = [99 1 0 99].



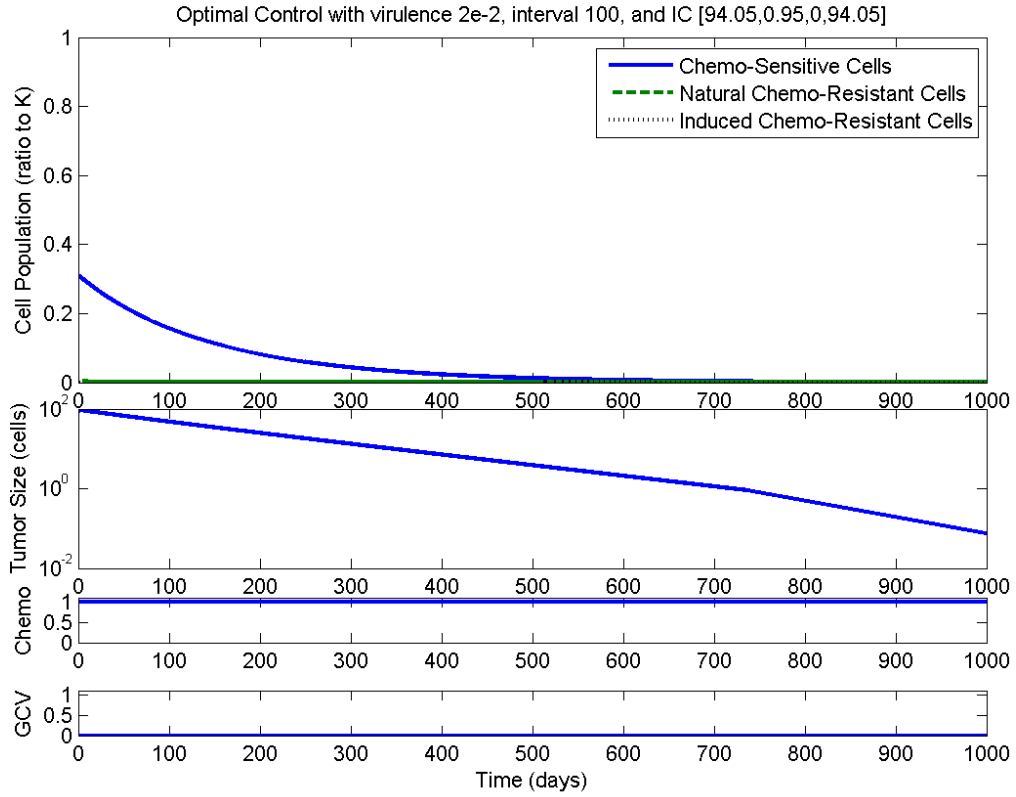
**Figure 4.23:** The simulation yields a treatment failure even though tumor size reaches one cell, because GCV is never applied.

ICs  $[123.75 \ 1.25 \ 0 \ 123.75]$ , factor of 1.25, gives a result where GCV is never applied. This is considered a treatment failure whereas the previous corresponding plot (fig 4.14) was a treatment success with GCV being applied at time = 900 days and tumor size decreasing to  $10^{-1}$ .



**Figure 4.24:** These model parameters cause a treatment success and GCV is applied at time = 800 days and tumor size reduces below one cell.

Using a factor of 1.1 results in a treatment improvement over the original case. In this case, the chemotherapy is only applied until time = 800 days. At this point GCV is applied and the bystander effect, while small as seen from the relatively constant negative slope of the tumor size plot, is enough to ensure that the tumor does not rebound.



**Figure 4.25:** The treatment switches back to a failure when we use ICs of (94.05 0.95 0 94.05) since GCV is never applied.

In the final figure using these parameters, the system reverts back to resulting in a treatment failure where GCV is never applied. While the tumor size does reach  $10^{-1}$ , it is susceptible to rebound because the chemoresistant  $Y$  type cells are never moved to extinction because no bystander effect ever occurs.

