

A Mathematical Journey of Cancer Growth

by

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ABSTRACT

Cancer is a major health problem in the world today and is expected to become an even larger one in the future. Although cancer therapy has improved for many cancers in the last several decades, there is much room for further improvement. Mathematical modeling has the advantage of being able to test many theoretical therapies without having to perform clinical trials and experiments. Mathematical oncology will continue to be an important tool in the future regarding cancer therapies and management.

This dissertation is structured as a growing tumor. Chapters 2 and 3 consider spheroid models. These models are adept at describing ‘early-time’ tumors, before the tumor needs to co-opt blood vessels to continue sustained growth. I consider two partial differential equation (PDE) models for spheroid growth of glioblastoma. I compare these models to *in vitro* experimental data for glioblastoma tumor cell lines and other proposed models. Further, I investigate the conditions under which traveling wave solutions exist and confirm numerically.

As a tumor grows, it can no longer be approximated by a spheroid, and it becomes necessary to use *in vivo* data and more sophisticated modeling to model the growth and diffusion. In Chapter 4, I explore experimental data and computational models for describing growth and diffusion of glioblastoma in murine brains. I discuss not only how the data was obtained, but how the 3D brain geometry is created from Magnetic Resonance (MR) images. A 3D finite-difference code is used to model tumor growth using a basic reaction-diffusion equation. I formulate and test hypotheses as to why there are large differences between the final tumor sizes between the mice.

Once a tumor has reached a detectable size, it is diagnosed, and treatment begins. Chapter 5 considers modeling the treatment of prostate cancer. I consider a joint model with hormonal therapy as well as immunotherapy. I consider a timing study to determine whether changing the vaccine timing has any effect on the outcome of

the patient. In addition, I perform basic analysis on the six-dimensional ordinary differential equation (ODE). I also consider the limiting case, and perform a full global analysis.

To my family: Mom, Dad, Lindsay, John, Eric, Sukhon, and Pretzel.

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Chapter 1

INTRODUCTION

1.1 Mathematics in Medicine

Mathematics has been used to model biological phenomena for centuries, although it was only recently that mathematics was applied to medicine. One of the earliest mathematical models in medicine is attributed to Bernoulli, who created a model to investigate the efficacy of inoculation against smallpox (Bernoulli, 1760).

Within the realm of epidemiology, Kermack and McKendrick formulated the well-known Susceptible-Infective-Recovered (S-I-R) model to explain the dynamics of communicable diseases, specifically the spread of the bubonic plague in early 20th century Bombay (Kermack and McKendrick, 1927). This S-I-R type model has birthed many related modeling frameworks such as the Susceptible-Exposed-Infected-Recovered (S-E-I-R) and Susceptible-Infected-Recovered-Susceptible (S-I-R-S) modeling frameworks. Researchers use these frameworks to model anything from current disease outbreaks such as SARS (Ng *et al.*, 2003) and H1N1 (Coburn *et al.*, 2009) to far more outlandish ideas like hypothetical zombie outbreaks (Munz *et al.*, 2009) and social disease dynamics from bulimia nervosa spread (González *et al.*, 2003) to drug addiction (Behrens *et al.*, 1999).

Apart from epidemiology, mathematics has been extremely useful in understanding disease dynamics on the sub-cellular, cellular, and tissue levels. There are many resources detailing the types of mathematical models available for disease progression, healthy organ function, and more (Bellman *et al.*, 1983; Hoppensteadt and Peskin, 2013).

1.2 Mathematical Oncology

Until approximately 50 years ago, researchers believed that tumor growth was governed by an exponential process (Laird, 1964). Mayneord (1932) noticed that in rat sarcomas, plotting the cube root of the total number of cancer cells against time generated a linear fit, implying that the overall growth slows in the latter stages of cancer. This finding was corroborated by other researchers for other types of cancers (Klein and Révész, 1953).

Due to the slowing growth in the late stages of cancer, Mendelsohn (1963) proposed a slight variation on the equation for exponential growth, creating the power-law growth. Ultimately, this is a generalization of exponential growth, which can produce sub-exponential growth curves. In fact, if you choose parameters wisely, your solution will have tumor volume growing approximately as time cubed, similar to that found in Mayneord (1932).

Shortly thereafter, Laird (1964) applied the Gompertz Equation (Gompertz, 1825), an equation that was initially used for mortality curves in actuarial practice, to tumor growth. She keenly observed that growth given by the Gompertz Equation exhibited retarding of the growth rate as time increased. Laird (1964) generated excellent fits to over 20 separate experimental cancer studies, fitting the data using least-squares methods. She used these fits to estimate doubling time and parameters.

Over twenty years prior to these works, von Bertalanffy (1938) developed a growth equation, originally meant to model the growth of *Lebistes Reticulatus*, or guppies, in terms of length and mass. This model has often been used sparingly in cancer models, although it was suggested by von Bertalanffy himself in the early 1950s (von Bertalanffy and Pirozynski, 1953). This is the only example of a biologically-derived growth model.

One other model was suggested for growth, although not specifically for cancer growth. The well-known logistic equation was first proposed by Verhulst (1845), although mainly forgotten until revived by Pearl and Reed (1920), who used the equation to model the population growth of the United States. The logistic curve remains prevalent in today's literature, modeling the growth of many tumor cells.

Recent work (Gerlee, 2013) argues that the von Bertalanffy model is most effective due to its biologically-driven derivation and the fact that it had fit tumor growth curves better than both the Gompertz and Logistic models, although only in animal data (Vaidya and Alexandro, 1982). In human tumor data, the logistic curve was found to be the best-fit (Vaidya and Alexandro, 1982). Recently Sarapata and de Pillis (2014) compared five phenomenological models (exponential, power law, Gompertz, logistic, and von Bertalanffy) to 10 types of cancer growths. They were unable to conclude that one type of phenomenological model was always the best fit, rather some methods fit the data better than others for certain types of cancers. They were able to show, however, that the von Bertalanffy model was inferior to the logistic and Gompertz models in 9 out of 10 cases (Sarapata and de Pillis, 2014). However, in the same year, Benzekry *et al.* (2014) compared logistic, exponential, Gompertz, and von Bertalanffy models (among others) and found the von Bertalanffy model able to describe tumor data as well as the Gompertz model, but found the logistic model to be lacking.

What conclusions can be drawn from such seemingly contradictory findings? Firstly, I would argue that these findings do *not* contradict one another. Every type of cancer is different. There is no one way to describe the growth of all types of cancers. Secondly, the drawback to the previously mentioned works is that, in general, there is no real physical or biological justification for the equations, although attempts were made for the Gompertz equation (Xu, 1987). These works, although highly influential, exhibit

some of the types of modeling which are phenomenological: models which are used to fit the data rather than explain the underlying processes. In order to try to understand the underlying processes, we must turn to mechanistic models.

Although mathematical oncology still represents a small amount of the work performed in the mathematical biology field, it is on the rise. Textbooks aimed at the upper undergraduate level and graduate level are becoming more commonplace (Kuang *et al.*, 2016; Wodarz and Komarova, 2014). For excellent reviews on the progression of cancer modeling in mathematics, see Araujo and McElwain (2004) and Byrne (2010).

In accordance with current Arizona State University policy, since portions of my thesis have already been published, or are in the process of being published, I will detail in this introduction the contributions I made to each of these papers and projects. Additionally, I will discuss the contributions made by each author within the publication and discuss the unique attributes I brought to the project and my interpretation of the results.

1.3 Density-Dependent Diffusion Model of *in vitro* Spheroid Glioblastoma Growth

The article published in this section, (Stepien *et al.*, 2015), is an article in which I am the second author. The project investigated a one-dimensional density-dependent diffusion model of glioblastoma multiforme (GBM) growth. A highly-cited paper, Stein *et al.* (2007a), argued that in order to accurately model GBM, the population of tumor cells must be split into proliferating cells and migrating cells. The explanation is that cells either phenotypically stay put and proliferate (Grow), or they migrate but do not proliferate (Go). This ‘Go or Grow’ hypothesis is widely used by mathematical

modelers in the GBM field.

Dr. Kuang proposed a single equation model based on the biology of GBM. We used a density-dependent diffusion which allowed for there to be high diffusion when there is a low population (the migratory cells) and low diffusion when there is a high population (the proliferating cells). This density-dependent diffusion was inspired by the data obtained by the experimentalists, which showed a heavy proliferative core that did not move much along with a very mobile, low-density region. The model was fully analyzed, by the first author, Dr. Tracy Stepien, including determining the minimum wave speed. The model was then used to fit the data presented in the Stein *et al.* (2007a) paper. We were able to obtain a much higher quality fit than the original two-equation model proposed by the experimentalists.

My part in this project was the numerical simulations. I performed a sensitivity analysis of all the parameters present in our model, showing that all were sensitive. I also estimated the parameters of our model in order to generate the best fit to the experimental data. Finally, we were interested in numerically corroborating the minimum wave speed found analytically, which I performed as well. Since I was not involved in the analysis, I did not include that portion of the work in my thesis, only stating the final result and citing the paper for those interested in reading the details. I did, however, perform the stability of the traveling wave solutions, which was not included in the original paper.

Overall, I initially wrote the introductory section, and the numerics section. All authors proof-read and edited the paper as necessary. As per ASU regulations, the full journal article appears in Appendix E.

1.4 2 Population Model of *in vitro* Spheroid Glioblastoma Growth

The article in this section is an article in which I will be the second author, currently in preparation for submission. As in the previous chapter, the project concerns the experimental data obtained in the Stein *et al.* (2007a) paper. The original model proposed by the experimentalists was more phenomenological, rather than mechanistic. As such, it included terms that were problematic for both analysis and computation. Dr. Kuang proposed a 2-equation model, with subpopulations of proliferating cells and migrating cells, which can exhibit switching. In this model we assume a constant diffusion. The model was analyzed by the first author, Dr. Tracy Stepien, who was able to determine the minimum wave speed. The model was then used to fit the experimental data. However, we were only able to obtain a similar fit as the model proposed by the experimentalists, and much larger than that of our density-dependent diffusion model, presented in Chapter 2.

My part in this project was again the numerics. I wrote a basic solver, based on the Crank-Nicolson method, which proved to be more efficient and accurate than MATLAB's built-in pde solver PDEPE. I performed sensitivity analysis to determine how each parameter affected the outcome of the solution. I also estimated the parameters necessary in order to obtain the best fit to the experimental data. In this paper, the analysis performed (not by me and not included in my thesis) informed us that there was a possible range of what we could expect the minimum wave speed to be. I then plotted the theoretical minimum wave speeds versus the computed minimum wave speeds for a variety of parameter values.

Lastly, we compared all proposed models in their ability to fit the experimental data. The argument for comparing all of these models is that we are unsure which type of model fits the data best. Most of the models are derived from biological assumptions

and conditions. Having a model fit the experimental data, with parameters in their biologically relevant ranges, leads credence to the underlying biological assumptions made in constructing the model. Although being able to fit experimental data to a model does not validate the model, it does help justify the assumptions. In performing this comparison, we are eager to see if the ‘Go or Grow’ hypothesis is truly biologically relevant.

Overall, I initially wrote the introductory section and the numerics section. All authors proof-read and edited the paper as necessary.

1.5 *in vivo* Murine Model of Glioblastoma Growth

The article published in this chapter is an article in which I am the first author. This intensive project included obtaining five immune-competent mice, injecting them with GL261-Luc2 cells (a cell line that shares many characteristics with the human GBM), imaging these mice at five separate time points after injection using Magnetic Resonance Imaging (MRI), processing these images to become computational domains, and finally performing parameter estimations to generate the best fit with our model.

Because this work is so intensive, there are many co-authors involved in this paper. I will specifically highlight the aspects of this project that I performed. I became involved in the project just as the imaging was being finished. For the experimental side, Dr. Adrienne C. Scheck grew the tumor cell lines, which were implanted into the mice by Eric C. Woolf. The MR protocol was designed by Dr. Gregory Turner, Dr. Qingwei Liu and Dr. Vikram Kodibagkar. Jonathan Plasencia performed the image acquisition and trained me for image processing, which was overseen by Dr. David Frakes. Dr. Mark C. Preul oversaw the experimental portion of this project. I was involved in the post-processing of the MR images into computational domains as well as formulating the hypotheses and performing the parameter estimation.

To start the post-processing of images, I used the medical software Mimics ® (Materials Inc, Leuven, Belgium). The raw images involved not just the brain, but also the skull, eyes, and other material that needed to be eliminated in order to obtain the brain geometry. Each mouse at each time point has approximately 20-25 2D images that can be stacked to make a 3D brain geometry. To eliminate non-brain matter, I used thresholding techniques in Mimics ® (Materials Inc, Leuven, Belgium) in order to segment out a rough brain outline. In order to ensure a computationally-friendly domain, I smoothed these edges pixel by pixel for each of the 20-35 images, for each of the five mice, at all five time points. In addition, I segmented the regions of visible tumor on the MR images, by hand, which was corroborated independently by another student, Jonathan Plasencia.

From these brain segmentations, I output the brain surface mesh into point-cloud form for each mouse at each time point. We decided that all brain geometries would be registered to their third time point. We assume that the overall volume of the brain does not change over the course of the experiment. However, the placement of the mouse for each MR scan could have been slightly different, so we must make sure to register all the brain volumes to their respective third time point. In order to do this, for each mouse, I input the outputted brain surface mesh from the first time point and from the third time point into Geomagic Studio (Geomagic Inc, NC), which calculated the affine matrix describing the rotation, scaling, reflection, and shearing needed to transform the brain surface at the first time point to match the brain surface at the registered brain at time point 3. This was done for each mouse at each time point and MATLAB was then used to apply the affine matrices, using code written by Jonathan Plasencia.

Once we began looking at the tumor sizes, we realized there was a lot of variation between the mice. I formulated three distinct hypotheses as to why the variation

existed. Using a 3D finite-difference code written by Barrett Anderies, I tested these three hypotheses by performing parameter optimization and comparing my results with the data. Other aspects of the paper, such as the 2D level set code were performed by Dr. Tracy Stepien and therefore not included in my dissertation. Dr. Yang Kuang and Dr. Eric Kostelich assisted with the modeling aspect of the project.

Overall, I initially wrote the majority of the paper, excepting the sections on experimental methods (written by Jonathan Plasencia) and the 2D level set results (written by Dr. Tracy Stepien). All authors assisted in proof-reading and improving the paper.

1.6 Global Dynamics of a Model of Joint Hormone Treatment with Dendritic Cell Vaccines for Prostate Cancer

The article published in this chapter is one in which I am the first author. This project concerns how the timing and dosage of treatment therapies for prostate cancer influence the dynamics of the disease. The model is an extension of a previous model, which the other author, Dr. Yang Kuang, suggested could be improved by incorporating a few key changes such as generalizing the functions. Previous work had only considered one dosage. Our paper examined how timing dosage affected the outcome of the disease. Based on our observation that frequent small injections were much more effective than infrequent large injections, we considered the special case where the patient is intravenously connected to the vaccine. From here we performed bifurcation analysis on a personalized parameter for which literature suggests there is a range of values. We analyzed this system, proving positivity and determining local stability of the disease-free equilibrium. Finally, we let the model go to quasi-steady state, which gave a system of two equations. I performed full global analysis on this system, including conditions necessary for the global stability of both the disease-free

equilibrium and the endemic equilibrium. We wrapped up the paper by translating our mathematical conditions for global stability back into biological meaning.

I performed both theoretical and computational work for this project. At the beginning of the project, I proposed growth functions for the androgen-dependent and androgen-independent cells that were more biologically likely. I generated all figures studying the vaccine timing using MATLAB code and the ODE45 solver. I created the bifurcation diagram through one of the parameters and examined the effect of continual dosing strategies.

In addition to the computational work, I also thoroughly analyzed the model. For the full model, I performed basic positivity analysis and proved local stability. I then translated this local stability condition back into a minimum dosage needed to theoretically eradicate the prostate cancer.

At Dr. Kuang's suggestion, I then let the model go to quasi-steady state, reducing the model to a system of two equations. I performed numerical simulation to show that, in the limit, the full system and the quasi-steady state system exhibit the same dynamics. I fully analyzed the quasi-steady state system and found global stability conditions. I also spearheaded the effort to translate these conditions back into biological meaning.

I wrote the majority of the paper, including background, model introduction and the results. Dr. Yang Kuang oversaw the writing and double-checked the proofs and analysis for correctness. This publication has been accepted and, per ASU regulations, is included in Appendix F.

Chapter 2

DENSITY-DEPENDENT DIFFUSION MODEL OF *IN VITRO* SPHEROID GLIOBLASTOMA GROWTH

2.1 Introduction

In the early stages of cancer, the tumor is initially somewhat self-sufficient. The tumor is small enough that it is able to acquire nutrients without the growth of new blood vessels. The growth and spread of this young tumor can be approximated by spheroids. There is no competition yet for space nor nutrients, and simple models can be used to approximate the growth of the tumor.

In this chapter, I present a spheroid model of glioblastoma growth, comparing and contrasting it's ability to fit experimental *in vitro* data. I also analyze the model to determine the stability of traveling waves of the growing spheroid and compare with the numerically obtained traveling wave speed. Some of the work contained in this chapter has been previously published and the full journal article is in Appendix E.

2.1.1 Glioblastoma Multiforme (GBM)

Glioblastoma multiforme (GBM) is a malignant form of brain cancer that has a very negative prognosis. Even with treatment, mean survival time from detection is less than 15 months (Norden and Wen, 2006). Symptoms of GBM include seizures, hemorrhaging, and tumor necrosis. Additionally tumors are difficult to treat because GBM are characterized not only by intense proliferation, but also by excessive migration. This is complicated by the fact that surgical resection is performed on what is visibly identified as ‘tumor’ in MR images. Only areas of intense proliferation, leaky vasculature, and

high tumor volume are often visible in MR images. Thus, surgical resection is able to remove the tumor core, but less effective at identifying areas where there are few tumor cells. After resection, secondary tumors often occur, which may be fatal.

The dichotomy between proliferation and invasive migration also makes GBM difficult to model. Early models of GBM growth include reaction-diffusion equations that are able to accurately capture the proliferating tumor core. Tracqui et al. formulated the earliest reaction-diffusion model to describe glioblastoma growth and diffusion, combined with therapeutic intervention (Tracqui *et al.*, 1995). Swanson et al continued with these reaction-diffusion models, accounting for a spatially-dependent diffusion function in an attempt to capture the migratory behavior of the tumor cells (Swanson *et al.*, 2003). However, none of these reaction-diffusion models were able to accurately model both the proliferating core and the invasive populations.

2.1.2 Stein et al. (2007a) Experimental Data and Model

An influential paper was published by Stein et. al in 2007 where an *in vitro* cell line experiment was performed. Two human astrocytoma U87 cell lines were implanted into gels—one with a wild-type receptor (EGFRwt) and one with an over-expression of the epidermal growth factor receptor gene (Δ EGFR). The resulting spheroids were left to grow over 7 days and imaged every day. A summary of the results of the experiment for the invasive and proliferating cell radii on days 0, 1, 3, and 7 is presented in Figure 2.1. The tumor core radius was measured to be where pixels had an intensity of < 0.12 and the invasive radius as the half-maximum for the image averaged over the azimuthal angle. From this data, the authors concluded that in order to accurately model the invasive radius, it was necessary to use two equations to model the tumor cells: one for the proliferating core cells and one for the migratory cells (Stein *et al.*, 2007a).

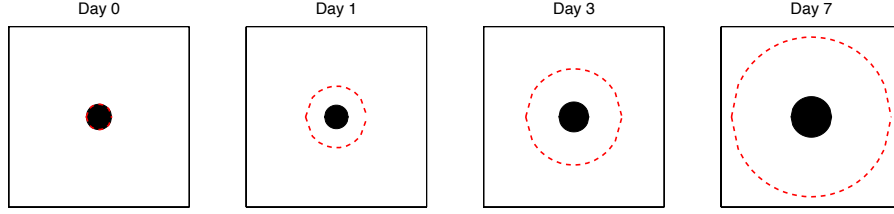


Figure 2.1: The radius of the core proliferating (black) and migratory (red dashed) cells for the experiment for EGFRwt strain from (Stein *et al.*, 2007a) on days 0, 1, 3, and 7. The domain is a 3 mm by 3 mm square.

The landmark mathematical model introduced by Stein *et al.* (Stein *et al.*, 2007a) advocated for the separation of glioblastoma cells into two separate populations: the proliferating core cells and the migratory cells. In addition to the standard reaction-diffusion terms in the equation for migratory cells, the model includes a radially biased motility term corresponding to convection to account for the situation where cells detect the location of the tumor core and actively move away from it (Stein *et al.*, 2007a).

The mathematical model proposed by the experimentalists describes the movement of the invasive, or migratory, cells (u_i) for the EGFRwt cell line and the Δ EGFR cell line based on the experiment described above. The radius of the tumor core, consisting of the less mobile cells, is modeled as increasing at a constant rate based on the *in vitro* experimental data (Stein *et al.*, 2007a). The model assumes that the proliferating tumor cells leave the tumor core and become migratory cells and invade the collagen gel (Stein *et al.*, 2007a). The behavior of invasive cells is described by the model in a way that can be quantitatively compared to the experimental measurements. The invasive cell population is governed by the following ad hoc partial differential

equation:

$$\frac{\partial u_i(r, t)}{\partial t} = \underbrace{D\nabla^2 u_i}_{\text{diffusion}} + \underbrace{gu_i \left(1 - \frac{u_i}{u_{\max}}\right)}_{\text{logistic growth}} - \underbrace{\nu_i \nabla_r \cdot u_i}_{\text{taxis}} + \underbrace{s\delta(r - R(t))}_{\text{shed cells from core}} \quad (2.1)$$

where u_i represents the invasive cells of the tumor at radius r and time t (Stein *et al.*, 2007a). The change in invasive cell population is governed by random diffusion, directed diffusion (taxis), logistic growth, and cells being ‘shed’ from the proliferating tumor core population. Taxis refers to the active biased motility of invasive cells away from the tumor core which the authors attribute to possibly chemotaxis or haptotaxis (Stein *et al.*, 2007a). This taxis term was found to be more necessary for the highly invasive EGFRwt strain compared to the less invasive Δ EGFR strain. For our model, I will only consider the EGFRwt strain, as it was more invasive, and thus, more difficult to model accurately. The shedding term represent the tumor cells at the front of the invasion: tumor cells which are proliferating may make a phenotypic switch from proliferating to migrating cells. This is a well-known hypothesis termed ‘Go or Grow’, which stipulates that glioma cells are either growing (proliferating), or migrating, but cannot be doing both (Hatzikirou *et al.*, 2010).

The parameters of the model are as follows: D is the random diffusion constant, g is the intrinsic growth rate, $u_{\max}$ is the carrying capacity of tumor cells, ν_i is the degree at which cells preferentially migrate away from the core, s is the number of proliferating cells shed per day, and δ is the Dirac delta function. The radius of the tumor core is modeled by $R(t) = R_0 + \nu_c t$, where R_0 is the initial radius of the tumor core on day 0 and ν_c is the constant velocity at which the tumor core radius increases. This value of ν_c was determined by linearly fitting the invasive radii through time. Additionally, the data on the invasive cell radius also appears to be somewhat linear, implying that traveling wave speed analysis could be beneficial. However, the existence of the Dirac delta function makes this model difficult to analyze, both theoretically

and numerically. Furthermore, the term is somewhat artificial. In order to perform traveling wave speed analysis on both proliferating and migrating cells, we propose a model which contains only one cell population, but uses a density dependent diffusion function.

2.2 Model Introduction

The model is inspired by the data presented in the paper, so we build our model with a similar base. The population of cells is governed mainly by logistic growth, taxis, and density-dependent diffusion. The cell-shedding term is not needed, as there is no separation between the populations of proliferating and migrating cells. Logistic growth is used because the tumor cells grow in number, and logistic growth is a sensible choice. The experimental results also suggest the core reaches a capacity of tumor cells. The undetermined taxis term is kept, since the experimental results suggest that glioblastoma tumor cells migrate preferentially away from the tumor core (Stein *et al.*, 2007b). As mentioned earlier, many current cancer cell migration models are based on reaction-diffusion equations and most of those models consider diffusion to be a constant or a function of space. There is no reason to assume that diffusion occurs at a constant rate. Also, many consider spatially dependent diffusion, to differentiate between cell movement in white matter and grey matter. Since there is no brain geometry in this model, spatially-dependent diffusion functions are not applicable. Instead, the diffusion function is based on the density of cells. Some cell migration models have included density-dependent diffusion, such as wound healing models (Cai *et al.*, 2007).

The governing equation is a density-dependent convective-reaction-diffusion equation, which implies that the amount of random diffusion depends on how many cells

are present. The equation for the tumor cells $u(x, t)$ is:

$$\frac{\partial u}{\partial t} = \underbrace{\nabla \cdot \left(D \left(\frac{u}{u_{\max}} \right) \nabla u \right)}_{\text{density-dependent diffusion}} + \underbrace{gu \left(1 - \frac{u}{u_{\max}} \right)}_{\text{logistic growth}} - \underbrace{\text{sgn}(x) \nu_i \nabla \cdot u}_{\text{taxis}} \quad (2.2)$$

where the equation is in Cartesian coordinates but assume there is radial symmetry. Parameters g and ν_i are as in equation (2.1), but now they are in relation to the entire tumor cell population instead of just the invasive cell population.

There are many functions that could serve as the density-dependent diffusion $D(u^*)$, where $u^* = \frac{u}{u_{\max}}$. Experimental work suggests that diffusion is large for areas where the cell density is small (the migrating tumor cells), but diffusion is small where the cell density is large (the proliferating tumor cells) (Stein *et al.*, 2007a). This relation could possibly be explained by cell-cell adhesion (Armstrong *et al.*, 2006). To capture this behavior, the diffusion is:

$$D(u^*) = D_1 - \frac{D_2(u^*)^n}{a^n + (u^*)^n}. \quad (2.3)$$

For biologically relevant parameters, positivity is assumed, $n > 1$, and $D_2 \leq D_1$ to avoid “negative” diffusion, which is a problem both biologically and numerically. Examples of the density-dependent diffusion function for varying a values and n values are shown in Figure 2.2. The parameter n governs how steeply the function decreases and the parameter a governs the u^* value at which the transition is occurring at half maximal rate. D_1 and D_2 govern the range of the function: if D_1 and D_2 are far apart, diffusion could be approximated by a constant, however if D_1 and D_2 are similar, diffusion is very different when cell populations are low compared to when cell populations are high.

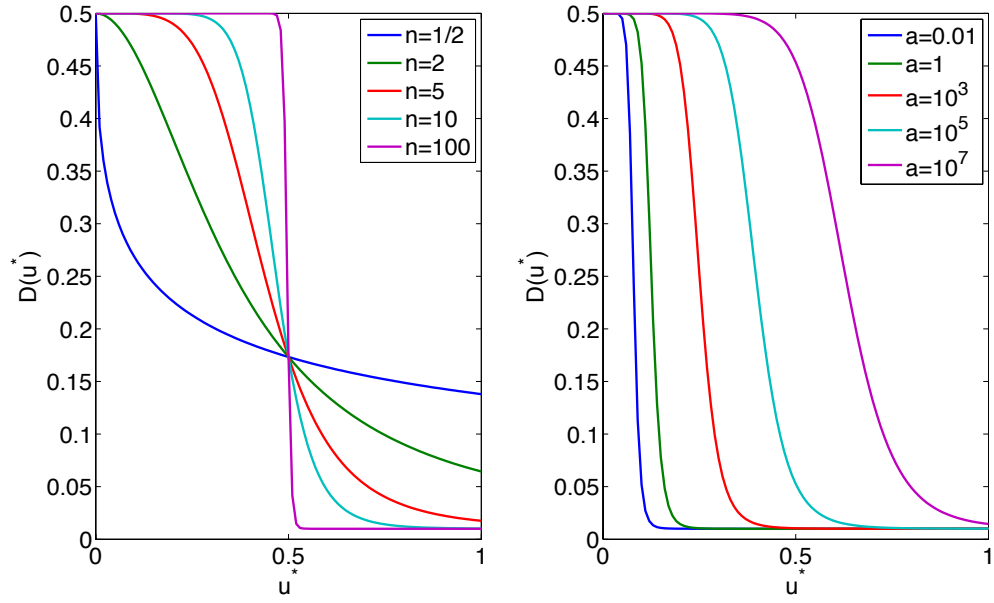


Figure 2.2: The density-dependent diffusion function $D(u^*)$, where $u^* = \frac{u}{u_{\max}}$, from Equation (2.3) for $D_1 = 0.5$, $D_2 = 0.4$, and various a and n . As u^* increases, $D(u^*)$ decreases from its maximum D_1 to its minimum $D_1 - D_2$.

2.3 Analysis

2.3.1 Existence of Traveling Waves

One of the fundamental questions with *in vitro* tumor growth is how fast is the tumor growing? Can we estimate how far the tumor will spread after a certain amount of time? We want to quantify tumor spread, not just computationally, but also analytically. To this end, we will need to analyze the traveling wave solutions.

Traveling wave solutions have been studied in models of glioblastoma growth with multiple cell populations and constant diffusion ((Pérez-García *et al.*, 2011), (Harko and Mak, 2015)). Traveling wave solutions also arise in density-dependent reaction-diffusion equations, and numerous density-dependent diffusion functions have

been studied ((Atkinson *et al.*, 1981), (Murray, 2002), (Witelski, 1994), (Sánchez-Garduño and Maini, 1995), (Harris, 2004), (Pedersen, 2005), (Maini *et al.*, 2006), (Sánchez-Garduño *et al.*, 2010), (Ngamsaad and Khompurngson, 2012), and (Kengne *et al.*, 2013)). More generally, traveling wave solutions for convective-reaction-diffusion equations have also been studied ((Malaguti and Marcelli, 2002), (Malaguti *et al.*, 2010), (Malaguti *et al.*, 2011), (Gilding and Kersner, 2005)). Minimal speeds for various diffusion and convective terms were estimated.

For the model (2.2) with density-dependent diffusion function (2.3), the minimum wave speed is:

$$k \geq k_{\min} = 2\sqrt{D_1 g} + \nu_i. \quad (2.4)$$

For full explanation and details regarding the proof, see Stepien *et al.* (2015).

2.3.2 Stability of Traveling Waves

I want to investigate the stability of the traveling waves. I follow the methods from Canosa (1973) to determine what conditions, if any, must be met for wave stability.

Rewriting (2.2) in one-dimensional Cartesian coordinates, the governing equation is

$$\frac{\partial u}{\partial t} = D \left(\frac{u}{u_{\max}} \right) \frac{\partial^2 u}{\partial x^2} + \frac{1}{u_{\max}} D' \left(\frac{u}{u_{\max}} \right) \left(\frac{\partial u}{\partial x} \right)^2 - \nu_i \frac{\partial u}{\partial x} + gu \left(1 - \frac{u}{u_{\max}} \right). \quad (2.5)$$

I rescale by writing

$$t^* = gt, \quad x^* = x\sqrt{g}, \quad u^* = \frac{u}{u_{\max}},$$

set

$$v = \frac{\nu_i}{\sqrt{g}},$$

and, omitting the asterisks and dividing through by $gu_{\max}$ for simplicity, the equation

(2.5) becomes

$$\frac{\partial u}{\partial t} = D(u) \frac{\partial^2 u}{\partial x^2} + D'(u) \left(\frac{\partial u}{\partial x} \right)^2 - v \frac{\partial u}{\partial x} + u(1 - u). \quad (2.6)$$

In order to investigate the stability of traveling wave solutions, I re-write the equations in terms of the moving coordinate system: $z = x - ct$, where $c \geq 0$ is the minimum wave speed. Then the calculated partial derivatives are:

$$\begin{aligned} \frac{\partial u}{\partial t} &= \frac{\partial u}{\partial t} + \frac{\partial u}{\partial z} \frac{\partial z}{\partial t} \\ &= \frac{\partial u}{\partial r} - c \frac{\partial u}{\partial z} \\ \frac{\partial u}{\partial x} &= \frac{\partial u}{\partial z} \\ \frac{\partial^2 u}{\partial x^2} &= \frac{\partial^2 u}{\partial z^2} \end{aligned}$$

I can then express the equation in the moving coordinate frame:

$$\begin{aligned} \frac{\partial u}{\partial t} - c \frac{\partial u}{\partial z} &= D(u) \frac{\partial^2 u}{\partial z^2} + D'(u) \left(\frac{\partial u}{\partial z} \right)^2 - v \frac{\partial u}{\partial z} + u(1 - u) \\ \frac{\partial u}{\partial t} &= D(u) \frac{\partial^2 u}{\partial z^2} + \frac{\partial u}{\partial z} \left(D'(u) \frac{\partial u}{\partial z} - v + c \right) + u(1 - u) \end{aligned}$$

Following the work in Canosa (1973), I assume there exists a solution for the traveling wave in the form $u(z, t) = u_c(z)$, meaning that $\frac{\partial u}{\partial t} = 0$, $\frac{\partial u}{\partial z} = u'_c$, $\frac{\partial^2 u}{\partial z^2} = u''_c$ and the equation becomes:

$$0 = D(u_c)u''_c + u'_c (D'(u_c)u'_c - v + c) + u_c(1 - u_c) \quad (2.7)$$

Now I assume a slight perturbation away from the traveling wave speed, $y(z, t)$, which I assume goes to zero far away from the wave front: $y(z, t) = 0$ if $|y| \geq L$. Then I can see what happens to the wave speed when it is perturbed by investigating $w = u_c + y$:

$$\begin{aligned} \frac{\partial w}{\partial t} &= \frac{\partial y}{\partial t} \\ \frac{\partial w}{\partial z} &= \frac{\partial u_c}{\partial z} + \frac{\partial y}{\partial z} \\ \frac{\partial^2 w}{\partial z^2} &= \frac{\partial^2 u_c}{\partial z^2} + \frac{\partial^2 y}{\partial z^2} \end{aligned}$$

We now look to plug in our findings for the equation in terms of $w(z, t)$:

$$\frac{\partial w}{\partial t} = D(w) \frac{\partial^2 w}{\partial z^2} + \frac{\partial w}{\partial z} \left(D'(w) \frac{\partial w}{\partial z} - v + c \right) + w(1 - w)$$

and note in terms of our u_c and y :

$$\begin{aligned} \frac{\partial y}{\partial t} = & D(u_c + y) \left[\frac{\partial^2 u_c}{\partial z^2} + \frac{\partial^2 y}{\partial z^2} \right] + \left[\frac{\partial u_c}{\partial z} + \frac{\partial y}{\partial z} \right] \left[D'(u_c + y) \left(\frac{\partial u_c}{\partial z} + \frac{\partial y}{\partial z} \right) - v + c \right] \\ & + (u_c + y)(1 - (u_c + y)) \end{aligned}$$

and by expanding all terms

$$\begin{aligned} \frac{\partial y}{\partial t} = & D(u_c + y) \frac{\partial^2 u_c}{\partial z^2} + \frac{\partial u_c}{\partial z} \left[D'(u_c + y) \frac{\partial u_c}{\partial z} - v + c \right] + u_c(1 - u_c) \\ & + D(u_c + y) \frac{\partial^2 y}{\partial z^2} + \frac{\partial u_c}{\partial z} D'(u_c + y) \frac{\partial y}{\partial z} + \frac{\partial u_c}{\partial z} D'(u_c + y) \frac{\partial y}{\partial z} \\ & \frac{\partial y}{\partial z} \left[D'(u_c + y) \frac{\partial y}{\partial z} - v + c \right] + y(1 - y) - 2u_c y \end{aligned}$$

Under the assumption that $D(u_c + y) \approx D(u_c)$ and $D'(u_c + y) \approx D'(u_c)$ and by Equation (2.7), this simplifies greatly to:

$$\begin{aligned} \frac{\partial y}{\partial t} = & D(u_c + y) \frac{\partial^2 y}{\partial z^2} + 2 \frac{\partial u_c}{\partial z} D'(u_c + y) \frac{\partial y}{\partial z} + \\ & \frac{\partial y}{\partial z} \left[D'(u_c + y) \frac{\partial y}{\partial z} - v + c \right] + y(1 - y) - 2u_c y \end{aligned}$$

Now, ignoring all terms which are higher than first order in y generates:

$$\frac{\partial y}{\partial t} = D(u_c + y) \frac{\partial^2 y}{\partial z^2} + \left[2D'(u_c + y) \frac{\partial u_c}{\partial z} - v + c \right] \frac{\partial y}{\partial z} + (1 - 2u_c)y \quad (2.8)$$

I will now assume a solution of the form $y(z, t) = g(z)e^{-\lambda t}$, which means the partial derivatives are given by the following

$$\begin{aligned} \frac{\partial y}{\partial t} &= -\lambda g(z)e^{-\lambda t} \\ \frac{\partial y}{\partial z} &= g'(z)e^{-\lambda t} \\ \frac{\partial^2 y}{\partial z^2} &= g''(z)e^{-\lambda t} \end{aligned}$$

which, when plugged into Equation (2.8), gives the following relationship:

$$-\lambda g(z)e^{-\lambda t} = D(u_c)g''(z)e^{-\lambda t} + g'(z)e^{-\lambda t} [2D'(u_c)u'_c - v + c] + g(z)e^{-\lambda t} [1 - 2u_c g''(z)] \quad (2.9)$$

which can be rearranged into terms of $g(z)$ as:

$$g''(z)D(u_c) + g'(z) [2u'_c D'(u_c) - v + c] + g(z) [1 - 2u_c + \lambda] = 0 \quad (2.10)$$

In order to simplify the calculation, we assume that $g(z) = h(z)e^{p(z)}$, where $p'(z) = \frac{1}{D(u_c)} (-u'_c D'(u_c) + \frac{v}{2} - \frac{c}{2})$. Then it is apparent that the derivatives are given by the following:

$$g(z) = h(z)e^{p(z)}$$

$$g'(z) = h'(z)e^{p(z)} + h(z)p'(z)e^{p(z)}$$

$$g''(z) = h''(z)e^{p(z)} + 2h'(z)p'(z)e^{p(z)} + h(z)p''(z)e^{p(z)} + h(z)(p'(z))^2 e^{p(z)}$$

These can then be substituted into Equation (2.10) to arrive at the expression involving $h(z)$:

$$h''(z)D(u_c) + 2h'(z)p'(z)D(u_c) + h(z)p''(z)D(u_c) + h(z)(p'(z))^2 D(u_c) + h'(z)[2u'_c D'(u_c) - v + c] + h(z)p'(z)[2u'_c D'(u_c) - v + c] + h(z)[1 - 2u_c + \lambda] = 0$$

We notice that $[2u'_c D'(u_c) - v + c] = -2D(u_c)p'(z)$ and we can simplify to:

$$h''(z) + h(z) \left[\lambda \frac{1}{D(u_c)} - q(z) \right] = 0 \quad (2.11)$$

where $q(z) = (p'(z))^2 - p''(z) + \frac{2u_c - 1}{D(u_c)}$.

Proposition 1. *Equation (2.11) is a regular Sturm-Liouville problem.*

Proof. A regular Sturm-Liouville problem is of the form:

$$\begin{aligned} \frac{d}{dz} \left[m(z) \frac{dh}{dz} \right] + [\lambda w(z) - q(z)]h &= 0, \quad a < z < b \\ \alpha_0 h(a) + \alpha_1 h'(a) &= 0 \\ \beta_0 h(b) + \beta_1 h'(b) &= 0 \end{aligned} \quad (2.12)$$

where the boundary conditions are unmixed and the following conditions are met:

1. (a, b) is finite
2. m, m', q, w are all real and continuous on the interval $[a, b]$
3. $m > 0, w > 0$ on $[a, b]$.

In our case, $m(z) = 1, w(z) = \frac{1}{D(u_c)}$, and $q(z) = (p'(z))^2 - p''(z) + \frac{2u_c-1}{D(u_c)}$. Let us examine each condition in detail.

1. (a, b) is finite.

Without loss of generality, the interval of interest for us is $(0, L)$. We could choose any arbitrary finite end-point to this interval, $L < \infty$.

2. m, m', q, w are all real and continuous on the interval $[a, b]$

Since $m(z) = 1$, both m and m' are real and continuous on our arbitrary interval $(0, L)$.

$q(z) = (p'(z))^2 - p''(z) + \frac{2u_c-1}{D(u_c)}$. We need to show that this is real and continuous.

We start by rewriting $q(z)$ by expanding and combining all terms to see exactly what $q(z)$ is comprised of:

$$\begin{aligned}
q(z) &= (p'(z))^2 - p''(z) + \frac{2u_c - 1}{D(u_c)} \\
&= \frac{1}{D(u_c)^2} \left[(u'_c D'(u_c))^2 - v u'_c D'(u_c) + c u'_c D'(u_c) - cv + \frac{c^2}{4} + \frac{v^2}{4} \right] \\
&\quad - \frac{1}{D(u_c)} [D(u_c)(-u_c'^2 D''(u_c) - u_c'' D'(u_c))] \\
&\quad + \frac{1}{D(u_c)^2} \left[\frac{D'(u_c) u'_c v}{2} - \frac{D'(u_c) u'_c c}{2} - (D'(u_c) u'_c)^2 \right] + \frac{2u_c - 1}{D(u_c)} \\
&= \frac{1}{D(u_c)^2} \left[\frac{D'(u_c) u'_c}{2} (c - v) - \frac{cv}{2} + \frac{1}{4} (v^2 + c^2) \right] \\
&\quad + \frac{1}{D(u_c)} [2u_c - 1 + (u'_c)^2 D''(u_c) + u_c'' D'(u_c)]
\end{aligned}$$

We know that $u_c, u'_c, D(u_c)$ and $D'(u_c)$ are all real and continuous functions. We also know that $D(u_c) > 0 \forall u_c \geq 0$, so we do not have discontinuities in our $q(z)$ function. The only possible sources of discontinuity would then be $D''(u_c) = -\frac{D_2 n a^n u_c^{n-2} (a^n (n-1) - u_c^n (n+1))}{(a^n + u_c^n)^3}$, which obviously does not have any discontinuities, and u_c'' itself, which there is no reason to believe is not continuous.

$w(z) = \frac{1}{D(u_c)}$ is continuous everywhere on our interval.

3. $m > 0, w > 0$ on $[a, b]$

$m(z) = 1 > 0$ everywhere and $w(z) = \frac{1}{D(u_c)} > 0$ everywhere since $D(u_c) > 0$.

Thus, Equation (2.11) is a regular Sturm-Liouville problem. $\square$

There are many benefits to a regular Sturm-Liouville problem, including that all eigenvalues are real and can form an infinite ordered sequence $\lambda_1 < \lambda_2 < \lambda_3 < \dots$. We will use these properties in order to prove the following main result.

Theorem 2.3.1. *Traveling wave solutions to Equation (2.6) are stable, assuming that*

$$\frac{\partial^2}{\partial z^2} (D(u_c(z))) + 2u_c(z) \geq 0.$$

Proof. We must show that eigenvalues of Equation (2.11) are all positive. If these eigenvalues are positive, then $y(z, t) = g(z)e^{-\lambda t} \rightarrow 0$ as $t \rightarrow \infty$. This will prove that the traveling wave solution is asymptotically stable.