## Chapter 5

### CONCLUSION

In our research we use an ordinary differential equation model for a novel gene-therapeutic approach to curing pancreatic cancer, which aims to completely eradicate cancer cells based on a combination of chemotherapy and gene therapy. The experiments conducted by Martinez-Quintanilla et al. (6,7) achieved a bystander effect but it was not sufficient enough to completely eradicate the tumor. In our results however, we ran many simulations using real human-relevant parameters and some resulted in complete eradication of the tumor while others merely partially reduced the size of the tumor.

When using a high virulence delivery virus (figure 4.2) we found that a successful treatment needed an interval of 100 days. With these parameters, the optimal treatment schedule was to apply chemotherapy for 900 days then switch to GCV. Since such a long administration of chemotherapy is biologically problematic we analyzed the system dynamics when using a low virulence delivery virus instead.

Switching to a delivery virus with a virulence of 0.02, our system found successful treatment schedules for both the cases of interval = 20 (figure 4.3) and interval = 100 (figure 4.4). The GCV application times of 160 days and 200 days respectively were a sizeable improvement over the high virulence delivery virus case, so we focused on utilizing the low virulence virus.

Minimum tumor size versus virus virulence (figure 4.5) concluded that the behavior of the data strongly changed depending on the model parameters we assigned. For the case of interval = 20 and initial conditions of  $[99 \ 1 \ 1 \ 99]$  we saw a general positive correlation with three distinct tiers and a large range in y-axis values.

When interval was switched to 100 days (figure 4.6) we saw that the data was now negatively correlated with a constant slope. The range of y-axis values was smaller than in the previous case (figure 4.5).

Realizing that the characteristics of the results changed depending on the interval, we looked into the extreme case where interval = 1 (figure 4.7). With a smaller interval we had to increase the value of the tumor size threshold to  $1e2$ . The figure appeared to be negatively correlated with a constant slope but noticing the small range of the y values we determined that the minimum tumor size isn't affected by the virulence much.

Time to achieve (number of days to reach either minimum tumor size or the threshold) vs. virulence was investigated next. Setting parameters of interval = 20 and IC [99 1 1 99] (figure 4.8) we see an overall positive correlation with three groupings of y values and a y-axis range of 138 to 178 days.

Changing the interval to equal 100 days (figure 4.9) we again see a negatively correlated series with relatively constant slope and a small range in y values. This behavior is consistent with the minimum tumor size vs. virulence plot with corresponding parameter values (figure 4.6).

The achieve time plot using interval = 1 (figure 4.10) requires a threshold of 100 cells and displays an initial increase in time to achieve, until virulence reaches approximately 0.012. After this point the series drops to the minimum value. The range of the y-axis is only 0.14 days.

Next we used the more biologically realistic initial conditions of [99 1 0 99]. Assuming there was no initial population of  $Z$  type cells we ran our simulations and analyzed their results. For the high virulence delivery virus we found that these initial conditions improved the treatment by requiring chemotherapy for 100 less days (figure 4.12) than when using interval = 100 and IC [99 1 1 99] (figure 4.2).

However, the low virulence virus results were made worse by the [99 1 0 99] ICs. With the interval set to 20 days (figure 4.13), the treatment now fails because GCV is never applied and when the interval equals 100 days (figure 4.14) the chemotherapy

schedule requires 900 days instead of only 200.

Minimum tumor size and achieve time are unaffected by virulence when using parameters  $[99 \ 1 \ 0 \ 99]$  and interval = 1, 20, or 100 (figures 4.15, 4.16, 4.17). Note the y-axis scales.

Performing sensitivity analysis entailed running our optimal control simulations again using scaled initial conditions to determine how it altered our treatment success and treatment schedules.

Since the greatest treatment success came from the parameters virulence = 0.02, interval = 20, and ICs =  $[99 \ 1 \ 1 \ 99]$  (figure 4.3), we started with this case. First we scale the ICs by a factor of 2, resulting in  $[198 \ 2 \ 2 \ 198]$  and run the optimal control simulation. The output (figure 4.18) is a treatment failure where chemotherapy is switched to GCV at 100 days. Due to the fact that the ratio of the  $Z$  type cells to the total tumor size is too small at this time, the bystander effect is too small to stop the tumor size from rebounding.

Similar behavior is observed when using ICs of  $[148.5 \ 1.5 \ 1.5 \ 148.5]$  but now the time at which GCV is applied is 80 days (figure 4.19). It isn't until we use a factor of 1.25 that we have a successful treatment again. Although it is possible that a multiplying factor between 1.5 and 1.25 could also lead to a successful treatment, we are concerned more with overall sensitivity analysis and not finding absolute bounds on factors. Using the 1.25 factor,  $[123.75 \ 1.25 \ 1.25 \ 123.75]$ , we have a successful treatment and GCV is applied at 180 days when  $Z$  cells are the dominant type (figure 4.20).

The induced chemo-resistant cells also reach dominance in the scenario of ICs  $[94.05 \ 0.95 \ 0.95 \ 94.05]$ . The treatment (figure 4.21) is a success and the bystander effect that takes place at 160 days is large enough to completely eradicate the tumor.

The treatment reverts back to a failure when we use a factor of 0.9, ICs of  $[89.1 \ 0.9 \ 0.9 \ 89.1]$  because GCV is never applied within the duration of the simulation (figure 4.22). This is because the tumor size continues to decrease as long as chemotherapy is applied, and simultaneously the tumor size never reaches the threshold where we could logically assume that the tumor would stochastically go extinct.



Next we moved to the sensitivity analysis of the case with parameters virulence = 0.02, interval = 100, ICs [99 1 0 99]. We scaled the ICs by a factor of 1.25 and used [123.75 1.25 0 123.75]. Consequently the simulation yielded (figure 4.23) a treatment failure even though tumor size reaches one cell, because GCV was never applied.

Multiplying the original ICs by a factor of 1.1 results in [108.9 1.1 0 108.9]. These model parameters cause a treatment success (figure 4.24) and GCV is applied at time = 800 days and tumor size reduces below one cell.

The treatment switches back to a failure when we use ICs of [94.05 0.95 0 94.05] since GCV is never applied (figure 4.25). While the tumor size does decrease below one cell, it is susceptible to rebounding to an incurable tumor because the natural chemoresistant cells never undergo apoptosis.

As seen from our results, the optimal chemotherapy schedule and system dynamics determined by our control algorithm is dependent on the initial conditions and properties of the tumor, as well as our other parameter values. For our biologically realistic test cases the approach of using both a positive and negative selection phase gave us desirable results. If the proportion of cell types were favorable when the ganciclovir was administered, the resulting bystander effect was great enough to completely eradicate the tumor. Thus, the development of a dynamic model that describes this biological process is a useful tool to study.

Real parameters of tumor growth and of the delivery virus found in previous work provided a more realistic behavior to our model built upon the work in (14). This thesis furthered previous research by combining the real-life parameter values with the model. We also improved several aspects of the previous system (i.e. changing the control algorithm) and added robustness through extra analysis tools. Our results show how even a relatively simple optimal control algorithm is useful in achieving the strongest probability of pancreatic tumor eradication, and the benefits and importance of using mathematical models and control analysis to analyze complex biological systems.