I follow along the guidelines set out by Kot (2001), and see that in order to have positive eigenvalues we need $q(z) > 0$ by the Raleigh quotient:

$$\lambda = \frac{\int_0^L [h'(z)^2 + q(z)h(z)^2] dz}{\int_0^L h(z)^2 dz} \quad (2.13)$$

$q(z) > 0$ is a sufficient, but not necessary condition. If we examine $q(z)$ in detail, in terms of signs:

$$\begin{aligned} q(z) &= (p'(z))^2 - p''(z) + \frac{2u_c - 1}{D(u_c)} \\ &= \underbrace{\frac{D'(u_c)u'_c}{2}[c-v]}_{\geq 0} \underbrace{- \frac{cv}{2}}_{\leq 0} + \underbrace{\frac{v^2}{4}}_{\geq 0} + \underbrace{\frac{c^2}{4}}_{\geq 0} + \underbrace{2D(u_c)u_c}_{\geq 0} \underbrace{- D(u_c)}_{\leq 0} \\ &\quad + \underbrace{(u'_c)^2}_{\geq 0} \underbrace{D''(u_c)}_{?} \underbrace{D(u_c)}_{\geq 0} + \underbrace{u''_c}_{?} \underbrace{D'(u_c)}_{\leq 0} \underbrace{D(u_c)}_{\geq 0} \end{aligned}$$

Note that these assumptions are based on the fact that our density-dependent diffusion $D(u)$ is positive and decreasing, as is our wave solution u_c .

According to our theorem assumptions, $\frac{\partial^2}{\partial z^2} (D(u_c(z))) + 2u_c(z) \geq 0$. which implies that $(u'_c)^2 D''(u_c) D(u_c) + u''_c D'(u_c) D(u_c) + 2D(u_c)u_c \geq 0$. Therefore, everything is nonnegative except $-\frac{cv}{2} - D(u_c)$. We use two other terms to balance this negativity out.

Under these assumptions, we can look for sufficient, but not necessary conditions for $q(z) > 0$.

$$\frac{v^2}{4} + \frac{c^2}{4} - \frac{cv}{2} - D(u_c) > 0$$

Recalling that $D(u) = D_1 - \frac{D2u^n}{a^n + u^n}$, we can see that the second part will end up being positive, so we just investigate $\frac{v^2}{4} + \frac{c^2}{4} - \frac{cv}{2} - D_1 > 0$. Let us look at what value

of c must be in order to have a positive $q(z)$.

$$v^2 + c^2 - 2cv > 4D_1$$

$$(c - v)^2 > 4D_1$$

$$(c - v) > \pm 2\sqrt{D_1}$$

$$c > \pm 2\sqrt{D_1} + v$$

We get the stability condition that $c > 2\sqrt{D_1} + v$, which we can translate back into our dimensionalized units to get $c > 2\sqrt{D_1 g} + \nu_i$. We use the positive root because we assume that $c > v$, meaning the wave speed is larger than the advection term.

Under this condition, all eigenvalues of the Sturm-Liouville problem are positive, which implies that the traveling wave solution is asymptotically stable.

□

2.4 Numerics

2.4.1 Parameter Estimation

In order to ensure that the model selection and traveling wave speed analysis is correct, it is necessary to compare numerical simulations with the experimental data, and observed wave speed with the minimum traveling wave speed generated in the wave speed analysis. Before beginning the parameter estimation, I will perform a sensitivity analysis to ensure that all parameters are indeed important to the model results. Once this is complete, a parameter optimization using MATLAB's `FMINSEARCH` is performed to determine the biologically relevant parameters that fit the experimental data best. Finally, using those optimized parameters, I will compare the simulated wave speed with the minimum wave speed (2.4) derived in the previous section.

The experimental data that is provided includes the density profile of cells on day

3 and the invasive radius of the cells measured from days 1 through 7 for both the U87WT and U87 Δ EGFR cell lines (Stein *et al.*, 2007a). As I do not have access to the true data, experimental results and the experimental model results were obtained via GRABIT (Doke, 2014), a MATLAB program which extracts data points from an image file. As mentioned previously, only the U87WT cell line is considered because it is necessary to show the model is effective even for the strongest migratory cells. All simulations are performed with the U87WT cell line in mind and are compared to the U87WT data.

The spatial domain for the numerical simulations is large to ensure that the boundaries do not interfere with the cell growth and diffusion process. The boundary conditions are $u(x, t) = 0$ when $x = 1$ cm, and reflective boundary conditions at $x = 0$. When considering the initial conditions, we recall that in the previous model, the tumor core cells are not modeled, and the invasive cells start out with a initial cell density of zero everywhere on the domain (Stein *et al.*, 2007a). For the density-dependent diffusion model, we must decide how to initialize the tumor cell counts. The initial cell count is not specified in the paper, but we can see that the initial core tumor radius is 210 μ m and the maximum cell density is $u_{\max} = 4.2 \times 10^8$ cells/cm³. Therefore, we assume that initial cell density is 95% of $u_{\max}$ for the initial core tumor radius of 210 μ m and zero elsewhere (Stein *et al.*, 2007a).

The governing equation (2.2) is solved numerically using MATLAB's PDEPE solver. A more original code was not used due to the complex nonlinearity of the diffusion term. The full code is available in Appendix C. I conducted a limited spatial study to determine the largest possible spatial step size which still produced accurate results. PDEPE determines the time-stepping scheme, so there was no need to conduct a time-step size test. The results of the spatial study are provided in Figure 2.3 and in Table 2.1. We use two different metrics to determine convergence: first visually in

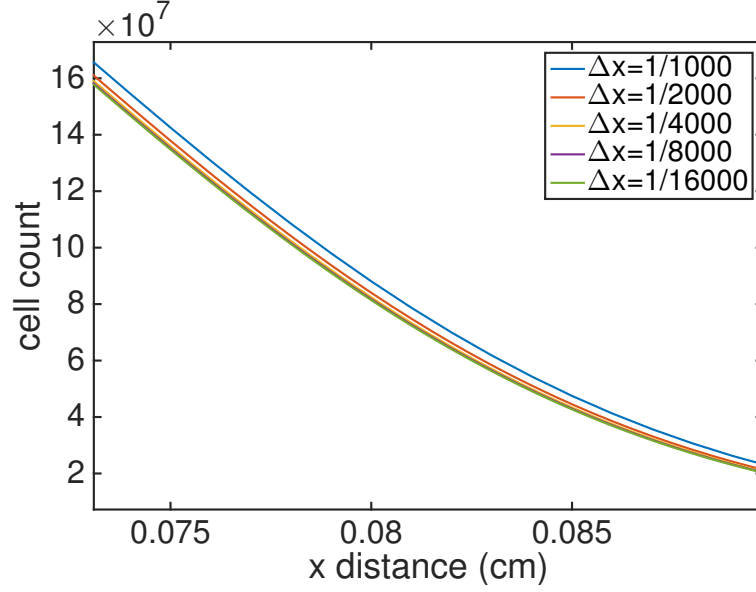


Figure 2.3: Spatial convergence study performed in PDEPE for various values of Δx . We see that the convergence is quick.

the Figure 2.3, secondly in comparing the errors generated by the error function in Equation (3.3).

Resolution (Δx)	Error Value	% decrease
$\frac{1}{1000}$	1.3842	
$\frac{1}{2000}$	1.3320	3.92
$\frac{1}{4000}$	1.3150	1.29
$\frac{1}{8000}$	1.3084	0.504
$\frac{1}{16000}$	1.3055	0.222

Table 2.1: Spatial convergence study in terms of error generated by Equation (3.3). As the size of Δx halves, the error also halves.

In Figure 2.3, the spatial convergence is very quick. Once $\Delta x = \frac{1}{4000}$, there is little difference in the corresponding solution curves. However, a visual cue is not

substantial enough to claim convergence, so I examine the errors generated by our solution to the data, using Equation (3.3) in Table 2.1. I can see that the decrease in Δx has a corresponding decrease in the error. I chose $\Delta x = \frac{1}{8000}$ since this generates a very good fit, and only differs by 0.22% from the solution obtained when halving Δx to $\frac{1}{16000}$. There is the expected cost associated with decreasing Δx , and the doubling of simulation time for a 0.22% decrease in error seemed fruitless.

In order to perform MATLAB's FMINSEARCH for optimization, we first must choose a function that we want to minimize. In this case we base our error function on the error function used in the original paper, which is based on the χ^2 error function (Stein *et al.*, 2007a). We could not use the exact function because it included information about the standard deviation in the measurements of cell densities, the value of which was not provided in the paper. We modified the error function to account for the experimental data that was made available. It uses relative errors for each point because the order of magnitude for the cell densities are approximately $10^7 - 10^8$, while the invasive radius measurements are on the order of 0.01 cm. The modified error function, like that of the original error function, takes into account both how fast the tumor is spreading, by measuring the invasive radius at each day, and also the density of cells by comparing the spatial profile of cells on day 3. The error function is given by:

$$\text{err} = \frac{1}{(N + M) - q - 1} \left[\sum_{t=1}^N \frac{|r_{\text{data}}(t) - r_{\text{simulation}}(t)|}{r_{\text{data}}(t)} + \sum_{i=1}^M \frac{|u_{\text{data}}(3, x_i) - u_{\text{simulation}}(3, x_i)|}{u_{\text{data}}(3, x_i)} \right], \quad (2.14)$$

where N is the total number of days for which there is invasive radius data, so $N = 7$, M is the total number of cell density data points at day 3, so $M = 17$, and q is the number of parameters being optimized which, in this case, $q = 6$. The

sums are broken into the comparison of invasive radii and the comparison of cell density profiles. The first sum compares the invasive radii of the experimental data, $r_{\text{data}}(t)$, and the simulation, $r_{\text{simulation}}(t)$. The second sum compares the cell density at day 3 at experimental data point x_i for the data, $u_{\text{data}}(3, x_i)$, and the simulation, $u_{\text{simulation}}(3, x_i)$.

The governing equation (2.2) with diffusion function (2.3) has six parameters that are unknown: D_1 , D_2 , a , n , g , and ν_i . There are estimated ranges for some of these parameters, which can be obtained from the experimental paper (Stein *et al.*, 2007a), but others have little biological measurements. In particular, the migratory diffusion (diffusion when the density u is small), D_1 , is estimated to be on the order of $10^{-4} \text{ cm}^2/\text{day}$. The growth rate $g \in (0, 1)/\text{day}$ and the taxis constant $\nu_i \in (0, 0.02) \text{ cm}/\text{day}$. However, for a and n , there is no precedent to compare with. We know that a^n represents the half-maximum of the saturation and that n determines the steepness of the curve which describes the diffusion for various cell densities. We perform a sensitivity study to determine if the model error is insensitive to any of the changing parameters, particularly those which we do not have biological information about. If we are able to determine that a parameter is insensitive, we will be able to fix its value and not include it as a free variable for parameter optimization, increasing the possible accuracy and decreasing the computational time.

The parameter sensitivity analysis was performed for the six parameters D_1 , D_2 , a , n , g , and ν_i such that each was in a physiologically relevant range. The set of base parameters chosen that result in a reasonable match to the data were:

$$\begin{aligned} D_1 &= 10^{-4} \text{ cm}^2/\text{day}, & D_2 &= 9.99 \times 10^{-5} \text{ cm}^2/\text{day}, & a &= 1 \text{ cells}/\text{cm}^2, \\ n &= 2, & g &= 1/\text{day}, & \nu_i &= 0.05 \text{ cm}/\text{day}. \end{aligned} \tag{2.15}$$

These base parameters are not chosen for their excellent fit to the data, rather they

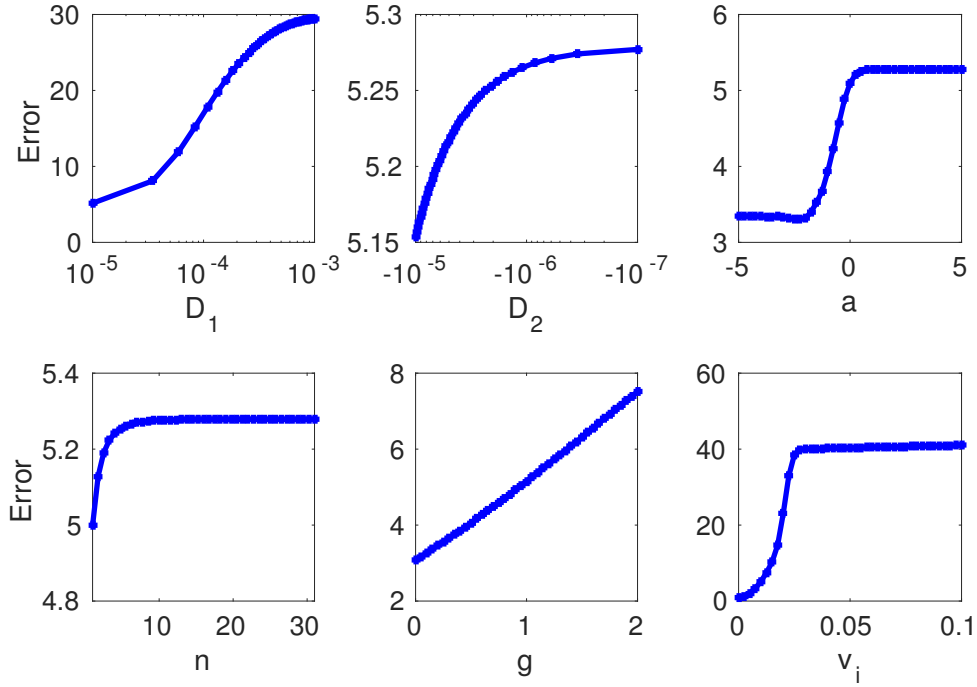


Figure 2.4: Results of a sensitivity analysis with base parameters (2.15) using error function (3.3) (vertical axis).

are chosen in order to have error that is not too large and to allow for variations to still remain in biologically relevant ranges. To test the sensitivity of one parameter, all the other parameters were held constant and the parameter in question was varied. The results of this sensitivity test is shown in Figure 2.4.

It is apparent that some parameters are much more sensitive than others, in particular, D_1 , g , and ν_i appear to generate the most sensitivity. The results of this sensitivity analysis inform us that all parameters are sensitive and we must take care when we perform our optimization.

The parameters are optimized via the MATLAB program FMINSEARCH (Lagarias *et al.*, 1998) by minimizing Equation (3.3). Various initial parameter guesses were used as input to ensure that the parameter space was adequately covered. Additionally,

for those parameters which had estimated ranges, we ensured that our parameters remain within those ranges. The optimized parameters found are:

$$\begin{aligned} D_1 &= 1.4247 \times 10^{-4}, \text{ cm}^2/\text{day}, & D_2 &= 1.4040 \times 10^{-4}, \text{ cm}^2/\text{day}, \\ a &= 0.0683 \text{ cells}/\text{cm}^3, & n &= 18.6547, \\ g &= 0.6855/\text{day}, & \nu_i &= 1.7843 \times 10^{-9} \text{ cm}/\text{day}. \end{aligned} \tag{2.16}$$

Though $D_1 - D_2$ is very close to 0, it is still the case that $D_1 > D_2$ as necessary. Having $D_1 - D_2$ close to zero implies that the diffusion is very different when the cell densities are low and high, which adds credence to our hypothesis. We also see that in our model, ν_i is very close to 0. We included this term, because the experimentalists claimed it was necessary to generate a good fit, arguing that there was some type of tactic term influencing the migratory cells (Stein *et al.*, 2007a). However, it appears that our density-dependent diffusion negates the need for this tactic term. To determine the comparable errors to the two population model simulations that use invasive cells as a separate population, we use GRABIT (Doke, 2014) to obtain the simulation data points from the experimental paper (Stein *et al.*, 2007a). We present the results of our simulation with optimized parameters compared to the experimental data and the model from the experimental paper in Figure 2.5.

In the top left panel of Figure 2.5, the cell density is plotted on the entire domain and it indicates that the model is successful in capturing the behavior of both the tumor core cells and the migratory cells. We want to ensure that the model does not just compare well with the migratory cell population area, but also with the proliferating core. It is apparent that our simulation matches qualitatively with both the core and expanding tail of cells. In the top right panel, the cell density is plotted on a smaller domain, containing the area where the bulk of the migratory cells exist, and further verifies that our model with one population is relatively as accurate as the

model which uses separate invasive cell population and proliferating cell population. The bottom panel displays the invasive radius of the migrating cells for the seven days that the experiment was performed. We can see that our fit is not as accurate as the simulations performed by the experimentalist, however, they are still reasonable. When we examine the fit as a whole, we can see that our simulations with one equation are indeed a qualitatively good fit. In fact, our total error from (3.3) is approximately one-half that of the model proposed by the experimentalists (0.19 compared to 0.38) (Stein *et al.*, 2007a). However, I will point out that our simulation has more points to fit, since we also fit the central tumor area, so the scaling is different (since $M + N$ is larger in our error calculation). If we examine just the points that Stein *et al.* (2007a) fits, our error is approximately 62% the error.

2.4.2 Numerical Minimum Wave Speed

Now that we have shown that our model can be utilized and fits the data quite well, we want to verify the analytic minimum wave speed (2.4) obtained in our analysis above. In order to do this, we compare with the wave speed observed in the simulations. However, as traveling wave speed analysis is meant to be on an infinite domain, there are several changes we must performed before we can measure the wave speed. Firstly, I rearrange the initial conditions. I use a point source at $x = 0$ of maximum cell density. Next, we increase the run-time of the simulation to 25 days, which ensures that the wave will no longer be ‘growing’ to carrying capacity, but rather will only be spreading. Similarly, we ignore the first few days of the simulation to ensure we are only capturing when the wave has already reached maximum cell density in the core. We also want to enlarge the spatial domain to more so mimic an infinite domain, however, limitations with PDEPE force us to keep the same domain. Additionally, this allows us to not worry about the boundary conditions affecting the wave shape and

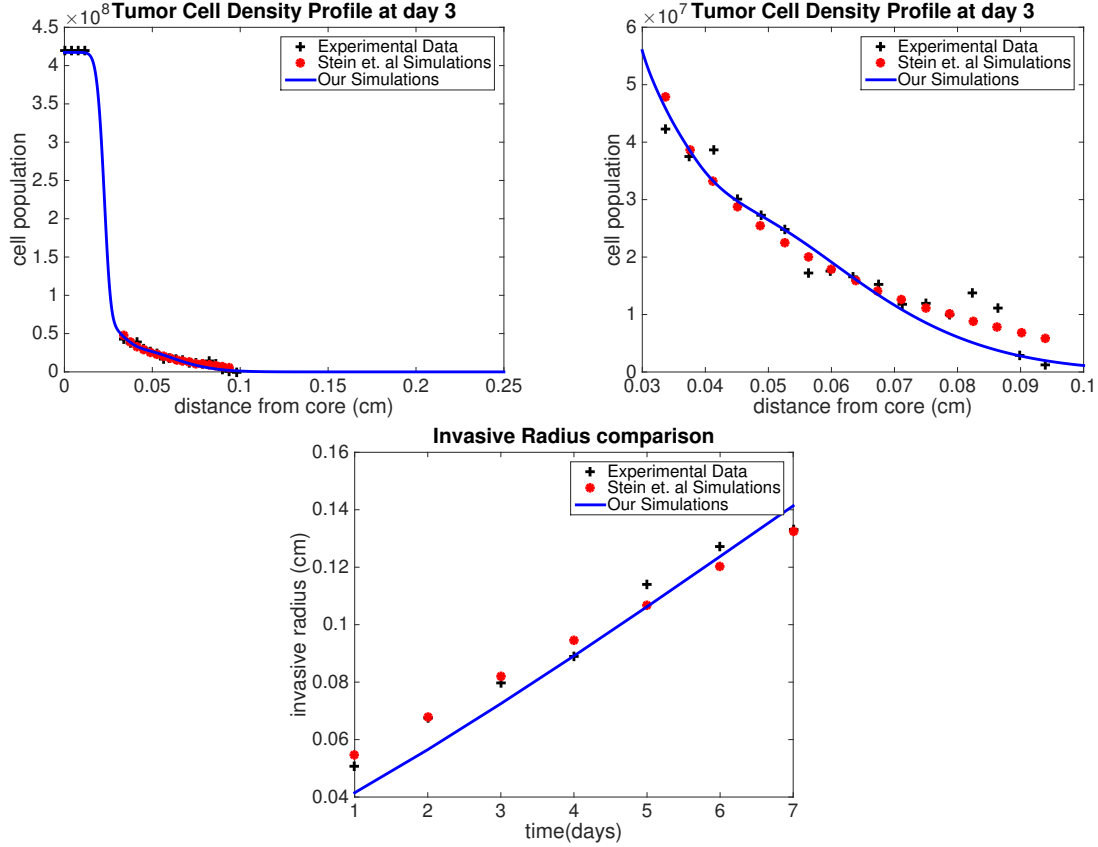


Figure 2.5: Numerical solution of the density-dependent diffusion glioblastoma model (2.2) with diffusion function (2.3) and optimized parameter values (2.16) compared to experimental data from Stein *et al.* (2007a) and their simulations. The density profile is from day 3 of the experimental data.

speed. To estimate the wave speed, we ignore the first half (in time) of the simulation so that the tumor core stably reaches maximum cell density, and then measure the x location where the cell density is last over 4.2×10^5 cells/cm³, approximately 1% of the maximum cell density. We measure this location throughout the time of the simulation and use the MATLAB's POLYFIT to estimate the slope of the curve for a linear fit.

The results of the wave speed simulation are shown in Figure 2.6. The leading

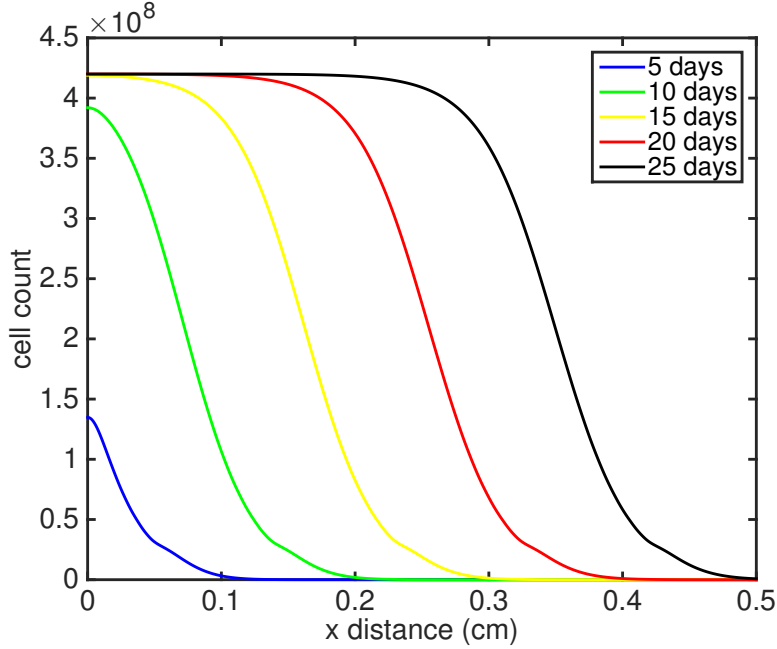


Figure 2.6: Numerical solution of the density-dependent diffusion glioblastoma model (2.2) with diffusion function (2.3) and optimized parameter values (2.16) for days 5, 10, 15, 20, and 25.

edge of the tumor maintains a constant shape and ultimately moves at a constant rate, once the wave speed shape is built. This is why we only use the latter portion of the simulation in order to estimate the traveling wave speed. Using the optimized parameters (2.16), the minimum wave speed (2.4) is $k_{\min} \approx 0.01977$ cm/day and the simulated wave speed $k = 0.01921$ cm/day. We can see that the simulated wave speed is very close to the theoretical wave speed, as expected.

Now that we have found our traveling wave profile, we can test the assumption that was made in Theorem 2.3.1 numerically. The assumption was:

$$\frac{\partial^2}{\partial z^2} (D(u_c(z))) + 2u_c(z) \geq 0 \quad (2.17)$$

In order to determine whether our condition is satisfied, we look towards the numerical solutions. Specifically, we use our numerical wave speed solution from

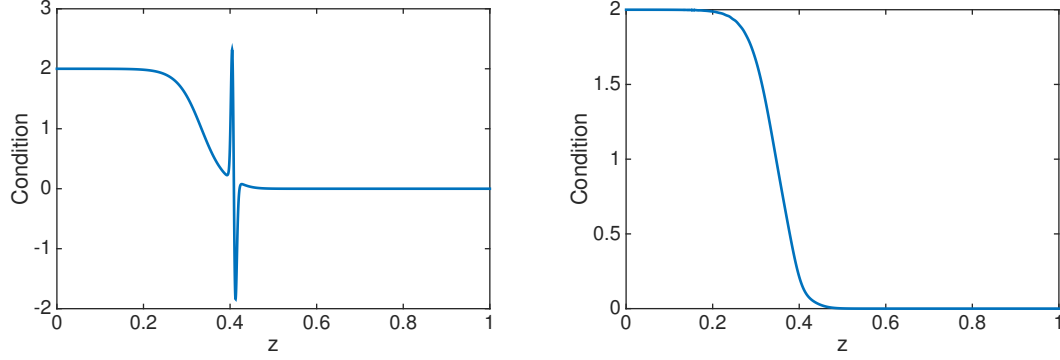


Figure 2.7: Examining the condition, given by Equation (2.17) using the wave speed solution of Figure 2.6 at 25 days as u_c . On the left, parameters given by (2.16) and on the right, parameters given by (2.16) with $n = 2, a = 0.25$.

Figure 2.6, at 25s as our u_c .

We display the condition, in Equation (2.17) in Figure 2.7. On the left, we have the condition using parameters given by (2.16). We can see that for our chosen parameter values, the condition does not hold. However, if we examine other sets of parameters, it is possible to have this condition hold and be guaranteed stability of wave solutions. On the right panel of Figure 2.7, we examine the case for parameters given by (2.16) with $n = 2$ and $a = 0.25$ in which the condition does hold.

Chapter 3

2 POPULATION MODEL OF *IN VITRO* SPHEROID GLIOBLASTOMA GROWTH

3.1 Introduction

In Chapter 2, I discussed the influential experimental and modeling paper which began the discussion of the ‘Go or Grow’ hypothesis. Stein *et al.* (2007a) discovered from his data that in order to model *in vitro* GBM-like growth, subpopulations of proliferating cells and migrating cells must be modeled separately. Our previous work concluded that a one-Equation model with density-dependent diffusion was able to fit the data better and was biologically justified.

In this Chapter, we consider a 2-population model of migratory cells and proliferating cells. We provide a justification for the proposed model, analyze the minimum traveling wave speed, and fit this model to the experimental data provided in Stein *et al.* (2007a). We compare our fit not only to the experimental data, but to the ad-hoc model proposed by Stein *et al.* (2007a), Equation (2.1), and to our 1-D density-dependent diffusion model, Equation (2.2).

3.1.1 Early Models

Early models of GBM invasion were inspired by reaction-diffusion Equations. Tracqui *et al.* (1995) formed one of the earliest models of GBM growth. Swanson has continued using reaction-diffusion Equations with great success. Swanson *et al.* discovered that the simple reaction-diffusion model was able to model the growth, diffusion, and treatment of GBM, noting that there are very differing dynamics when

low D and high ρ versus high D and low ρ , although these may show identical visible tumors at the same time point (Swanson *et al.*, 2003). Building upon this idea, they discovered a method using the two most common types of MR images to estimate the ‘invasivity ratio’ given by $\frac{D}{\rho}$ and from this be able to classify the tumors and their expected survival (Swanson *et al.*, 2008; Baldock *et al.*, 2014).

3.1.2 Go or Grow?

While this work was being performed, other researchers decided that the model was too simple to accurately describe the dynamics of GBM. Based on the Stein *et al.* (2007a) paper, researchers were convinced that in order to accurately describe the behavior of GBM, they needed to separate the tumor population into a migratory subpopulation and a proliferating subpopulation. Hatzikirou *et al.* (2010) coined the term ‘Go or Grow’ to represent the hypothesis that the cells are either proliferating or migrating, but not both. They argued that the transformation from the proliferating phenotype to the migrating phenotype was not solely due to mutation, but driven by hypoxia, which was corroborated experimentally (Godlewski *et al.*, 2010). Other researchers have posited that a variety of mechanisms might trigger the phenotypic switch, from chemotaxis to haptotaxis to self-adhesion (Kim *et al.*, 2009). Others have investigated how changing aspects of the switching rates from proliferating to migrating and migrating to proliferating affected the dynamics of the tumor growth (Gerlee and Nelder, 2012). Pham *et al.* investigated a basic model which only feature diffusion and phenotypic switching (for the migrating cells) and growth and phenotypic switching (for the proliferating cells) and were able to observe dynamics such as fingering (Pham *et al.*, 2012). While these models exhibit behavior consistent with the GBM invasion, experimentalists have been unable to directly prove the ‘Go or Grow’ hypothesis (Garay *et al.*, 2013).

Although the previously cited works have investigated the truth of the so-called ‘Go or Grow’ hypothesis, current researchers often use the hypothesis in their works. Eikenberry *et al.* (2009) used two separate sub-populations of tumors in their simulations of growth, diffusion, and treatment of GBM in mathematical models. Kostelich *et al.* (2011) used two subpopulations along with extracellular matrix to forecast glioma growth using data assimilation techniques. Martínez-González *et al.* (2012) uses the terms ‘normoxic’ and ‘hypoxic’ to signify the differing dynamics of proliferative and migratory cells, respectively, while adding necrotic cells and based their switching rates on the level of oxygen available. Tanaka *et al.* (2009) investigated a similar model which had migrating, proliferating and necrotic regions depending on the tumor volume. Chauviere *et al.* (2009) used the two subpopulations to characterize cell migration through the extracellular matrix. Recently Saut *et al.* (2014) used the two populations to characterize how GBM would respond to anti-angiogenesis drugs. Böttger *et al.* (2012) determined how invasive, measured in invasion speed and width of the infiltration zone, glioma can be for the ‘Go or Grow’ hypothesis versus the ‘Go and Grow’ hypothesis, showing much richer and complex dynamics with the former.

3.2 Mathematical Model

Our model is based on two subpopulations which exhibit vastly differing behavior: proliferating cells which mainly grow, and migrating cells which mainly migrate. There is, of course, phenotypic switching from subpopulation to subpopulation, as has been the norm in many of the previously mentioned models.

Our model concerns migratory cells ($M(x, t)$), proliferating cells ($P(x, t)$), and the

total number of cells ($T(x, t) = M(x, t) + P(x, t)$):

$$\frac{\partial M}{\partial t} = \underbrace{D_1 \nabla^2 M}_{\text{diffusion}} + \underbrace{\epsilon k \frac{T^n}{T^n + K_M^n} P}_{\text{switch from proliferating to migratory}} - \underbrace{k \frac{K_P^n}{T^n + K_P^n} M}_{\text{switch from migratory to proliferating}} - \underbrace{\mu M}_{\text{death}} \quad (3.1a)$$

$$\frac{\partial P}{\partial t} = \underbrace{g \left(1 - \frac{T}{T_{\max}} \right) P}_{\text{growth}} - \underbrace{\epsilon k \frac{T^n}{T^n + K_M^n} P}_{\text{switch from proliferating to migrating}} + \underbrace{k \frac{K_P^n}{T^n + K_P^n} M}_{\text{switch from migrating to proliferating}} \quad (3.1b)$$

In this model, we are assuming that the migratory cells are governed by diffusion and changing to and from proliferating cell populations as well as a death term. As for the proliferating cells, we assume they are growing logistically and switch to and from migratory cells, but do not diffuse. This model exhibits the ‘Go or Grow’ hypothesis in which cells are either growing (proliferating) or going (migrating). According to the premise from Stein *et al.* (2007a), this model should more accurately fit their experimental data than a reaction-diffusion Equation.

As in the previous model, the boundary conditions are $M(x, t) = 0 = P(x, t)$ when $x = \pm 1$ cm, which states that there can be no tumor cells at the boundaries. The initial cell density of the proliferating core cells is 95% of $u_{\max}$ for the initial core tumor radius of 210 μm and zero elsewhere.

3.3 Numerical Method

The numerics for this section of work was implemented in MATLAB. Due to the prohibitive overhead costs of using PDEPE, the code for the resulting numerics was written by me, featuring Crank-Nicolson for the diffusion term and first-order finite differencing for the remaining terms, including an advective term, possibly some taxis term as in Equation (2.2). This was also implemented to have the freedom to include a diffusion term in the proliferating population, if necessary.

The Crank-Nicholson method is a stable finite difference method for solving PDE's. It is implemented by using the trapezoidal rule, which gives second-order convergence in time (as opposed to Euler's method which would only give first-order convergence in time). For an Equation $\frac{\partial u}{\partial t} = F\left(u, x, t, \frac{\partial u}{\partial x}, \frac{\partial^2 u}{\partial x^2}\right)$ the Crank-Nicolson method is written as:

$$\frac{u_i^{n+1} - u_i^n}{\Delta t} = \frac{1}{2} \left[F_i^{n+1} \left(u, x, t, \frac{\partial u}{\partial x}, \frac{\partial^2 u}{\partial x^2} \right) + F_i^n \left(u, x, t, \frac{\partial u}{\partial x}, \frac{\partial^2 u}{\partial x^2} \right) \right] \quad (3.2)$$

where the subscript denotes spatial coordinates and the superscript denotes the time steps.

3.3.1 Derivation of Code

We can now discretize the derivatives using centered finite differencing in order to implement our Crank-Nicolson scheme. We recall the well-known approximations for first and second derivatives here:

$$\frac{\partial u}{\partial t} = \frac{u^{j+1} - u^j}{\Delta t} \quad \frac{\partial u}{\partial x} = \frac{u_{i+1} - u_{i-1}}{2\Delta x} \quad \frac{\partial^2 u}{\partial x^2} = \frac{u_{i+1} - 2u_i + u_{i-1}}{(\Delta x)^2}$$

And now we plug the terms into the Crank-Nicolson method:

$$\begin{aligned} \frac{u_i^{j+1} - u_i^j}{\Delta t} &= \frac{1}{2} \left[F_i^{j+1} \left(u, x, t, \frac{\partial u}{\partial x}, \frac{\partial^2 u}{\partial x^2} \right) + F_i^j \left(u, x, t, \frac{\partial u}{\partial x}, \frac{\partial^2 u}{\partial x^2} \right) \right] \\ \frac{u_i^{j+1} - u_i^j}{\Delta t} &= \frac{1}{2} \left[D \frac{\partial^2 u^{j+1}}{\partial x^2} + D \frac{\partial^2 u^j}{\partial x^2} \right] \\ \frac{u_i^{j+1} - u_i^j}{\Delta t} &= \frac{1}{2} \left[D \left(\frac{u_{i+1}^{j+1} - 2u_i^{j+1} + u_{i-1}^{j+1}}{(\Delta x)^2} \right) + D \left(\frac{u_{i+1}^j - 2u_i^j + u_{i-1}^j}{(\Delta x)^2} \right) \right] \\ u_i^{j+1} &= u_i^j + \frac{D\Delta t}{2\Delta x^2} \left[u_{i+1}^{j+1} - 2u_i^{j+1} + u_{i-1}^{j+1} + u_{i+1}^j - 2u_i^j + u_{i-1}^j \right] \end{aligned}$$

In order to see more clearly, we set $c = \frac{\Delta t}{2(\Delta x)^2}$ and separate into the $j, j + 1$ time points:

$$u_i^{j+1} - cD \left(u_{i+1}^{j+1} - 2u_i^{j+1} + u_{i-1}^{j+1} \right) = u_i^j + cD \left(u_{i+1}^j - 2u_i^j + u_{i-1}^j \right)$$

Now we can organize by spatial points to get a tridiagonal matrix:

$$u_{i+1}^{j+1} [-cD] + u_i^{j+1} [1 + 2cD] + u_{i-1}^{j+1} [-cD] = u_{i+1}^j [cD] + u_i^j [1 - 2cD] + u_{i-1}^j [cD]$$

This can be solved easily as it is a tridiagonal matrix.

In order to model the full Equation, we want there to be a growth terms, mutation terms, and the allowance for a chemotactic term as well, so we add these terms so our final Equation is:

$$\frac{\partial u}{\partial t} = D \nabla^2 u + \epsilon k \frac{(u + v)^n}{(u + v)^n + K_M^n} u - k \frac{K_P^n}{(u + v)^n + K_P^n} u - \mu M + gu \left(1 - \frac{u}{u_{\max}} \right) - \nu_i \nabla \cdot u$$

which effects our computational work by first having adding in the extra terms. We know that for consistency and stability we need to express the chemotactic term using forward differencing. We introduce $k = \frac{\Delta t}{2\Delta x}$, and our final solve becomes:

$$\begin{aligned} & u_{i+1}^{j+1} [-cD] + u_i^{j+1} [1 + 2cD] + u_{i-1}^{j+1} [-cD] \\ & = u_{i+1}^j [cD - k\nu_i] + u_i^j [1 - 2cD] + u_{i-1}^j [cD + k\nu_i] + \Delta t f(u) \end{aligned}$$

where $f(u)$ is all the remaining terms which do not contain a derivative (growth, mutation, and death terms).

Our code is set up to handle a system of Equations (since we have proliferating and migrating subpopulations) and is set up so each of the subpopulations can specify the value of all parameters. For example, if we restrict the proliferating subpopulation to be unable to diffuse ($D = 0$) this can easily be done in the code. The full code is available in Appendix D.

3.3.2 Comparison of codes

In order to ensure that the code is accurate, I ran a comparison using MATLAB's PDEPE and our Crank-Nicolson code. The following simulations use the initial condition listed above for which the cell density of the proliferating core cells is 95% of $u_{\max}$ for the initial core tumor radius of 210 μm and zero elsewhere. The domain for PDEPE is $x \in (0, 1)$, and the boundary conditions are reflective at $x = 0$ and 0 far away from the tumor. For the Crank-Nicolson code, we run on a full domain $x \in (-1, 1)$ with boundary conditions of 0 at the ends of the domain.

We compare several sets of parameters to ensure that we did not luckily choose a parameter set which happened to match both codes. Figure 3.1 shows examples of comparisons for various parameter sets modeled with both PDEPE and the Crank-Nicolson code, plotted on top of each other at the final time of day 7. It is apparent that the Crank-Nicolson solver and the PDEPE solve share a high amount of agreement in every one of the cases for which we have tested. One thing that differs between the two simulations is the appearance of some numerical oscillations only present in the PDEPE simulations, as seen in the fourth panel of Figure 3.1.

Why do we choose to use our Crank-Nicolson code? Ultimately, we notice that there are some numerical artifacts that arise from MATLAB's PDEPE. Figure 3.2 shows the numerical oscillations at the border of the migratory and proliferating cells in PDEPE versus the Crank-Nicolson code. We can minimize the oscillations but we would require a much finer resolution. Even choosing $\Delta x = \frac{1}{128000}$ does not completely clear up the numerical instabilities that arise. For our purposes, running the Crank-Nicolson code is much faster and does not contain these numerical artifacts. Although they appear to not affect our simulation results too much, we cannot be sure that this artifact will not affect the outcome in every type of parameter set. To

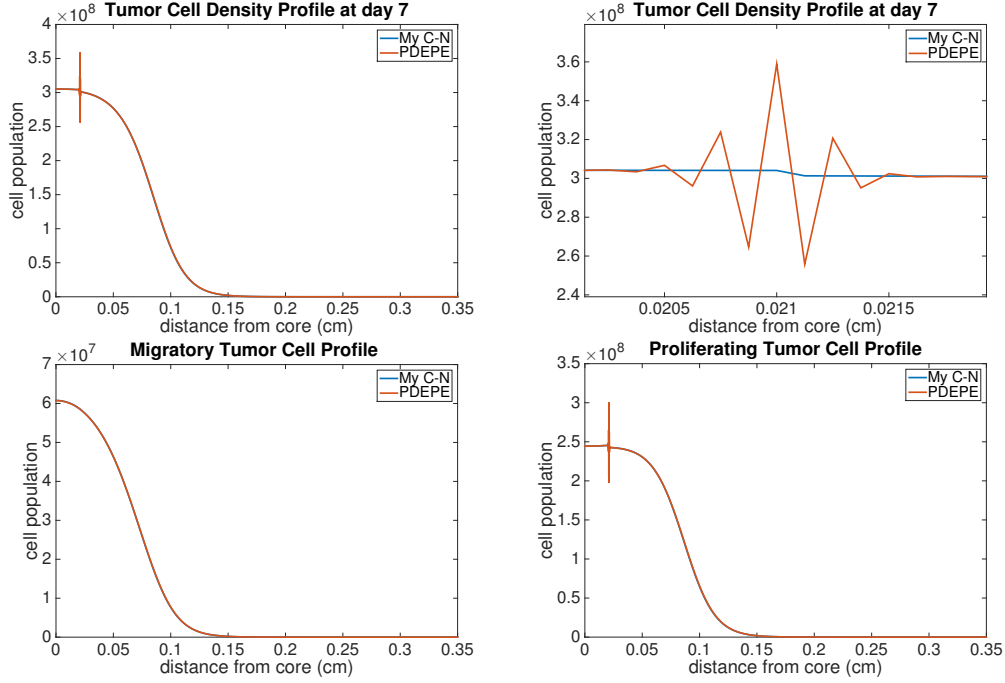


Figure 3.1: Comparisons of MATLAB's PDEPE and the Crank-Nicolson solver. We see agreement between the two codes, but some issues at the edge where the proliferating and migrating cells seem to be switching. In the upper left-hand corner solutions are compared on day 7. The upper right-hand corner shows a zoomed-in portion where we can see that PDEPE generates oscillatory behavior, while our code does not. The bottom left-hand corner shows the migratory cell profile, which does not have oscillatory behavior. The bottom right-hand corner shows the proliferating cell population, which is where the oscillations occur.

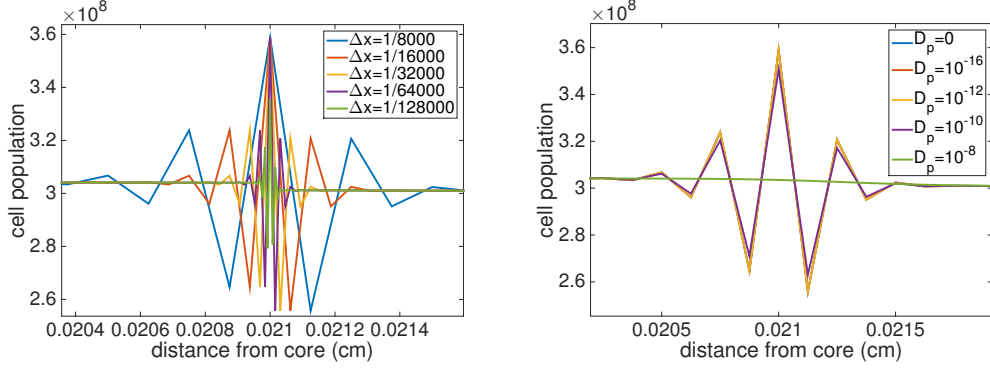


Figure 3.2: We determine the cause of numerical oscillations using PDEPE, by decreasing Δx (right) and introducing diffusion to proliferating population (left). As we decrease the value of Δx in the PDEPE simulations, the numerical oscillations decrease slightly, although they never disappear. When we introduce a small diffusion coefficient into proliferating population, we are able to eliminate the oscillations.

be safe, we only use the Crank-Nicolson code.

As PDEPE is a black-box code, we are unable to exactly determine why these artifacts arise. However, my hypothesis is that since PDEPE is supposed to solve *PDE's* and our proliferating cell population is, in fact, an ODE, this might be where the issue arises. It appears to be in effect at the boundary of the proliferating cells and the migrating cells. If we examine the lower panel of Figure 3.1, it is apparent that the perturbation is only in the proliferating cell population, not the migrating cell populaion. In order to test this hypothesis, I introduce a very small diffusion constant for the proliferating population. I am not able to fully eliminate the oscillations until I allow this diffusion constant to be approximately $D = 10^{-8}$, which slightly changes the dynamics of the simulation. Therefore, to simulate our 2 population model (Equations 3.1), we use our code instead of PDEPE.

3.4 Numerical Results

3.4.1 Sensitivity Analysis

We begin our numerical exploration by determining how sensitive the solutions are to perturbations in the parameters. The reason to investigate this is that if some parameters are insensitive, we may eliminate them from our parameter estimation. Our parameter estimation is performed by varying the nominal value of each parameter from 1/100* nominal value to 100* nominal value. The nominal values were chosen to have a reasonable fit to the data and are listed below:

$$D = 3.5 * 10^{-4} \text{ cm}^2/\text{day}, g = 1/\text{day},$$

$$K_m = 1 \times T_{\max}, K_p = 0.1 \times T_{\max},$$

$$k = 1, \epsilon = 0.5,$$

$$\mu = 0.01\text{day}$$

From these nominal values, we vary them and calculate the error generated by Equation (3.3). This allows us to see that there is change, although we do not characterize exactly what that change entails.

In Figure 3.3, we can see that all parameters appear to be sensitive to solutions. Although all parameters appear to be sensitive, some are far more sensitive than others, such as g, ϵ , and D . However, we do notice that unless we have large values of μ , on the order of approximately 0.1 or larger, the solutions remain insensitive for smaller values of μ .

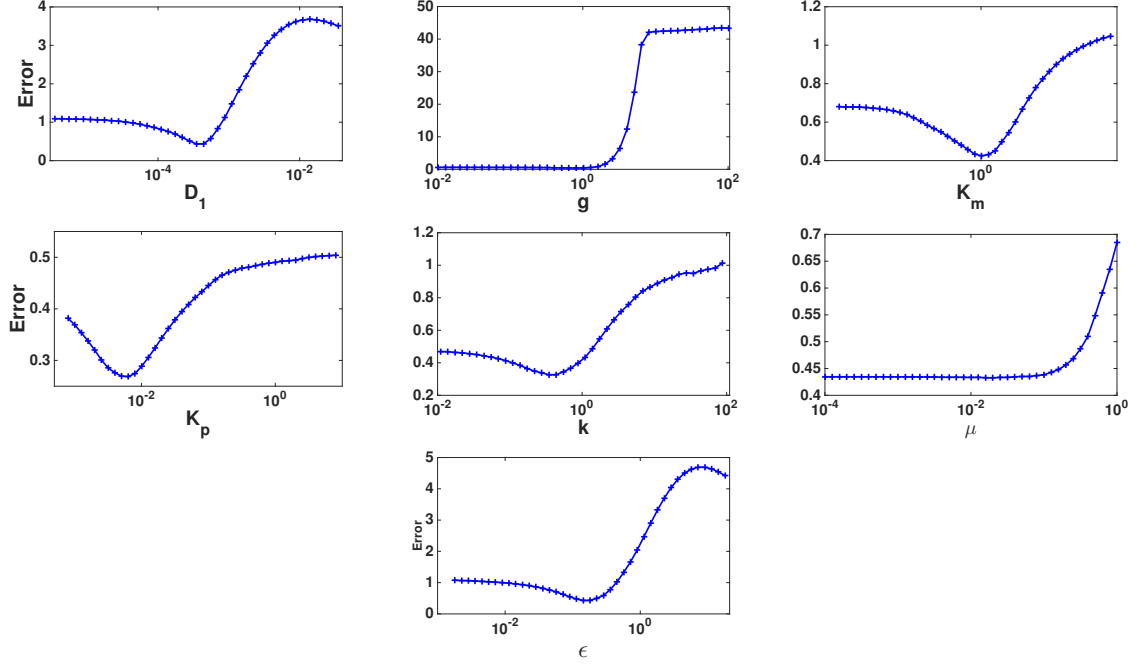


Figure 3.3: Sensitivity analysis for all parameters varying from 1/100 nominal value to 100*nominal value. All parameters appear to be sensitive.

3.4.2 Parameter Estimation

Now that we have ensured that all parameters are sensitive, we begin our parameter estimation. The error function that we use for the parameter estimation is:

$$\text{err} = \frac{1}{(N + M) - q - 1} \left[\sum_{t=1}^N \frac{|r_{\text{data}}(t) - r_{\text{simulation}}(t)|}{r_{\text{data}}(t)} + \sum_{i=1}^M \frac{|u_{\text{data}}(3, x_i) - u_{\text{simulation}}(3, x_i)|}{u_{\text{data}}(3, x_i)} \right], \quad (3.3)$$

where N is the number of data points available on the day 3 cell density profile, M is the number of invasive radius measurements, and q is the number of parameters we optimize over. This is the same error function that was given in Chapter 2.

In order to obtain the best-fit for parameter estimations, we minimize the error

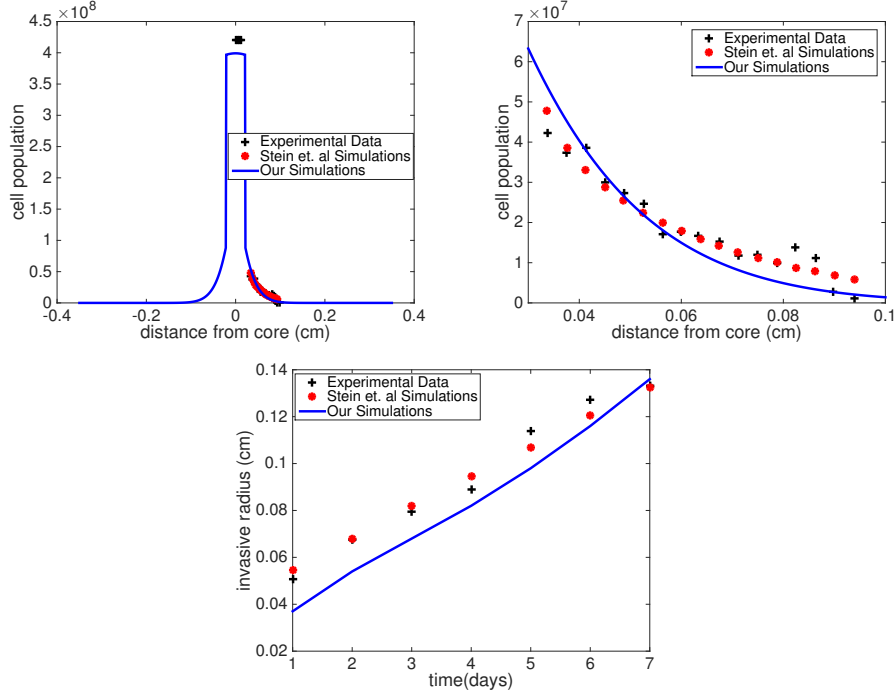


Figure 3.4: Numerical solution of 2-Equation GBM model with optimized parameter values compared to experimental data and simulations from Stein *et al.* (2007a)

given by Equation (3.3) using the FMINSEARCH algorithm in MATLAB. In order to lead credence to our solutions, we make sure to run the parameter estimation from a variety of input parameter guesses. Our best fit is given in Figure 3.4

The parameters that generate this fit are:

$$D = 3.512 \times 10^{-4} \text{ cm}^2/\text{day}, \quad g = 1.020/\text{day},$$

$$k = 1.091, \quad \mu = 0.01228/\text{day},$$

$$K_M = 0.8120 \times T_{\max}, \quad K_P = 0.006282 \times T_{\max},$$

$$\epsilon = 0.1794$$

We can see that in this instance, we do not have as good of a fit as our one-Equation density-dependent diffusion model. In fact, our error is on par with that of the model

proposed in Stein *et al.* (2007a). Although our model (Equation 3.1) is built upon biological understanding, as opposed to the rather ad-hoc Stein *et al.* (2007a), we still do not generate as good of a fit. We do note, however, that the best-fit generated by a generic reaction-diffusion Equations is still much, much higher.

3.4.3 Comparison of Multiple Methods

In this next section, we compare all methods used so far in their ability to fit the experimental data, as well as introduce two more models in necessary for examining the best fit for the model. Is the ‘Go or Grow’ hypothesis really necessary or accurate?

The whole argument from Stein *et al.* (2007a) was that the basic reaction-diffusion Equation was unable to fit the data and so the populations had to be separated into migrating or proliferating subpopulations. However, the fit for the reaction-diffusion Equation was never actually shown in the Stein *et al.* (2007a) paper, so we introduce the reaction-diffusion Equation for fitting here:

$$\frac{\partial u}{\partial t} = \underbrace{D\nabla^2 u}_{\text{diffusion}} + \underbrace{gu \left(1 - \frac{u}{u_{\max}}\right)}_{\text{growth}} \quad (3.4)$$

In order to simulate this Equation, we use the numerical method introduced in Section 3.3.

Since our generic 2 population model did not generate such a good fit, we merge the ideas of the ‘Go or Grow’ hypothesis with our density-dependent diffusion. We also want to look at whether it is just the density-dependent diffusion that generated such a wonderful fit. Therefore, we replace the diffusion constant in Equation (3.1) with the density-dependent diffusion function in Equation (2.3) in order to get a

2-Equation, density-dependent diffusion model given by:

$$\begin{aligned}\frac{\partial M}{\partial t} &= \nabla \cdot \left(\left(D_1 - \frac{D_2(M)^n}{a^n + (M)^n} \right) \nabla M \right) + \epsilon k \frac{T}{T + K_M} P - k \frac{K_P}{T + K_P} M - \mu M \\ \frac{\partial P}{\partial t} &= g \left(1 - \frac{T}{T_{\max}} \right) P - \epsilon k \frac{T}{T + K_M} P + k \frac{K_P}{T + K_P} M\end{aligned}\tag{3.5}$$

In order to simulate the model given by Equations (3.5), I use PDEPE, since the density-dependent diffusion term complicates the numerical method described in Section 3.3.

In order to compare the efficacy of all models, I present not only the error results for the Stein *et al.* (2007a) mathematical model and the two-population model proposed here, but also the Stepien *et al.* (2015) density-dependent diffusion model as well as the basic reaction-diffusion Equation.

Table 3.1 allows us to see how effective the various models are to fitting the experimental data in three ways: firstly a weighted error that penalizes additional parameters given by Equation (3.3), secondly a raw error that is just the summation of the differences between data and experiment, and finally the raw error divided by the number of data points fitted (essentially the Equation (3.3) but without the parameter penalty). This last measure is necessary because the Stein *et al.* (2007a) model only fits to the migratory data, not including the proliferating data in the center (it is fitted to four less points). In any case, the one Equation density-dependent diffusion model generates the least error from the experimental data. We note that, as expected, the reaction-diffusion Equation (3.4) generates the largest error. Coming in at approximately half the error of the reaction-diffusion Equation is the ad-hoc model proposed by Stein *et al.* (2007a), given by Equation (2.1). The one-Equation density-dependent diffusion Equation (2.2) has one quarter of the error of the reaction-diffusion Equation. Finally, our 2-Equation models with and without density-dependent diffusion have approximately one third the error of the reaction

Model Type	Error (Weighted)	Error (Raw)	Error (per data point)
Reaction-Diffusion Equation (3.4)	0.7816	16.4140	0.6079
Stein <i>et al.</i> (2007a) Equation (2.1)	0.3805	7.229	0.3143
Stepien <i>et al.</i> (2015) Equation (2.2)	0.1918	4.0279	0.1492
2-Equation Model Equation (3.1)	0.2782	5.5630	0.2060
2-Equation Model with DDD Equation (3.5)	0.3170	5.3888	0.2000

Table 3.1: Error generated by Equation (3.3) for various models. Error is given weighted by the number of parameters as well as unweighted (raw) and the error per data point (raw error/# of data points).

diffusion Equation.

In Table 3.2, we are only examining the migratory cell populations, instead of both proliferating and migrating cells. Again, we examine both the weighted error given by Equation (3.3) as well as the raw error. The results are very similar to the previous table. If we are just interested in examining the migrating cell population, we can still see that our one-dimensional density-dependent diffusion Equation is still the best fit (whether weighted or raw). Our proposed 2-Equation models have similar error as to each other, and generate less error than the Stein *et al.* (2007a) model, although when introducing the parameter penalization, the error is not much better.

Model Type	Error (Weighted)	Error (Raw)
Reaction-Diffusion Equation (3.4)	0.8975	15.2573
Stein <i>et al.</i> (2007a) Equation (2.1)	0.3805	7.229
Stepien <i>et al.</i> (2015) Equation (2.2)	0.2353	4.0005
2-Equation Model Equation (3.1)	0.3334	5.3347
2-Equation Model with DDD Equation (3.5)	0.3035	5.1595

Table 3.2: Error generated by Equation (3.3) for various models over the migratory cell population only. Error is given weighted by the number of parameters as well as unweighted (raw).

3.5 Wave Speed

3.5.1 Analysis

As with our previous model, we would like to determine the traveling wave speed. Through some analysis using methods similar to Chapter 2, a range of minimum wave speed is estimated:

$$c_{m1} = g\sqrt{\frac{D}{g+k+\mu}} \leq c_m \leq (g+k)\sqrt{\frac{D}{g+2k+\mu}} = c_{m2} \quad (3.6)$$

3.5.2 Numerics

As in Chapter 2, we are interested in whether we can observe the minimum wave speed in our simulations. We found a range of minimum wave speeds, contingent

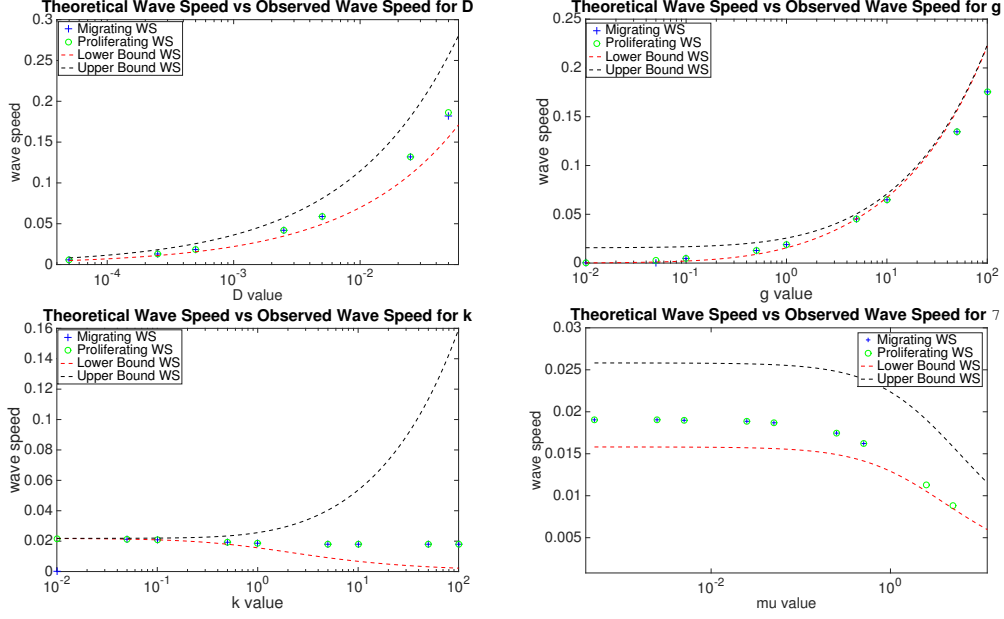


Figure 3.5: Wave speed bounds given by Equation (3.6), the lower bound in black, the upper bound in red. The circles represent the migrating wave speed and the plusses are the proliferating wave speed.

on what the fraction of proliferating cells to migrating cells, in Equation (3.6). The results of the numerical wave speeds are presented in Figure 3.5.

Figure 3.5 has the lower bound of minimum wave speed, $c_{m1} = g\sqrt{\frac{D}{g+k+\mu}}$ given in the red dashed line and an upper bound of the minimum wave speed $c_{m2} = (g+k)\sqrt{\frac{D}{g+2k+\mu}}$ given in the black dashed line. The calculated numerical wave speed for the migrating population is given by blue plusses, and the numerical wave speed for the proliferating wave speed is given by green circles. Our results indicate that the true minimum wave speed does lie between the ranges given by Equation (3.6). There are a few that fall outside the range of the minimal theoretical wave speed range, for very large g values. Whether this is an artifact of numerics remains to be seen.

Chapter 4

IN VIVO MURINE MODEL OF GLIOBLASTOMA GROWTH

4.1 Introduction

Once the tumor reaches a certain size, it is no longer able to function independently. At this time, tumor cells begin to interact with their surrounding environment more. Specifically, cancer cells begin emitting pro-angiogenic factors which encourage blood vessels to grow towards them, facilitating further growth and development. As the tumor grows larger, it begins to compete with the surrounding healthy tissue cells and its own tumor cells. At this point, the environment and competition for resources available begin to influence the growth of the cancer. In order to begin to accurately model this phenomena, we need to consider *in vivo* data and modeling.

In this chapter, we consider a murine model for glioblastoma growth and diffusion. Five mice were injected cranially with GL261-Luc2 cell line and imaged over a series of time points. We then converted the MR images into a computational domain upon which to run simulations. We observed a large variance between final tumor sizes and use a simple model to determine whether or not we can easily explain this discrepancy.

4.1.1 GBM

Glioblastoma multiforme (GBM) is an aggressive form of brain cancer with mean survival time following diagnosis ranging from about 4 months with supportive care only (Schapira, 2007) to 15 months with standard treatment (Norden and Wen, 2006; Johnson and O'Neill, 2012). GBM cells have both proliferative and migratory characteristics. Although surgical resection may remove the bulk of the original tumor,

it has long been recognized that GBM cells migrate away from the main mass and penetrate the brain parenchyma (Scherer, 1940), forming recurrent tumors adjacent to and/or extending from the previous surgical resection bed.

It is difficult to capture both the invasive behavior as well as the proliferating core of the tumor in mathematical models. Compounding this issue is the fact that only high-density proliferative cell regions that are usually considered to be analogous to areas of increased contrast (i.e., gadolinium) signal are visible on magnetic resonance imaging (MRI). Further, areas of increased signal from gadolinium may also indicate edema surrounding the tumor or tumor necrosis.

GBM has been the subject of many previous mathematical modeling attempts (more details are provided below). One early effort used a reaction–diffusion model to capture the gross behavior of GBM tumors as well as the effects of treatment (Tracqui *et al.*, 1995). Work by Hermann Frieboes, John Lowengrub, and their collaborators (Frieboes *et al.*, 2006, 2007; Bearer *et al.*, 2009) demonstrates the angiogenic and invasive behavior of growing gliomas at the microscale. Kristin Swanson and her collaborators have explored the utility of a two-parameter reaction–diffusion equation, which can be expressed in terms of a normalized tumor cell density as:

$$\frac{\partial u}{\partial t}(\mathbf{x}, t) = \underbrace{\nabla \cdot (D \nabla u(\mathbf{x}, t))}_{\text{diffusion}} + \underbrace{\rho u(\mathbf{x}, t) (1 - u(\mathbf{x}, t))}_{\text{growth}}, \quad (4.1)$$

to explore the potential utility of ‘patient specific’ mathematical models to estimate patient survival (Swanson *et al.*, 2007) and optimize treatment strategies (Jackson *et al.*, 2015). The parameters D and ρ can be estimated from successive magnetic resonance (MR) images of individual patients (Jackson *et al.*, 2015).

Mathematical modeling of GBM in human patients is an inherently challenging enterprise. For one thing, GBM may arise *de novo* or as a malignant progression from a preexisting lower-grade lesion (Ohgaki and Kleihues, 2007), and the extent

of GBM invasion away from the main mass is difficult to ascertain from MRI (Giese *et al.*, 2003). Thus, the initial conditions that should be used to model an individual patient case are uncertain. In addition, multiple genetic pathways appear to be involved in the development and progression of GBM (Ohgaki and Kleihues, 2007), and genomic expression may affect the rate at which GBM tumors proliferate and spread (Jackson *et al.*, 2015). GBM invasion may be facilitated by other cell types, including macrophages and microglia (Coniglio and Segall, 2013). Thus, the ability to estimate growth and diffusion constants in any mathematical model of an individual tumor is limited. Finally, models like Equation (4.1) do not explicitly account for immune response, genetic drift in the tumor, or the effects of treatment. For all of these reasons, mathematical models of human GBM tumors may fail to replicate the observed growth patterns in individual patient cases.

These considerations motivated the following laboratory experiment. Five genetically similar, immunocompetent mice were injected with similar initial doses of murine glioma tumor cells from the same cell line (details provided Section 4.2). The progression of each tumor was followed in five successive MR imaging sessions designed to yield high-quality images. Due to issues in processing the images for two of the mice, imaging data and the resulting mathematical analyses are presented here for the remaining three. Under these carefully controlled conditions we have sought to determine how well simple models like Equation (4.1) could predict the future growth of each mouse’s tumor based on various assumptions of the initial conditions estimated from the images.

Figure 4.1 shows a representative T2-weighted MR image of each mouse at the last imaging session, 25 days after implantation. Although each mouse was inoculated with nearly the same number of cells from the same culture, the visible volumes of the final tumors range from approximately 12 mm^3 to 62 mm^3 , with considerable differences in

mass effect. Table 4.1 shows estimated visible tumor volumes in T2-weighted images for each mouse at each of the five imaging time points. The sizes of the tumors diverge considerably during the last ten days of growth.

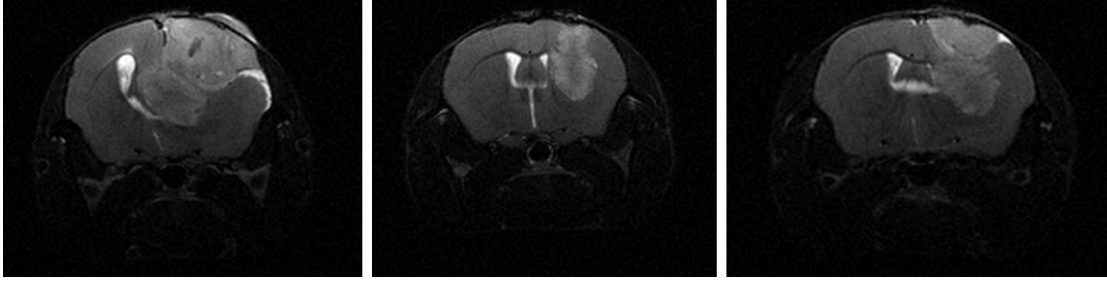


Figure 4.1: T2-weighted MR images taken 25 days after inoculation at the same location in the brain with respect to the initial needle track of the three mice used in this study. Despite inoculations with the same volume of tumor cells, the final tumors varied considerably in size.

Mouse	Day 11	Day 15	Day 18	Day 22	Day 25
	volume	volume	volume	volume	volume
	(mm ³)	(mm ³)	(mm ³)	(mm ³)	(mm ³)
1	0.715	1.155	4.980	15.570	25.460
2	0.595	3.025	4.320	8.095	12.340
3	0.970	3.855	16.485	30.675	61.490

Table 4.1: Experimental volumes of visible tumor on MRI of each mouse a each time point.

The purpose of this chapter is the use the laboratory imaging data to test a 3-dimensional (3D) finite difference method code in order to predict the growth and diffusion of the tumor. If we are successful in this endeavor, we can build on this

preliminary work, adding further complexities to the model in the form of mass effect and anisotropic diffusion (using diffusion tensor imaging). In this way, we hope to obtain insight into the model errors that arise when attempting to model the more complicated case of human GBM.

4.2 Methods

Animals and Care

The study followed the guidelines and regulations set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals Council (2011) and approved by the Institutional Animal Care and Use Committee at the Barrow Neurological Institute and St. Joseph’s Hospital and Medical Center. All aspects of the experiments including animal housing, surgeries, imaging, and euthanasia were carried out at the Barrow Neurological Institute at St. Joseph’s Hospital and Medical Center. The animals had continuous free access to food and water.

GL261-Luc2 Model

The GL261 tumor model in the C57BL/6J mouse strain was chosen because it is an established glioma tumor cell line that is recommended for preclinical GBM therapy studies Jacobs *et al.* (2011). GL261 is a syngeneic tumor cell line originally developed within the immunocompetent C57BL/6J mouse strain Seligman *et al.* (1939); Ausman *et al.* (1970); Jacobs *et al.* (2011); Martirosyan *et al.* (2011); Mohammed G *et al.* (2011). Seligman and Shear originally promoted the GL261 tumor cell line in the C57BL/6J mouse by implanting 3-methylcholanthrene pellets into the brains of mice in the 1930’s Jacobs *et al.* (2011); Seligman *et al.* (1939); Mohammed G *et al.* (2011). The GL261 cell line within the immunocompetent C57BL/6J mouse strain thus produces a

more typical immune response to the glioma and is thought to involve a pathological progression of malignant glioma akin to that in humans. The GL261 cell line is an aggressive tumor that shares many characteristics similar to the human GBM: tumor necrosis, hemorrhages, angiogenesis, cellular proliferation, tumoral cell rim, invasion, and inflammation Mohammed G *et al.* (2011); Candolfi *et al.* (2007); Drevelegas (2010). Although GL261 has a tumoral cell rim, it is not the typical pseudopalisading architecture seen in human GBM Candolfi *et al.* (2007). However, gross cellular proliferation and invasion are the most important biological characteristics for this study in the context of modeling cellular growth and migration.

GL261 cells were obtained from the DCTD Tumor Repository (NCI, Frederick, MD) and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) at 37°C with 5% CO₂. To facilitate a quantitative measurement of tumor growth rate GL261 cells were stably transfected with the gene encoding LUC2 using the pGL4.51[luc2/CMV/Neo] vector (Promega Corp, Madison, WI) and FuGENE ® 6 Transfection Reagent (Roche Applied Science, Indianapolis, IN) following conditions specified by the manufacturer. Stable transfectants were selected and maintained in DMEM containing 10% FCS and 100 µg/ml Geneticin ® (G418, Invitrogen Corp, Carlsbad, CA). These cells were designated GL261-Luc2; their growth rate is equivalent to the parental GL261 cell line Mohammed G *et al.* (2011); Abdelwahab *et al.* (2012).

Implantation

GL261-Luc2 cells were harvested by trypsinization, washed and resuspended at a concentration of $1-2 \times 10^7$ cells/ml in DMEM without FCS and implanted into ten-week-old immunocompetent C57BL/6-cBrd/cBrd/Cr (albino C57BL/6) mice (The Jackson Laboratory, Bar Harbor, ME, USA) with an average weight of 20 g. Briefly, the

animals were anesthetized by an intraperitoneal injection of ketamine (10 mg/kg) and xylazine (80 mg/kg), placed in a stereotactic apparatus, and an incision was made over the cranial midline. A burrhole was made 0.1 mm posterior to the bregma and 2.3 mm to the right of the midline. A needle was inserted to a depth of 3 mm and withdrawn 0.4 mm to a depth of 2.6 mm. Two μl of GL261-Luc2 cells ($1-2 \times 10^4$ cells/ μl) were infused over the course of 3 minutes. The burrhole was closed with bonewax and the incision was sutured. Mice were housed in groups of five in the animal care facility at St. Joseph’s Hospital and Medical Center in rooms with controlled temperature and humidity under a 12-hour light-dark cycle according to the guidelines outlined in the NIH Guide for Care and Use of Laboratory Animals Council (2011). Complete methods describing the GL261-Luc2 implantation into C57BL/6J mice have been previously published Mohammed G *et al.* (2011).

Imaging

Tumor bioluminescence and MRI were performed under isoflurane anesthesia on days 11, 15, 18, 22, and 25, after initial injection of cells. These imaging intervals were chosen to obtain frequent snapshots of the growing tumor in a manner that, in the experimentalists’ judgment, would not be detrimental to the health of the mice. For each imaging session, anesthesia was induced and maintained under isoflurane (1–2%) in oxygen. Respiration was continually monitored with a pillow sensor position under the abdomen (SA Instruments, Stony Brook, NY), and normal body temperature was maintained with a circulating warm water blanket (Thermo Scientific, Rockford, IL). Animals were stabilized with ear and tooth bars to minimize any motion and to ensure accurate image-to-anatomy registration. We present full details of the laboratory imaging here, although we only use the T2W images for this paper. Subsequent work will use this information to formulate more complex model frameworks.

Prior to MR imaging, bioluminescent tumor signal was measured. Animals received a subcutaneous injection of 150 μg luciferin/kg body weight 15 min prior to *in vivo* imaging using an IVIS® Spectrum *in vivo* imaging system (Perkin Elmer, Waltham, MA). Images were acquired and analyzed using the system’s Living Image ® 4.0 software.

MR images were acquired using a Bruker BioSpin 7T system in the following sequential order for each imaging session: high resolution T2-weighted (T2W), diffusion weighted (DW), T2W series, T1-weighted (T1W) series, dynamic contrast enhancement (DCE), and post contrast T1W. In the following discussion regarding imaging, we report the dimensions as axial $\times$ sagittal $\times$ coronal. High resolution T2W images (0.1 mm $\times$ 0.1 mm $\times$ 0.5 mm voxels) were acquired as a reference between time points. The DWI data series was acquired in 6 diffusion directions with a resolution of 0.2 mm $\times$ 0.2 mm $\times$ 1.0 mm. The T1W and T2W image series were acquired for T1 and T2 mapping, respectively. The resolution for these images was 0.2 mm $\times$ 0.2 mm $\times$ 0.5 mm. The DCE was continuously acquired for approximately 21 min, with the scientist needing to enter the MR room at 1.5 min into the acquisition to deliver a subcutaneous injection of a 1 ml bolus of contrast agent (i.e., Gd-DPTA (0.04 mol/ml)) through a preset catheter in the neck. The first 1.5 min of the DCE scan series was used as a pre-contrast baseline. The injections were completed approximately 2 min into the DCE acquisition. The resolution for these images was 0.2 mm $\times$ 0.2 mm $\times$ 0.5 mm. The full set of MRI parameters will be provided in detail in the full paper.

Representative examples of the MR images are displayed in Fig. 4.1.

Tissue Procurement and Histology

The mice were euthanized on day 26 using deep anesthesia with isoflurane followed by decapitation. The brains were carefully removed to minimize damage and were

immediately placed in a 4% paraformaldehyde solution for 48 hours. The sample was then placed in a phosphate-buffered saline and submitted to St. Joseph’s Hospital and Medical Center’s histology lab for dehydration and paraffin embedding.

Image Preprocessing

The brain, ventricle, and tumor spaces were segmented from the high resolution T2W images using the medical image processing software Mimics® (Materials Inc, Leuven, Belgium). The segmented spaces were then exported as STL files, i.e., as surface meshes.

The brain surface meshes were used in two subsequent steps. First, the brain surface mesh from a given time point was imported back into Mimics to identify and ultimately export point clouds of the intensity values included in all original images acquired during the same time point’s MR session. Second, brain meshes for a single animal at different time points were co-registered using the best-fit function in Geomagic Studio (Geomagic Inc, NC). Specifically, the brain volumes were registered to the mid time point, day 18. For each time point and for each mouse, Geomagic exported an affine matrix describing the necessary rotation, scaling, reflection, and shearing necessary to transform the surface of a given brain to the registered brain (which is the identity matrix for the third time point, day 18).

The exported affine matrices were then used to transform the corresponding resulting point clouds in MATLAB, so that the location of the brain for each mouse was constant. The transformed data points, containing intensity values, were then linearly interpolated to a uniform grid. The spacing of the grid is $0.1\text{ mm} \times 0.1\text{ mm} \times 0.5\text{ mm}$ to match the T2W image spacing, which allows for easy comparisons. This uniform grid is then used for the 3D finite difference described below and also in the 2D level set method that is in the full paper but outside the scope of this thesis.

4.3 Mathematical Model

Early mathematical modeling of GBM growth and diffusion were based on reaction–diffusion equations. Tracqui *et al.* (1995) formulated the earliest reaction–diffusion model applied to GBM and were able to incorporate the effect of treatment. Others expanded upon this basic model, incorporating a spatially-dependent diffusion coefficient that depended on whether the material was gray or white matter, as cell diffusion occurs preferentially in white matter (Swanson *et al.*, 2002; Eikenberry *et al.*, 2009). These models were effective in determining the proliferating core of the tumor but may not have been as successful at estimating the invasive front. Later modeling efforts incorporated a ‘Go or Grow’ hypothesis, in which GBM tumor cell populations are assumed to consist of two phenotypes: a migratory subpopulation and a stationary, proliferative subpopulation (Stein *et al.*, 2007a; Hatzikirou *et al.*, 2012). A recent paper showed that *in vitro* this behavior was able to be explained using one equation with density-dependent diffusion (Stepien *et al.*, 2015). Recent *in vivo* modeling efforts have incorporated anisotropic diffusion and make use of diffusion weighted imaging (DWI), which maps the diffusion of water molecules. From the 6 (or more) directional DWI, a diffusion tensor image (DTI) can be created, which is a method to view the three-dimensional ways in which water diffuses through the brain (Westin *et al.*, 2002). It is unclear how to directly relate water diffusion from DTI to cell diffusion, though several groups have proposed various techniques to relate these quantities (Jbabdi *et al.*, 2005; Clatz *et al.*, 2005; Bondiau *et al.*, 2008; Konukoglu *et al.*, 2010a,c; Painter and Hillen, 2013). This is still an area for further investigation. For a recent review on GBM modeling efforts, please see Martirosyan *et al.* (2015).

4.3.1 Computational Setup

The mathematical model that we are considering is the dimensionless reaction-diffusion equation given by:

$$\frac{\partial u}{\partial t}(\mathbf{x}, t) = \underbrace{D\nabla^2 u(\mathbf{x}, t)}_{\text{diffusion}} + \underbrace{u(\mathbf{x}, t)(1 - u(\mathbf{x}, t))}_{\text{growth}}, \quad \mathbf{x} \in \Omega \quad (4.2)$$

where Ω represents the segmented brain geometry. Ω is the brain tissue with the ventricles segmented out. We use the last scanned time point (day 25) for the computational domain. This is because for many of the mice, the growing tumor altered this brain geometry – specifically the location of the ventricles. Thus, at each time point, the brain geometry would differ. Therefore we chose the last time point, which aids in the ease of comparing our final tumor with the images of the final tumor. For this model Ω represents a 3D volume of the entire domain, and $\mathbf{x}$ is representative of a spatial vector, containing three components $\mathbf{x} = (x, y, z)$.

The boundary conditions are given by:

$$\frac{\partial}{\partial n} u(\mathbf{x}, t) = 0, \quad \mathbf{x} \in \partial\Omega \quad (4.3)$$

where $\partial\Omega$ is the boundary of the segmented brain geometry: this would be the outline of the brain and the outline of the ventricles. Essentially, we are assuming there is no diffusion of tumor cells outside of the brain or into the ventricle space.

The initial condition is:

$$u(\mathbf{x}, 0) = f(\mathbf{x}), \quad \mathbf{x} \in \Omega \quad (4.4)$$

where $f(\mathbf{x})$ represents the initial tumor at a given time. We have two proposed methods for initializing the tumor: firstly, we can estimate the amount of cells initially injected into the mouse brain, or we can start our simulations on the day of the first scan (day 11), where tumor is visible. There are advantages and draw backs to

each method. For the initialization at day 0, there are many uncertainties associated. Firstly, we must consider that as the tumor cells are injected into the brain, there may or may not be backslash occurring, meaning that some of the tumor cells might ooze out. Secondly, we are not sure what proportion of those cells are actually viable and, of those that are viable, what proportion will actually take hold and grow into a tumor. It is possible that these proportions might vary drastically between each mouse. However, we would be able to compare our computational tumor with the MR image for all five time points. By using initialization at day 11, we can only use four time points to compare simulation with MR images. However, there is more ability to be consistent since we are using the actual image to generate the initial condition.

For the simulations that use day 11 as an initial condition, we consider that any part that registers ‘tumor’ is in fact at half the carrying capacity. While this may seem somewhat arbitrary, we must consider that there is currently not much work performed which relates what percentage of carrying capacity is ‘visible’ in the MR image. Once histology is returned, we anticipate quantifying this relationship better to more accurately model the growth and diffusion of the glioblastoma. A section below will talk about sensitivity of our solutions to the initial condition cell-density.

When the simulations are complete, we create synthetic T2W MR images. Previous work indicates that 16% of maximum tumor cells is considered the ‘visible’ tumor on a T2W MR image (Swanson *et al.*, 2008). We display everywhere above 16% carrying capacity to compare with the MR image.

When segmenting the murine brains and tumors from the MR image, we also calculated the volume of the tumor present in each mouse at each time point. We noticed a large variance in the final tumor sizes in the mice, from 12 mm^3 to 62 mm^3 . We have several hypotheses which we believe might account for the discrepancy in these final tumor sizes.

4.3.2 Simulation Hypotheses

We investigate the values of D and ρ necessary to generate the various experimental tumor volumes. From the segmented tumors in the MR images, we calculated the volume of the visible tumor in Mimics ® based on the number of pixels that were segmented as tumor (as seen in Table 4.1). In the mice injected with GL261-Luc2 cell line, the final tumor size on day 25 varied from approximately 12 mm³ to 62 mm³, so we explore several hypotheses that may explain this large discrepancy between mice.

- **Hypothesis 1:** The natural variance in the growth rate ρ and diffusion rate D account for the large difference in final tumor size. In the first set of simulation results described below, we initialize the model based on the first MR imaging on day 11 following implantation. Our goal is to determine how well an optimized choice of constant (averaged) parameter values fits the observed images on day 25 and how well they agree with values estimated elsewhere in the literature.
- **Hypothesis 2:** The values of D and ρ are time dependent, corresponding to morphological changes during the growth of the tumors. A small tumor may be highly proliferative, but as the tumor grows and resources become scarce, the growth dynamics may be dominated by cell migration away from the main mass. In the second set of simulation results below, we attempt to optimize the values of D and ρ from time point to time point and see whether all of the mice display similar trends in the variation of these parameters.
- **Hypothesis 3:** In addition to the selection of optimized model parameters from one imaging time to the next, updates to the initial conditions at each imaging time point are made based on the visible tumor in each scan. This ‘predictor-corrector’ approach is analogous to that used in operational weather forecasting,

where the initial conditions of the weather model are updated periodically in view of incoming observations.

To test these hypotheses, we optimize the model parameters D and ρ for each mouse individually using the `FMINSEARCH` command in MATLAB. We minimize an error function based on the average of the sum of the Jaccard distances (4.6):

$$\text{error} = \frac{1}{n} \sum_{k=1}^n d_{J,k}(A, B) \quad (4.5)$$

over all n imaging time points used in a given simulation. In the simulations, modeled cell densities in regions of the computational domain that exceed the assumed detection threshold are ‘visible tumor’ (shown in red in Figures 4.3-4.9, corresponding to set A) in the synthetic MR images below, and the Jaccard distance is obtained from comparison with the visible tumor on the actual MR images (set B).

We do not include a comparison between the simulated and experimental boundaries of the tumor in the error function (4.5) because the extent to which the glioma cells infiltrate the brain beyond the visible tumor margin in T2W images is unknown. Konukoglu *et al.* (2010b) have suggested an estimation procedure based on Equation (4.1) to extrapolate the tumor cell density distribution beyond the visible margin in human GBM. However, since we do not have histological data with which to compare our extrapolations, we concern ourselves with only visible tumor. For the purposes of the simulations here, we assume that the detection threshold is 0.16 (Swanson *et al.*, 2008). As the results below suggest, however, there is considerable uncertainty in this value. In future work, we plan to digitally analyze all the histology slides from the mouse brains and correlate them to the final images to address this question, which may provide insight into human cases.

4.3.3 Relevant Background

The modeling efforts in this paper focus on Equation (4.1), as described in the introduction. Although Equation (4.1) is simple, it has proven effective in clinical settings. Tracqui *et al.* (1995) were able to show agreement with both the initial growth of the tumor as well as the response to chemotherapy, and Alvord Jr (1991) was able to estimate the recurrence of GBM after resection using similar models.

Recently, Baldock *et al.* (2014) estimated the ratio ρ/D , a measure of tumor invasiveness relative to growth, from successive imaging studies of individual patients. This measure was used to predict whether resection would significantly prolong the life of the patient. Other authors have considered a spatially dependent diffusion coefficient, with different values for regions of gray and white matter (Swanson *et al.*, 2003). However, since mouse brains have a relatively uneven distribution of gray and white matter as compared to humans, we consider only a diffusion constant that is uniform in space.

In Chapter 2, we discussed how the migratory and proliferating core could not be modeled using Equation (4.1), because it worked well at modeling the core, but not the migratory cells. Our justification for using this simple model for our current simulations is that with the MR image, we are only seeing the higher-density material of the tumor – most likely the proliferating core. Until we receive back the histology, Equation (4.1) will be adequate for generating a prediction of the *visible* tumor on an MR image.

4.4 Computational Methods

As discussed in Section 4.2, the computational domain Ω for each mouse is the linearly interpolated uniform grid. The uniform grids of the brain and ventricles are

imported into MATLAB. Then the ventricles are segmented out of the brain geometry to produce the computational domain. Since the ventricles have been segmented out, they are ignored in the discretization and no diffusion or growth occurs in the ventricular space, consistent with the boundary conditions in Section 4.3.1. As the tumor grows, it begins to displace the surrounding brain tissue. At each scan session, the ventricles of each mouse brain have shifted slightly from the previous session due to mass effect. The gross anatomy of the brain has been registered; however, the internal structures have shifted due to mass effect of the growing tumor. Because the ventricles are necessary to determine the computational boundary, we set the ventricles to be constant throughout the simulation. We use the ventricle locations of the last MR scan in our computational geometry.

The model is discretized using centered finite differences in space, and for our purposes, MATLAB's `ode45` proved to be a suitable solver. (Explicit schemes for finite difference methods are not always stable.) The space discretization is chosen to correspond to the pixel width in the postprocessed images (the scans have a resolution of $0.1 \text{ mm} \times 0.1 \text{ mm} \times 0.5 \text{ mm}$). The computation is vectorized for efficiency. Code was written by Barrett J. Anderies, a CSUMS student in the summer of 2014 and is publicly available in the murine-GL261-tumors repository located on github, <https://github.com/banderie/murine-GL261-tumors>.