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## Appendix

### MATLAB CODE

```
1 %run twice to avoid error
2 clc
3 N = 10;
4 NN = 2^N;
5 cost1 = 1000000;
6 % cost1 = 0;
7 costold = cost1;
8 Tmax = 0;
9 vir = 0.02;
10
11 interval = 100;
12 IC = 1.25*[99 1 0 99];
13
14 chemoArray = [0, 512, 768, 896, 960, 992, ...
15             1008, 1016, 1020, 1022, 1023]; %decimal for
16             binary 0000000000...1000000000...11...
17 virArray = [0.005, 0.006, 0.007, 0.008, 0.009, 0.010, 0.011,
18            0.012, 0.013, ...
19            0.014, 0.015, 0.016, 0.017, 0.018, 0.019, 0.020];
20 tumorSizeArray = [0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0];
```

```

21 achieveTimeArray = [0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0];
22
23 for aLoop = 1:1:16
24     for ii = 1:11
25         seq1 = dec2bin(chemoArray(ii),N);
26         [cost1, Tmax1, minTumorSize1] = PancOptimTest(seq1,
27             virArray(aLoop));
28         if cost1 < costold %minSize
29             seqoptim = seq1;
30             costold = cost1;
31             Tmax = Tmax1;
32             minTumorSize = minTumorSize1;
33         end
34     end
35     tumorSizeArray(aLoop) = minTumorSize;
36     achieveTimeArray(aLoop) = Tmax;
37     costoptim = costold;
38     cost1 = 1000000;
39     costold = cost1;
40 end
41
42 % for ii = 1:11
43 %     seq1 = dec2bin(chemoArray(ii),N);
44 %     [cost1, Tmax1, minTumorSize1] = PancOptimTest(seq1, vir
45 %         );
46 %     if cost1 < costold %minSize
47 %         seqoptim = seq1;
48 %         costold = cost1;

```

```

48 %           Tmax = Tmax1;
49 %           minTumorSize = minTumorSize1;
50 %       end
51 % end
52
53 Tmaxround = interval*(ceil(Tmax/interval));
54 cutoff = Tmaxround/interval;
55 if seqoptim == dec2bin(chemoArray(11),N)
56     if cutoff == 1
57         seqoptim = dec2bin(chemoArray(2),N);
58     end
59     if cutoff == 2
60         seqoptim = dec2bin(chemoArray(3),N);
61     end
62     if cutoff == 3
63         seqoptim = dec2bin(chemoArray(4),N);
64     end
65     if cutoff == 4
66         seqoptim = dec2bin(chemoArray(5),N);
67     end
68     if cutoff == 5
69         seqoptim = dec2bin(chemoArray(6),N);
70     end
71     if cutoff == 6
72         seqoptim = dec2bin(chemoArray(7),N);
73     end
74     if cutoff == 7
75         seqoptim = dec2bin(chemoArray(8),N);
76     end

```

```

77     if cutoff == 8
78         seqoptim = dec2bin(chemoArray(9),N);
79     end
80     if cutoff == 9
81         seqoptim = dec2bin(chemoArray(10),N);
82     end
83 end
84
85 costoptim = costold;
86 cost1 = 1000000;
87 costold = cost1;
88
89 PancPlotOptim(seqoptim, Tmax, vir);
90
91 figure;
92 semilogy(virArray, tumorSizeArray);
93 xlabel('Virulence')
94 ylabel('Minimum Tumor Size (cells)')
95 str2=sprintf('Minimum Tumor Size with interval %d, and IC [%d
96             ,%d,%d,%d]', interval, IC(1), IC(2), IC(3), IC(4));
97
98 figure;
99 plot(virArray, achieveTimeArray);
100 xlabel('Virulence')
101 ylabel('Time to Achieve (days)')
102 str3=sprintf('Time to Achieve with interval %d, and IC [%d,%d
103             ,%d,%d]', interval, IC(1), IC(2), IC(3), IC(4));

```



```

1 function [Cost ,Tmax,minTumorSize] = PancOptimTest(seq1 , a1)
2 tttt=cputime;
3 length = max(size(seq1));
4
5 %

```

---

```

6
7 %Model Parameter Definitions
8 global Tmax seq r e K deathX n C I lambda deathY b s a deathZ
   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
9
10 seq = seq1;
11 q = 0.0676;      % uninfected tumor growth rate mm3/day
12 e = 1;          % B-R exponent

```

??

```

13 K = 302.3935;   % B-R carrying capacity [((1-d/r)^(1/e))*
   Carrying capacity]
14 deathX = 0.009464; % natural death rate uninfected tumor
   ?? dX(t)
15 n = .0007;     % rate of change to chemoresistant tumor
16 C = .95;       % Chemotherapy treatment efficacy
17 Cn = 0;        % Chemotherapy treatment efficacy (natural chemo
   resistant)
18 Ci = 0;        % Chemotherapy treatment efficacy (induced chemo
   resistant)
19 I = 1.1;       % Beta-a ratio

```

```

20 f = 0.0676;      % growth rate of natural chemo resistant tumor
    cells
21 deathY = 0.009464; % death rate of natural chemo resistant
    tumor cells
22 b = 2;      % death rate due to bystander effect
23 j = 0.0676;      % growth rate of induced chemo resistant tumor
    cells
24 deathZ = 0.009464; % death rate of induced chemo resistant
    tumor cells
25 % a = 0.06; % frequency of infected cell lysing required for
    viral proliferation
26 a = a1; % virulence
27 G = 0.9;      % death rate of due to ganciclovir (GCV)
    sensitivity
28 R = 3.73;
29 density = 1e6;
30 Beta = (R*p)/(k*density); % virus mass action rate
31 p = 24;      % virus death rate
32 L = 0.0000; % Lytic rate
33 k = 1e4;      % Burst size PFU/cell
34 u = .0;      % Chemotherapy efficacy on chemoresistant
35 w = .0;      % Chemotherapy efficacy on Induced Resistant
    resistant back to sensitive term?
36
37 %

```

---

```

38 for ti = 0:0
39 % Treatment Times

```

```

40 %ti = 15; % Pulse time increment
41 t0 = 0; % Chemo treatment beginning time (day)
42 t1 = 0; % Chemo treatment end time (day)
43 t2 = 0; % GCV treatment begin time (day)
44 t3 = 0; % GCV treatment end time (day)
45 interval = 100; %Fastest switch time in days
46
47 %

```

---

```

48
49 % ODE
50 Tr = interval*length;
51 IC = 1.25*[99 1 0 99];
52 sol = ode45(@go, [0.01, Tr], IC);
53
54 ymax=max(sol.y(2,:));
55 zmax=max(sol.y(3,:));
56 [Cost, Teemax] = min(sol.y(1,:)+sol.y(2,:)+sol.y(3,:));
57 [minTumorSize] = min(sol.y(1,:)+sol.y(2,:)+sol.y(3,:));
58 % [Cost, Teemax] = max(sol.y(3,:)./(sol.y(1,:)+sol.y(2,:)+sol.y
    (3,:)));
59 Tmax = sol.x(Teemax);
60 if Tmax == interval*10
61     Tmax = sol.x(find((sol.y(1,:)+sol.y(2,:)+sol.y(3,:))<1e
        -5,1, 'first'));
62 end

```

```

63 %


---


64 end
65 % Plots
66 lw = 2;
67 cputime-tttt;
68
69 % Differential Equation Functions
70 function ddt = go(t,y)
71 global seq Tmax r e K deathX n C I lambda deathY b s a deathZ
    G t0 t1 t2 t3 q j bb Beta p L k f u w GG CCi CCn Cn Ci t4
    t5 ti interval
72 X = (y(1));
73 Y = (y(2));
74 Z = (y(3));
75 V = (y(4));
76
77 % Floor Functions
78 if X < .9
79     r = 0;
80 else if X > .9
81     r = q;
82     end
83 end
84
85 if Y < 1.5
86     lambda = 0;
87 else if Y > 1.5

```

```

88         lambda = f;
89     end
90 end
91
92 if Z < .9
93     s = 0;
94 else if Z > .9
95     s = j;
96     end
97 end
98
99 rn = round(rand);
100
101 if str2num(seq(ceil(t/interval))) == 1
102     CC = C;
103     CCn = Cn;
104     Cci = Ci;
105     GG = 0;
106     bb = 0;
107 end
108
109 if str2num(seq(ceil(t/interval))) == 0
110     CC = 0;
111     CCn = 0;
112     Cci = 0;
113     GG = G;
114     bb = b;
115 end
116