As mentioned above, we obtained usable time sequences of images for three mice, called Mouse 1, 2, and 3 in the results presented below. Due to software difficulties, it was not possible to segment out the ventricles for the first two time points (days 11 and 15) for Mouse 3, for which the associated simulations are carried out only for a truncated time period. (It was possible, however, to estimate the total visible tumor volume in all mice at each time point, so Table. 4.1 gives a complete set of imaging volumes.)

We compare the visible tumor volume on the synthetic MR images with that of the actual T2W MR images using an error function based on the Jaccard distance at each imaging time point. The Jaccard distance is a measure of the overlap between two sets A and B at time t , given by

$$d_{J,t}(A, B) = 1 - \frac{|A \cap B|}{|A \cup B|}. \quad (4.6)$$

In this application, A is the set of voxels in the simulated MR image corresponding to tumor and B is the voxels corresponding to visible tumor. If A and B are disjoint, then $d_{J,t}(A, B) = 1$, and if $A = B$, then $d_{J,t}(A, B) = 0$. Figure 4.2 shows the nonlinear relationship between Jaccard distance and the percentage of overlap between two sets.

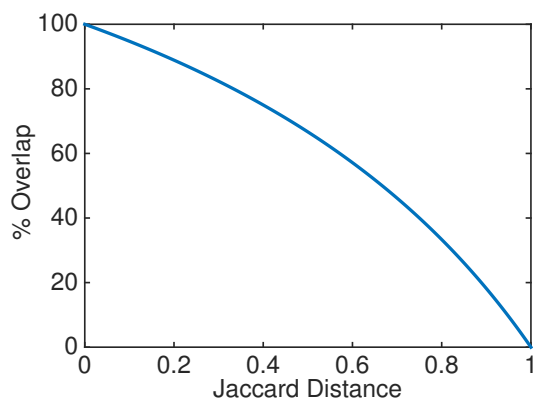


Figure 4.2: Relationship between the Jaccard distance measure and the overlap percentage between two sets.

Since the Jaccard distance is a measure of similarity of two sets, we must have a binary operator which to compare. In our case, for each pixel, if the density is higher than the predetermined threshold (0.16), that pixel is considered ‘tumor’ (value =1). Otherwise, that pixel is considered ‘brain’ (value = 0). We then define the intersection as the number of pixels for which simulation and data both have a value of 1 (representing tumor). The union is the number of pixels for which simulation or

data have a value of 1. These are the values which are used to calculate our Jaccard distance.

4.5 Simulation Results

Hypothesis 1

Hypothesis 1 states that natural variance in parameters ρ , D , or both account for the large difference in final tumor size. To test this hypothesis, we vary the parameters and use parameter estimation techniques to determine best-fit parameters for individual mouse cases where simulations are initialized using the MR image from time point 1.

As mentioned above, we select initial conditions corresponding to the visible tumor at the first MR imaging session (day 11 following implantation). We use MATLAB's FMINSEARCH function to determine values of D and ρ that minimize the Jaccard distance over all subsequent images, Equation (4.5). The resulting values of ρ vary from 0.0167 h^{-1} to 0.0188 h^{-1} among the three mice, but D varies from $319.22 \mu\text{m}^2/\text{h}$ (Mouse 2) to $651.17 \mu\text{m}^2/\text{h}$ (Mouse 3). (See Tables 4.2, 4.3, and 4.4 for Mouse 1, 2, and 3, respectively, at the end of this section.)

Figure 4.3 superimposes the simulated tumor in red over representative MR images at each subsequent imaging time point for Mouse 1. The Jaccard distance error given by Equation (4.5) is 0.4524, corresponding to a 70.8 percent overlap between the simulated and actual visible tumors, based on the MR data.

The simulation results of the optimization under Hypothesis 1 are shown in Figure 4.4 for Mouse 2 and Figure 4.5 for Mouse 3. Similar results were obtained for Mouse 2 and Mouse 3: unsurprisingly mouse 3, which had the largest final tumor size, also had larger ρ values than Mouse 1 and Mouse 2.

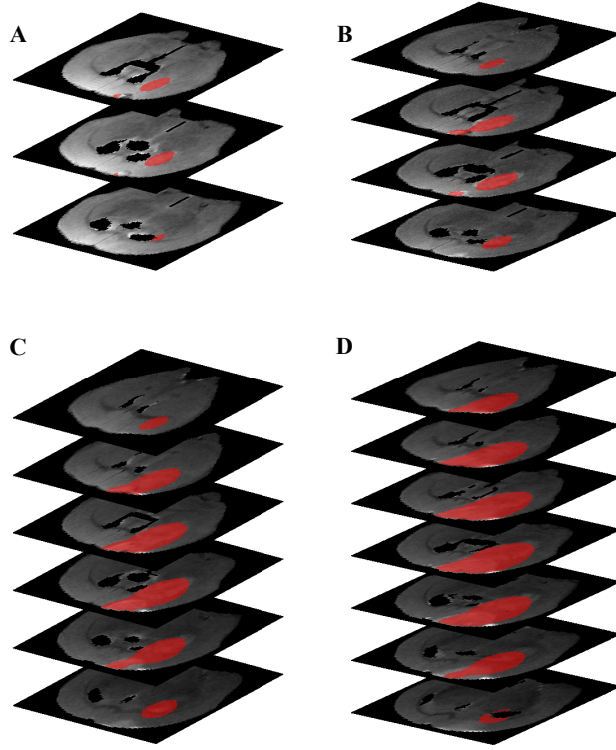


Figure 4.3: Simulated tumor (red) superimposed on MR images for Mouse 1 at the second through fifth imaging time points, using initial conditions estimated from the first set of MR images. The model parameters D and ρ are assumed constant (Hypothesis 1). (A) Day 15; (B) Day 18; (C) Day 22, and (D) Day 25 following implantation.

Hypothesis 2

Hypothesis 2 states that there may be a morphological change that occurs sometime within the tumor growth process which might change the values of D and ρ . When the tumor is small, it may be able to grow much faster. However, as time increases and resources become scarce, we may see instead the migration increase and the

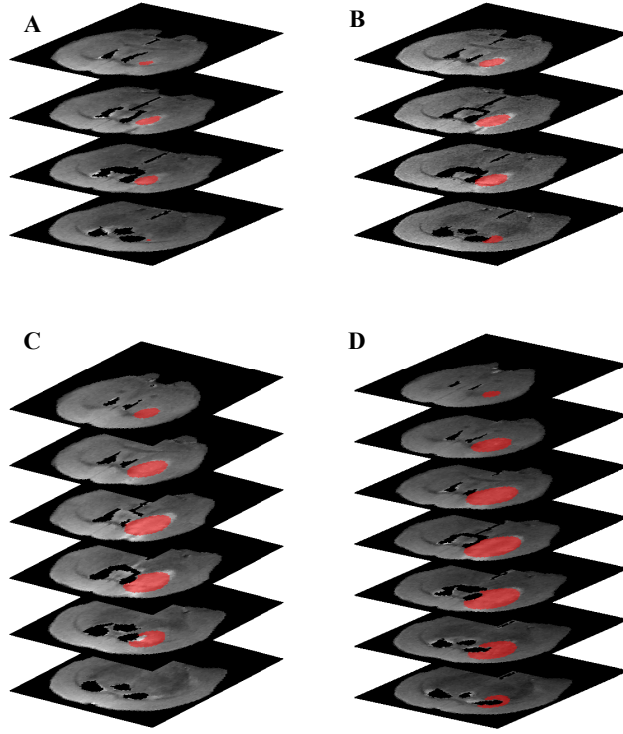


Figure 4.4: Representative slices for Mouse 2 under Hypothesis 1. The simulations are otherwise the same as in Fig. 4.3.

proliferation decrease.

The procedure to testing this hypothesis is comparable to that in Hypothesis 1, except that the parameter optimizations are done between successive imaging time points. That is, we take the same initial conditions on Day 11, then run FMINSEARCH to minimize the error measure in Equation (4.5) at Day 15 only. Then, starting with the simulated tumor at Day 15, we select parameter values to minimize the Jaccard distance at Day 18, and so on. Tables 4.2, 4.3, and 4.4 show all the results for hypothesis 2, for Mouse 1, Mouse 2 and Mouse 3 respectively. For Mouse 1, the estimates are $\rho = 0.0182, 0.0248, 0.0192, 0.0082 \text{ h}^{-1}$, respectively, for the Day 11–15,

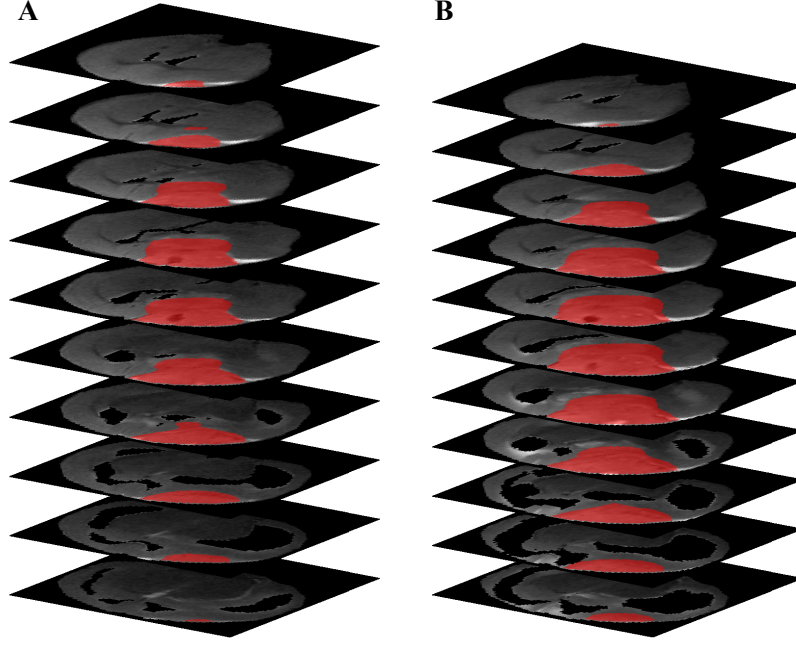


Figure 4.5: Representative slices for Mouse 3 under Hypothesis 1. We use time point 3 (day 18) as the initial condition. The simulations are otherwise the same as in Fig. 4.3. (A) Time point 4 (day 22); (B) time point 5 (day 25).

15–18, 18–21, and 21–25 time intervals. The respective estimates for diffusion are $D = 139.24, 839.93, 1047.6, 968.75 \mu\text{m}^2/\text{h}$. The sum of the individual Jaccard error measures (Equation 4.5)) is 0.4365, and the overlap between simulated and actual tumors varies from 68.6 to 76.6 percent. Figure 4.6 shows the results for Mouse 1.

The results suggest that there is a significant temporal variation in the model parameters as the tumor evolves. However, the model’s ability to track the visible tumor is only slightly improved compared to Hypothesis 1. Figures 4.7 and 4.8 are the results for Hypothesis 2 for Mouse 2 and 3, respectively.

We observe that, similar to Mouse 1, all the remaining mice also exhibit a better fit using hypothesis 2 than hypothesis 1. This supports our idea that a constant D and ρ does not accurately describe the behavior. Although we see that the optimal D

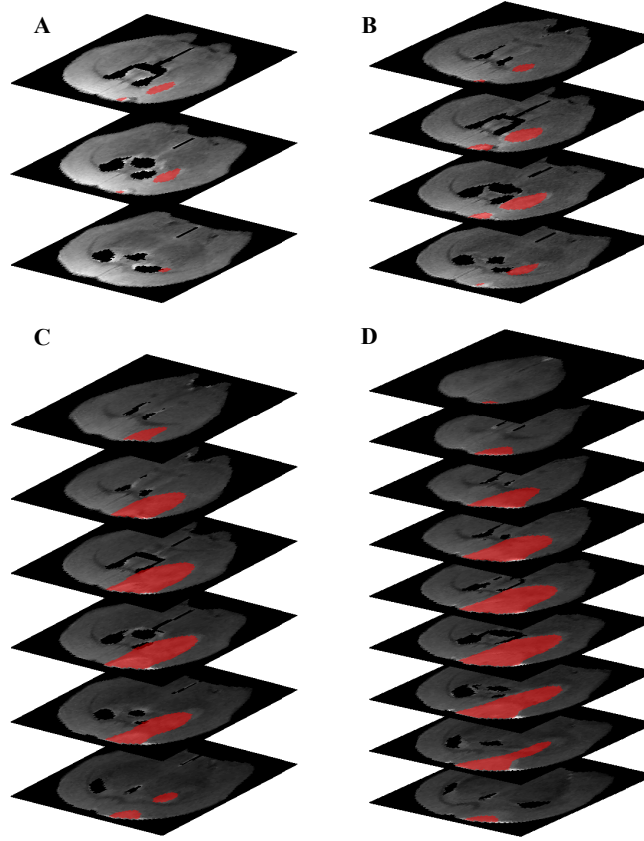


Figure 4.6: Representative simulation results for Mouse 1 under Hypothesis 2. (A) Using the optimized parameter values from Day 11–15; (B) Day 15–18; (C) Day 18–21; (D) Day 21–15.

and ρ change for each time point, the pattern in which they change does not remain constant. For Mouse 1, the diffusion increases drastically from time point 2 to time point 3 and slightly to time point 4. The growth increases from time point 2 to time point 3, then falls for the remainder of the simulation. On the other hand, for Mouse 2, the diffusion starts larger and falls from time point 2 to time point 3, increasing thereafter. Mouse 2 starts with a large growth, falling quickly until rebounding from time point 4 to time point 5. For Mouse 3, diffusion decreases and the growth increases as time increases. Even though all optimized parameters changed from time point to

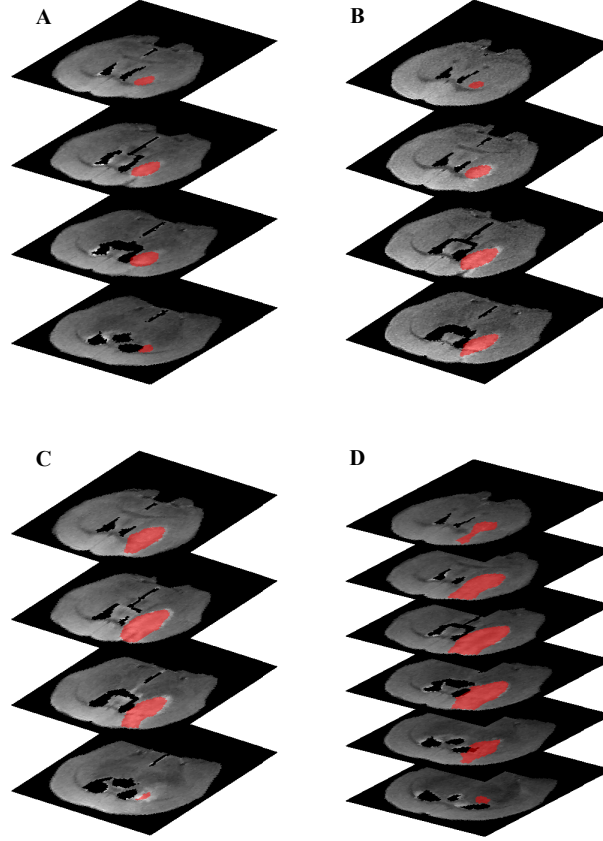


Figure 4.7: Representative slices for Mouse 2 under Hypothesis 2. The simulations are otherwise the same as in Fig. 4.6.

time point, between mice, the result was inconsistent.

Hypothesis 3

Hypothesis 3 also considers allowing D and ρ to vary with time; however, these simulations are corrected by using actual data to initialize each time point. In this approach, we optimize the parameters from time point to time point as in Hypothesis 2, but in addition, we reset the initial conditions at each imaging time point, assuming as usual that tumor cell densities at and above 0.16 correspond to visible tumor on T2W images. The optimized estimates for Mouse 1 for the growth rate are

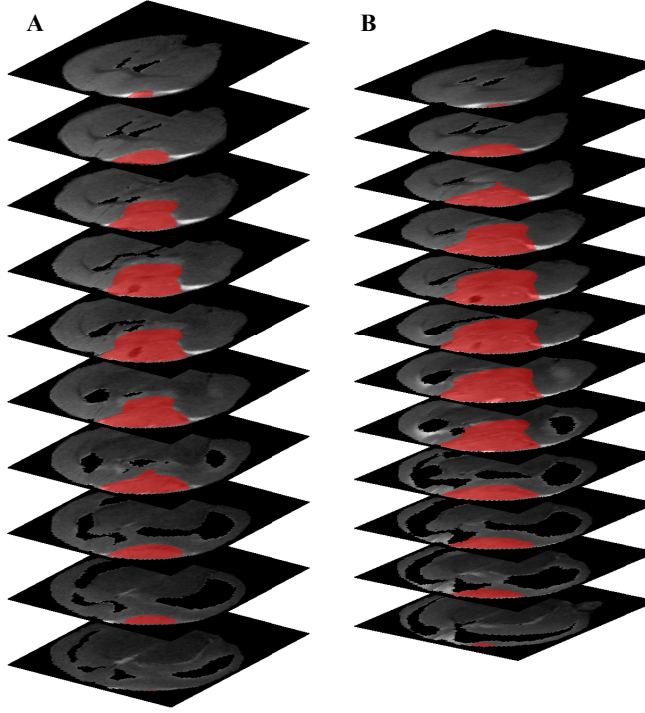


Figure 4.8: Representative slices for Mouse 3 under Hypothesis 2. We use time point 3 (day 18) as the initial condition. The simulations are otherwise the same as in Fig. 4.6. (A) Time point 4 (day 22); (B) time point 5 (day 25).

$\rho = 0.0182, 0.0499, 0.0178, 0.0105 \text{ h}^{-1}$ respectively for the Day 11–15, 15–18, 18–21, and 21–25 time intervals, and the respective estimates for diffusion coefficient are $D = 139.24, 233.97, 1156.2, 1305.6 \text{ } \mu\text{m}^2/\text{h}$.

The sum of the individual Jaccard error measures given by Equation (4.5), based on the predicted images prior to the initial condition updates, is 0.3663, and the overlap between simulated and actual tumors by Day 25 is 85.2 percent. Figure 4.9 shows the results for Mouse 1. Figures 4.10 and 4.11 are the results for Hypothesis 3 for Mouse 2 and 3, respectively.

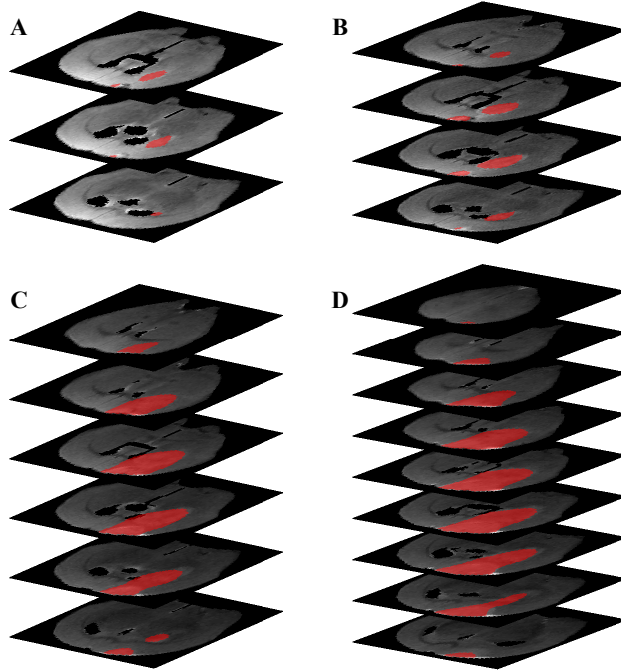


Figure 4.9: Representative simulation results for Mouse 1 under Hypothesis 3, using parameter values optimized over each imaging time interval followed by an update of the initial conditions: (A) Day 11–15; (B) Day 15–18; (C) Day 18–21; (D) Day 21–15.

For two of the three mice (Mouse 1 and Mouse 3), the diffusion coefficient increased over time, suggesting that the tumor growth dynamics become more diffusive with time. However, for Mouse 2, the optimized values for D decrease significantly over the last two time points, but the growth parameter ρ increased sharply. As for the growth of the other mice, Mouse 1 had large growth early in tumor development, and then growth decreased in the later stages of the simulation (time points 4 and 5). For Mouse 3, there was increasing ρ . This result may reflect a biological difference in the growth dynamics of the Mouse 2 tumor compared to the others.

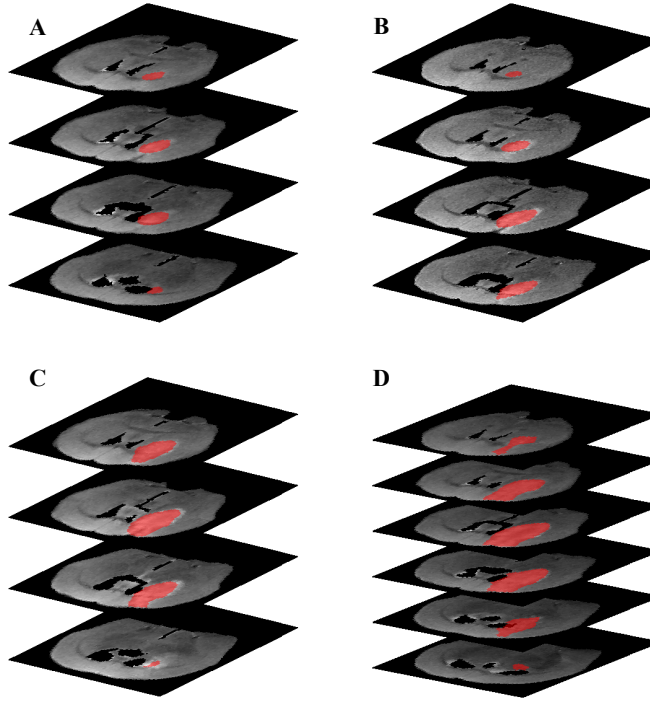


Figure 4.10: Representative slices for Mouse 2 under Hypothesis 3. The simulations are otherwise the same as in Fig. 4.9.

Velocity of Tumor Boundary

Since our analysis of Hypotheses 1–3 in the previous sections indicates that assuming constant D and ρ is inadequate to accurately model tumor growth and diffusion, we examine the dependence of the velocity of the visible tumor boundary on the volume to determine if the estimated parameters correlate with the volume of the tumor.

Originally, we had hypothesized that we would observe similar dynamics in the changing of D and ρ over time for all three of the mice. However, our analysis when examining the results of hypothesis 2 and hypothesis 3 indicated that for each

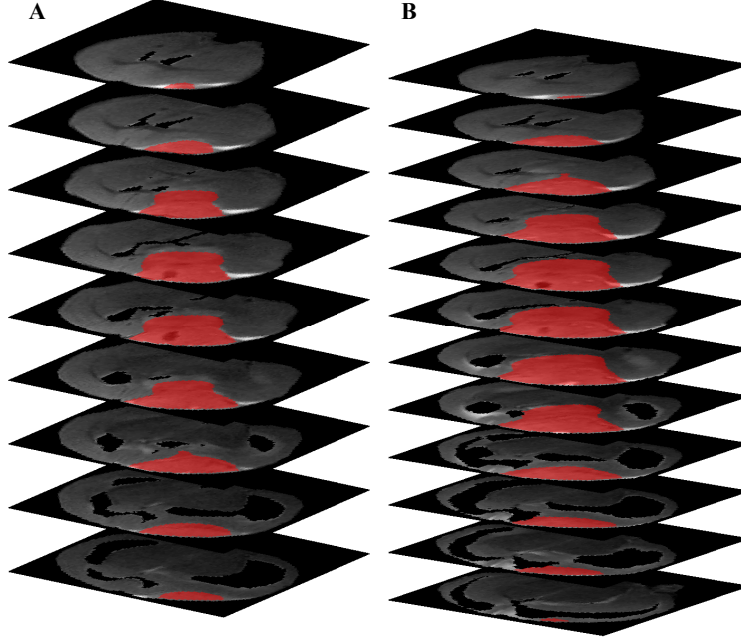


Figure 4.11: Representative slices for Mouse 3 under Hypothesis 3. We use time point 3 (day 18) as the initial condition. The simulations are otherwise the same as in Fig. 4.9. (A) Time point 4 (day 22); (B) time point 5 (day 25).

mouse, there was no consistency in variation of D and ρ over time. The rationale for re-optimizing the parameters from time point to time point is that there is some morphological change which occurs, changing how the tumor spreads. The idea is that when the tumor is small, it should have a very large growth, or ρ value. As the tumor grows, however, it needs to start spreading in order to gain nutrients so we would expect a corresponding increase in diffusion, D . However, with this idea, it makes more sense that the values of D and ρ are dependent on the *volume* of the tumor, rather than the time since tumor implantation. Since our mice exhibited such large differences in tumor volume through time, it makes sense that we do not have matching dynamics at the same time point.

Model (4.1) implies that the tumor boundary moves as a traveling wave whose

Hypothesis	Time	D	ρ (h^{-1})	velocity	Error	Overlap
	Point	($\mu\text{m}^2/\text{h}$)		$2\sqrt{D\rho}$ ($\mu\text{m}/\text{h}$)		(%)
1	–	413.77	0.0188	5.5781	0.4524	70.8
2	2	139.24	0.0182	3.1838	0.1196	68.6
	3	839.93	0.0248	9.1280	0.1191	68.7
	4	1047.6	0.0192	8.9697	0.1029	74.1
	5	968.75	0.0082	5.6369	0.0949	76.6
3	2	139.24	0.0182	3.1838	0.1196	68.6
	3	233.97	0.0499	6.8338	0.1145	70.3
	4	1156.2	0.0178	9.0731	0.0688	84
	5	1305.6	0.0105	7.4051	0.0644	85.2

Table 4.2: Values of estimated parameters for Mouse 1 for each hypothesis at each time point using the 3D finite difference method. Minimization is performed with respect to Equation (4.5).

wave speed (velocity) is $2\sqrt{D\rho}$. Figure 4.12 shows a plot of the predicted wave speed versus the visible tumor boundaries under each set of modeling hypotheses. The data points are separated by shape and color: blue markers correspond to the wave speeds calculated for Hypothesis 2 and red markers correspond to the wave speeds generated from Hypothesis 3. Within these colors, the markers signify the separate mice: Mouse 1 is represented by triangles, Mouse 2 with plusses, and Mouse 3 with circles. At first glance, it may appear that there is little correlation between the two variables. However, if we examine the Hypothesis 3 data (red markers), we can see there does appear to be some relationship between wave speed and visible tumor size. More data is necessary to quantify the relationship, however.

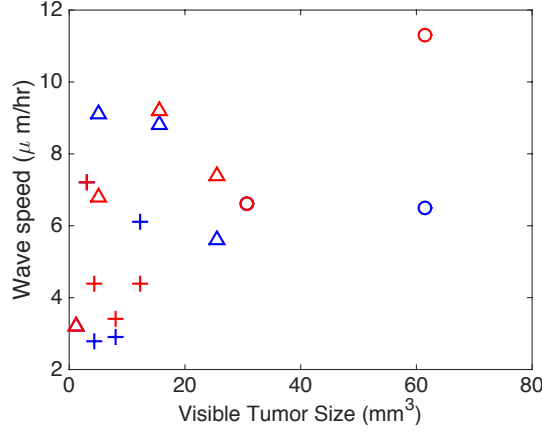


Figure 4.12: Dependence of wave speed of tumor on tumor size. The wave speed (velocity) of the tumor is calculated by $2\sqrt{D\rho}$. Blue markers show results from parameters optimized under Hypothesis 2; red markers, Hypothesis 3. Triangles: Mouse 1; pluses: Mouse 2; circles: Mouse 3.

4.5.1 Initial Condition Sensitivity Analysis

We performed additional analysis to determine the sensitivity of the parameter estimation procedure to the choice of initial condition. Using Mouse 1, we re-ran the parameter estimation procedure assuming initial cell densities of 0.3 and 0.7 in voxels corresponding to visible tumor on MRI. We discovered that our parameter estimations were rather insensitive to initial starting densities, the results of which are displayed in a Table 4.5. Although our values of D increase as we increase the initial cell density, we have a corresponding decrease in ρ . This is logical, because when we have a high initial density of cells, we would expect more diffusion and less growth. The result of this is that the optimized wave speed given by $2\sqrt{D\rho}$ are all within a very close value to each other. Additionally, our errors given by the Jaccard distance and the percentage overlap are very consistent.

Hypothesis	Time	D	ρ (h^{-1})	velocity	Error	Overlap
	Point	($\mu\text{m}^2/\text{h}$)		$2\sqrt{D\rho}$ ($\mu\text{m}/\text{h}$)		(%)
1	–	319.22	0.0167	4.6178	0.4528	70.7
2	2	558.74	0.0235	7.2472	0.1151	70.1
	3	206.21	0.0100	2.8720	0.1067	72.9
	4	346.35	0.0055	2.7604	0.1042	73.7
	5	886.07	0.0104	6.0713	0.0979	75.6
3	2	558.74	0.0235	7.2472	0.1151	70.1
	3	950.79	0.0051	4.4041	0.0846	79.6
	4	77.734	0.0369	3.3873	0.0621	85.8
	5	94.161	0.0520	4.4255	0.0643	85.2

Table 4.3: Values of estimated parameters for Mouse 2 for each hypothesis at each time point using the 3D finite difference method. Minimization is performed with respect to Equation (4.5).

The same dynamics are observed for Mouse 2. However, Mouse 3 appears to have a slight difference for the 30% initial cell density case. It is apparent that the wave speed is much larger for this case. Upon further investigation, I discovered that there were many sets of parameters that gave approximately the same amount of error for the 30% initial cell density case for Mouse 3. For the remaining sets of parameters, they were approximately $D = 588.1633 - 664.1250 \mu\text{m}/\text{h}$, and $\rho = 0.0202 - 0.0214 (\text{h}^{-1})$, which gives a wave speed of $7.0955 - 7.3254$, which is much closer to the remaining wave speeds for Mouse 3. This indicates that there are many parameter sets which could optimize the fit of simulated data to experimental data.

Hypothesis	Time	D	ρ (h ⁻¹)	velocity	Error	Overlap
	Point	($\mu\text{m}^2/\text{h}$)		$2\sqrt{D\rho}$ ($\mu\text{m}/\text{h}$)		(%)
1	–	651.17	0.0177	6.7899	0.2833	83.5
2	4	859.70	0.0127	6.6085	0.1408	83.6
	5	454.29	0.0236	6.5487	0.1364	84.2
3	4	859.704	0.0127	6.6085	0.1408	83.6
	5	1552.1	0.0200	11.1431	0.1027	88.6

Table 4.4: Values of estimated parameters for Mouse 3 for each hypothesis at each time point using the 3D finite difference method. Minimization is performed with respect to Equation (4.5).

4.6 Discussion

In this study, we examine a laboratory murine model of GBM tumor growth, in which genetically similar, immunocompetent mice received identical intracranial injections from the same culture of a GL261 tumor line. After 25 days, the growth patterns of the resulting tumors are significantly different. From experimental MR images, we investigate two mathematical models and examine their ability to simulate the laboratory results under various choices of model parameters. The reaction–diffusion model, Equation (4.1), is numerically integrated using a finite difference method in three dimensions.

Previous estimates of the growth parameter ρ is approximately in the range 0.014–0.018 h⁻¹ for *in vitro* GL261-Luc2cells (Hercbergs *et al.*, 2009) and 0.0096–0.011 h⁻¹ for *in vivo* GL261-Luc2 cells (Doblas *et al.*, 2010). The estimated values for ρ in our simulations, presented in Tables 4.2, 4.3, and 4.4 is comparable with these previous ranges. Although some of our values are larger and smaller than this range, our

Mouse	Initial Condition	D	ρ (h^{-1})	velocity	Error
	Cell Density (%)	($\mu\text{m}^2/\text{h}$)		$2\sqrt{D\rho}$ ($\mu\text{m}/\text{h}$)	
1	30	316.43	0.0226	5.3484	0.4510
	50	413.77	0.0188	5.5781	0.4524
	70	441.52	0.0168	5.4476	0.4592
2	30	220.0783	0.0220	4.4008	0.4678
	50	319.22	0.0167	4.6178	0.4528
	70	350.2452	0.0146	4.5226	0.4421
3	30	861.3994	0.0190	8.0911	0.2789
	50	651.17	0.0177	6.7899	0.2833
	70	630.4063	0.0173	6.6049	0.2880

Table 4.5: Values of estimated parameters for Mouse 1, 2, and 3 using various initial condition cell densities for Hypothesis 1. Minimization is performed with respect to Equation (4.5).

average values of ρ are in this range.

Measurements of the apparent diffusion constant (ADC) for *in vivo* GL261-Luc2 cells, which is calculated from diffusion weighted imaging that maps the diffusion of water molecules, range from 2.2–3.3 mm^2/h (Seshadri and Ciesielski, 2009; Laufer *et al.*, 2014). These values are orders of magnitude larger than the diffusion constant D estimated from our simulations in Tables 4.2, 4.3 and 4.4, which models the movement of tumor cells. A more meaningful comparison between the two types of measurements requires a quantitative correlation between the diffusion of water in DWI images and the growth of the tumor over time, which likely will require cell density measurements from histology. Our largest value of D is still considerably less

than previously published values, which suggests that errors in the initial conditions and in the assumptions on the correspondence between tumor cell densities and enhancing regions on MR images are contributing factors. Our assumption regarding the minimum cell density that is visible on MR may need to be revised significantly.

The 3D finite difference method applied to Equation (4.1) implies that the velocity of the visible tumor boundary is $2\sqrt{D\rho}$. Converting to units of centimeters per year from Table 4.2, 4.3, and 4.4 the 3D finite difference method yields velocity estimates from 2.789–7.996 cm/yr for Mouse 1, 2.516–6.348 cm/yr for Mouse 2, and 5.737–9.761 cm/yr for Mouse 3. The velocities are somewhat larger than those obtained from tumors in rat brains with implanted human glioblastoma cells (Massey *et al.*, 2012) and are on the low end of the human velocity range (Harpold *et al.*, 2007; Szeto *et al.*, 2009). Our results suggest that, in this respect, GL261-Luc2 cells implanted in mice brains appears to be a reasonable proxy of human GBM growth.

In the original work, we also ran simulations in 2D, which are not chronicled here as they were written and performed by someone else. We noticed that in two and three dimensions, we find comparable estimates of the model parameters D and ρ from the experimental data, implying that there does not need to be a scaling factor between the 2D and 3D simulations. As far as we are aware, this relationship and possible scaling factor between 2D and 3D simulations has not been previously investigated.

GLOBAL DYNAMICS OF A MODEL OF JOINT HORMONE TREATMENT
WITH DENDRITIC CELL VACCINES FOR PROSTATE CANCER

5.1 Introduction

Once cancer has reached a large enough size that the patient begins to experience symptoms, they are often diagnosed with the disease, at which point they are offered standard treatment. Depending on the type of cancer, these treatments may include, but are not limited to, surgical resection, chemotherapy, radiation therapy, and immunotherapy. Dosing schedules and amounts are rarely questioned by physicians for these treatments, but they are of great interest to mathematicians. Is there an optimal dosing strategy for a certain type of cancer? Do we need to individualize dosing strategies?

An emerging field among mathematical oncology is determining this optimal dosing strategy. The goal is no longer necessarily to eradicate cancer, but rather to render it manageable, as in diabetes or HIV. Metronomic therapy, or the use of much lower dosages of medication, but more frequently, has been mathematically investigated as an alternative to the popular types of maximal-dosing strategies (Ledzewicz and Schättler, 2002; Ledzewicz *et al.*, 2012). The benefits that these therapies offer can not be overstated: they lower toxicity of the treatment, improving quality of life for the patient, they lower costs, and increase time until treatment resistance occurs.

In this chapter, we explore the various treatment strategies for late-stage prostate cancer. We investigate hormonal therapy, both continual and intermittent, in conjunction with immunotherapy in the form of dendritic cell vaccines. We consider discrete

injections of dendritic cell vaccines, and determine whether dosing schedules influences the time to rise of androgen independent cancer relapse. The work contained in this chapter has been accepted for publication and the accepted version of the article is located in Appendix F.

5.1.1 Prostate Cancer and Current Treatment

Prostate cancer is one of the most ubiquitous cancers that affect males in the United States, with an expected one in six men being diagnosed in their lifetime (Jemal *et al.*, 2008). Current treatment protocol is hormone therapy in the form of androgen deprivation therapy. Androgens are the male sex hormones, the most known of which is testosterone. The prostate requires androgens, such as testosterone and 5 α -dihydrotestosterone (DHT) to function and continue proliferation. Thus, restricting the production of these androgens will lead to inhibited growth of cancerous cells as well. Although the initial response to this hormonal treatment is excellent, eventually an androgen-independent form of prostate cancer, which can be extremely fatal (Nelson, 2012).

Androgen deprivation therapy is an unpleasant therapy with many undesirable side effects such as loss of libido, loss of muscle, and osteoporosis. Recent research and clinical trials have begun to question whether an intermittent hormone therapy schedule might be more effective in terms of both quality of life of the patient, as well as delaying the rise to treatment resistance (Jain and Friedman, 2013; Portz *et al.*, 2012; Crook *et al.*, 1999; Larry Goldenberg *et al.*, 1995). Intermittent androgen deprivation therapy considers the level of prostate specific antigen (PSA), a biomarker of the disease. A patient is kept on hormone therapy until their PSA levels drop below a pre-determined threshold. Once the patient has reached that threshold, they are taken off treatment and have their PSA levels monitored. If their PSA levels rise

above a second predetermined threshold, they are placed back on treatment, and the cycle begins anew. This treatment still results in overall suppression of androgen, but can improve the quality of life of the patient (Crook *et al.*, 2012; Hussain *et al.*, 2013).

It is often suggested that IAD may stave off the emergence of castration-resistant prostate cancer, although this has yet to be shown in clinical trials and meta studies, although it is accepted that IAD is non inferior to continual ablation therapy (Crook *et al.*, 2012; Botrel *et al.*, 2014; Sciarra *et al.*, 2013; Tsai *et al.*, 2013). However, there is still little consensus within the medical community about who and how to administer IAD therapy to. Certain studies suggest that IAD is suitable for patient groups which exhibit certain disease characteristics, but determining which characteristics remains a work in progress (Wolff *et al.*, 2014; Klotz and Toren, 2012). In addition to the confusion over who may benefit from IAD, there is still no accepted norm within the medical community regarding the timing of IAD (Gleave *et al.*, 2009). Even recently, the debate rages on, with many questioning the findings of the previous clinical trials, saying the studies used to draw conclusions are flawed (Park and Eisenberger, 2016; Hussain *et al.*, 2016).

5.1.2 Immunotherapy

In recent years, immunotherapy has emerged as a viable disease management strategy. Immunotherapy relies on using the body's own immune system to fight the cancer. There are many different types of immunotherapy, such as nonspecific antibodies to boost the immune system response, synthetic antibodies, and targeted therapies. Non-specific antibodies that boost the immune system response can be suppressive or boosting. One such example are cytokines which can help the immune system cells grow and divide. However, there are unpleasant side effects such as flu-like symptoms and, in the case of too high a dose, possibly fatal heart conditions. Synthetic

antibodies are lab-designed antibodies which specifically attack target antigens found on cancer cells. However, for many cancers and diseases, the big question is, which antigen should these synthetic antibodies attack? Finally, targeted therapies use the patient's specific tumor to induce a response. In our model, we will use this last type of immunotherapy.

First, however, some background on the immune system. We begin with cytokines, which are small proteins which effect cell signaling. Cytokines are produced by immune cells such as macrophages, T cells, and B cells. They are released to influence other types of cells and are known to affect maturation, growth, and death of cell populations. There are several types of cytokines such as chemokines, interferons and interleukins, among others. Chemokines induce chemotactic responses in nearby cells. Interferons are released to cause nearby cells to activate their viral defenses, and also interfere with viral replications. Interleukins stimulate the development and differentiation of T and B cells. Large doses of these cytokines, however, can have adverse side effects and can be fatal in large enough doses. The different types of cytokines interact with each other, keeping the body in homeostasis. We are investigating an interleukin, specifically interleukin-2 (IL-2). IL-2 directly promotes the differentiation of immature T cells into regulatory T cells, which prevents attacking of healthy cells. Additionally, IL-2 promotes the differentiation of native T cells into effector T cells and memory T cells. Like other cytokines, IL-2 is dangerous if too much exists in the body, eliciting responses such as seizures, heart complications, and difficulty breathing.

Another class of immune cells are antigen-presenting cells. Antigen-presenting cells work by traveling through the blood stream in an immature state. Once they encounter an antigen, they ingest it, and are activated. Once activated, these cells migrate to the lymph nodes to interact with T and B cells. The antigen presenting cells then present the antigen material on their cell surface to naive and memory T

cells, which then target that antigen. The most potent of these antigen-presenting cells are dendritic cells. Additionally, they secrete co-stimulatory signals.

The actual therapeutic practice that we investigate in this model is dendritic cell vaccines. These are created first by extracting blood from a patient. The monocytes are then differentiated into dendritic cells. These dendritic cells are then loaded with tumor-derived antigens. Ideally, these tumor-derived antigens are proteins from autologous tumor extracts, i.e. from the patient's own tumor. If these autologous tumor extracts are unavailable, as in a case with an inoperable tumor, synthetic, lab-derived peptides can be used instead. Once these dendritic cells have had the opportunity to ingest the antigens, they are activated by cytokines. Finally, these tumor antigen-presenting dendritic cells are re-injected into the patient. For prostate cancer, the target antigen used in clinical trials is Prostatic Acid Phosphatase (PAP) (Fong *et al.*, 2001; Small *et al.*, 2000; Burch *et al.*, 2004). PAP is an enzyme that is produced by the prostate and tends to be elevated in prostate cancer patients. In fact, PAP used to be a biomarker of the disease until the switch was made to PSA Taira *et al.* (2007). The highest levels of PAP are found in patients for whom the cancer has already metastasized Whitesel *et al.* (1984). Currently, Spuleucel-T (Provenge) is the only FDA-approved dendritic cell vaccine treatment for prostate cancer, which has been clinically shown to extend the life of patients (Cheever and Higano, 2011; Higano *et al.*, 2009). DC vaccines tend to be very safe with mild limited side effects, such as flu-like symptoms. DC vaccine efficacy is mitigated by radiation and chemotherapy, which makes prostate cancer an ideal candidate to test these vaccines on, as treatment tends to be hormonal. Currently, Provenge is used for advanced prostate cancer that is no longer helped by hormonal therapy, the androgen-independent cancer. We question whether it may be more effective if used in conjunction with hormonal therapy, as opposed to sequentially.

5.2 Previous and Proposed Models

Many previous mathematical models have been formulated to estimate evolution and treatment of prostate cancer using androgen deprivation therapy. In a pioneering paper published in 2004, Jackson formulated a partial differential equation model that featured both androgen independent and androgen dependent cancer cells. The resulting dynamics were consistent with experimental data which exhibited androgen independent relapse (Jackson, 2004). From this landmark paper, many sequential papers have considered variations on the model to explore further dynamics. We concentrate on three previous papers: one which models immunotherapy interaction with tumors, one which models intermittent hormone therapy for prostate cancer, and one which marries the two models. Our proposed model is a variation on this last model, with more realistic terms.

5.2.1 Kirschner and Panetta (1998)

Kirschner and Panetta (1998) formulated a model quantifying the anti-tumor response due to the immune system. Their model contained effector T cell population E , tumor cell population T , and IL-2 concentration I_L . The governing equations are:

$$\frac{dE}{dt} = \underbrace{\frac{cT}{\text{recruitment from tumor}}}_{\text{recruitment from tumor}} - \underbrace{\mu_2 E}_{\text{death}} + \underbrace{\frac{p_1 E I_L}{g_1 + I_L}}_{\text{stimulation from IL2}} + \underbrace{s_1}_{\text{immunotherapy}} \quad (5.1)$$

$$\frac{dT}{dt} = \underbrace{\frac{r_2(T)T}{\text{growth and death}}}_{\text{growth and death}} - \underbrace{\frac{aET}{g_2 + T}}_{\text{death from T cells}} \quad (5.2)$$

$$\frac{dI_L}{dt} = \underbrace{\frac{p_2 ET}{g_3 + T}}_{\text{secretion by tumor}} - \underbrace{\mu_3 I_L}_{\text{degradation}} + \underbrace{s_2}_{\text{immunotherapy}} \quad (5.3)$$

The population of effector T cells is governed by a tumor recruitment term, a death term, a stimulation from IL-2 term, and an additional immunotherapy term.

The tumor recruitment term is based on the antigenicity of the tumor (represented by the parameter c), or how different the tumor is from the healthy tissue. The more ‘different’ the tumor is, the faster the growth of T cells. The stimulation term from IL-2 is a standard Michaelis-Menten term. The last term allows for effector T-cells to be injected into the patient, as a form of immunotherapy. For standard immunological response, we would assume $s_1=0$. For the tumor cell population, we see a growth function $r_2(T)$ which could be something like a logistical growth term. The death term is due to interaction between tumor cells and T cells and is saturating. The equation for IL-2 concentration has a secretion by the tumor, which also displays Michaelis-Menten kinetics. As mentioned earlier, cytokines are regulated by the body, so it is necessary for there to be self-limiting production of IL-2. There is also the option for IL-2 to be directly injected as a form of immunotherapy, but for a standard immunological response, we would take $s_2 = 0$.

5.2.2 Ideta et al. (2008)

In 2008, Ideta et. al created a prostate cancer model that would incorporate not only continual androgen deprivation therapy, but also intermittent androgen deprivation therapy. The model incorporated both AD (X_1) and AI (X_2) cells, as well as androgen concentration (A). Within the model, AD cells are governed by proliferation, death, and mutation to AI, which are all dependent on the androgen concentration. The AI cells are governed by mutation from AD, proliferation and death terms, which can also depend on androgen concentration. The androgen level itself is governed by a homeostasis term and a term which represents whether the

patient is on or off treatment. Ideta *et al.* (2008) model is given by:

$$\frac{dX_1}{dt} = \underbrace{\alpha_1 p_1(A) X_1}_{\text{proliferation}} - \underbrace{\beta_1 q_1(A) X_1}_{\text{apoptosis}} - \underbrace{m(A) X_1}_{\text{mutation to AI}} \quad (5.4)$$

$$\frac{dX_2}{dt} = \underbrace{\alpha_2 p_2(A) X_2}_{\text{proliferation}} - \underbrace{\beta_2 q_2(A) X_2}_{\text{apoptosis}} + \underbrace{m(A) X_1}_{\text{mutation from AD}} \quad (5.5)$$

$$\frac{dA}{dt} = \underbrace{\gamma(a_0 - A)}_{\text{homeostasis of androgen}} - \underbrace{\gamma a_0 u(t)}_{\text{depletion of androgen if on therapy}} \quad (5.6)$$

where $u(t)$ represents the state of treatment: $u(t) = 1$ when treatment is on, $u(t) = 0$ when treatment is off. The value of $u(t)$ depends on the PSA level $y(t)$, which is calculated depending on the populations of AD and AI cells in addition to how that level is changing (i.e., a patient would remain on treatment even if they go under the threshold if their PSA levels are increasing). The summary of this interaction is shown below:

$$y(t) = c_1 X_1 + c_2 X_2$$

$$u(t) = \begin{cases} 0 \rightarrow 1 & \text{if } y(t) > L_1 \text{ and } \frac{dy}{dt} > 0 \\ 1 \rightarrow 0 & \text{if } y(t) < L_0 \text{ and } \frac{dy}{dt} < 0 \end{cases} \quad (5.7)$$

Numerical simulations performed by the authors were able to display the cyclical behavior expected with intermittent therapy.

5.2.3 Portz and Kuang (2012)

The Portz and Kuang model is based on marrying these two previous models together with an additional equation that represents the dendritic cell levels. Their model is given by:

$$\frac{dX_1}{dt} = \underbrace{r_1(A)X_1}_{\text{growth and death}} - \underbrace{m_1 \left(1 - \frac{A}{a_0}\right) X_1}_{\text{mutation to AI}} - \underbrace{\frac{e_1 X_1 T}{g_1 + X_1}}_{\text{death by T cell}}, \quad (5.8)$$

$$\frac{dX_2}{dt} = \underbrace{r_2 X_2}_{\text{growth}} + \underbrace{m_1 \left(1 - \frac{A}{a_0}\right) X_1}_{\text{mutation from AD}} - \underbrace{\frac{e_2 X_2 T}{g_1 + X_2}}_{\text{death by T cell}}, \quad (5.9)$$

$$\frac{dT}{dt} = \underbrace{\frac{eD}{g + D}}_{\text{activation of T cell by DC}} - \underbrace{\mu T}_{\text{death}} + \underbrace{\frac{e_3 T I_L}{g_3 + I_L}}_{\text{activation of T cell by cytokines}}, \quad (5.10)$$

$$\frac{dI_L}{dt} = \underbrace{\frac{e_4 T (X_1 + X_2)}{g_4 + X_1 + X_2}}_{\text{secretion by tumor}} - \underbrace{\omega I_L}_{\text{degradation}}, \quad (5.11)$$

$$\frac{dA}{dt} = \underbrace{\gamma(a_0 - A)}_{\text{homeostasis of androgen}} - \underbrace{\gamma a_0 u(t)}_{\text{depletion of androgen if on therapy}}, \quad (5.12)$$

$$\frac{dD}{dt} = - \underbrace{cD}_{\text{death}}. \quad (5.13)$$

where $r_1(A) = \alpha_a \frac{A}{A+k_1} - \beta_1 \left(k_2 + (1 - k_2) \frac{A}{A+k_3} \right)$.

The equation describing the dendritic cells is just a degradation term, as the dendritic cells are added only through immunotherapy. This model adequately describes the tumor-immune interaction, however, there are some shortcomings that inhibit the reality and analysis of the model. Firstly, growth of the androgen independent cancer cells is assumed to be exponential, which is unrealistic. Secondly, the terms governing the death of the AD and AI cells with respect to T should be saturating over the entire tumor population, as opposed to the individual AD or AI cell populations. For this reason, we make some changes to create our model.

5.2.4 Previous Mathematical Models

Many have continued the work of the Ideta et al model. Hirata *et al.* (2010a) extended the model to include two differing types of androgen independent cells. This

included a subpopulation which has a reversible mutation to AI and subpopulation of AI for which the mutation is irreversible. They used this model to then characterize patients into three sub-groups in order to determine if IAD would be a suitable therapy (Hirata *et al.*, 2012). They have also used this model to optimize patient protocols (Hirata *et al.*, 2010b, 2014). Recently, for the patient group which is most likely to benefit from IAD, they found conditions necessary for non-trivial periodic orbits (Hirata and Aihara, 2015).

Portz *et al.* (2012) considered androgen to be a limiting nutrient and introduced stoichiometric ideas into the model using cell quotas. This model incorporated the dependence of both AI and AD cell populations on the concentration of intracellular androgen. This model was furthered and compared to the Hirata *et. al* model described above (Everett *et al.*, 2014).

In addition to deterministic models, Tanaka *et al.* (2010) incorporated stochasticity into the Ideta *et. al* model, formulating more realistic PSA data.

There has not been as much mathematical modeling work performed on immunotherapy in the context of prostate cancer, however. Kronik *et al.* (2010) investigated the interaction of prostate cancer with dendritic cell vaccines, using patient data, but they only considered one population of prostate tumor cells, and did not incorporate hormone therapy into their treatment model.

5.2.5 Our Model

Our model is heavily influenced by the Portz *et. al* model, but we incorporate several key changes which allow more realistic assumptions. Their results showed that combining dendritic cell vaccines with the hormone treatment was able to delay the onset of castration-resistant prostate cancer, however they did not consider altering the dosing schedule or amounts of dendritic cell vaccine injections. A previous

mathematical model showed very different dynamics, including increase to AI relapse by changing the dosages and frequencies of the dendritic cell vaccine (Kronik *et al.*, 2010).

We still consider six populations: androgen-dependent cancer cells (X_1), androgen independent cancer cells (X_2), activated T cells (T), concentration of cytokines (I_L), concentration of androgen (A), and number of dendritic cells (D). However, in our case now, we consider logistic growth functions, instead of exponential growth. Additionally, we generalize many of the functions for interactive terms. Finally, we consider that androgen-independent cells may mutate back to using more androgen if there is an androgen-rich environment, similar to Hirata *et al.* (2010a).

$$\frac{dX_1}{dt} = \underbrace{r_1(A, X_1, X_2)X_1}_{\text{growth and death}} - \underbrace{m_1(A)X_1}_{\text{mutation to AI}} + \underbrace{m_2(A)X_2}_{\text{mutation from AI}} - X_1 \underbrace{f_1(X_1, X_2, T)}_{\text{death by T cell}}, \quad (5.14)$$

$$\frac{dX_2}{dt} = \underbrace{r_2(X_1, X_2)X_2}_{\text{growth and death}} + \underbrace{m_1(A)X_1}_{\text{mutation from AD}} - \underbrace{m_2(A)X_2}_{\text{mutation to AD}} - X_2 \underbrace{f_2(X_1, X_2, T)}_{\text{death by T cell}}, \quad (5.15)$$

$$\frac{dT}{dt} = \underbrace{\frac{eD}{g+D}}_{\text{activation of T cell by DC}} - \underbrace{\mu T}_{\text{death}} + \underbrace{Tf_3(I_L, T)}_{\text{activation of T cell by cytokines}}, \quad (5.16)$$

$$\frac{dI_L}{dt} = \underbrace{Tf_4(X_1, X_2)}_{\text{secretion by tumor}} - \underbrace{\omega I_L}_{\text{degradation}}, \quad (5.17)$$

$$\frac{dA}{dt} = \underbrace{\gamma(a_0 - A)}_{\text{homeostasis of androgen}} - \underbrace{\gamma a_0 u(t)}_{\text{depletion of androgen if on therapy}}, \quad (5.18)$$

$$\frac{dD}{dt} = - \underbrace{cD}_{\text{death}}. \quad (5.19)$$

The population of androgen-dependent cancer cells is influenced by a growth and death term, mutation to and from androgen-independent cancer cell populations, and a death due to interactions with T cells. The population of androgen-independent cancer cells has a different growth and death term, can also mutate to and from androgen-dependent cancer cell population, and can also be killed by interacting with

T cells. The T cell population is activated by the antigen-presenting dendritic cells as well as the cytokines, and dies linearly. The concentration of IL-2 has a natural degradation term but is produced by the tumor and T cell interactions. The androgen concentration depends on whether the patient is on treatment or off treatment, but has a homeostatic term as well as a depletion term if patient is on therapy. Finally, the dendritic cells naturally die.

The modeling of the IAD is exhibited in the interaction of $u(t)$, the switch from on-treatment to off-treatment, which depends on the patient's PSA levels, $y(t)$. We note that $u(t)$ governs the androgen deprivation therapy: when $u(t) = 1$ treatment is on, when $u(t) = 0$, treatment is off. We also use the PSA levels to govern whether or not the patient is put on or off treatment. Following the work of Swanson, we model the PSA as simple linear relationship for the number of tumor cells. When the PSA levels decrease below a certain threshold, labeled L_0 , the patient is taken off androgen deprivation therapy. When the PSA levels rise above a certain threshold, L_1 , the patient is put back on treatment. Note that we require $L_0 < L_1$. The equations governing treatment and PSA are those listed in Ideta *et al.* (2008), Equations (5.7).

Our model differs from Portz and Kuang (2012) in three major ways: the inclusion of more realistic growth and death terms, mutation from not only AD to AI but also from AI to AD, and generalizing the immune system interaction functions. Each of these changes, and their reasoning, are explained below.

In the original model by Portz and Kuang (2012), the growth for AD cells only depended on androgen concentration, not current amount of cancer cells and the growth for AI cells is exponential. In order to consider a more realistic growth function, we assume both AD and AI cancer cells grow logistically. The growth of AD cells is also dependent on the current level of androgen: if there is a_0 , the homeostatic level of androgen, the AD cells grow at their intrinsic growth rate r_1 . As the level of androgen

decreases, it represents a corresponding decrease in how fast the AD cell population is growing, finally reaching no growth in the absence of androgen ($A = 0$). The growth of AI cells is very much the same, although it always grows at its intrinsic growth rate r_2 , not being scaled by androgen levels. We also assume that not only does the absence of androgen effect the growth, but that it actively kills and AD cells (the second term below). This assumption is realistic and has been used in other prostate cancer models (Eikenberry *et al.*, 2010; Jain *et al.*, 2011). We have no extra death when we are at homeostatic levels, but as the androgen level decreases, we start having active cell death due to lack of androgen. In the absence of androgen, the growth rate of the androgen dependent cells is negative, which represents natural death due to lack of androgen. As the amount of androgen increases, the growth rate also increase, resulting in it's maximum when $A = a_0$. The equations are displayed below:

$$\begin{aligned} r_1(A, X_1, X_2) &= r_1 A X_1 \left(1 - \frac{X_1 + X_2}{K} \right) - d_1(a_0 - A) \\ r_2(X_1, X_2) &= r_2 X_2 \left(1 - \frac{X_1 + X_2}{K} \right) \end{aligned} \tag{5.20}$$

The next main change is the mutation functions: the original model only had mutation from AD cells to AI cells in an androgen-depleted environment. It is, however, a reasonable assumption that in the case of an an androgen-rich environment, cells might mutated from AI to AD. Hirata *et al.* (2010a) also included an assumption that there is a ‘reversible’ phenotype, which may be able to mutate back into AD cells. We assume that when $A = a_0$, there is no mutation from androgen dependent to androgen independent because there is no need for the mutation to occur, as androgen is abundant. As A decreases, the rate at which the cells mutate grows higher and higher. On the other hand, for mutation from androgen independent to androgen dependent, we assume that if there is no androgen, $A = 0$, we will not have any mutation from androgen independent to androgen dependent. However, as we increase

the amount of androgen present, the rate at which androgen independent cells mutate to androgen dependent cells increases as well. The mutation functions are listed below.

$$\begin{aligned} m_1(A) &= m_1 \left(1 - \frac{A}{a_0} \right) \\ m_2(A) &= m_2 \left(\frac{A}{A + k_4} \right) \end{aligned} \tag{5.21}$$

Our last deviation concerns the immune system interactions. As there is little published about the immune system and the fact that the immune system is a vast network of many interacting cells, we are at a loss as to how to form a hypothesis on how the components of the immune system interact with our cancer cells and with each other. Due to this fact, we use generic functions so that if, in the future, more information becomes available, our theoretical results will still hold. Although we lack knowledge about the specifics of these interactions, we do assume that these functions have certain properties which we would expect. Firstly, we assume that if the cell populations and concentrations are non-negative, the functions will also be non-negative. Additionally, if the populations and concentrations are zero, the function will also be zero: $f_1(X_1, X_2, 0) = f_2(X_1, X_2, 0) = f_3(0, T) = f_4(0, 0) = 0$. We also assume that the functions are monotonic and have the following partial derivative conditions: $\frac{\partial f_1}{\partial X_1} \leq 0$, $\frac{\partial f_2}{\partial X_2} \leq 0$, and $\frac{\partial f_1}{\partial T} \geq 0$, $\frac{\partial f_2}{\partial T} \geq 0$. Similarly, we have $\frac{\partial f_3}{\partial I_L} \geq 0$, $\frac{\partial f_4}{\partial X_i} \geq 0$. These assumptions allow for many classes of functions traditionally used in mathematical biology models such as mass action and Holling type I, II, and III functions.

With this proposed model, we have both biological and mathematical questions that need to be answered. We want to quantify how timing of the dendritic cell vaccine dose effects the time to independent relapse. Since dendritic cell vaccines are currently used to treat cancers which no longer respond to hormone therapy, we want to ask if they could be effective in treating cancers which are not androgen

independent. Mathematical questions we would like to know include whether we can determine an optimal dosing quantity to stabilize or eradicate the disease. We would also like to determine global stability conditions mathematically, and then translate those conditions back into biological meaning.

5.3 Simulation Results

Although we can perform analysis on the system with generalized functions, in order for simulation, we must choose our f_i functions. We propose the following functions:

$$\begin{aligned} f_1(X_1, X_2, T) &= \frac{e_1 T}{g_1 + X_1 + X_2} \\ f_2(X_1, X_2, T) &= \frac{e_2 T}{g_2 + X_1 + X_2} \\ f_3(I_L, T) &= \frac{e_3 I_L}{g_3 + I_L} \\ f_4(X_1, X_2) &= \frac{e_4 (X_1 + X_2)}{g_4 + X_1 + X_2} \end{aligned} \tag{5.22}$$

We note that these functions all satisfy our requirements. There are many parameters, yet we have no data to use to estimate these parameters. Some of the parameters have been estimated in literature or other mathematical papers, so we present a table of parameters values as Table 5.1. In the remaining simulations, we will assume that $e_1 = e_2$: T-cells are just as cytotoxic to AD cells as they are to AI cells. For those which do not have estimates, we study how sensitive these parameters are to the solutions obtained.

P	Biological Meaning	Value	Source
r_1	AD proliferation rate	0.025/day	(Berges <i>et al.</i> , 1995)
β_1	AD cell death rate	0.008/day	(Berges <i>et al.</i> , 1995)
K	cell carrying capacity	11 billion	

k_4	AI to AD mutation half-saturation	1.7	
r_2	AI net cell growth rate	0.006/day	(Berges <i>et al.</i> , 1995)
m_1	maximum mutation rate from AD to AI	0.00005/day	(Ideta <i>et al.</i> , 2008)
m_2	maximum mutation rate from AI to AD	0.00015/day	(Portz <i>et al.</i> , 2012)
a_0	base level androgen concentration	30 ng/ml	(Ideta <i>et al.</i> , 2008)
γ	androgen clearance and production rate	0.08/day	(Ideta <i>et al.</i> , 2008)
ω	cytokine clearance rate	10/day	(Rosenberg and Lotze, 1986)
μ	T cell death rate	0.03//day	(Kirschner and Panetta, 1998)
c	DC death rate	0.14/day	(Lotze and Thomson, 2001)
e_1	max rate T cells kill AD cancer cells	0-1/day	(Kirschner and Panetta, 1998)
g_1	AD cell saturation level for T cell kill rate	10 x 10 ⁹ cells	(Kirschner and Panetta, 1998)
e_2	max rate T cells kill AI cancer cells	0-1/day	(Kirschner and Panetta, 1998)
g_2	AI cell saturation level for T cell kill rate	10 x 10 ⁹ cells	(Kirschner and Panetta, 1998)
e	T cell max activation rate	20 x 10 ⁶ cells/day	(Kirschner and Panetta, 1998)
g	DC saturation level	400 x 10 ⁶ cells	(Small <i>et al.</i> , 2000)

	for T cell activation		
e_3	max clonal expansion rate	0.1245/day	(Kirschner and Panetta, 1998)
g_3	saturation level for T cell clonal expansion	1000 ng/ml	(Kirschner and Panetta, 1998)
e_4	max rate T cells produce IL-2	5×10^{-6} ng/ml/cell/day	(Kirschner and Panetta, 1998)
g_4	saturation level for T cell stimulation	10×10^9 cells	(Kirschner and Panetta, 1998)
D_1	DC vaccine dosage	300×10^6 cells	(Small <i>et al.</i> , 2000)
c_1	AD cell PSA level correlation	1×10^{-9} ng/ml/cell	(Ideta <i>et al.</i> , 2008)
c_2	AI cell PSA level correlation	1×10^{-9} ng/ml/cell	(Ideta <i>et al.</i> , 2008)

Table 5.1: Values of parameters (P), explanations, and cited sources of every parameter used in this mathematical model.

The parameters which do not have literature-estimated values are K , the carrying capacity of the tumor cells and k_4 , the half-saturation of the AI to AD mutation. Our value for K was obtained by a brief calculation, observing that the mean weight of a male prostate is 11 grams (7-16 grams) and the average human cell has a mass of 1 ng. Therefore, we arrive at the carrying capacity of 11 billion cells. As for k_4 , we perform some simulations to see how sensitive the parameter is to change, the results of which are in Figure 5.3.

Now that we have fully explained the model, we can begin the simulations. The

simulations are run using MATLAB, with the basic solver ODE45. We have a constant initial condition, with has an initial androgen dependent cancer cell count of 14 billion cells, androgen independent cancer cell count of 0.1 billion cells, 0 activated T cells, 0 (ng/mL) concentration of cytokines, 30 nmol/mL concentration of androgen, and 0 dendritic cells. We set the thresholds for treatments: $L_0 = 5$ ng/mL turns treatment off, and $L_1 = 15$ ng/mL turns treatment back on. We run simulations out past 4000 days in order to more accurately capture the end behavior.

The code I used was adapted from a previous code written by Travis Portz [?], so I will describe the numerical method rather than include it in the appendices. ODE45 is used to solve the continuous portions of the equation, however there are two discrete components: the intermittent androgen suppression therapy switch, and the dendritic cell vaccine injection. The maximum time length the continuous portion of the code can run is from one dendritic cell vaccine injection to the next. We also use the 'events' function in MATLAB in order to simulate the intermittent androgen suppression therapy. The events function measures $\text{PSA} - L_0$ if treatment is on ($u = 1$) and $\text{PSA} - L_1$ if treatment is off ($u = 0$). This way, the numerical integration stops if PSA levels drop below L_0 , when the patient should be switched off therapy (changed to $u = 0$), and starts therapy if PSA levels rise above L_1 , ($u = 1$). The numerical integration is then continued until it reaches the next discrete injection of dendritic cells (stopping to switch on or off androgen deprivation therapy as needed). At this point, only the value of the D (dendritic cell) equation is changed, to the number of cells (in billions) injected in each therapy session.

5.3.1 Discrete Injections

In the previous iteration of this immunotherapy model, the hypothetical vaccines were administered every 30 days with a total dosage of 0.3 billion dendritic cells. In

order to test accurately the effect timing has, we must keep the total dosage over a period of time constant, while varying how often and what dosage they receive at each injection. We considered timing ranging from daily injections to once every 120 days.

Figure 5.1 displays the PSA levels, androgen dependent (X_1), and androgen independent (X_2) cell populations through time. From the graph, we can see that the more frequent injections result in a much longer delay of onset for AI relapse, although all of these still result in eventual AI relapse.

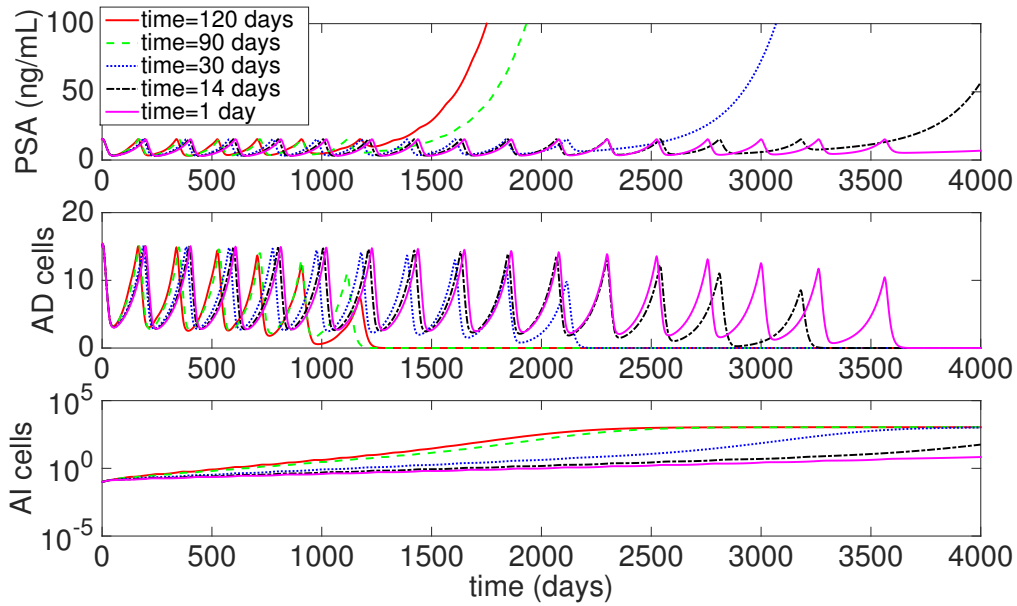


Figure 5.1: PSA serum concentration, androgen dependent (AD), and androgen independent (AI) cell concentrations for various dendritic cell vaccine injection times with an $e_1 = e_2$ of 0.75. More frequent injections result in delay of the rise of fatal androgen independent cancer.

We would like to better understand this relationship, so we turn to our personalized parameters e_1 and e_2 . We recall that these are a measure of cytotoxicity of the T-cells and represent how many cancer cells a T-cell can kill in one day. This is a personalized parameter that may vary person to person, which is why a range (0-1) is given in

Table 5.1. We investigate the minimal e_1 value for which we have stable disease cycles – limit cycles – for the various vaccine timings, shown in Figure 5.2. These limit cycles are determined numerically.

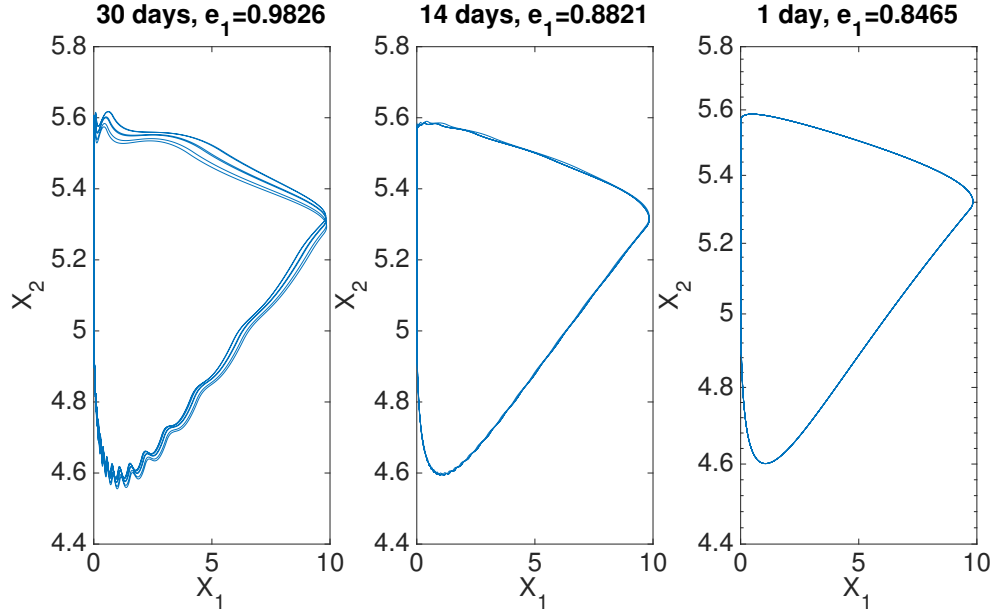


Figure 5.2: Limit cycle solutions for androgen dependent X_1 , and androgen independent X_2 cancer cells. The minimal value of $e_1 = e_2$ required to produce limit cycle behavior is noted above each solution. As vaccine timing decreases, minimal $e_1 = e_2$ necessary to have stable disease state decreases.

We can see that the minimal value of $e_1 = e_2$ necessary to obtain steady disease cycling decreases the more frequently we inject the dendritic cell vaccine. Lower, more frequent doses of dendritic cell vaccines appear to be more effective at delaying the rise to castration-resistant prostate cancer. In Figure 5.2, we notice that only for injections which are monthly, or more frequent, do we observe limit cycle behavior. For less frequent injections, we would need an $e_1 = e_2$ value larger than literature suggests in order to obtain disease cycles. Thus, more frequent injections of smaller doses appears to increase efficacy of treatment.

We also examine the sensitivity of the solutions with respect to k_4 , a parameter that has no previous literature assumptions. Figure 5.3 shows solution trajectories for various values of k_4 . It is apparent that although we vary k_4 from our nominal value by two orders of magnitude in either direction, the outcome of the solution remains very similar. In conclusion, solutions are insensitive to values of k_4 , so we are content with the value given in Table 5.1.

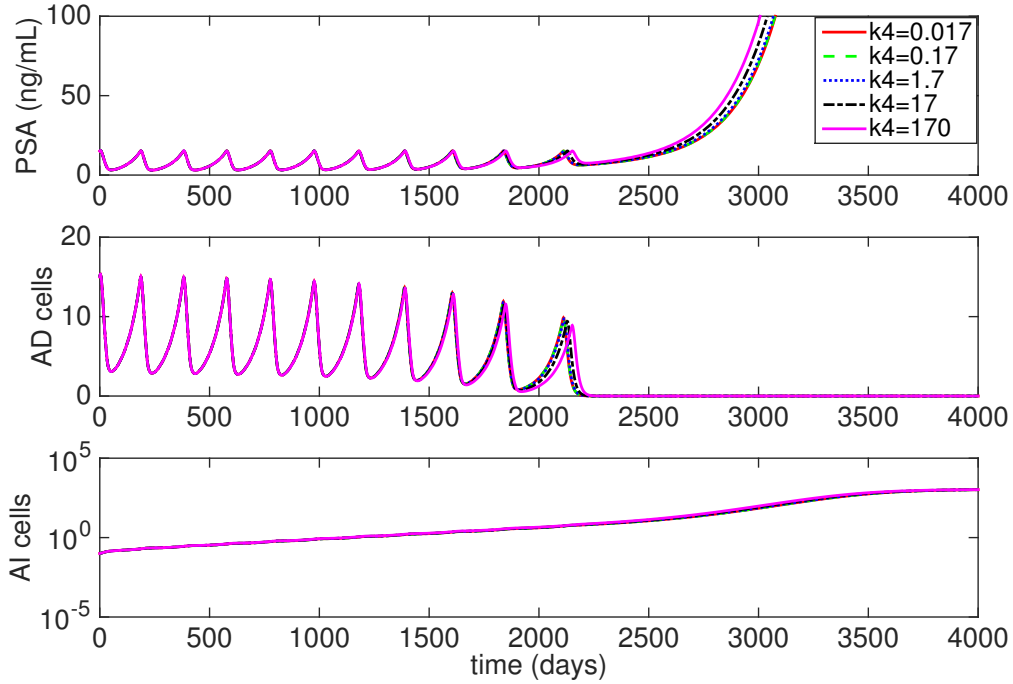


Figure 5.3: PSA serum concentration, androgen dependent (AD), and androgen independent (AI) cell concentrations for various values of k_4 . We can see that the solutions are not very sensitive to large changes in k_4

5.3.2 Continual Injections

Keeping the total amount of vaccine constant, we notice that more frequent dendritic cell vaccine administrations are more effective at delaying the onset of AI

relapse. Now we consider the natural extension of this model: continuous injections. We propose that the patient is always connected to the vaccine through an IV system. In order to allow for this in our model, we slightly modify the equation representing dendritic cell population to:

$$\frac{dD}{dt} = v - cD \quad (5.23)$$

where v is the continual injection rate.

We are interested ultimately in how the continual injection rate influences the emergence of AI prostate cancer. We keep our injection rate constant, but vary the parameter e_1 , which can be thought of as the cytotoxicity of the T cell, or rather, how many tumor cells a T cell is able to kill on average per day. We have a biologically relevant range for e_1 , from 0 to 1, but it is reasonable to assume this is a somewhat personalized parameter. We can see in Figure 5.4, that for the wide range of biologically relevant values of e_1 , we are able to observe all different outcomes of the disease. For small values of e_1 , around 0, we observe that the AI relapse occurs. For slightly larger values of e_1 , we see that there is stable cyclical disease. As we increase e_1 , the cycles become longer, meaning more off-treatment time for the patient. Finally, if we have high enough e_1 values, we observe eradication of the disease.

We see an interesting switch in dynamics as we increase through $e_1 = e_2$, so we create a bifurcation diagram for the parameter, shown in Figure 5.5. When we have $e_1 = 0$, the carrying capacity equilibrium is stable, for small values of e_1 we have stable disease cycles, and for larger values of e_1 , those cycles collapse into a single steady state solution which is stable. For the highest values of e_1 , this steady state solution becomes zero, representing the disease-free equilibrium. For smaller values of e_1 we also see jagged solutions, which we think may be due to long transition times before approaching the limiting periodic state. We also would like to examine $e_1 \approx 0.24$ in more detail, as it appears the solution may have a steady state before switching into

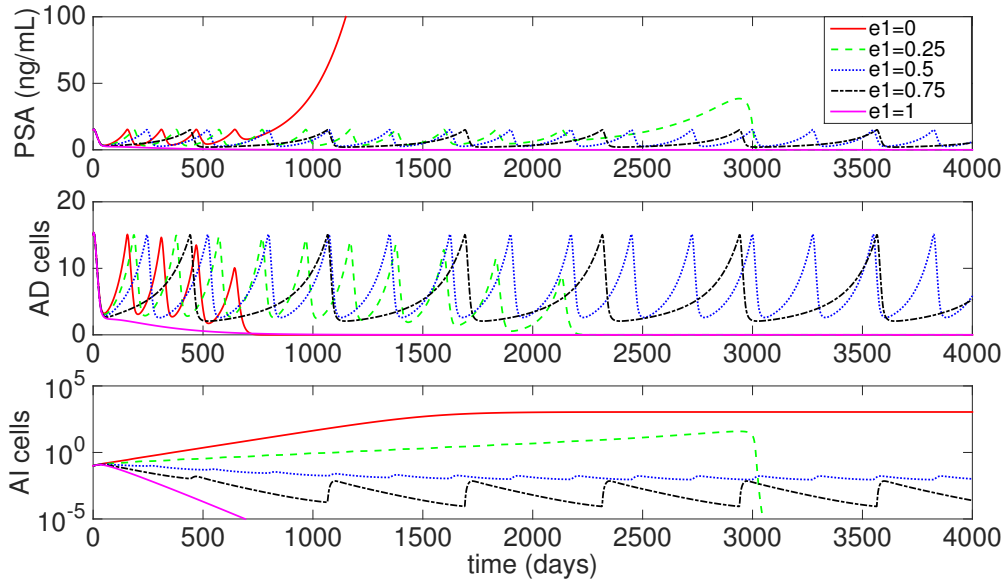


Figure 5.4: PSA serum concentration, androgen dependent (AD), and androgen independent (AI) cell concentrations for various values of e_1 . We see stable disease cycles, disease eradication, and disease growth.

cyclical behavior.

Our model is unique in that we allow mutations back from androgen independent to androgen dependent. It is a logical assumption that although some cancer cells mutate to function without androgen, when androgen is resupplied, they may go back to using androgen to grow. As this is a parameter which there is no accepted literature value, we can examine the effect in more detail. We vary m_2 widely, because we assume that androgen independent cells did originate from androgen dependent cells, meaning that it is possible for $m_2 \gg m_1$. Figure 5.6 displays the results of this analysis of varying m_2 with respect to the value of m_1 . We examine how the PSA levels, AD cells, and AI cells change when examining our shift through m_2 . For these simulations, we assume $e_1 = 0.05$, which is before stable limit cycles emerge, rather the behavior is almost chaotic, hence why these simulation have a long run-time. As

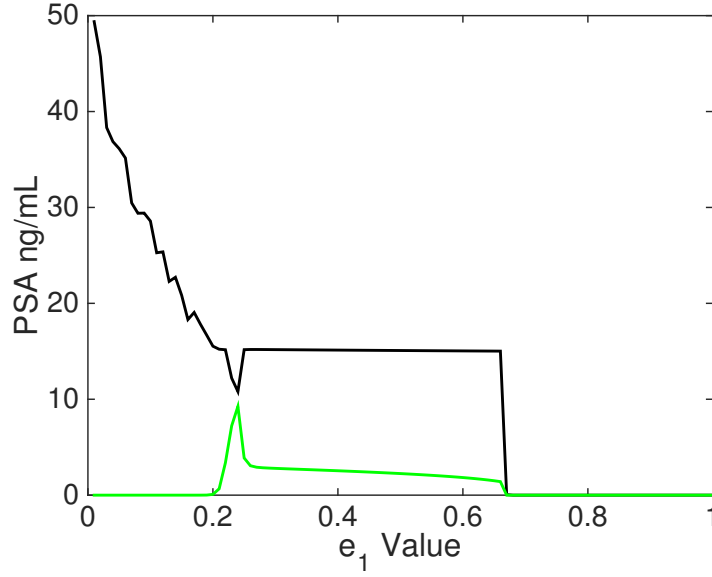


Figure 5.5: Bifurcation digram for parameter e_1 , a measure of cytotoxicity of T-cells. Maximum PSA level in black, minimum PSA level in green. Carrying capacity is stable only for $e_1 = 0$. Immediately after, we have a what appears to be a Hopf bifurcation and limit cycles, until we reach $e_1 \approx 0.66$, after which the disease-free steady state is stable.

we increase to large enough m_2 , we can see that we are able to stabilize our cyclical behavior – a trend which holds for larger values of m_2 as well. Although the value of m_2 does not drastically alter the outcome of the simulations, we do notice slight differences in terms of the cycles of on-treatment off-treatment.

We notice that as we increase the ability for an androgen independent cancer cell to become androgen dependent, m_2 , we elongate the time until androgen independent relapse occurs. For small values of m_2 , on the order of m_1 , we do not notice any improvement. However, when $m_2 \gg m_1$, we can see that androgen independent cancer does not arise.

It also appears that m_2 is only sensitive for some values of e_1 : when we have

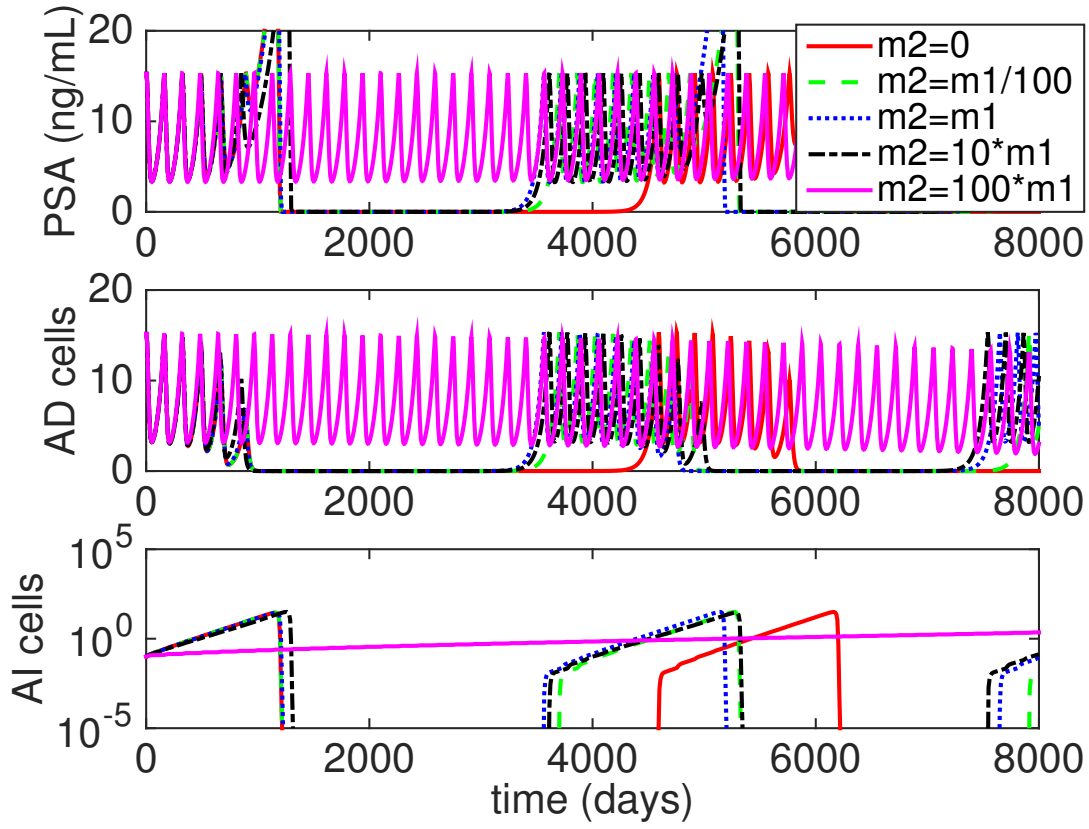


Figure 5.6: Concentration of PSA levels, AD cell density, and AI cell density in blood for varying values of m_2 , mutation from androgen independent back to androgen dependent. High values of m_2 prevent androgen independent cancer.

stable limit cycles or stable disease-free steady-states, m_2 does not appear to affect the solution trajectories much. When we have stable disease-free steady state, the only difference is that for large values of m_2 , time to elimination of cancer is slightly quicker.

5.3.3 Chaotic Regime

During a small subset of parameter values, we observe what appears to be chaotic behavior. When $e_1 = e_2 < 0.25$, before the stable limit cycles emerge, what appears

to be chaotic behavior emerges. In fact, for certain values of e_1 , near 0.2, we observe what appears to be chaotic behavior, where the PSA is fluctuating, but without any discernible pattern. In order to determine if this is indeed chaotic behavior, we use the 0-1 chaos test proposed in Gottwald and Melbourne (2004).

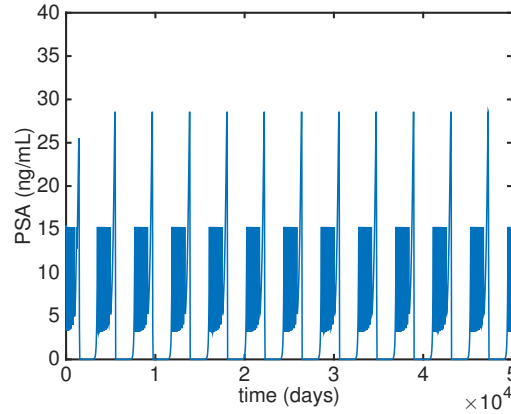


Figure 5.7: Concentration of PSA levels for $e_1 = 0.1$. The solutions appear somewhat erratic and chaotic.