```

```

117 % Differential Equations
118 ddt = [r*X*(1-CC)*(1 - (X + Y + Z)/K) - X*(deathX + Beta*V +
        GG*bb*(Z/(X+Y+Z)))
119       Y*(1-CCn)*lambda*(1 - (X + Y + Z)/K) - Y*(deathY + GG*bb*(
        Z/(X+Y+Z)))
120       Beta*X*V + Z*(1-CCi)*s*(1 - (X + Y + Z)/K) - Z*(deathZ +
        GG + a)
121       k*a*Z - p*V];

1 function PancPlotOptim(seq1, Tmax, a1)
2 tttt=cputime;
3 % length = max(size(seq1));
4
5 %

```

---

```

6
7 %Model Parameter Definitions
8 global LL ss CGGG CCCC CCC GGG h Tmax seq r e K deathX n C I
        lambda deathY b s a deathZ 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
9
10 seq = seq1;
11 q = 0.0676; % uninfected tumor growth rate mm3/day
12 e = 1; % B-R exponent
13 K = 302.3935; % B-R carrying capacity [((1-d/r)^(1/e))*
        Carrying capacity]
14 deathX = 0.009464; % natural death rate uninfected tumor
        dX(t)

```

```

15 n = .0007;    % rate of change to chemoresistant tumor
16 C = .95;     % Chemotherapy treatment efficacy
17 Cn = 0;      % Chemotherapy treatment efficacy (natural chemo
               resistant)
18 Ci = 0;      % Chemotherapy treatment efficacy (induced chemo
               resistant)
19 I = 1.1;     % Beta-a ratio
20 f = 0.0676;  % growth rate of natural chemo resistant tumor
               cells
21 deathY = 0.009464; % death rate of natural chemo resistant
               tumor cells
22 b = 2;       % death rate due to bystander effect
23 j = 0.0676;  % growth rate of induced chemo resistant tumor
               cells
24 deathZ = 0.009464; % death rate of induced chemo resistant
               tumor cells
25 % a = 0.06; % frequency of infected cell lysing required for
               viral proliferation
26 a = a1; % virulence
27 G = 0.9;     % death rate of due to ganciclovir (GCV)
               sensitivity
28 R = 3.73;
29 density = 1e6;
30 Beta = (R*p)/(k*density); % virus mass action rate
31 p = 24;      % virus death rate
32 L = 0.0000;  % Lytic rate
33 k = 1e4;     % Burst size PFU/cell
34 u = .0;      % Chemotherapy efficacy on chemoresistant

```

```

35 w = .0;      % Chemotherapy efficacy on Induced Resistant
      resistant back to sensitive term?
36
37 %

```

---

```

38 for ti = 0:0
39 % Treatment Times
40 %ti = 15; % Pulse time increment
41 t0 = 0; % Chemo treatment beginning time (day)
42 t1 = 0; % Chemo treatment end time (day)
43 t2 = 0; % GCV treatment begin time (day)
44 t3 = 0; % GCV treatment end time (day)
45 interval = 100; %Fastest switch time in days
46
47 %

```

---

```

48
49 % ODE
50 Tr = interval*max(size(seq1));
51 IC = 1.25*[99 1 0 99];
52 sol = ode45(@go, [0.01, Tr], IC);
53
54 ymax=max(sol.y(2,:));
55 zmax=max(sol.y(3,:));
56 Cost = min(sol.y(1,:)+sol.y(2,:)+sol.y(3,:));
57 % Cost = max(sol.y(3,:)./(sol.y(1,:)+sol.y(2,:)+sol.y(3,:)));