In order to test ensure that I am applying the 0-1 chaos test correctly, I look at the value of $e_1 = 0.5$, which has periodic solutions. Using the 0-1 test, I get a value of 0.06420, signifying that the results are not chaotic. Using this same methodology, we investigate the 0-1 chaos test for $e_1 = 0.1$, the solution which is shown in Figure 5.7. We obtain values close to 1 (0.9901), which signifies chaotic behavior.

5.4 Analytical Results

It is necessary to study the basic properties of this system. As informative as simulations can be, it is imperative to fully understand the system. Simulations can sometimes be misleading, so we would like to analyze the properties that ensure stability of the disease-free and diseased steady states. For our analytical results, we

will assume that we are constantly suppressing androgen, so the treatment is always on. This assumption changes our equation governing the concentration of androgen, so the full analyzable system becomes:

$$\frac{dX_1}{dt} = r_1(A, X_1, X_2)X_1 - m_1(A)X_1 + m_2(A)X_2 - X_1f_1(X_1, X_2, T), \quad (5.24)$$

$$\frac{dX_2}{dt} = r_2(X_1, X_2)X_2 + m_1(A)X_1 - m_2(A)X_2 - X_2f_2(X_1, X_2, T), \quad (5.25)$$

$$\frac{dT}{dt} = \frac{eD}{g + D} - \mu T + Tf_3(I_L, T), \quad (5.26)$$

$$\frac{dI_L}{dt} = Tf_4(X_1, X_2) - \omega I_L, \quad (5.27)$$

$$\frac{dA}{dt} = -\gamma A, \quad (5.28)$$

$$\frac{dD}{dt} = v - cD. \quad (5.29)$$

5.4.1 Full System

We start with basic properties of the full system before we prove any results for local stability.

Proposition 2. *Solutions of (5.24)-(5.29) that start positive remain positive.*

Proof. Solutions for D and A can be explicitly solved for, and it is clear that these solutions will remain positive when the initial conditions are positive. If the solutions for the remaining parameters do not remain positive, there must be some time $t_1 > 0$ such that $X_1(t_1) = 0$, $X_2(t_1) = 0$, $T(t_1) = 0$ or $I_L(t_1) = 0$. We examine the first case where $X_1(t_1) = 0$. Then $X_1'(t) \geq -m(A)X_1$, $\forall t \in [0, t_1]$, or more specifically, $X_1(t_1) \geq X_1(0)e^{-m(A)t_1} > 0$, which is a contradiction. Similar arguments can be extended to the remaining variables, ensuring positivity of all solutions.

□

We would also like to analyze the existence of equilibria and their corresponding stability from the system. By examining the conditions necessary for stability, and their biological translation, we hope to understand underlying biological processes which affect prostate cancer.

Proposition 3. *The model system (5.24)-(5.29) has a disease-free equilibrium $E_0^* = (0, 0, \frac{ev}{\mu(cg+v)}, 0, 0, \frac{v}{c})$ with growth functions (5.20), mutation functions (5.21), and generic functions. E_0^* is unstable if $r_2 > f_2(0, 0, T_0^*)$, where $T_0^* = \frac{ev}{\mu(cg+v)}$, and locally asymptotically stable if $r_2 \leq f_2(0, 0, T_0^*)$. When $r_2 > f_2(0, 0, T_0^*)$, a positive endemic equilibrium $E^* = (X_1^*, X_2^*, T^*, I_L^*, A^*, D^*)$ emerges, stability unknown.*

Proof. All variables except X_2 are easily solved and have only one steady state. For X_2 , we can either have $X_2^* = 0$, which exists always, or an X_2 that solves $g(X_2) := r_2 - \frac{r_2 X_2}{K} - f_2(0, X_2, T^*) = 0$. We examine this quantity in more detail by calculating its sign at $X_2 = 0$:

$$g(0) = r_2 - f_2(0, 0, T_0^*)$$

where $T_0^* = \frac{ev}{\mu(cg+v)}$ is the corresponding T^* value when $X_2^* = 0$. Next we calculate the sign as $X_2 \rightarrow K$, its maximum possible value:

$$g(K) = -f_2(0, K, T_K^*) < 0$$

Thus, if $r_2 - f_2(0, 0, T_0^*) < 0$, there is no biologically relevant X_2 value that solves $g(X_2^*) = 0$, since the function f_2 is monotonically decreasing in X_2 . Therefore, there is only the trivial, disease-free equilibrium $E_0^* = (0, 0, \frac{ev}{\mu(cg+v)}, 0, 0, \frac{v}{c})$ in our domain.

On the other hand, if $r_2 - f_2(0, 0, T_0^*) > 0$ then, by the Intermediate Value Theorem, there must be some $X_2^* \in (0, K)$ which solves $g(X_2^*) = 0$, giving us an

endemic equilibrium $E_1^* = (0, X_2^*, T_1^*, I_{L1}^*, 0, \frac{v}{c})$, representing androgen-independent relapse.

We would now like to examine the stability of the disease-free steady state, which is exhibited in the Jacobian. For E_0^* , we have the following Jacobian matrix:

$$\begin{pmatrix} -d_1 a_0 - m_1 - f_1(0, 0, T^*) & 0 & 0 & 0 & 0 & 0 \\ m_1 & r_2 - f_2(0, 0, T^*) & 0 & 0 & 0 & 0 \\ 0 & 0 & -\mu & T^* \frac{\partial}{\partial I_L} f_3(0, T^*) & 0 & \frac{ceg}{cg+v} \\ T^* \frac{\partial}{\partial X_1} f_4(0, 0) & T^* \frac{\partial}{\partial X_2} f_4(0, 0) & 0 & -\omega & 0 & 0 \\ 0 & 0 & 0 & 0 & -\gamma & 0 \\ 0 & 0 & 0 & 0 & 0 & -c \end{pmatrix}$$

The eigenvalues of this matrix are along the diagonal and are given by $(-d_1 a_0 - m_1 - f_1(0, 0, T^*), r_2 - f_2(0, 0, T^*), -\mu, -\omega, -\gamma, -c)$. Since parameters are positive and given our constraints on our generic functions, all eigenvalues are negative except $\lambda_2 = r_2 - f_2(0, 0, T^*)$. In order for local stability, we require $r_2 < f_2(0, 0, T^*)$. For an unstable equilibrium we would have the opposite: $r_2 > f_2(0, 0, T^*)$ $\square$

If we examine what our condition for local stability entails – $r_2 < f_2(0, 0, T^*)$ – we can see that it boils down to the intrinsic growth rate of AI cells must be less than the death rate due to T-cells. This is inherently sensible: if the AI cells are being killed faster than they are reproducing, we would expect the AI cells to die out.

Since our generic functions are monotone, we can take the inverse of f_2 in order

to find an equivalent condition in terms of T^* . If we recall that $T^* = \frac{ev}{\mu(cg+v)}$, this means we can solve directly for a condition based on v , the injection rate. If we can measure all other parameters, some of which may be personalized, we could concoct a dosage in order to have local stability of the cancer-free equilibrium. If we examine the function given in Equation (5.22), we can directly solve our condition for stability to become $v > \frac{cgg_2r_2\mu}{e_2e-g_2r_2\mu}$, which is the critical dosage value.

5.4.2 Reduced System

Global analysis is extremely difficult to perform on large systems of ordinary differential equations, so we consider the limiting system. We create the quasi-steady state system since the dynamics of concentrations such as androgen, cytokines, and dendritic cells, operate on much faster time scales. We use Thieme (1992) to assert that the asymptotic behavior of the limiting system is identical to the full system.

Again, we assume continual androgen deprivation therapy, and let A, D, I_L approach quasi-steady state to end up with the following equations:

$$\frac{dX_1}{dt} = -d_1a_0X_1 - m_1X_1 - X_1f_1(X_1, X_2, T), \quad (5.30)$$

$$\frac{dX_2}{dt} = r_2X_2 \left(1 - \frac{X_1 + X_2}{K}\right) + m_1X_1 - X_2f_2(X_1, X_2, T), \quad (5.31)$$

$$\frac{dT}{dt} = \frac{eD}{g + D} - \mu T + Tf_3(X_1, X_2, T), \quad (5.32)$$

$$I_L = \frac{Tf_4(X_1, X_2)}{\omega}, \quad (5.33)$$

$$A = 0, \quad (5.34)$$

$$D = \frac{v}{c}. \quad (5.35)$$

Further, since all parameters and functions are non-negative, it is apparent that,

in the limit, $\lim_{t \rightarrow \infty} X_1(t) = 0$, as seen below:

$$\begin{aligned} \frac{dX_1}{dt} &= X_1 [-d_1 a_0 - m_1 - f_1(X_1, X_2, T)] \\ &\leq X_1 [-d_1 a_0 - m_1] \\ &\leq -aX_1. \end{aligned} \tag{5.36}$$

Therefore we can reduce the full system of (5.30)-(5.35) to that of two equations on the subdomain $\bar{\Omega} = \{(X_2, T) : X_2 \geq 0, T \geq 0\}$:

$$\begin{aligned} \frac{dX_2}{dt} &= r_2 X_2 \left(1 - \frac{X_2}{K}\right) - X_2 f_2(X_2, T), \\ \frac{dT}{dt} &= \frac{eD}{g + D} - \mu T + T f_3(I_L, T), \end{aligned} \tag{5.37}$$

Here we eliminate X_1 from f_2 for easier notation, since $X_1 = 0$ in the limit.

We would like to ensure, before delving into the analysis of the reduced system, that the dynamics and behavior of the full and reduced system are equivalent. To that end, we ran the full system ((5.30)-(5.35)) and the quasi-steady state subsystem (5.37) for various values of $e_1 = e_2$ and compared the dynamics. In Figure 5.8, we can clearly see that for most values of $e_1 = e_2$, we observe the same dynamics. With the exception of $e_1 = e_2 = 0.15$, there are no discernible differences between any of the dynamics. Now that we have determined that long-term dynamics are very similar for the full system and the quasi-steady state system, we are able to confidently proceed with our analysis.

Theorem 5.4.1. *The disease-free steady state of (5.30)-(5.35) is globally asymptotically stable under the following conditions:*

- i) $\mu > f_3(I_L, T)$,
- ii) $r_2 < f_2(0, 0, T_0^*)$,
- iii) $\frac{\mu^2(cg+v)}{ev} > \frac{\partial}{\partial T} f_3(0, T_0^*)$.

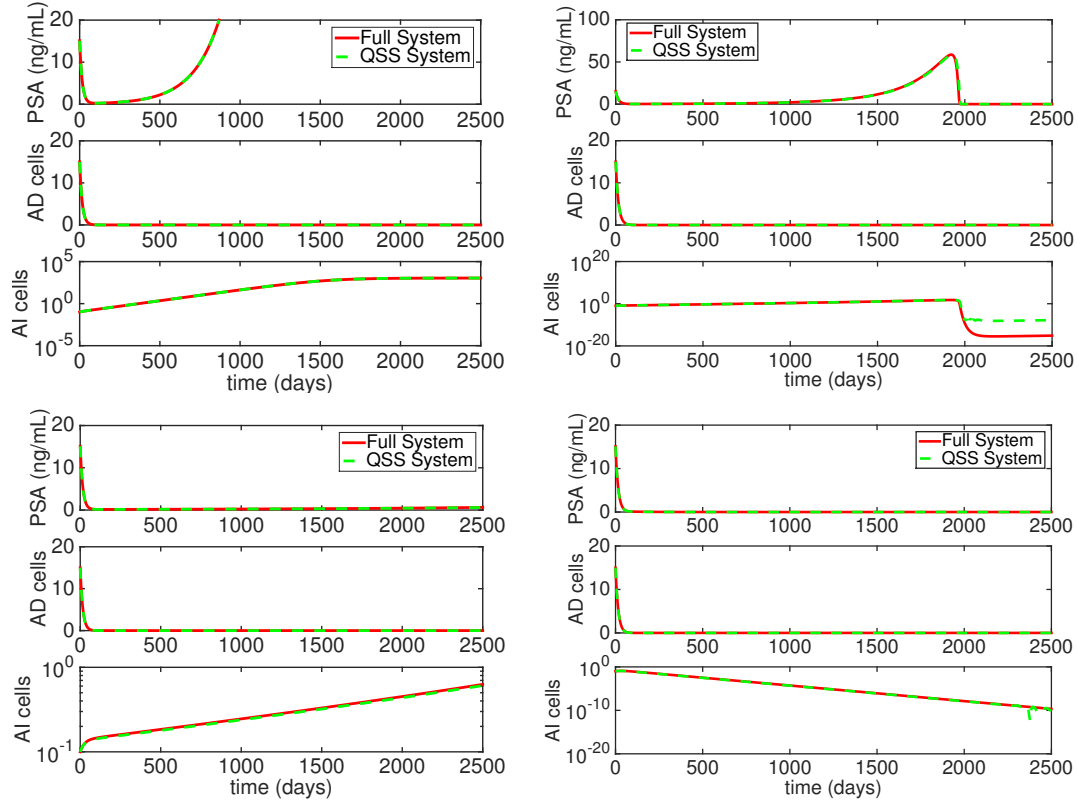


Figure 5.8: Comparisons of the full system and quasi-steady state system for various values of e_1 : 0, 0.15, 0.25, and 0.65, assuming androgen deprivation therapy is constantly on. These values of e_1 show many differing dynamics. The quasi-steady state system closely approximates the full system in every case, but shows slight differences in the case of $e_1 = 0.15$

In order to simply prove the theorem, we will break the proof into several propositions: positivity and boundedness, local stability, and global asymptotic stability. We start with positivity and boundedness:

Proposition 4. *If $\mu > f_3(I_L, T)$ then solutions of (5.30)-(5.35) remain positive and bounded.*

Proof. We begin with the proof for positivity, examining T first. We note that since we are assuming that $T(t_0) \geq 0$, in order for $T(t) < 0$ for some t , we would require that $\frac{dT}{dt} < 0$ when $T = 0$. However, $\frac{dT}{dt}|_{T=0} = \frac{eD}{g+D} > 0$ since all parameters are positive. For X_2 , since we are assuming that $X_2(t_0) \geq 0$, in order for $X_2(t) < 0$ for some t , we would require that $\frac{dX_2}{dt} < 0$ when $X_2 = 0$. However, $\frac{dX_2}{dt}|_{X_2=0} = m_1 X_1 > 0$ since we have already proved that X_1 stays positive and all parameters are positive.

We have already proven that solutions remain nonnegative, so we now look to boundedness. We begin with T :

$$\begin{aligned} \frac{dT}{dt} &= \frac{eD}{g+D} + T(f_3(I_L, T) - \mu) \\ &\leq c - \bar{g}T \end{aligned} \tag{5.38}$$

where $\bar{g} = \min[\mu - f_3(I_L, T)] > 0$ by assumption. This implies that T is bounded.

We look towards the boundedness of X_2 now. We immediately see that X_2 is bounded above by K .

□

Next, we examine local stability of the quasi-steady state system:

Proposition 5. *The limiting system (5.37) contains two equilibria: the disease-free equilibrium and a negative equilibrium, under assumption ii): $r_2 < f_2(0, 0, T_0^*)$. The disease-free equilibrium is locally asymptotically stable under assumption ii) and iii): $\frac{g+D}{eD} > \frac{\partial}{\partial T} f_3(0, T_0^*)$.*

Proof. There are two possible equilibria of the system. Following the proof from Proposition 3: if $r_2 < f_2(0, 0, T_0^*)$, only the disease-free equilibrium exists, $E_0^* = (0, T_0^*)$. We get that $X_2^* = 0$. Correspondingly, we get $T^* = \frac{eD}{\mu(g+D)}$, since $f_3(0, T^*) = 0$ by our assumption for our generalized functions. Thus, we only concern ourselves with the disease-free steady state $E_0^* = (0, \frac{eD}{\mu(g+D)})$, where $D = \frac{v}{c}$.

In order to determine the stability of the disease-free equilibrium, we need to compute the Jacobian evaluated at E_0^* , which is shown below:

$$\begin{pmatrix} r_2 - f_2(0, T^*) & 0 \\ T^* \frac{\partial}{\partial X_2} f_3(0, T^*) & -\mu + T^* \frac{\partial}{\partial T} f_3(0, T^*) \end{pmatrix}$$

which has eigenvalues along the diagonal, $\lambda_1 = r_2 - f_2(0, T^*)$ and $\lambda_2 = -\mu + T^* \frac{\partial}{\partial T} f_3(0, T^*)$. We see that our conditions (ii) and (iii) from our theorem force these eigenvalues to be negative, showing local stability for the disease-free equilibrium. $\square$

Now we are ready to prove the main result of our theorem, stating the disease-free equilibrium is globally asymptotically stable under our conditions.

Proof of Theorem 5.4.1. Our physically relevant equilibrium is a boundary equilibrium and since solutions to the system are positive, we conclude that there can be no limit cycles in our biologically relevant range ($X_2 \geq 0, T \geq 0$). Since solutions are positive, bounded, and contain no limit cycles, by Poincare-Bendixson, all solutions tend towards our disease free steady state. E_0^* is globally asymptotically stable. $\square$

Now that we have proven the conditions under which we have global asymptotic stability, we would like to see what these conditions mean when translated back into biological meaning. We look at the conditions and their corresponding meaning below:

- i) $\mu > f_3(I_L, T)$, : The death of T-cells is greater than the production of T cells due to the cytokine IL_2 . This helps keep our T cells at a temperate value.
- ii) $r_2 < f_2(0, T)$, : The intrinsic growth rate of AI cells needs to be smaller than the killing rate of AI cells by T cells. This was a condition also present in the full system and is biologically sensible.
- iii) $\frac{\mu^2(g+D)}{eD} > \frac{\partial}{\partial T} f_3(0, T^*)$: Unsure how to interpret this condition in a biological manner.

In addition to examining the disease-free equilibrium, we would also like to consider the case when we have an endemic equilibrium and the conditions necessary for global stability.

Theorem 5.4.2. *The diseased steady state of (5.30)-(5.35) is globally asymptotically stable under the following conditions:*

- i) $\mu > f_3(I_L, T)$,
- ii) $r_2 > f_2(0, 0, T_0^*)$,
- iii) $\mu - f_3(I_L, T) > -X_2 \frac{\partial}{\partial X_2} f_2(X_1, X_2, T) + T \frac{\partial}{\partial T} f_3(I_L, T) - \frac{r_2 X_2}{K} \forall X_2, T \geq 0$.

As with the previous proof, we will prove the result with the help of several propositions in order to be clear: first we will tackle positivity and boundedness, then local asymptotic stability, followed by the elimination of limit cycles, then we finish with tying everything together. We begin with positivity and boundedness.

Proposition 6. *If condition i) is satisfied, then solutions of (5.30)-(5.35) remain positive and bounded.*

Proof. The proof is identical to that of Proposition 4, assuming condition i). □

We now look to the equilibria and their assorted local stability:

Proposition 7. *The limiting system (5.37) contains two equilibria: the disease-free equilibrium, E_0^* , and a secondary equilibrium, E_1^* . The secondary equilibrium is positive (assuming condition ii). The disease-free equilibrium is a saddle point (under conditions ii) and iii)).*

Proof. The existence of the equilibria follow from Proposition 3, so we know that under condition ii), if $r_2 > f_2(0, T_0^*)$, we will have two biologically relevant equilibria: $E_0^* = (0, T_0^*) = (0, \frac{ev}{\mu(cg+v)})$ and $E_1^* = (X_2^*, T_1^*)$.

We will start by showing stability of the disease-free steady state E_0^* by analyzing the following Jacobian:

$$\begin{pmatrix} r_2 - f_2(0, T_0^*) & 0 \\ T_0^* \frac{\partial}{\partial X_2} f_3(0, T_0^*) & -\mu + T_0^* \frac{\partial}{\partial T} f_3(0, T_0^*) \end{pmatrix}$$

and the eigenvalues are given by $\lambda_1 = r_2 - f_2(0, T_0^*) > 0$ by condition ii) and $\lambda_2 = -\mu + T_0^* \frac{\partial}{\partial T} f_3(0, T_0^*) < 0$, by condition iii). Thus, the disease-free equilibrium is a saddle point.

We now turn towards the stability of the endemic equilibrium $E_1^* = (X_2^*, T_1^*)$, which is determined by the Jacobian:

$$\begin{pmatrix} -\frac{r_2 X_2^*}{K} - X_2^* \frac{\partial}{\partial X_2} f_2(X_2^*, T_1^*) & -X_2^* \frac{\partial}{\partial T} f_2(X_2^*, T_1^*) \\ T_1^* \frac{\partial}{\partial X_2} f_3(I_L^*, T_1^*) & -\mu + T_1^* \frac{\partial}{\partial T} f_3(I_L^*, T_1^*) + f_3(I_L^*, T_1^*) \end{pmatrix}.$$

We look towards the trace τ and determinant Δ to ascertain the stability of E_1^* . In order to have a locally stable E_1^* , we need $\tau < 0, \Delta > 0$. Thus, the trace is given by

$$\tau = -\frac{r_2 X_2^*}{K} - X_2^* \frac{\partial}{\partial X_2} f_2(X_2^*, T_1^*) - \mu + T_1^* \frac{\partial}{\partial T} f_3(I_L^*, T_1^*) + f_3(I_L^*, T_1^*) \quad (5.39)$$

and the determinant is given by

$$\begin{aligned} \Delta = & \left(-\frac{r_2 X_2^*}{K} - X_2^* \frac{\partial}{\partial X_2} f_2(X_2^*, T_1^*) \right) \left(-\mu + f_3(I_L^*, T_1^*) + T_1^* \frac{\partial}{\partial T} f_3(I_L^*, T_1^*) \right) \\ & + \left(X_2^* \frac{\partial}{\partial T} f_2(X_2^*, T_1^*) T_1^* \frac{\partial}{\partial X_2} f_3(I_L^*, T_1^*) \right). \end{aligned} \quad (5.40)$$

In order for E_1^* to be stable we require $\tau < 0, \Delta > 0$. Notice that $\tau < 0$ is given by assuming condition iii). However, we have no idea about the sign of Δ . Given our information of $\tau < 0$, we know that E_1^* is either a saddle point or a stable node/spiral. $\square$

Next we move on to proving the preclusion of period orbits and limit cycles.

Proposition 8. *The limiting system (5.37) has no limit cycles as long as condition iii) is satisfied.*

Proof. To show that there are no periodic orbits, we will be using the Dulac criterion. We set $h(X_2, T) = \frac{1}{X_2}$, and prove that $\Delta = \frac{\partial}{\partial X_2} \left(h(X_2, T) \frac{\partial X_2}{\partial t} \right) + \frac{\partial}{\partial T} \left(h(X_2, T) \frac{\partial T}{\partial t} \right)$ stays positive or stays negative.

$$\begin{aligned} \Delta &= \frac{\partial}{\partial X_2} \left[\frac{1}{X_2} \left(r_2 X_2 \left(1 - \frac{X_2}{K} \right) - X_2 f_2(X_2, T) \right) \right] \\ &\quad + \frac{\partial}{\partial T} \left[\frac{1}{X_2} \left(\frac{eD}{g+D} - \mu T + T f_3(I_L, T) \right) \right] \\ &= \frac{\partial}{\partial X_2} \left[r_2 - \frac{r_2 X_2}{K} - f_2(X_2, T) \right] + \frac{\partial}{\partial T} \left[\frac{eD}{X_2(g+D)} - \frac{\mu T}{X_2} + \frac{T(f_3(I_L, T))}{X_2} \right] \\ &= -\frac{r_2}{K} - \frac{\partial}{\partial X_2} f_2(X_2, T) - \frac{\mu}{X_2} + \frac{f_3(I_L, T)}{X_2} + \frac{T}{X_2} \frac{\partial}{\partial T} f_3(I_L, T) \end{aligned}$$

To ensure that there are no periodic orbits, we must prove that this quantity Δ does not change sign. We begin by re-writing this condition:

$$X_2\Delta = -\frac{r_2X_2}{K} - X_2\frac{\partial}{\partial X_2}f_2(0, X_2, T) - \mu + f_3(I_L, T) + T\frac{\partial}{\partial T}f_3(I_L, T). \quad (5.41)$$

We know that for $X_2, T \geq 0$, $\Delta < 0$ by condition iii). Thus, the Dulac criterion has ensured that we will have no periodic orbits in our domain. $\square$

We are now ready to prove the main result for global asymptotic stability.

Proof of Theorem 5.4.2. By Proposition 8, we can see that there are no limit cycles present. Solutions are positive and bounded, and the model system has two fixed points, one of which is a saddle point. By the Poincare-Bendixson theorem, we know that there are three possibilities: all solutions tends to a fixed point, all solutions tend to a periodic orbit, or there is a homoclinic or heteroclinic orbit which connects our two fixed points. We have ruled out periodic orbits by Dulac criterion using condition iii).

Now we must show there is no homoclinic or heteroclinic orbits connecting our fixed points.

In order to show there is no heteroclinic orbit connecting the two fixed points, we look towards the stable manifold of the saddle point. Looking back towards our Jacobian, we can clearly see that the stable manifold is given by the T -axis

$$\begin{pmatrix} X_2 \\ T \end{pmatrix} = \begin{pmatrix} 0 \\ 1 \end{pmatrix}. \quad (5.42)$$

Due to assumption ii), we notice that near the disease free steady state E_0^* , we have $dX_2(t)/dt > 0$. These observations preclude the existence of a heteroclinic orbit connecting E_0^* and E_1^* .

The fact that $dX_2(t)/dt > 0$ near E_0^* also implies that there is no homoclinic orbit originating from E_0^* . There can not be any homoclinic orbits originating from E_1^* if it is stable. The Dulac criteria also rules out homoclinic orbits originating from E_1^* if it is a saddle.

By Poincare-Bendixson Theorem, the only option remaining is that all solutions of (5.37) converge to E_1^* . Thus, E_1^* is globally asymptotically stable. $\square$

Now that we have proven the conditions under which we have global asymptotic stability, we would like to see what these conditions mean when translated back into biological meaning. We look at the conditions and their corresponding meaning below:

- i) $\mu > f_3(I_L, T)$, : The death of T-cells is greater than the production of T cells due to the cytokine IL_2 . This helps keep our T cells at a temperate value. This showed up as a condition in Theorem 5.4.1 as well.
- ii) $r_2 > f_2(0, 0, T_0^*)$, : The intrinsic growth rate of AI cells needs to be larger than the killing rate of AI cells by T cells. This is a logical assumption considering that we want stability of an equilibrium that has non-zero X_2 values.
- iii) $\mu - f_3(I_L, T) > -X_2 \frac{\partial}{\partial X_2} f_2(X_1, X_2, T) + T \frac{\partial}{\partial T} f_3(I_L, T) - \frac{r_2 X_2}{K} \forall X_2, T \geq 0$.: Unsure how to interpret this condition in a biological manner.

CONCLUSIONS AND FURTHER DIRECTIONS

In accordance with current Arizona State University policy, since portions of my thesis have already been published, or are in the process of being published, I will detail in this conclusion the conclusions of each of these papers and projects. Additionally, I will discuss how these papers have shaped and will shape my future research thoughts and ideas.

6.1 Density-Dependent Diffusion Model of *in vitro* Spheroid Glioblastoma Growth

We derived a one-equation density-dependent diffusion model for *in vitro* glioblastoma tumor growth that was validated by existing experimental data (Stein *et al.*, 2007a). With the density-dependent diffusion model, we were able to capture the behavior of both the proliferating tumor core as well as the invading migratory cells. Since analysis of one equation is much more accessible, this model has the opportunity to simplify glioblastoma tumor modeling. Additionally, a one-population model will simplify and hasten numerical simulations.

The one-population model used phase plane analysis to prove the existence of traveling wave solutions. The minimum traveling wave speed was corroborated by simulations and I proved stability of the traveling waves. Finally, the model incorporated a sensitivity analysis to show that all parameters needed to be optimized. Parameter optimizations were performed to fit the model to the experimental data and compared with the original model proposed by the experimentalists.

The governing equation for the one-population model contains density-dependent diffusion, logistic growth, and taxis which could possibly be due to chemotaxis or

haptotaxis. The chosen density-dependent diffusion function (2.3) ensures that when the cell density is large, random diffusion is small, and when the cell density is small, random diffusion is large, which could possibly be explained by cell-cell adhesion and is observed in experimental data. This function matches the behavior observed, like how the migratory cells move further away from the tumor core at a large speed, while the core remains proliferating and slowly expanding. The logistic term describes the growth of the total number of cells. The taxis term describes the active and directed motility of the tumor cells away from the tumor core, possibly in search of nutrients.

The performed sensitivity analysis indicated that all parameters largely influenced the numerical solutions for the model. We performed a parameter optimization for all parameters of the model and showed that the error for the best fit was approximately half that of the error generated by the two-population model of the experimentalist. This implies that monotone decreasing density-dependent diffusion may better explain the behavior of tumor spheroid cell migration as opposed to cell shedding of the tumor core, as was argued by the experimentalist..

Through wave speed analysis we were able to determine a minimum wave speed and conditions necessary for the existence of traveling wave solutions. I was also able to show that this condition generated stable traveling wave solutions. Numerical simulations indicated that the observable wave speed was extremely similar to the analytic minimum wave speed.

Future studies of glioblastoma growth will focus on comparing this model to *in vivo* data. The model will need to be extended to include brain geometry, and furthermore, comparing the model to *in vivo* data instead of *in vitro* data may result in the need to extend the equations to describe more complex behavior such as tumor cell necrosis, brain tissue type differentiation, and mass effect. Instead of density-dependent diffusion, it may be more appropriate to implement anisotropy through

diffusion tensor imaging (DTI), as has been done in other GBM models ((Jbabdi *et al.*, 2005), (Bondiau *et al.*, 2008), and (Painter and Hillen, 2013)), depending on the availability of experimental data. Another direction is to consider a non-local reaction-diffusion equation instead of density-dependent diffusion with proliferating and dispersing cell groups in which dispersing cells convert proliferating cells into dispersing ones.

Although I was not involved in the actual analysis of determining the minimum wave speed, I did follow along with the mathematics. I also performed the stability of the traveling wave solutions, which ended up being somewhat complicated and very messy. The fact that I was able to be present and suggestive during the process has shaped my future in understanding PDE's. I now feel confident that I can at least start the analysis for traveling wave speeds for non-traditional PDE's, and would feel comfortable examining the stability of those traveling wave solutions..

6.2 2 Population Model of *in vitro* Spheroid Glioblastoma Growth

The ‘Go or Grow’ hypothesis has often been suggested as a means for explaining why GBM are so invasive. The idea is that tumor cells are either going (migrating) or growing (proliferating). However our model suggests that such a distinction is still too simple to explain the underlying dynamics. While the model fits better than a basic reaction-diffusion model, it is on par with the Stein *et al.* (2007a) model. It falls far short, however, as compared to the one-equation density-dependent diffusion model results. This suggests that merely incorporating a mutation switch is insufficient.

We also examined the fits of several models: the reaction-diffusion equation, Stein *et al.* (2007a), our one equation density-dependent diffusion mode, the two-population model, and our two population model assuming density-dependent diffusion. Most of the results were unsurprising: the reaction-diffusion equation generated the worst fit,

followed by the Stein *et al.* (2007a) model, and the two population model. However, we were astonished to see that the one-equation density-dependent diffusion model generated less error than the two equation model which incorporated density-dependent diffusion. This result lends credence to our original hypothesis: diffusion of GBM is governed by the surrounding density. The reason the two-equation density-dependent diffusion does not fit well is that only the migratory cell population includes density-dependent diffusion. Although the ‘go or grow’ hypothesis does describe the data better than the reaction-diffusion equation, it is not the best hypothesis. There is no biological evidence that there are separate phenotypes. Our work and experimental data suggest that the movement of cells is controlled by the cell density: those cells which have a predilection towards proliferation will be in high-density areas and less likely to diffuse, while those cells which tend to migrate will be in lower-density areas and will diffuse at a much higher rate.

We also compared our theoretical minimum wave speed calculations with simulated wave speeds. We discovered bounds for the theoretical minimum wave speed and were able to show good agreement with our calculated minimum wave speed bounds. More work will need to be performed in order to tweak our analysis to obtain a theoretical minimum wave speed.

This project forced me to question the established literature assumptions. As a mathematician early in my career, I constantly feel overwhelmed by the research and information that has already been performed, and am intimidated to question the status quo. Just because a model is able to fit data does not mean that it is an effective or sensible model. This is compounded by the fact that more complicated equations generally involve more parameters, increasing the likelihood of a good fit, regardless of model assumptions. I have now reached the point where I will question the assumptions that I see made in papers as well as whether or not those assumptions

are correct. I will also not make the mistake of thinking that just because a model involves more complications and assumptions, it must be a better model that generates a good fit.

6.3 *in vivo* Murine Model of Glioblastoma Growth

We obtained experimental data from injecting mice with GL261-Luc2 cells to mimic the growth of human GBM. MR images provided information on the location and movement of tumor boundaries, which we used to fit parameters in mathematical models based on a reaction–diffusion equation. We found that the murine tumors did not move symmetrically in general, and that their growth patterns differed considerably between mice; however, they appeared to match the behavior of rat models of GBM as well as those of human patient GBM.

Of course, model (4.1) is only an approximation of the tumor dynamics. It does not account for interactions with the immune system, genetic drift within the tumor cells, or anisotropy in the brain parenchyma, among other factors. Although the model error probably is substantial, with reasonable choices of parameters and a crude inference about the correspondence between tumor visibility on T2W imaging and local cell density, the model nevertheless produces reasonable short-term predictions of the future size and location of the tumor. If, after a future careful examination of all the histology slides derived from each mouse, it proves possible to provide a quantitative correspondence between the local tumor cell density and MR imaging, then more sophisticated data assimilation methods can be applied to infer the distribution of tumor cells in regions beyond the visible tumor boundary (Kotelich *et al.*, 2011).

An improved future modeling framework will incorporate mass effect and spatial heterogeneity of the model parameters. Associated quantitative histology data may make possible (via MR imaging) a reliable statistical correlation between tumor

enhancement and tumor cell densities in and near the visible tumor.

From this project, I learned about the realities of dealing with data. My involvement in this project gave me a sympathetic understanding for the experimentalists who have provided data to me in the past. I had hands-on working experience with transforming the MR images into a computational domain. This long, and often frustrating, process required me to smooth individual images by hand, ensuring that there are uncertainties in brain geometry. I had naively assumed, as one that does not perform experiments, that experimental data is always true. I had not considered these little uncertainties, which occur for even the most experienced experimentalists. Because of this project, I have a greater appreciation and understanding of the sheer difficulties involved in any type of experimental foray. This understanding will continue to influence my work, and I hope to learn more sophisticated methods for coping with these uncertainties in the future.

6.4 Global Dynamics of a Model of Joint Hormone Treatment with Dendritic Cell Vaccines for Prostate Cancer

Current treatment options for late-stage prostate cancer are sub-optimal in terms of survival and quality of life. By examining a model of intermittent hormone therapy coupled with dendritic cell vaccines, we are able to prolong both the life and quality of life of the patient. We found that, keeping total yearly dosages the same, more frequent injections are conducive to managing prostate cancer for a longer period of time. We extrapolate this idea by modifying the model to include a ‘continual’ dosage, as if administered through an intravenous fluid.

In our model, there are several parameters which are patient specific, or do not have accepted literature values. We examined the effect of varying values of e_1 , the killing T-cell efficiency. Predictably, increasing e_1 led from androgen-independent

relapse, to stable limit cyclical behavior, and when increased enough, total eradication of the disease. This parameter measures how effective dendritic cell vaccine therapy will be - if e_1 is small, the therapy will be negligible. e_1 also acts to elongate the cycle times for a stable cyclical disease, which means increased time for the period when the patient is not undergoing androgen deprivation therapy. We performed bifurcation analysis on parameter e_1 to examine the various behaviors that exist in the system. As our simulations only considered the case where $e_1 = e_2$, we could also investigate how the dynamics change when we allow these values to be different. Additionally, we examined m_2 , the rate at which androgen independent cells switch back to androgen dependent cells in an androgen-rich environment, such as when a patient is removed from androgen suppression therapy. We notice that increasing m_2 , the rate at which androgen independent cancer cells may switch back to becoming androgen dependent has the effect of delaying the eventual onset of fatal androgen independent cancer.

Additionally, for mathematical analysis, we simplify the model to have continuous androgen suppression, in order to determine the effect of continual dosages. We notice there are two possible equilibria – cancer-free equilibrium and an androgen-independent (fatal) cancer equilibrium. If our continual dosage is greater than a determined critical value, the cancer-free equilibrium exists and it is stable. If our continual dosage is lower than that critical value, the cancer-free equilibrium is unstable and a cancerous equilibrium is born (stability unknown). These findings have biological significance. In previous papers it has been determined that for a 30-day vaccine, it is necessary for a large values of e_1 to ensure cancer-free progression (Portz and Kuang, 2012). In this analysis, we could have smaller values of e_1 that still resulted in stable disease-free equilibrium. For patients with less effective immune systems (lower e_1 values), it is possible to eradicate cancer with higher presence of dendritic cells. As dendritic cell vaccines are not known to have an adverse effect on the human body, it is possible that

for patients with weakened immune systems, a tailored dose could be administered.

We further examined the limiting cases of behavior for this system, by allowing several parameters to go to quasi-steady state. We were able to determine requirements for global stability – or the guarantee of elimination of prostate cancer in some cases. Additionally, we were able to determine further conditions for the global stability of the endemic equilibrium.

Despite the insights that this model has afforded us, there is still much to be done. Future work may include comparing the model with available patient data. This will allow us to determine the efficacy of this model at predicting behavior of the prostate cancer. We will also be able to explore the patient-specific parameters and the effect these have on final outcome. Additionally, we would like to be able to compare our model with other patient-data validated models. Local stability of the endemic equilibrium of the full system should also be studied in detail. Additionally, we could further investigate how the simulation outcomes are affected by allowing $e_1 \neq e_2$.

This section of my thesis was truly the only chapter that involved much analysis. This project taught me the importance of keeping strong with your mathematical roots, even if it is tempting to only work on projects that involve real data. There is no data available to corroborate my model because this combination of treatments has not yet been studied. This is where the power of mathematics lies: we can fully investigate the expected potency of altering dosing structures without requiring clinical data. We can form hypotheses as to what combination, and in what amounts, can have a strong effect on disease outcome. Of course, I would have loved to test my models with data, and I hope that one day mathematicians can work more closely with physicians, in hopes of predicting patient trial outcomes before they happen.

6.5 The Future of Mathematics in Oncology

Cancer is a very complex process that involves knowledge from the molecular levels up through cellular levels and tissue. Additionally, depending on the *type* of cancer, certain models are more applicable than others. As you examine the work performed in previous chapters, it is apparent that the tie binding my work together is the fact that the models are applied to cancer. The models and methods are markedly different: single PDE, systems of PDE's and systems of ODE's are all incorporated in separate chapters. Additionally, the methods for analyzing these models were very different, depending on what data (if any) was available. The heterogeneity of cancer growth requires us researchers to *think* and to be able to determine which models are most effective for modeling a specific type of cancer and why.

Mathematics will continue to play a large role in oncological understanding and practice. With a public push towards personalized medicine, mathematics may become a more important tool in modeling treatment options. These models can be used by doctors *and* patients, allowing patients to once again regain control of their treatment. Models can bridge the gap between physician and patient: if a physician can show the patient what their tumor is expected to look like in six months if they choose no treatment, treatment A, or treatment B, both can make an effective decision together. Although this point is still very far into the future, the work performed in this thesis aims to assist the future mathematicians who strive to achieve this goal.

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APPENDIX A
CO-AUTHOR PERMISSIONS

I certify that my co-authors, Dr. Tracy L. Stepien and Dr. Yang Kuang have given me permission, in writing, to include all material in my PhD thesis for Chapter 2.

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APPENDIX B

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APPENDIX C
COMPUTER CODE FOR CHAPTER 2

```

1 function [errortot] = x0parest_pdepe_vardiff_later(params,necinfo)
2
3 % XOPAREST_PDEPE_VARDIFF_LATER returns an error estimate from the
4 % Stein et al data compared with the parameters that are input for
5 % density-dependent diffusion
6 %
7 % ERRTOT = XOPAREST_PDEPE_VARDIFF_LATER(PARAMS,NECINFO) solves the
8 % density-dependent diffusion equation
9 %  $u' = d/dx(D1 - D2 * u^n / (a^n + u^n) du/dx) - vi du/dx + g(1 - u/umax)$ ,
10 % for parameters PARAMS given in order (D1, D2, a, n, g, vi), and
11 % necinfo basic settings for simulations -- in order
12 % (a, b, dx, T, dt), where x goes from a to b in with stepsize dx,
13 % final time T, incrementing by dt. The initial condition assumes
14 % day 0 with 210 microns at 95% capacity. The solution is obtained
15 % using pdepe
16
17 % This code requires the user to have 'ic.mat', which is a matrix
18 % of Stein et. al data cell density profile on day 3 (location,
19 % cell count) as well as 'invasiverad3c.mat' whcih is a matrix of
20 % Stein et. al data on invasive radius location (day, location).
21 % Either can be generated by using grabit.
22
23 % Written by Erica Rutter (Sept 2014)
24
25
26 m=0;
27
28 % Parameters
29 D1 = params(1);
30 D2 = params(2);
31 aval = 10^params(3);
32 nval = params(4);
33 g = params(5);
34 vi = params(6);
35 umax = 4.2*10^8;
36
37 % Necessary info to set up simulation
38 a = necinfo(1);
39 b = necinfo(2);
40 dx = necinfo(3);
41 T = necinfo(4);
42 dt = necinfo(5);
43 nt=ceil(1/dt);
44
45 % Ensuring the optimized aparameters are non-negative and D1>D2
46 if(D1<0|abs(D1)<abs(D2)|g<0|vi<0|aval<0|nval<0)
47     errortot=10^15;
48     return
49 end
50
51 x=a:dx:b;
52 t=0:dt:T;
53
54 % loading data taken from Stein et. al paper using grabit
55 load('day0starter.mat') %uniform blob density at 210 microns
56 load('ic.mat') %radius at day 3
57 load('invasiverad3c.mat') %invasive radius from Stein et. al paper

```

```

58
59 invraddata=invasiverad3c(:,2)/10000; % correcting units
60 celldensity3=ic(5:21,:); % radius at day 3 for invasive cells only
61
62 ic(1:4,2)=umax; % ensuring that the middle does not go above umax
63
64 umax=1; % simulations are scaled form 0 to 1
65 sol = pdepe(m,@pdex1pde,@pdex1ic,@pdex1bc,x,t,[],aval,nval,D1,D2,g,
    umax,vi);
66
67 % Extract the first solution component as u.
68 u = sol(:,:,1);
69
70 soln=u*4.2*10^8; % rescaling
71
72 % Find invasive radius front
73 n=length(invraddata);
74 solndata=zeros(n,1);
75 for k =1:n
76     index=max(find(soln(1+nt*(k),:) < 1*10^7,1,'first'),1);
77     solndata(k)=x(index-1);
78 end
79
80 % Find error in density profile at day 3.
81 for j=1:length(ic(:,2))-1
82     index1=find(x < ic(j,1),1,'last');
83     indexs=find(x > ic(j,1),1,'first');
84     if((x(index1)-ic(j,1)) < (x(indexs)-ic(j,1)));
85         index2=index1;
86     else
87         index2=indexs;
88     end
89     if (j < 5)
90         diff(j)=sqrt((ic(j,2)-soln(151,index2))^2/ic(j,2));
91     else
92         diff(j)=sqrt((ic(j,2)-soln(151,index2))^2/ic(j,2));
93         steincomp(j-4)=sqrt((ic(j,2)-soln(151,index2))^2/ic(j,2));
94         % can only compare to stein for migratory cells
95     end
96
97 end
98
99 % caclulate total error
100 errrad=sum(sqrt((invraddata-solndata).^2)./invraddata);
101 errdens=(sum(diff));
102 errortot=1/(length(invraddata)+length(ic(1:21,2))-7)*(errrad+errdens
    );
103
104
105 %calculate the errors for just the migratory cells
106 errdenscomp=(sum(steincomp));
107 errortotviastein=1/(length(invraddata)+length(celldensity3(:,2))-7)
    *(errrad+errdenscomp);
108 errortotviasteinnoscale=(errrad+errdenscomp);
109
110
111 % Calculate Error Generated by Stein et. al mdoel

```

```

112 load('steinsimdensity.mat')
113 load('steinsim3c.mat')
114 errdenstein=(sum(sqrt((steinsimdensity(:,2)-celldensity3(:,2)).^2)
    ./(celldensity3(:,2))));
115 errradstein=(sum((sqrt(((invraddata-steinsim3c(:,2)/10000)).^2)./(
    invraddata))));
116 errortotstein=1/(length(invraddata)+length(celldensity3(:,2))-5)*(
    errradstein+errdenstein);
117
118
119 %% Plotting the graphs
120
121 load('steinsimdensity.mat')
122 load('steinsim3c.mat')
123 figure;
124 figureHandle=gcf;
125 set(gca,'FontSize',20);%,'fontWeight','bold')
126 set(findall(gcf,'type','text'),'FontSize',30);%,'fontWeight','bold')
127 plot(1:7,invraddata,'k+',1:7,steinsim3c(:,2)/10000,'r*',1:7,solndata
    ,'b-','linewidth',3)
128 xlabel('time(days)')
129 ylabel('invasive radius (cm)')
130 xlim([1,7])
131 title('Invasive Radius comparison')
132 legend('Experimental Data','Stein et. al Simulations','Our
    Simulations',2)
133 set(gca,'FontSize',20);%,'fontWeight','bold')
134
135
136
137 figure;
138 figureHandle=gcf;
139 set(gca,'FontSize',20);%,'fontWeight','bold')
140 set(findall(gcf,'type','text'),'FontSize',30);
141 plot(celldensity3(:,1),celldensity3(:,2),'k+',steinsimdensity(:,1)
    /10000,steinsimdensity(:,2),'r*',x,soln(151,:),'b-','linewidth'
    ,3)
142 xlim([0.03,0.1])
143 xlabel('distance from core (cm)')
144 ylabel('cell population')
145 title('Tumor Cell Density Profile at day 3')
146 legend('Experimental Data','Stein et. al Simulations','Our
    Simulations')
147 set(gca,'FontSize',20);%,'fontWeight','bold')
148
149
150
151 figure;
152 set(gca,'FontSize',20);%,'fontWeight','bold')
153 set(findall(gcf,'type','text'),'FontSize',30);
154 plot(ic(:,1),ic(:,2),'k+',steinsimdensity(:,1)/10000,steinsimdensity
    (:,2),'r*',x,soln(151,:),'b-','linewidth',3)
155 %xlim([0.03,0.1])
156 xlabel('distance from core (cm)')
157 ylabel('cell population')
158 title('Tumor Cell Density Profile at day 3')
159 legend('Experimental Data','Stein et. al Simulations','Our

```

```

    Simulations')
160 set(gca,'FontSize',20);%,'fontWeight','bold')
161
162
163 % -----
164 function [c,f,s] = pdex1pde(x,t,u,DuDx,aval,nval,D1,D2,g,umax,vi)
165 c = 1;
166 f = (D1-(D2*u.^nval)./(aval^nval+u.^nval)).*DuDx;
167 s = g*u.*(1-u)-vi*DuDx;
168 % -----
169 function u0 = pdex1ic(x,aval,nval,D1,D2,g,umax,vi)
170 load('day0starter.mat');
171
172 u0=0.95*interp1(day0starter(:,1),day0starter(:,2),x)/(4.2*10^8);
173
174 % -----
175 function [p1,q1,pr,qr] = pdex1bc(xl,ul,xr,ur,t,aval,nval,D1,D2,g,
    umax,vi)
176 p1 = 0;
177 q1 = 1;
178 pr = ur;
179 qr = 0;

```

APPENDIX D
COMPUTER CODE FOR CHAPTER 3

```

1 function [errortot] = updated_error_twoequations_ode_parest(newparam
    ,necinfo)
2
3 % UPDATED_ERROR_TWOEQUATIONS_ODE_PAREST returns an error estimate
4 % from the Stein et al data compared with the parameters that are
5 % input for a 2 population go or grow model.
6 %
7 % ERRTOT = UPDATED_ERROR_TWOEQUATIONS_ODE_PAREST(PARAMS,NECINFO)
8 % solves the 2 population equations
9 %
10 %  $M' = D(d^2M/dx^2) + \epsilon * k * T / (T + K_M) * P - k * K_P / (T + K_P) * M - \mu * M$ 
11 %  $P' = g * P * (1 - T/T_{max}) - \epsilon * k * T / (T + K_M) * P + k * K_P / (T + K_P) * M$ 
12 %
13 % for parameters params given in order (D1,g,k,mu,Km,Kp,epsilon),
14 % and necinfo for basic settings for simulations -- in order
15 % (a, b, dx, T, dt), where x goes from a to b in with stepsize
16 % dx, final time T, incrementing by dt. The initial condition
17 % assumes day 0 with 210 micron spread. The solution is obtained
18 % using Crank-Nicholson for the diffusion term, and forward
19 % differencing for logistic growth and switching
20
21 % Written by Erica Rutter (Nov 2014)
22
23 %% parameters
24 param.Tmax = 4.2*10^8;
25 param.D1 = newparam(1);
26 param.g = newparam(2);
27 param.k = newparam(3);
28 param.mu = newparam(4);
29 param.Km = newparam(5)*param.Tmax;
30 param.Kp = newparam(6)*param.Tmax;
31 param.epsilon = newparam(7);
32 n=1;
33
34 if(param.D1<0|param.g<0|param.k<0|param.mu<0| param.Km <0|param.Kp
    <0|param.epsilon<0)
35     errortot=10^15;
36     return
37 end
38
39 %% initialization parameters
40 a = necinfo(1);
41 b = necinfo(2);
42 dx = necinfo(3);
43 T = necinfo(4);
44 dt = necinfo(5);
45 nx=ceil(b/dx);
46 nt=ceil(1/dt);
47
48
49 % % load data/IC
50 load('day0starter.mat') %uniform blob density at 210 microns
51 load('ic.mat') %radius at day 3
52 load('invasiverad3c.mat')
53
54 %
55 invraddata=invasiverad3c(:,2)/10000; % scaling to cm

```

```

56  celldensity3=ic(5:21,:); % radius at day 3 for invasive radius in
    figures
57  ic(1:4,2)=param.Tmax;
58
59  dim=2;
60  x=a:dx:b;
61  t=0:dt:T;
62  dims=[x;x];
63
64  Uplot=zeros(length(t),length(x),dim);
65  X = length(x);
66
67  % migratory functions
68  mig_diffusion = @(u) param.D1+0.*u;
69  mig_diffusiondiff = @(u) 0.*u;
70  migtoprofswitch=@(up,um) param.k*((param.Kp).^n./((up+um).^n+param.
    Kp^n)).*um;
71  mig_fisher = @(up,um) -param.mu*um;
72
73  advection=@(u) 0*u;
74
75  % proliferating functions
76  prof_diffusion = @(u) 0*u;
77  prof_diffusiondiff= @(u) 0.*u;
78  prof_fisher = @(up,um) param.g*up.*(1-(up+um)/param.Tmax);
79  proftomigswitch=@(up,um) param.epsilon*((up+um).^n./((up+um).^n+
    param.Km^n)).*up;
80
81  % IC and BC functions
82
83
84  prof_IC = @(x) 0.95*interp1(day0starter(:,1),day0starter(:,2),x);
85  mig_IC = @(x) 0*x;
86  leftBC = @(t,u) 0*t
87  rightBC = @(t) 0*t;
88
89  migUprev=feval(mig_IC,x);
90  profUprev=feval(prof_IC,x);
91  Umig=migUprev;
92  Uprof=profUprev;
93
94  migImain_diagonal=zeros(length(x)-2,length(x)-2);
95  migIlower_diagonal=zeros(size(migImain_diagonal));
96  migIupper_diagonal=zeros(size(migImain_diagonal));
97  migImatrix=zeros(size(migImain_diagonal));
98  migEmain_diagonal=zeros(length(x)-2,length(x)-2,2);
99  migElower_diagonal=zeros(size(migImain_diagonal));
100  migEupper_diagonal=zeros(size(migImain_diagonal));
101  migEmatrix=zeros(size(migImain_diagonal));
102
103  Uplot(1,:,1)=profUprev;
104  Uplot(1,:,2)=migUprev;
105
106  %parameters for simulation
107  mu = 0.5; % 0.5 for Crank-Nicholson, 1 for FE, 0 for BE
108  c1 = dt/dx^2;
109  c3 = dt/dx;

```

```

110 soln=zeros(length(t)-1,length(x));
111
112 for m = 1:length(t)
113
114     b1mig = feval(mig_diffusion,Umig);
115     advmig = feval(advection,Umig);
116     fishmig = feval(mig_fisher,Uprof,Umig);
117     switchpopmig=feval(migtoprofswitch,Uprof,Umig);
118
119     b1prof = feval(prof_diffusion,Uprof);
120     advprof = feval(advection,Uprof);
121     fishprof= feval(prof_fisher,Uprof,Umig);
122     switchpopprof=feval(proftomigswitch,Uprof,Umig);
123
124
125     migImain_diagonal=sparse(diag(1+mu*(2*c1*b1mig(2:end-1))));
126     migIlower_diagonal= sparse(diag((-mu*(c1*b1mig(3:end-1))
127     ,-1)));
128     migIupper_diagonal = sparse(diag((-mu*(c1*b1mig(2:end-2))
129     ,1)));
130     migImatrix = sparse(migImain_diagonal + migIupper_diagonal +
131     migIlower_diagonal);
132
133     migEmain_diagonal = sparse(diag(-(1-mu)*(2*c1*b1mig(2:end-1)
134     )-c3*advmig(2:end-1)));
135     migElower_diagonal = sparse(diag(((1-mu)*(c1*b1mig(3:end-1))
136     ),-1)));
137     migEupper_diagonal = sparse(diag(((1-mu)*(c1*b1mig(2:end-2))
138     +c3*advmig(2:end-2)),1)));
139     migEmatrix = sparse(migEmain_diagonal + migEupper_diagonal +
140     migElower_diagonal);
141
142     Umig(2:X-1) = migImatrix \ (migUprev(2:X-1)' + migEmatrix*
143     migUprev(2:X-1)' ...
144     + dt*fishmig(2:X-1)'-dt*switchpopmig(2:X-1)'+dt*
145     switchpopprof(2:X-1)');
146
147     Uprof(2:X-1) = profUprev(2:X-1)'+dt*fishprof(2:X-1)''-dt*
148     switchpopprof(2:X-1)'+dt*switchpopmig(2:X-1)';
149
150     % Ensure boundary conditions
151     Uprof(1)=0;
152     Uprof(end)=0;
153     Umig(1)=0;
154     Umig(end)=0;
155
156     Uplot(m, :, 1)=Uprof;
157     Uplot(m, :, 2)=Umig;
158
159     migUprev=Umig;
160     profUprev=Uprof;
161
162 end
163
164 soln=Uplot(:, :, 1)+Uplot(:, :, 2);
165
166 % %Find invasive radius front

```

```

157 migfront=1*10^7;
158 n=length(invraddata);
159 solndata=zeros(n,1);
160 for k =1:n
161     index=max(find(soln(1+nt*(k),nx:end) < migfront,1,'first'),1);
162     solndata(k)=x(index+nx-1);
163 end
164
165 % %Find density profile at day 3.
166 for j=1:length(ic(:,2))-1
167     indexl=find(x < ic(j,1),1,'last');
168     indexs=find(x > ic(j,1),1,'first');
169     if((x(indexl)-ic(j,1)) < (x(indexs)-ic(j,1)));
170         index2=indexl;
171     else
172         index2=indexs;
173     end
174     if (j < 5)
175         diff(j)=sqrt((ic(j,2)-soln(151,index2))^2/ic(j,2);
176     else
177         diff(j)=sqrt((ic(j,2)-soln(151,index2))^2/ic(j,2);
178         steincomp(j-4)=sqrt((ic(j,2)-soln(151,index2))^2/ic(j,2);
179     end
180 end
181
182 % %caclulate errors
183 errrad=(sum(sqrt((invraddata-solndata).^2)./invraddata));
184 errdens=(sum(diff));
185 errdenscomp=(sum(steincomp));
186 errortot=1/(length(invraddata)+length(ic(1:21,2))-8)*(errrad+errdens
    )
187
188 %stein cmpare
189 errortotviastein=1/(length(invraddata)+length(celldensity3(:,2))-8)
    *(errrad+errdenscomp)
190
191 %stein err
192 load('steinsimdensity.mat')
193 load('steinsim3c.mat')
194 errdenstein=(sum(sqrt((steinsimdensity(:,2)-celldensity3(:,2)).^2)
    ./((celldensity3(:,2))));
195 errradstein=(sum(sqrt(((invraddata-steinsim3c(:,2))/10000).^2)./(
    invraddata))));
196 errortotstein=1/(length(invraddata)+length(celldensity3(:,2))-5)*(
    errradstein+errdenstein)
197
198
199
200 %% Plotting the graphs
201
202 figure;
203 figureHandle=gcf;
204 set(gca,'FontSize',20);
205 set(findall(gcf,'type','text'),'FontSize',30);
206 plot(1:7,invraddata,'k+',1:7,steinsim3c(:,2)/10000,'r*',1:7,solndata
    ,'b-','linewidth',3)
207 xlabel('time(days)')

```

```

208 ylabel('invasive radius (cm)')
209 xlim([1,7])
210 %title('Invasive Radius comparison')
211 legend('Experimental Data', 'Stein et. al Simulations','Our
        Simulations',2)
212 set(gca,'FontSize',20);
213
214
215
216 figure;
217 figureHandle=gcf;
218 set(gca,'FontSize',20);
219 set(findall(gcf,'type','text'),'FontSize',30);
220 plot(cellldensity3(:,1),cellldensity3(:,2),'k+',steinsimdensity(:,1)
        /10000,steinsimdensity(:,2),'r*',x,soln(151,:), 'b-', 'linewidth'
        ,3)
221 xlim([0.03,0.1])
222 xlabel('distance from core (cm)')
223 ylabel('cell population')
224 %title('Tumor Cell Density Profile at day 3')
225 legend('Experimental Data', 'Stein et. al Simulations','Our
        Simulations')
226 set(gca,'FontSize',20);
227
228
229 figure;
230 set(gca,'FontSize',20);
231 set(findall(gcf,'type','text'),'FontSize',30);
232 plot(ic(:,1),ic(:,2),'k+',steinsimdensity(:,1)/10000,steinsimdensity
        (:,2),'r*',x,soln(151,:), 'b-', 'linewidth',3)
233 xlabel('distance from core (cm)')
234 ylabel('cell population')
235 %title('Tumor Cell Density Profile at day 3')
236 legend('Experimental Data', 'Stein et. al Simulations','Our
        Simulations')
237 set(gca,'FontSize',20);
238
239 end

```

APPENDIX E

PUBLISHED PAPER FOR CHAPTER 2

A DATA-MOTIVATED DENSITY-DEPENDENT DIFFUSION MODEL OF IN VITRO GLIOBLASTOMA GROWTH

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ABSTRACT. Glioblastoma multiforme is an aggressive brain cancer that is extremely fatal. It is characterized by both proliferation and large amounts of migration, which contributes to the difficulty of treatment. Previous models of this type of cancer growth often include two separate equations to model proliferation or migration. We propose a single equation which uses density-dependent diffusion to capture the behavior of both proliferation and migration. We analyze the model to determine the existence of traveling wave solutions. To prove the viability of the density-dependent diffusion function chosen, we compare our model with well-known in vitro experimental data.

1. Introduction. Glioblastoma multiforme is a malignant form of brain cancer with an especially grim prognosis—mean survival time from detection is less than 15 months (Norden and Wen [20]). Glioblastoma are characterized not only by intense proliferation, but also by excessive migration. This leads to an inability to effectively treat the tumors, as surgical resection is able to remove the core of the tumor, but not the migratory cells. This erratic behavior makes modeling all aspects of glioblastoma growth difficult.

Early models of glioblastoma growth include reaction-diffusion equations which are able to accurately capture the proliferating tumor core. Tracqui et al. [30] formulated the earliest reaction-diffusion model to describe glioblastoma growth and diffusion, also with therapeutic intervention. Swanson et al. [29] continued with these reaction-diffusion models, accounting for a spatially dependent diffusion model in an attempt to model more heavily the migratory behavior of the tumor cells.

A landmark mathematical model introduced by Stein et al. [27] advocated for the separation of glioblastoma cells into two separate populations: the proliferating core cells and the migratory cells. Their model was based off of observations from in vitro experiments involving the spreading of two human astrocytoma cell lines that form tumor spheroids. In addition to the standard reaction-diffusion terms in the equation for migratory cells, the Stein et al. [27] model includes a radially biased motility term corresponding to convection to account for the situation where cells detect the location of the tumor core and actively move away from it. Our goal is to formulate a single equation which captures both the migratory and core tumor

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characteristics as accurately as the dual-equation approach, by using a density-dependent diffusion term.

One of the fundamental questions with in vitro tumor growth is how fast is the tumor growing? Can we estimate how far the tumor will spread after a certain amount of time? We want to quantify tumor spread, not just computationally, but also analytically. To this end, we will need to analyze the traveling wave solutions.

Traveling wave solutions have been studied in models of glioblastoma growth with multiple cell populations and constant diffusion, such as in Pérez-García et al. [23] and Harko and Mak [8]. Traveling wave solutions also arise in density-dependent reaction-diffusion equations, and numerous density-dependent diffusion functions have been studied by, for example, Atkinson et al. [2], Murray [18], Witelski [31], Sánchez-Garduño and Maini [24], Harris [9], Pedersen [22], Maini et al. [13], Sánchez-Garduño et al. [25], Ngamsaad and Khompurngson [19], and Kengne et al. [11]. More generally, traveling wave solutions for convective-reaction-diffusion equations were studied in Malaguti et al. [14, 15, 16] and Gilding and Kersner [7]. Minimal speeds for various diffusion and convective terms were estimated.

In this article, we study a nondegenerate convective-reaction-diffusion equation model of glioblastoma tumor growth. The existence of traveling waves is analyzed and the minimum wave speed is corroborated by simulations. We perform a sensitivity analysis on the parameters in the model to detect how variations in parameters effect the numerical solution. Lastly, we optimize the parameters in order to validate the model with in vitro experimental data. We show that this single equation model fits the data as well as the previously posed two-equation model of Stein et al. [27].

2. Model formulation. Multiple mathematical models have attempted to explain and predict the proliferation and migration of the glioblastoma tumor cells in vitro and in vivo with varying success (see the review paper by Martirosyan et al. [17] and references therein). The growth and diffusion of malignant glioma are governed by many processes including, but not limited to, random diffusion, chemotaxis, haptotaxis, cell-cell adhesion, cell-cell signaling, and microenvironmental cues such as oxygen and glucose. Our proposed model is a variation on the in vitro mathematical model of Stein et al. [27]. We briefly discuss the original experiment and mathematical model and then the reasoning behind our alterations.

In Stein et al. [27], two human astrocytoma U87 cell lines are implanted into gels—one with a wild-type receptor (EGFRwt) and one with an over expression of the epidermal growth factor receptor gene (Δ EGFR). The resulting spheroids were left to grow over 7 days and imaged every day. We show a summary of the results of the experiment for the invasive and proliferating cell radii on days 0, 1, 3, and 7 in Figure 1. The tumor core radius was measured to be where pixels had an intensity of < 0.12 and the invasive radius as the half-maximum for the image averaged over the azimuthal angle.

The mathematical model proposed by Stein et al. [27] describes the movement of the invasive, or migratory, cells (u_i) for the EGFRwt cell line and the Δ EGFR cell line based on the experiment described above. The radius of the tumor core, consisting of the less mobile cells, is modeled as increasing at a constant rate based on the in vitro experimental data. The model of Stein et al. [27] assumes that the tumor cells leave the tumor core and become invasive cells to invade the collagen gel. The behavior of invasive cells is described by the model in a way that can

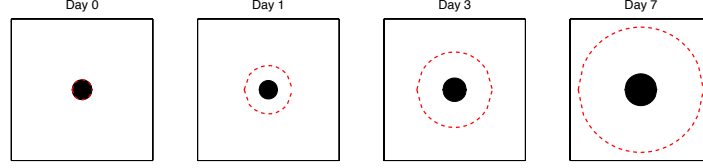


FIGURE 1. The radius of the core proliferating (black) and migratory (red dashed) cells for the experiment for EGFRwt strain from Stein et al. [27] on days 0, 1, 3, and 7. The domain is a 3 mm by 3 mm square.

be quantitatively compared to the experimental measurements. The invasive cell population is governed by the following ad hoc partial differential equation

$$\frac{\partial u_i(r, t)}{\partial t} = \underbrace{D \nabla^2 u_i}_{\text{diffusion}} + \underbrace{g u_i \left(1 - \frac{u_i}{u_{\max}} \right)}_{\text{logistic growth}} - \underbrace{\nu_i \nabla_r \cdot u_i}_{\text{taxis}} + \underbrace{s \delta(r - R(t))}_{\text{shed cells from core}}, \quad (1)$$

where u_i represents the invasive cells of the tumor at radius r and time t (Stein et al. [27]). The forces acting upon the invasive cell population are random diffusion, logistical growth, taxis, and cells being shed from the core of the tumor. Taxis refers to the active biased motility of invasive cells away from the tumor core which Stein et al. [27] attributes to possibly chemotaxis or haptotaxis. This taxis term was found to be more necessary for the highly invasive EGFRwt strain compared to the less invasive Δ EGFR strain.

As the core of the tumor increases, cells are shed from the front of the expanding core to become invasive cells. Parameter D is the diffusion constant, g is the growth rate, $u_{\max}$ is the carrying capacity, ν_i is the degree at which cells migrate away from the core, s is the amount of cells shed per day, and δ is the Dirac delta function. The radius of the tumor core is modeled by $R(t) = R_0 + \nu_c t$, where R_0 is the initial radius of the tumor core and ν_c is the constant velocity at which the tumor core radius increases.

The experimental data in Stein et al. [27] suggests that the invasive cell radius also spreads at a constant velocity. However, the assumption that the tumor core radius increases at a constant rate and the existence of the Dirac delta function in equation (1) is rather artificial and makes traveling wave analysis of the invasive cells very difficult. We therefore extend the model of Stein et al. [27] to alleviate some of these issues.

We build our model from a similar base. We keep the logistic growth term, as we know that the tumor cells grow in number, and logistic growth is sensible. We also keep the taxis term, since it is apparent that glioblastoma tumor cells do migrate away from the tumor core (Stein et al. [28]). Since we are trying to model the tumor core in addition to the invasive cells, we do not need to consider the cell shedding term. Although many current cancer cell migration models are based on reaction-diffusion equations, most of those models consider diffusion to be constant or as a function of space. Some cell migration models have included density-dependent diffusion, such as the wound healing model of Cai et al. [4].

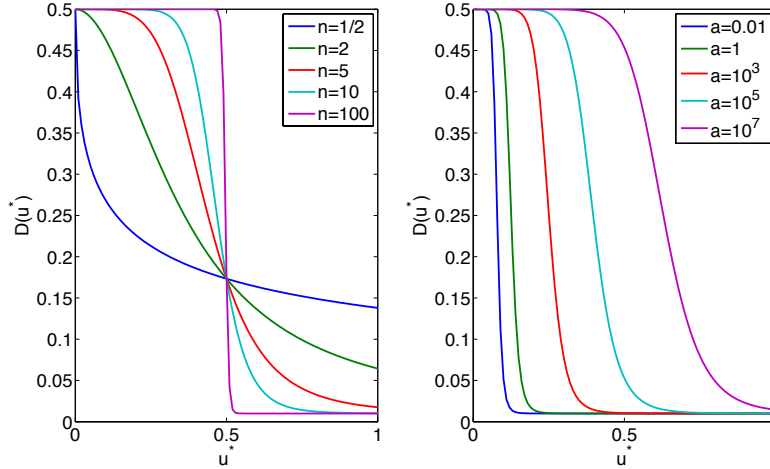


FIGURE 2. The density-dependent diffusion function $D(u^*)$, where $u^* = \frac{u}{u_{\max}}$, from Equation (3) for $D_1 = 0.5$, $D_2 = 0.4$, and various a and n . As u^* increases, $D(u^*)$ decreases from its maximum D_1 to its minimum $D_1 - D_2$.

We consider a density-dependent convective-reaction-diffusion equation, which implies that the amount of random diffusion depends on how many cells are present. Our governing equation for the tumor cells $u(x, t)$ is thus

$$\frac{\partial u}{\partial t} = \underbrace{\nabla \cdot \left(D \left(\frac{u}{u_{\max}} \right) \nabla u \right)}_{\text{density-dependent diffusion}} + \underbrace{gu \left(1 - \frac{u}{u_{\max}} \right)}_{\text{logistic growth}} - \underbrace{\text{sgn}(x) \nu_i \nabla \cdot u}_{\text{taxis}}, \quad (2)$$

where we consider the equation in Cartesian coordinates but assume there is radial symmetry. Parameters g and ν_i are as in equation (1), but now they are in relation to the entire tumor cell population instead of just the invasive cell population.

There are many functions that could serve as the density-dependent diffusion $D(u^*)$, where $u^* = \frac{u}{u_{\max}}$. Experimental work from Stein et al. [27] suggests that diffusion is large for areas where the cell density is small (the migrating tumor cells), but diffusion is small where the cell density is large (the proliferating tumor cells). This relation could possibly be explained by cell-cell adhesion (Armstrong et al. [1]). To capture this behavior, we set

$$D(u^*) = D_1 - \frac{D_2(u^*)^n}{a^n + (u^*)^n}. \quad (3)$$

For biologically relevant parameters, we assume that D_1 , D_2 , g , a , and ν_i are all positive, $n > 1$, and $D_2 \leq D_1$ to avoid “negative” diffusion, which is a problem both biologically and numerically. See Figure 2 for an illustration of the density-dependent diffusion function (3) for various a and n . The parameter n governs how steeply the function decreases and the parameter a governs the u^* value at which the transition is occurring at half maximal rate. D_1 and D_2 govern the range of the function.

As we consider the biology of tumor growth, we recall that glioblastoma tumor cells must be considered as two differing populations. The proliferative cells are assumed to remain somewhat stationary and diffuse slowly but grow in population quickly. On the other hand, the migrating cells diffuse very quickly, traveling very far in a short amount of time, but do not grow in population as quickly. Since the proliferating cells are the tumor core cells, they occur when cell density is very high, and the migrating cells are where the cell density is very low. For our application, we choose $D_2 \leq D_1$, which allows diffusion to be small for proliferating cells and larger for migrating cells.

3. Traveling wave speed analysis. In this section we analyze the existence of traveling wave solutions of (2) using phase plane analysis.

Rewriting (2) in one-dimensional Cartesian coordinates, the governing equation is

$$\frac{\partial u}{\partial t} = D\left(\frac{u}{u_{\max}}\right) \frac{\partial^2 u}{\partial x^2} + \frac{1}{u_{\max}} D'\left(\frac{u}{u_{\max}}\right) \left(\frac{\partial u}{\partial x}\right)^2 - \nu_i \frac{\partial u}{\partial x} + gu \left(1 - \frac{u}{u_{\max}}\right). \quad (4)$$

Rescale by writing

$$t^* = gt, \quad x^* = x\sqrt{g}, \quad u^* = \frac{u}{u_{\max}}, \quad (5)$$

set

$$v = \frac{\nu_i}{\sqrt{g}}, \quad (6)$$

and, omitting the asterisks and dividing through by $gu_{\max}$ for simplicity, the equation (4) becomes

$$\frac{\partial u}{\partial t} = D(u) \frac{\partial^2 u}{\partial x^2} + D'(u) \left(\frac{\partial u}{\partial x}\right)^2 - v \frac{\partial u}{\partial x} + u(1 - u). \quad (7)$$

A traveling wave solution of (7) is a solution of the form

$$u(x, t) = w(x - kt), \quad (8)$$

where $k \geq 0$ is the speed of the traveling wave and the function $w(z)$ is defined on the interval $(-\infty, \infty)$ and satisfies the boundary conditions

$$\lim_{z \rightarrow -\infty} w(z) = 1, \quad \lim_{z \rightarrow \infty} w(z) = 0. \quad (9)$$

Substituting ansatz (8) into (7) results in the second-order ordinary differential equation

$$w''(z) + \frac{1}{D(w(z))} \left((k - v)w'(z) + D'(w(z))(w'(z))^2 + w(z)(1 - w(z)) \right) = 0, \quad (10)$$

which we may write in this form because the function D as in equation (3) is always positive.

Rewriting (10) as a system of first-order ordinary differential equations by setting $y := dw/dz$,

$$w' = y, \quad (11a)$$

$$y' = \frac{-1}{D(w)} \left((k - v)y + D'(w)y^2 + w(1 - w) \right). \quad (11b)$$

This system has two equilibrium points, $(w, y) = (0, 0)$ and $(w, y) = (1, 0)$.

The Jacobian matrix evaluated at $(1, 0)$ is

$$J(1, 0) = \begin{pmatrix} 0 & 1 \\ \frac{1}{D(1)} & \frac{-(k-v)}{D(1)} \end{pmatrix}, \quad (12)$$

from which we have $\det J(1, 0) = \frac{-1}{D(1)} < 0$, and thus $(1, 0)$ is a saddle equilibrium point.

The Jacobian matrix evaluated at $(0, 0)$ is

$$J(0, 0) = \begin{pmatrix} 0 & 1 \\ \frac{-1}{D(0)} & \frac{-(k-v)}{D(0)} \end{pmatrix}, \quad (13)$$

from which we have $\det J(0, 0) = \frac{1}{D(0)} > 0$ and assuming that $k > v$, then $\text{tr } J(0, 0) = \frac{-(k-v)}{D(0)} < 0$ and $(0, 0)$ is a stable node or spiral. Since a stable spiral cannot result in physiologically relevant solutions, we obtain the condition

$$k \geq k_{\min} = 2\sqrt{D_1} + v, \quad (14)$$

which in terms of the original dimensional equation (4) is

$$k \geq k_{\min} = 2\sqrt{D_1 g} + \nu_i. \quad (15)$$

We will prove the following main result.

Theorem 3.1. *There exists a traveling wave solution (8) of the partial differential equation (7) with boundary conditions $u(x, t) \rightarrow 1$ as $x \rightarrow -\infty$ and $u(x, t) \rightarrow 0$ as $x \rightarrow \infty$ with $0 < u(x, t) < 1$, whose orbit connects the steady states $u \equiv 0$ and $u \equiv 1$ if and only if (14) is satisfied.*

To do so, we need to construct a positively invariant region in which to trap the unstable manifold of the saddle point. Figure 3 illustrates a positively invariant trapping region and the heteroclinic orbit connecting the two equilibrium points for a set of physiologically relevant parameters.

Since the horizontal ($w' = 0$) nullcline is the horizontal axis $\{y = 0\}$, the flow across this line (when $w \in (0, 1)$) is perpendicular in the negative y direction. Define the line

$$\mathcal{T}_1 = \{(w, y) : 0 \leq w \leq 1, y = 0\}. \quad (16)$$

Next, consider the line that corresponds to the eigenvector corresponding to the more negative eigenvalue of the linearized system at $(0, 0)$, i.e.,

$$y(w) = \alpha_0 w, \quad (17)$$

where we define

$$\alpha_0 = \frac{1}{2D(0)} \left(-(k-v) - \sqrt{(k-v)^2 - 4D(0)} \right). \quad (18)$$

Note that $\alpha_0 < 0$.

Lemma 3.2. *The flow at any point along the line $y(w) = \alpha_0 w$, for $w \in (0, 1]$, crosses that line in the positive y direction for k sufficiently small.*

Proof. The normal vector to the graph of $(w, \alpha_0 w)$ pointing in the positive y direction is $(-\alpha_0, 1)$. Restricting the vector field to points along the line $y(w) = \alpha_0 w$

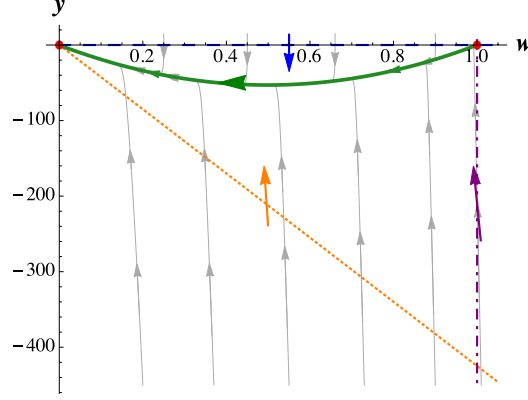


FIGURE 3. Phase Portrait of the system (11) with parameter values (37). The solid green curve is the unstable manifold, the dashed blue curve is $\mathcal{T}_1$ (16) the vertical nullcline, the dotted orange line is $\mathcal{T}_2$ (28) corresponding to the eigenvector of linearized system at $(0, 0)$, and the dash-dotted purple line is $\mathcal{T}_3$ (30). Arrows show direction of flow. The nondimensional wave speed $k = 2\sqrt{D_1} + v = 2\sqrt{D_1} + \nu_i/\sqrt{g} \approx 0.0047746$.

results in the system

$$w' = \alpha_0 w, \quad (19a)$$

$$y' = \frac{-1}{D(w)} \left((k - v)\alpha_0 w + D'(w)\alpha_0^2 w^2 + w(1 - w) \right). \quad (19b)$$

We choose k such that the inner product $(-\alpha_0, 1) \cdot (w', y') \geq 0$ along the graph of $(w, \alpha_0 w)$, and thus

$$-\alpha_0^2 w - \frac{1}{D(w)} \left((k - v)\alpha_0 w + D'(w)\alpha_0^2 w^2 + w(1 - w) \right) \geq 0, \quad (20)$$

which can be rearranged as

$$-\frac{\alpha_0 w}{D(w)} \left(\alpha_0 D(w) + (k - v) + D'(w)\alpha_0 w + \frac{1 - w}{\alpha_0} \right) \geq 0. \quad (21)$$

Since $\alpha_0 < 0$ and $D(w) > 0$ for $w \in (0, 1]$, then $-\frac{\alpha_0 w}{D(w)} > 0$ and the inequality becomes

$$\alpha_0 D(w) + (k - v) + D'(w)\alpha_0 w + \frac{1 - w}{\alpha_0} \geq 0. \quad (22)$$

Since (22) must hold for all $w \in [0, 1]$, then we obtain the condition

$$k \geq v - \max_{w \in [0, 1]} \left\{ \frac{d}{dw} (D(w)(\alpha_0 w)) - \frac{w(1 - w)}{\alpha_0 w} \right\}. \quad (23)$$

However, since α_0 depends on k , substitute (18) into (23), and after tedious algebraic manipulation the inequality becomes

$$k \leq v + \min_{w \in [0, 1]} \left\{ \frac{D(w) + wD'(w) - D_1(1 - w)}{\sqrt{w(D(w) + wD'(w) - D_1)}} \right\}. \quad (24)$$

Consider the function

$$h(w) = \frac{D(w) + wD'(w) - D_1(1-w)}{\sqrt{w(D(w) + wD'(w) - D_1)}}. \quad (25)$$

The minimum of $h(w)$ is attained in the interior of the domain $[0, 1]$ if $h'(w) = 0$ for some $w \in (0, 1)$. This means that either (i) $D_1(1+w) - \frac{d}{dw}(wD(w)) = 0$ or (ii) $D_1 - D(w) + w\frac{d}{dw}(wD'(w)) = 0$. In case (i), this means that the diffusion function must be of the form $D(w) = D_1(1+w) + \frac{C_1}{w}$, where C_1 is an arbitrary constant. In case (ii), the diffusion function must be of the form $D(w) = D_1 + C_1\frac{w^2-1}{w} + iC_2\frac{w^2-1}{2}$, where C_1 and C_2 are arbitrary constants. Since our diffusion function (3) is of neither of these forms, the minimum cannot be attained in the interior of $[0, 1]$ and must be attained at either of the endpoints $w = 0$ or $w = 1$.

Since $h(w)$ tends to infinity as $w \rightarrow 0$ (assuming that parameter $n > 1$), then the minimum occurs at $w = 1$, and thus if the condition

$$k \leq v + \frac{D(1) + D'(1)}{\sqrt{D(1) + D'(1) - D_1}} = v + \frac{(a+1)^2 D_1 + (1+a+an)D_2}{(a+1)\sqrt{(1+a+an)D_2}} \quad (26)$$

is satisfied, then the lemma holds. $\square$

Since

$$2\sqrt{D_1} \leq \frac{(a+1)^2 D_1 + (1+a+an)D_2}{(a+1)\sqrt{(1+a+an)D_2}}, \quad (27)$$

the flow across the line

$$\mathcal{T}_2 = \{(w, y) : 0 \leq w \leq 1, y = \alpha_0 w\} \quad (28)$$

is in the positive y direction for at least the minimum wave speed k . For larger speeds, the nonlinearities of the system require that $\mathcal{T}_2$ be nonlinear such that

$$k \leq v - \max_{w \in [0, 1]} \left\{ \frac{d}{dw}(D(w)f(w)) - \frac{w(1-w)}{f(w)} \right\}, \quad (29)$$

is satisfied for some function f where $f(0) = 0$ and $f(w) \leq 0$ for $w \in (0, 1]$. The minimum wave speed is determined by taking the infimum on the set of functions f (Sánchez-Garduño et al. [25]).

Also due to the nonlinearities of the system and the behavior of the system near $(1, 0)$, it is difficult to use the eigenvector of the linearized system at $(1, 0)$ as a portion of the boundary of the trapping region, as is standard for the Fisher-Kolmogorov equation (Chicone [5]). Instead, define

$$\mathcal{T}_3 = \{(w, y) : w = 1, \alpha_0 \leq y \leq 0\}. \quad (30)$$

The flow across this line is in the negative w direction. Thus, if we define the triangle $\mathcal{T}$ defined by the boundaries $\mathcal{T}_1$, $\mathcal{T}_2$, and $\mathcal{T}_3$, then $\mathcal{T}$ is a positively invariant set.

We now prove our main result.

Proof of Theorem 3.1. We first show that the unstable manifold of the saddle point $(w, y) = (1, 0)$ has nonempty intersection with $\mathcal{T}$ for all time.

The vertical ($y' = 0$) nullclines are the solutions to the quadratic equation

$$D'(w)y^2 + (k-v)y + w(1-w) = 0, \quad (31)$$

which are

$$y_+(w) = \frac{1}{2D'(w)} \left(-(k-v) + \sqrt{(k-v)^2 - 4D'(w)w(1-w)} \right), \quad (32a)$$

$$y_-(w) = \frac{1}{2D'(w)} \left(-(k-v) - \sqrt{(k-v)^2 - 4D'(w)w(1-w)} \right). \quad (32b)$$

Since $k > v$, the slope of the y_+ nullcline at $w = 1$ is

$$y'_+(1) = \frac{1}{k-v} > 0, \quad (33)$$

and the eigenvector of the linearized system corresponding to the positive eigenvalue at the saddle point $(1, 0)$ is

$$\vec{\eta} = \left(1, \frac{1}{2} \left(\frac{-(k-v)}{D(1)} + \sqrt{\left(\frac{k-v}{D(1)} \right)^2 + \frac{4}{D(1)}} \right) \right)^T. \quad (34)$$

All trajectories that leave the point $(1, 0)$ in the region $\mathcal{R} = \{(w, y) : 0 \leq w \leq 1, y \leq 0\}$ have the tangent vector $\vec{\eta}$ at $(1, 0)$. Comparing the slope of eigenvector $\vec{\eta}$ and the slope of the y_+ nullcline at the point $(1, 0)$, we find that the slope of $\vec{\eta}$ is less than the slope of y_+ . Therefore, trajectories leaving point $(1, 0)$ leave above y_+ . Since the flow across the nullcline y_+ is perpendicular in the negative w direction, and y_+ is contained in $\mathcal{T}$ near $w = 1$, the unstable manifold of the saddle at $(1, 0)$ has nonempty intersection with $\mathcal{T}$.

Thus, the unstable manifold of the saddle at $(1, 0)$ remains in the region $\mathcal{T}$ for all time, and furthermore, the ω -limit set of the corresponding orbit is also in $\mathcal{T}$. Since $w' = y \leq 0$ within $\mathcal{T}$, by the Poincaré–Bendixson theorem, there are no periodic orbits or equilibrium points in the interior of $\mathcal{T}$. The ω -limit set must be contained in the boundary of $\mathcal{T}$, and therefore, the ω -limit set is $(0, 0)$.

Hence, there exists a heteroclinic orbit connecting the equilibrium points $(w, y) = (0, 0)$ and $(w, y) = (1, 0)$ as long as the condition (14) is satisfied, which implies that a traveling wave solution exists. $\square$

Thus, we should expect in our numerical simulations that the wave speed of the invasive cells is constant and satisfies condition (15).

4. Computational results. In order to show that the proposed model is viable, we compare numerical simulations with experimental data and the traveling wave analysis. A sensitivity analysis as well as a parameter optimization to fit the experimental data is performed. Finally, the simulated wave speed is compared with the minimum wave speed (15) derived in the previous section.

Experimental data provided in Stein et al. [27] includes the density profile of cells on day 3 and the invasive radius of the cells measured from days 1 through 7 for both the U87WT and U87ΔEGFR cell lines. The data was obtained via GRABIT [6], a MATLAB program which extracts data points from an image file. We concentrate on the U87WT cell line because we want to show our model is effective even for the strongest migratory cells. All simulations are performed with the U87WT cell line in mind and are compared to the U87WT data.