```



```

58 %


---


59 end
60 % Plots
61 lw = 2;
62
63 Gr = sol.y(1,:) + sol.y(2,:) + sol.y(3,:);
64 XGr = sol.y(1,:) .* (Gr.^-1);
65 YGr = sol.y(2,:) .* (Gr.^-1);
66 ZGr = sol.y(3,:) .* (Gr.^-1);
67
68 KXGr = sol.y(1, :)/K;
69 KYGr = sol.y(2, :)/K;
70 KZGr = sol.y(3, :)/K;
71
72 MAXIMUMNCR = max(KYGr);
73 MAXIMUMICR = max(KZGr);
74 MAXIMUMBRATIO = max(ZGr);
75
76 t = sol.x;
77 chemo = ceil(t/interval);
78 ganc = ceil(t/interval);
79 for ind = 1:length(chemo)
80     if chemo(ind) <= length(seq)
81         chemo(ind) = str2num(seq(chemo(ind)));
82     elseif chemo(ind) > length(seq)
83         chemo(ind) = 0;
84     end

```

```

85     if chemo(ind) == 1
86         ganc(ind) = 0;
87     elseif chemo(ind) == 0
88         ganc(ind) = 1;
89     end
90 end
91
92 figure;
93 subplot(8,1,1:4)
94 plot(sol.x, KXGr, '-', 'LineWidth', lw);
95 hold all
96 plot(sol.x, KYGr, '—', 'LineWidth', lw);
97 hold all
98 plot(sol.x, KZGr, 'k:', 'LineWidth', lw);
99 % xlabel('time');
100 ylabel('Cell Population (ratio to K)');
101 legend('Chemo-Sensitive Cells', 'Natural Chemo-Resistant
        Cells', 'Induced Chemo-Resistant Cells')
102 axis([0 Tr 0 1])
103 str=sprintf('Optimal Control with virulence %d, interval %d,
        and IC [%d,%d,%d,%d]', a, interval, IC(1), IC(2), IC(3), IC
        (4));
104 title(str)
105 subplot(8,1,5:6)
106 semilogy(sol.x, Gr, '-', 'LineWidth', lw)
107 % xlabel('Time')
108 ylabel('Tumor Size (cells)')
109 % axis([0 Tr min(Gr) 100])
110 % axis([0 Tr 0 K])

```

```

111
112 subplot(8,1,7)
113 plot(t,chemo,'Linewidth',lw)
114 ylabel('Chemo')
115 axis([0 Tr 0 1.1])
116
117 subplot(8,1,8)
118 plot(t,ganc,'Linewidth',lw)
119 xlabel('Time (days)')
120 ylabel('GCV')
121 axis([0 Tr 0 1.1])
122 %

```

---

```

123
124 % Differential Equation Functions
125 function ddt = go(t,y)
126 global LL ss CGGG CCCC CCC GGG h Tmax seq r e K deathX n C I
      lambda deathY b s a deathZ G t0 t1 t2 t3 q j bb Beta p L k
      f u w GG CCi CCn Cn Ci t4 t5 ti interval
127 X = (y(1));
128 Y = (y(2));
129 Z = (y(3));
130 V = (y(4));
131
132 % Floor Functions
133 if X < .9
134     r = 0;
135 else if X > .9

```

```

136         r = q;
137     end
138 end
139
140 if Y < 1.5
141     lambda = 0;
142 else if Y > 1.5
143     lambda = f;
144     end
145 end
146
147 if Z < .9
148     s = 0;
149 else if Z > .9
150     s = j;
151     end
152 end
153
154 if str2num(seq(ceil(t/interval))) == 1
155     CC = C;
156     CCn = Cn;
157     Cci = Ci;
158     GG = 0;
159     bb = 0;
160 end
161
162 if str2num(seq(ceil(t/interval))) == 0
163     CC = 0;
164     CCn = 0;

```

```

165     CCI = 0;
166     GG = G;
167     bb = b;
168 end
169
170 % Differential Equations
171 ddt = [r*X*(1-CC)*(1 - (X + Y + Z)/K) - X*(deathX + Beta*V +
        GG*bb*(Z/(X+Y+Z)))
172       Y*(1-CCn)*lambda*(1 - (X + Y + Z)/K) - Y*(deathY + GG*bb*(
        Z/(X+Y+Z)))
173       Beta*X*V + Z*(1-CCi)*s*(1 - (X + Y + Z)/K) - Z*(deathZ +
        GG + a)
174       k*a*Z - p*V];

```