The numerical simulations are run over a large spatial domain and boundary conditions specify that there are no tumor cells at the boundaries, in other words, $u(x, t) = 0$ when $x = \pm 1$ cm. This ensures that the tumor can freely move within the domain. Stein et al. [27] does not model the tumor core cells in the invasive cell

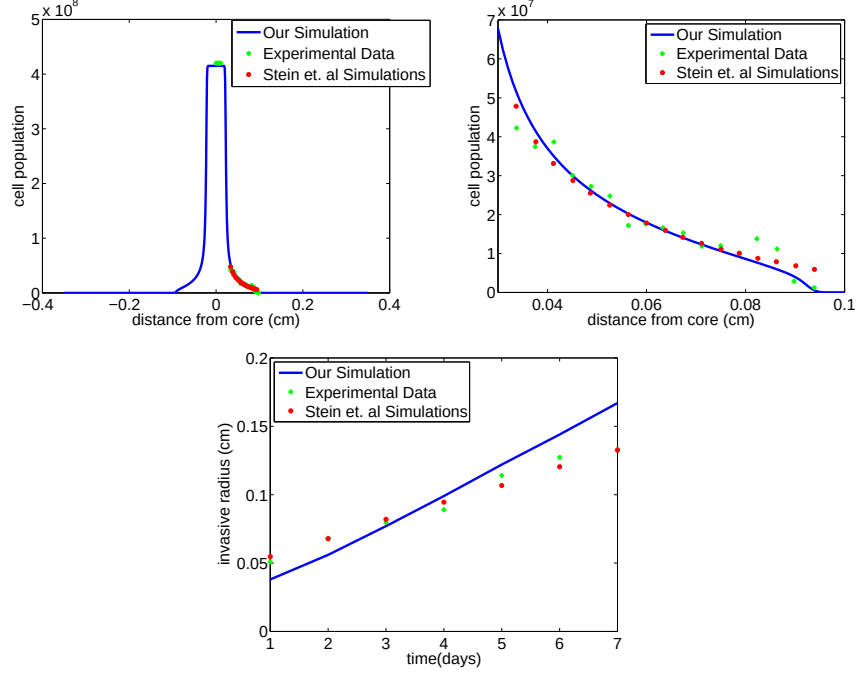


FIGURE 4. Numerical solution of the density-dependent diffusion glioblastoma model (2) with diffusion function (3) and optimized parameter values (37) compared to experimental data from Stein et al. [27] and their simulations. The density profile is from day 3 of the experimental data.

equation (1), and the initial cell density for their simulations was zero. However, our model (2) contains all tumor cells. From Stein et al. [27], the initial core tumor radius is $210\mu\text{m}$ and the maximum cell density is $u_{\max} = 4.2 \times 10^8 \text{ cells/cm}^3$, and thus we assume that initially the cell density is 95% of $u_{\max}$ for the initial core tumor radius of $210\mu\text{m}$ and zero elsewhere.

The governing equation (2) is discretized using the Crank-Nicolson method for the density-dependent diffusion term and first-order forward differencing for the advection and logistic growth terms. For the advection term on the left hand side, first-order backward differencing is used. A limited spatio-temporal study was conducted to determine the largest possible spatial step and time steps which would still produce accurate results. Our method was compared with results from the MATLAB program pdepe [26] to ensure the solutions were accurate. See Figure 4 for a typical simulation that is compared to the experimental data and model of Stein et al. [27].

The governing equation (2) with diffusion function (3) has six parameters that are unknowns: D_1 , D_2 , a , n , g , and ν_i . Estimated ranges for some of these parameters can be obtained from Stein et al. [27]. In particular, the migratory diffusion (diffusion when the density u is small), D_1 , is estimated to be on the order of $10^{-4} \text{ cm}^2/\text{day}$. The growth rate $g \in (0, 1)/\text{day}$ and the taxis constant

$\nu_i \in (0, 0.02)$ cm/day. However, for a and n , there is no precedent to compare with. We perform a sensitivity study to determine if the model error is insensitive to any of the changing parameters. If we are able to determine that a parameter is insensitive, we will be able to fix its value and not include it as a free variable for parameter optimization.

The error function that we aim to minimize is based on the χ^2 error function used in Stein et al. [27] but modified to account for the experimental data that was made available and uses relative errors for each point. The modified error function that takes into account both how fast the tumor is spreading, by measuring the invasive radius at each day, and also the density of cells by comparing the spatial profile of cells on day 3 is

$$\text{err} = \frac{1}{(N + M) - q - 1} \left[\sum_{t=1}^N \frac{|r_{\text{data}}(t) - r_{\text{simulation}}(t)|}{r_{\text{data}}(t)} + \sum_{i=1}^M \frac{|u_{\text{data}}(3, x_i) - u_{\text{simulation}}(3, x_i)|}{u_{\text{data}}(3, x_i)} \right], \quad (35)$$

where N is the total number of days for which there is invasive radius data, so $N = 7$, M is the total number of cell density data points at day 3, so $M = 17$, and q is the number of parameters being optimized which, in this case, $q = 6$. The first sum in (35) compares the invasive radii of the experimental data, $r_{\text{data}}(t)$, and the simulation, $r_{\text{simulation}}(t)$. The second sum compares the cell density at day 3 at experimental data point x_i for the data, $u_{\text{data}}(3, x_i)$, and the simulation, $u_{\text{simulation}}(3, x_i)$. We use relative errors because the data covers many different orders of magnitude: the cell density is on the order of 10^7 – 10^8 cells/cm³ and the invasive radius is on the order of 0.01 cm.

4.1. Parameter sensitivity. The parameter sensitivity analysis was performed for the six parameters D_1 , D_2 , a , n , g , and ν_i such that each was in a physiologically relevant range. The set of base parameters chosen that result in a reasonable match to the data were

$$\begin{aligned} D_1 &= 10^{-4} \text{ cm}^2/\text{day}, & D_2 &= 9.99 \times 10^{-5} \text{ cm}^2/\text{day}, & a &= 0.1 \text{ cells/cm}^2, \\ n &= 1, & g &= 0.5/\text{day}, & \nu_i &= 0.01 \text{ cm/day}. \end{aligned} \quad (36)$$

These base parameters are not chosen for their excellent fit to the data, rather they are chosen in order to have error that is not too large and to allow for variations to still remain in biologically relevant ranges. To test the sensitivity of one parameter, all the other parameters were held constant and the parameter in question was varied. The results of this sensitivity test is shown in Figure 5.

It is apparent that some parameters are much more sensitive than others, in particular, D_1 , g , and ν_i appear to generate the most sensitivity. The results of this sensitivity analysis inform us that all parameters are sensitive and we must take care when we perform our optimization.

4.2. Parameter estimation. The parameters are optimized via the MATLAB program `fminsearch` [12] by minimizing equation (35). Various initial parameter guesses were used as input to ensure parameter values were optimal. The optimized

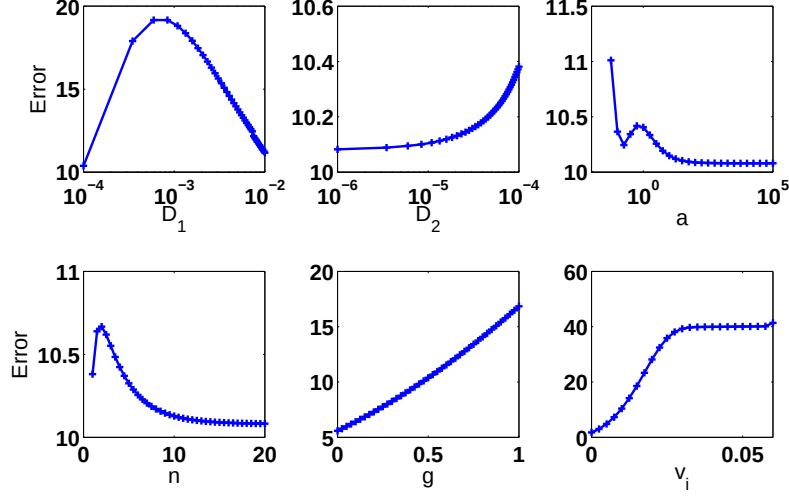


FIGURE 5. Results of a sensitivity analysis with base parameters (36) using error function (35) (vertical axis).

parameters found are

$$\begin{aligned} D_1 &= 5.5408 \times 10^{-6}, \text{ cm}^2/\text{day}, & D_2 &= 5.3910 \times 10^{-6}, \text{ cm}^2/\text{day}, \\ a &= 0.021188 \text{ cells}/\text{cm}^3, & n &= 1.2848, \\ g &= 0.49120/\text{day}, & \nu_i &= 4.6801 \times 10^{-5} \text{ cm}/\text{day}. \end{aligned} \quad (37)$$

Though $D_1 + D_2$ is very close to 0, it is still the case that $|D_1| > |D_2|$ as necessary. To determine the comparable error to the Stein et al. [27] simulations that use invasive cells as a separate population, we use GRABIT [6] to obtain the simulation data points. Figure 4 shows the results of our simulation with the optimized parameters compared to the Stein et al. [27] simulations and the experimental data.

In the top left panel of Figure 4, the cell density is plotted on the entire domain and it indicates that the model is successful in capturing the behavior of both the tumor core cells and the migratory cells. In the top right panel, the cell density is plotted on a smaller domain and further verifies that our model with one population is relatively as accurate as the model of Stein et al. [27] with the separated invasive cell population and proliferating cell population. In fact, our total error is approximately one-half that of Stein et al. [27] (0.21 compared to 0.45). Even though the simulated invasive radius (in the bottom panel) does not appear to match the experimental data as well, overall there is good agreement between our simulations and the experimental data.

4.3. Wave speed comparison. Now that we have shown that our model can be utilized, we compare the analytic minimum wave speed (15) to the wave speed observed in the simulations. To measure the wave speed of tumor spread accurately, we increase the run-time of the simulation to 200 days and enlarge the spatial domain to more so mimic an infinite domain, as is used in the traveling wave

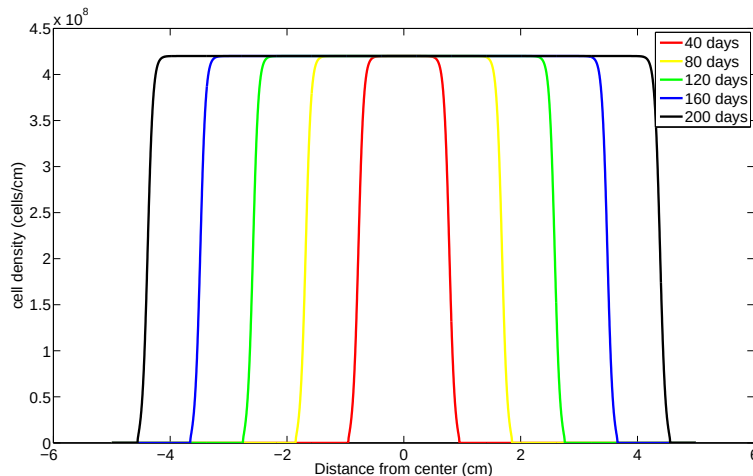


FIGURE 6. Numerical solution of the density-dependent diffusion glioblastoma model (2) with diffusion function (3) and optimized parameter values (37) for days 40, 80, 120, 160, and 200.

analysis. The results of this simulation are shown in Figure 6. The leading edge of the tumor maintains a constant shape and ultimately moves at a constant rate. To estimate the wave speed, we ignore the first few days of the simulation so that the tumor core stably reaches maximum cell density, and then measure the x location where the cell density is last over 2×10^8 cells/cm³. This is tracked against time, and the MATLAB program polyfit is used to estimate the slope of the curve for a linear fit. Using the optimized parameters (37), the minimum wave speed (15) is $k_{\min} \approx 0.003346$ cm/day and the simulated wave speed $k = 0.02255$ cm/day. While often the observed simulated wave speed is approximately equal to the analytic minimum wave speed, here the simulated wave speed is on the order of 10 times larger than the minimum wave speed.

5. Discussion and further directions. We derived a density-dependent diffusion model for in vitro glioblastoma tumor growth that was validated by existing experimental data from Stein et al. [27]. We accurately modeled both the proliferating tumor core as well as the invading migratory cells using only one equation. This model has the potential to simplify glioblastoma tumor modeling—with only one equation the analysis is simpler and simulations can be faster. The existence of traveling waves for this model was studied using phase plane analysis and was corroborated by simulations. We performed a sensitivity analysis and parameter optimization to ensure the model describes the behavior of the tumor.

The governing equation has density-dependent diffusion, logistic growth, and taxis which could possibly be due to chemotaxis or haptotaxis. The chosen density-dependent diffusion function (3) ensures that when the cell density is large, random diffusion is small, and when the cell density is small, random diffusion is large, which could possibly be explained by cell-cell adhesion. This behavior matches how the migratory cells move further away from the tumor core at a large speed, while

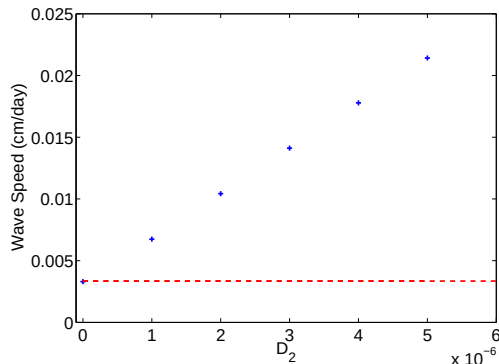


FIGURE 7. The observed simulated wave speed when varying parameter D_2 with all other parameters values (37) fixed. The theoretical minimum wave speed is shown in the red dashed line.

the core remains proliferating and slowly expanding. The logistic term describes the growth of the total number of cells. Taxis describes the active and directed movement of the invasive cells from the tumor core.

A sensitivity analysis indicated that all parameters largely influenced the numerical solutions. We performed a parameter optimization on all six parameters and showed that the error for the best fit was less than the error generated by the two-population model of Stein et al. [27]. This implies that monotone decreasing density-dependent diffusion may better explain the behavior of tumor spheroid cell migration as opposed to cell shedding of the tumor core.

Through wave speed analysis we were able to determine a minimum wave speed and conditions necessary for the existence of traveling wave solutions. Numerical simulations indicated that the observable wave speed is much larger than the analytic minimum wave speed. We conjecture that for monotone decreasing density-dependent diffusion functions, the traveling wave solution with minimum wave speed is unstable.

Furthermore, the observable wave speed appears to depend on other parameters besides D_1 , g , and ν_i . Figure 7 indicates that there is a linear relation between parameter D_2 and the observed wave speed, and parameter n also affects the wave speed while a does not affect it as much (results not shown). We also note that when $D_2 = 0$, the observable wave speed equals the analytic minimum wave speed, as expected. A possible explanation for the difference between the analytic and numerical wave speeds could be that nonlinear diffusion can be considered as contributing convection with a “velocity” $-D'(u)\frac{\partial u}{\partial x}$ (in one dimension) but the analytic minimum wave speed was obtained after linearizing the system (11). Further work can be done to investigate finding an expression for the observable wave speed.

Future studies of glioblastoma growth will focus on comparing this model to in vivo data. The model will need to be extended to include brain geometry, and furthermore, comparing the model to in vivo data instead of in vitro data may result in the need to extend the equations to describe more complex behavior such as tumor cell necrosis, brain tissue type differentiation, and mass effect. Instead of density-dependent diffusion, it may be more appropriate to implement anisotropy

through diffusion tensor imaging (DTI) such as in the models of Jbabdi et al. [10], Bondiau et al. [3], and Painter and Hillen [21], depending on the availability of experimental data. Another direction is to consider a non-local reaction-diffusion equation instead of density-dependent diffusion with proliferating and dispersing cell groups in which dispersing cells convert proliferating cells into dispersing ones.

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APPENDIX F

PUBLISHED PAPER FOR CHAPTER 5

GLOBAL DYNAMICS OF A MODEL OF JOINT HORMONE TREATMENT WITH DENDRITIC CELL VACCINE FOR PROSTATE CANCER

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ABSTRACT. Advanced prostate cancer is often treated by androgen deprivation therapy, which is initially effective but gives rise to fatal treatment-resistant cancer. Intermittent androgen deprivation therapy improves the quality of life of patients and may delay resistance towards treatment. Immunotherapy alters the bodies immune system to help fight cancer and has proven effective in certain types of cancer. We propose a model incorporating androgen deprivation therapy (intermittent and continual) in conjunction with dendritic cell vaccine immunotherapy. Simulations are run to determine the sensitivity of cancer growth to dendritic cell vaccine therapy administration schedule. We consider the limiting case where dendritic cells are administered continuously and perform analysis on the full model and the limiting cases of the model to determine necessary conditions for global stability of cancer eradication.

1. Introduction. Prostate cancer is one of the most ubiquitous cancers in males in the United States, with an expected one in six men diagnosed in their lifetime [23]. The prostate requires androgens, especially testosterone and 5α -dihydrotestosterone (DHT) to function and continue proliferation. The current treatment protocol is to suppress androgen, which should lead to inhibited growth of cancer cells as well. However, although the initial response rates to therapy are excellent, eventually androgen-independent prostate cancer arises and is most often fatal [33].

Recent research and clinical trials have begun to question whether the efficacy and comfort of this treatment could be increased by intermittent androgen deprivation (IAD) therapy [6, 22, 28, 37]. Intermittent androgen deprivation therapy works by administering androgen deprivation therapy until the patient reaches a certain threshold of prostate specific antigen (PSA), which is a biomarker of the disease. Upon reaching this threshold, the patient is removed from therapy until their PSA levels rise above a second threshold, when they are once again put on androgen

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deprivation therapy. This treatment still results in overall androgen suppression and may improve the quality of life of the individual patient, lessening the unpleasant side effects of androgen deprivation therapy [5, 18]. Although it has often been proposed that intermittent androgen deprivation therapy may increase time to androgen-independent relapse, this statement remains unproven in clinical trials, including meta-studies [2, 5, 39, 43]. Of course, there are studies which suggest that only certain patient groups may benefit from intermittent androgen deprivation therapy, but determining those groups remains a work in progress [25, 44]. Adding to the confusion is the fact that there is no consensus in the medical community on the duration or intervals of the treatment [10]. Some have concluded that the findings determining that intermittent androgen deprivation therapy is non-inferior to continual androgen deprivation therapy, are inconclusive due to flawed or inconsistent studies [17, 34].

Immunotherapy treatments use the body's immune system to fight against cancer by enhancing or repressing an immune response in the patient. Dendritic cells are strongest of the antigen-presenting cells, meaning they ingest antigens and present the antigen material to naive and memory T cells in the system. These T cells then target the specific antigen for removal. Dendritic cell vaccines are created by extracting dendritic cells from the patient, loading the cells with antigens and re-injecting the dendritic cells into the patient. The target antigen in this case is PAP (Prostatic acid phosphatase), which has been used in clinical trials [3, 9, 40]. The dendritic cells then serve to activate the T cells into an immune response against PAP. Dendritic cell vaccines have been suggested as a method to improve the efficacy of hormone therapy treatment of advanced prostate cancer. In fact, Provenge is an FDA-approved dendritic cell vaccine for advanced prostate cancer which has been shown to extend the life of patients [4, 11].

An emerging field among mathematical oncology is determining optimal dosing strategies in order to manage cancer. Metronomic therapy, or the use of much lower dosages of medication more frequently, has been mathematically investigated as a possible alternative to large dosage strategies [29, 30]. There are many advantages towards this type of approach: lowering cytotoxicity, lowering costs, and increasing time until treatment resistance. This strategy aims to manage cancer, rather than eradicate it.

This research examines the effect of varying the frequency in which the patient receives dendritic cell vaccines on long term behavior of prostate cancer. We examine not only the case of discrete injections, but also consider a continuous injection, as through an intravenous (IV) therapy. We perform analysis on the model to determine biologically realistic parameter values which could result in stable condition of the disease or the elimination of the prostate cancer, and analytical results are corroborated with simulations. The parameters which do not have existing literature values, or are patient-specific, are thoroughly investigated with simulations to determine what changes to dendritic cell vaccine therapy must be implemented to delay the onset of androgen independent prostate cancer. Additionally, we examine the quasi-steady state system and perform a full analysis of the steady states, determining what conditions are necessary to generate global stability.

2. Model Formulation. There have been many mathematical models that discuss the evolution and treatment of prostate cancer using androgen deprivation therapy. In 2004, Jackson [20] formulated a partial differential equation model which featured

both androgen independent and androgen dependent cancer cells. This model exhibited the effects of androgen independent relapse consistent with experimental data [20]. Ideta et. al [19] formulated a mathematical model comprised of ordinary differential equations designed to determine prostate cancer growth while on intermittent androgen therapy. Their model featured androgen concentration, androgen independent, androgen dependent cancer cells, with a term to model the mutation rate from androgen dependent to androgen independent in the absence of androgen.

Many have extended upon the model proposed by Ideta et. al. Hirata et. al [15] extended their model to include two sub-classes of androgen-independent cancer cells: a subpopulation whose mutation to androgen-independent was reversible, and a subpopulation whose mutation to androgen-independent was irreversible. From this model, they have investigated how to optimize patient treatment protocols [14, 16]. Additionally, they were able to use the model to classify patients and determine whether intermittent androgen deprivation therapy would be more or less effective for each type of patient [13]. Most recently, they have analyzed their model to find conditions for existence non-trivial periodic orbits for one of the types of patients [12].

Tanaka et. al [41] extended the Ideta et. al model by incorporating stochasticity, for more realistic PSA level data. Portz et. al [37] introduced cell quotas to model how dependent the androgen-dependent and androgen-independent cancer cells are on androgen concentration. This model has been furthered by other researchers and compared to previous models [8, 32].

Immunotherapy has also been formulated by mathematicians. Kirschner and Panetta [24] established a model which quantified the anti-tumor immune response using populations of T cells, IL2, and tumor cells. Their model also allowed for incorporation of therapy. In the context of prostate cancer, Kronik et. al [26] formulated a mathematical model which investigated the response of prostate cancer to dendritic cell vaccines, corroborated with patient data. However, they only considered one type of tumor cell population and did not account for hormonal therapy.

There has been little work in investigating the combination of dendritic cell vaccines with any type of androgen deprivation therapy. Portz and Kuang [27, 36] examined immunotherapy in conjunction with IAD for prostate cancer which combined the Ideta et. al model [19] with the Kirschner and Panetta [24] model. Their system of 6 equations included androgen independent cells, androgen dependent cells, androgen, cytokines (IL-2), activated T cells, and dendritic cells. Recently, Peng et. al [35] used a 10-dimensional ordinary differential equation model to investigate how androgen deprivation therapy combines with immunotherapy, including dendritic cell vaccines. They were able to determine how to synergistically combine ADT with immunotherapy and fit their model with mouse data. However, their model did not take into account intermittent androgen deprivation therapy.

Our model is based on the Portz and Kuang [27, 36] model described above with some appropriate modifications. Their results showed that adding dendritic cell vaccines resulted in an increase in time to androgen independent relapse. However, the study did not consider how the dynamics of dosage amounts and frequencies for the dendritic cell vaccines might influence the outcome of the treatment. It has been hypothesized in a mathematical model that changing the dosages and frequency of administration of the dendritic cell vaccine may drastically alter the time to androgen-independent relapse [26].

Our mathematical model is a population-style model of the interaction between androgen-dependent cancer cells (X_1), androgen independent cancer cells (X_2), activated T cells (T), concentration of cytokines (I_L), concentration of androgen (A), and number of dendritic cells (D).

$$\frac{dX_1}{dt} = \underbrace{r_1(A, X_1, X_2)X_1}_{\text{growth and death}} - \underbrace{m_1(A)X_1}_{\text{mutation to AI}} + \underbrace{m_2(A)X_2}_{\text{mutation from AI}} - \underbrace{X_1 f_1(X_1, X_2, T)}_{\text{death by T cell}}, \quad (1)$$

$$\frac{dX_2}{dt} = \underbrace{r_2(X_1, X_2)X_2}_{\text{growth and death}} + \underbrace{m_1(A)X_1}_{\text{mutation from AD}} - \underbrace{m_2(A)X_2}_{\text{mutation to AD}} - \underbrace{X_2 f_2(X_1, X_2, T)}_{\text{death by T cell}}, \quad (2)$$

$$\frac{dT}{dt} = \underbrace{\frac{eD}{g+D}}_{\text{activation of T cell by DC}} - \underbrace{\mu T}_{\text{death}} + \underbrace{\frac{Tf_3(I_L, T)}{T}}_{\text{activation of T cell by cytokines}}, \quad (3)$$

$$\frac{dI_L}{dt} = \underbrace{Tf_4(X_1, X_2)}_{\text{secretion by tumor}} - \underbrace{\omega I_L}_{\text{degradation}}, \quad (4)$$

$$\frac{dA}{dt} = \underbrace{\gamma(a_0 - A)}_{\text{homeostasis of androgen}} - \underbrace{\gamma a_0 u(t)}_{\text{depletion of androgen if on therapy}}, \quad (5)$$

$$\frac{dD}{dt} = - \underbrace{cD}_{\text{death}}. \quad (6)$$

The androgen-dependent cancer cells (AD) are governed by their proliferation and death (given by $r_1(A, X_1, X_2)$), their mutation to androgen independent cancer cells (AI), the mutation from androgen independent cells, and the number killed by T cells. The AI cancer cells are also governed by proliferation and death, independent of androgen, their mutation from AD cells, their mutation to AD cells, and the number killed by the T cells. The T cell counts are determined by the number activated by the dendritic cells, their natural death, and clonal expansion. The concentration of cytokines is determined by their production by stimulated T cells and a clearance rate. The concentration of androgen in the blood is described by homeostasis term and deprivation therapy term. The dendritic cells are governed by their death rate.

The modeling of the intermittent androgen deprivation therapy is governed by the $u(t)$. Note that if $u(t) = 1$, we are modeling the ‘on-treatment’ portion of the therapy, and when $u(t) = 0$, we model the ‘off-treatment’ therapy. Below are the detailed equations:

$$y(t) = c_1 X_1 + c_2 X_2, \quad (7)$$

$$u(t) = \begin{cases} 0 \rightarrow 1 & \text{if } y(t) > L_1 \text{ and } \frac{dy}{dt} > 0, \\ 1 \rightarrow 0 & \text{if } y(t) < L_0 \text{ and } \frac{dy}{dt} < 0. \end{cases} \quad (8)$$

In this case, $y(t)$ represents the serum PSA level. When the PSA level decreases below a certain threshold, L_0 , the androgen deprivation therapy begins. When the PSA level increases above another threshold level, L_1 , the ‘on-treatment’ therapy starts. Note that $L_0 < L_1$.

Although our model is similar to the Portz and Kuang model, we include three major changes which allow for a more realistic model. Firstly, we change the growth and death functions. The original model incorporated an exponential growth rate for the androgen independent cell population, which is very unrealistic. We modified

this growth function to be logistic. Similarly, we change the growth and death for the androgen dependent cell population. We assume that the lack of androgen affects the androgen dependent cell population in two ways: lack of androgen lowers the growth rate, and the lack of androgen actively kills the cell population. This is a realistic assumption which has been used in other prostate cancer models with great success [7, 21]. We note that when androgen is at its homeostatic level, a_0 , the growth rate of androgen-dependent cells is at its highest. As the levels of androgen decrease, the growth rate of androgen-dependent cells also decreases, and the death due to lack of androgen increases. When androgen is at its lowest value, 0, there is no growth of androgen dependent cells, and the highest death rate due to lack of androgen happens.

$$\begin{aligned} r_1(A, X_1, X_2) &= r_1 A \left(1 - \frac{X_1 + X_2}{K} \right) - d_1(a_0 - A), \\ r_2(X_1, X_2) &= r_2 \left(1 - \frac{X_1 + X_2}{K} \right). \end{aligned} \quad (9)$$

The second major change considers the mutation functions. The original model assumed that AD cells mutated to AI cells in an androgen-depleted environment. However, they did not consider the option that AI cells might mutated back to AD cells in an androgen-rich environment. We include this second mutation term in our model. If $A = a_0$, we note that there would be no mutation from AD to AI cell population, as we are at the homeostatic androgen levels. As we decrease the level of androgen, we increase the rate at which AD to AI mutations occur, until we reach $A = 0$, which gives the largest mutation rate m_1 . For mutation from AI to AD, we assume a similar, but opposite function. When we have low levels of androgen, we assume that there is no mutation from AI to AD. However, as we increase through androgen levels, the mutation rate from AI to AD increases accordingly. The mutation rate functions are listed below.

$$\begin{aligned} m_1(A) &= m_1 \left(1 - \frac{A}{a_0} \right), \\ m_2(A) &= m_2 \left(\frac{A}{A + k_4} \right). \end{aligned} \quad (10)$$

The third major change we consider is using a generic class of functions for the four immune system interactions. As the immune system is extremely complicated with many interacting cells, there is not much data to help form a hypothesis on how these various components of the immune system interact together. In order to combat this, we consider functions that are generic, but we do want some basic properties for these functions. Specifically, we assume the following.

- (A1): $f_1(X_1, X_2, 0) = f_2(X_1, X_2, 0) = f_3(0, T) = f_4(0, 0) = 0$.
- (A2): $\frac{\partial f_1}{\partial X_1} \leq 0$, $\frac{\partial f_2}{\partial X_2} \leq 0$, and $\frac{\partial f_1}{\partial T} \geq 0$, $\frac{\partial f_2}{\partial T} \geq 0$.
- (A3): $\frac{\partial f_3}{\partial I_L} \geq 0$, $\frac{\partial f_4}{\partial X_i} \geq 0$, $i = 1, 2$.

3. Simulations and Observations. There are many potential functions which could be excellent candidates for our f_i , but a logical choice would be a Holling Type II function. It is reasonable to assume that after a large enough presence of cancer cells, the death rate would approach a maximum death rate. It is not sensible

to use a linear function which would assume the death rate is proportional to the number of cancer cells. The functions that we propose and use for simulations are:

$$\begin{aligned}
 f_1(X_1, X_2, T) &= \frac{e_1 T}{g_1 + X_1 + X_2}, \\
 f_2(X_1, X_2, T) &= \frac{e_2 T}{g_2 + X_1 + X_2}, \\
 f_3(I_L, T) &= \frac{e_3 I_L}{g_3 + I_L}, \\
 f_4(X_1, X_2) &= \frac{e_4(X_1 + X_2)}{g_4 + X_1 + X_2}.
 \end{aligned} \tag{11}$$

Explanations for the various parameters and their chosen values, as well as their respective sources are displayed in Table 1.

As we were unable to find a literature estimate on what the carrying capacity of the tumor cells would be, we decided to estimate with a brief calculation. The mean weight of a male prostate is 11 grams (7-16 grams) and the average human cell has a mass of 1 ng. Thus, we can conjecture that the carrying capacity is on the order of 10 billion cells.

In previous iterations of the immunotherapy mathematical model, hypothetical vaccines were administered every 30 days consisting of 0.3 million dendritic cells. In order to preserve the correct dosage, when the length between vaccinations was altered, the dosage was also altered accordingly. For the discrete case, variances from daily vaccinations to vaccinations separated by 120 days were considered. Simulations are extended to 4000 days to be able to determine long-term behavior of the prostate cancer. A continuous dose of dendritic cell vaccines was also considered, as if constantly administered through an IV. All simulations have the same initial conditions with initial AD count of 15 million cells, AI count of 0.1 million cells, 0 activated T cells, 0 concentration of cytokines (ng/mL), 30 nmol/mL concentration of androgen, 0 dendritic cells. We have set the threshold to turn off treatment L_0 as 5 and the threshold to begin treatment L_1 as $15 \frac{ng}{mL}$.

Once graphical results are obtained, it is advantageous to determine numerically and analytically whether the more successful dendritic cell vaccination timings have an overall effect.

3.1. Discrete Case. For all numerics, we consider that $e_1 = e_2$, which means that T cells are able to kill AD and AI cancer cells equivalently. We begin by keeping $e_1 = e_2$, the effectiveness with which the T-cells eliminate cancer cells steady at a value of 0.75, which is reasonable within the range given in Table 1. We run a series of simulations, out to 4000 days, varying only the dosage level and frequency of the dendritic cell vaccine. The vaccination frequencies are varied from daily vaccinations to 120 days between each injection, and the dosages are varied from 0.01 to 1.20 million cells, respectively.

Figure 1 displays the PSA levels, androgen dependent (X_1) and androgen independent (X_2) cell densities versus time in days. We can see from the graph that the androgen suppression is triggered when the level reaches about $15 \frac{ng}{mL}$, and is discontinued when the level reaches about $5 \frac{ng}{mL}$. As can be seen, the PSA levels eventually skyrocket, indicating rise of androgen independent cancer. When the PSA levels grow drastically, it is clear that androgen suppression therapy is no longer effective. The effect is clear in the corresponding AD and AI graphs: the

P	Biological Meaning	Value	Source
r_1	AD cell proliferation rate	0.025/day	[1]
β_1	AD cell death rate	0.008/day	[1]
K	cancer cell carrying capacity	11 billion	
k_4	AI to AD mutation half-saturation	1.7	
r_2	AI net cell growth rate	0.006/day	[1]
m_1	maximum mutation rate from AD to AI	0.00005/day	[19]
m_2	maximum mutation rate from AI to AD	0.00015/day	[37]
a_0	base level androgen concentration	30 ng/ml	[19]
γ	androgen clearance and production rate	0.08/day	[19]
ω	cytokine clearance rate	10/day	[38]
μ	T cell death rate	0.03//day	[24]
c	dendritic cell death rate	0.14/day	[31]
e_1	max rate T cells kill AD cancer cells	0-1/day	[24]
g_1	AD cancer cell saturation level for T cell kill rate	10×10^9 cells	[24]
e_2	max rate T cells kill AI cancer cells	0-1/day	[24]
g_2	AI cancer cell saturation level for T cell kill rate	10×10^9 cells	[24]
e	T cell max activation rate	20×10^6 cells/day	[24]
g	DC saturation level for T cell activation	400×10^6 cells	[40]
e_3	max clonal expansion rate	0.1245/day	[24]
g_3	IL-2 saturation level for T cell clonal expansion	1000 ng/ml	[24]
e_4	max rate T cells produce IL-2	5×10^{-6} ng/ml/cell/day	[24]
g_4	cancer cell saturation level for T cell stimulation	10×10^9 cells	[24]
D_1	DC vaccine dosage	300×10^6 cells	[40]
c_1	AD cell PSA level correlation	1×10^{-9} ng/ml/cell	[19]
c_2	AI cell PSA level correlation	1×10^{-9} ng/ml/cell	[19]

TABLE 1. Values of parameters (P), explanations, and cited sources of every parameter used in this mathematical model.

AD cancer cells are eliminated in the infrequent injections, which gives rise to the more fatal androgen independent cancer. This is exhibited through the AI count, which skyrockets in the infrequent injection case. The rapid increase of AI cells indicates that the more fatal androgen resistant prostate cancer has begun. From these graphs, it is apparent that more frequent injections of the dendritic cell vaccine can help the effectiveness of the intermittent hormone therapy, delaying the rise of androgen independent cancer.

We can see that by increasing the frequency of the injections, but keeping the total dosage identical, there are vast improvements in the survival time of the patient. In order to quantify this relationship, we perform numerical analysis to determine

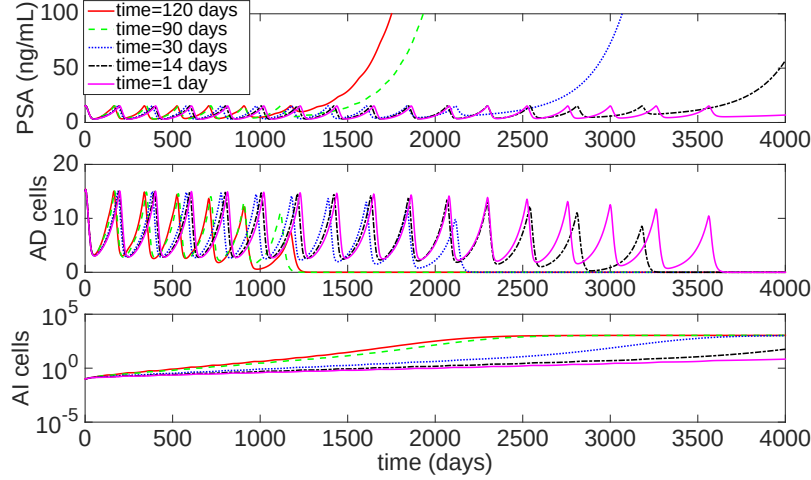


FIGURE 1. PSA serum concentration, androgen dependent (AD), and androgen independent (AI) cell concentrations for various dendritic cell vaccine injection times with an $e_1 = e_2$ of 0.75. More frequent injections result delay of the rise of fatal androgen independent cancer.

the lowest value of e_1 required for the solution to produce a limit cycle. A limit cycle in this case would represent a stable disease equilibrium, where the cancer is indefinitely responsive to IAD treatment. Recall that e_1, e_2 represent the cytotoxicity of the T-cells, a measure of how efficient a T cells is at killing the tumor cells. The biological range, as stated in 1 ranges from 0-1, cancer cells killed per day. A summary of the limit cyclical behavior is charted in figure 2.

We immediately notice that as vaccine timing is shortened, the minimal value of e_1 necessary to exhibit a stable disease state also decreases. We recall that e_1 could be a patient-specific parameter, as it is the maximum rate that T cells kill cancer cells per day. Thus, for those with weaker immune systems, more frequent injections could be much more effective. We note that for cases where the vaccine timing is greater than 30 days, even with values of maximal $e_1 = 1$, there is no stable disease state. Therefore, infrequent large doses of vaccine are much less effective at stabilizing the disease. Additionally, we can examine the shape of the limit cycles: as we increase the frequency of the dosage, we notice that the limit cycles are much smoother.

3.2. Continuous Case. We have shown that, even for total amount of vaccine being constant, more frequent dendritic cell vaccine administrations are more effective than infrequent administrations. If we consider the limiting case of this behavior, we arrive at the case of a continual injection, as if the patient is always connected to the vaccine through an IV system. In order to accommodate for the continual injection, we slightly modify the equation (6) representing the dendritic cell numbers to: $\frac{dD}{dt} = v - cD$, where v is the continual injection rate.

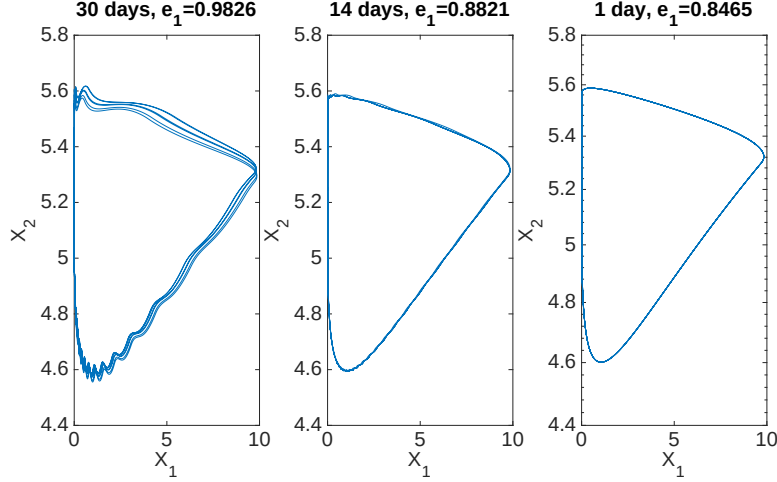


FIGURE 2. Limit cycle solutions for androgen dependent X_1 , and androgen independent X_2 cancer cells. The minimal value of e_1 required to produce limit cycle behavior is noted above each solution. As vaccine timing decreases, minimal e_1 necessary to have stable disease state decreases.

The simulation begins in this case with an initial injection of 0.04 billion dendritic cells, which is then kept constant throughout the duration of the simulation. For this continuous case, a variety of values of e_1 were considered, to determine if continuous vaccinations may help eliminate cancer. Figure 3 displays the PSA concentration for the different cases. We can see that contrary to the discrete case, it is apparent that even at lower values of $e_1 = 0.25$, the cancer is manageable. In fact, we even see elimination of cancer for very large values of e_1 , for $e_1 > 0.75$.

In order to clearly see if the cancer is manageable for lower values of e_1 we turn to the cell counts for AD and AI cancer cells. Figure 3 displays these counts for the continual dendritic cell vaccinations. It is apparent for the duration of the simulation, AI cells only become dominant for the e_1 values of 0 and 0.25. This implies that androgen independent cancer is avoidable for a larger range of e_1 values than in the discrete case. This means that despite a weaker auto-immune response, it may be possible to suppress the growth of androgen independent cancer cells. Additionally, the treatment cycle lengths are very different depending on the e_1 value: as we increase through our e_1 value, we see that the length of ‘off-treatment’ is much longer, resulting in more comfort for the patient.

Since we see a full range of behavior as we increase through e_1 , we take a closer look at a bifurcation diagram for the parameter. The resulting bifurcation diagram is shown in Figure 4. We can see that during most biologically relevant parameter values of e_1 , which is a range of 0-1, we have cyclical behavior. At the smallest range of $e_1 = 0$, we have stability of carrying capacity equilibrium. However, once we increase past $e_1 = 0$, we can see that we have stable cyclical behavior. As observed in Figure 3, as we increase through e_1 we continue observing stable disease cycles. Finally, for higher values of e_1 , we notice that the cycles collapse into a single steady state solution, which is stable. As we continue increasing e_1

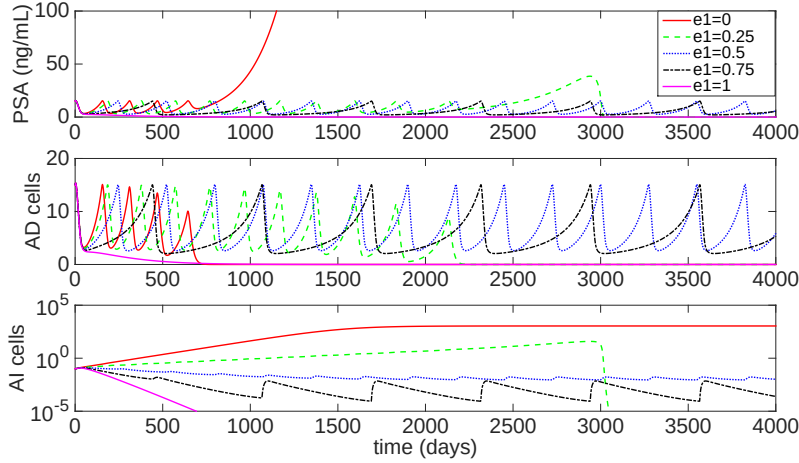


FIGURE 3. PSA serum level, AD cell density, and AI cell density for continual dendritic cell vaccinations, with an injection rate 0.04 billion cells for various values of e_1 . It is apparent that in this continuous case, a wider range of e_1 is able to suppress the growth of cancer and elongate the cycles of IAD.

past this point, the steady state approaches zero, which represents the eradication of the disease. We note that in examining the solutions for small values of e_1 , the graph appears to be zigzagging. We believe this may be due to the model dynamics having a long transition time before approaching the limiting periodic state. We also have interesting dynamics at $e_1 = 0.24$, where we seem to approach a steady state, before the solution switches back into cyclical behavior.

4. Basic Properties of the System. Although numerically studying the behavior of equations is useful, further information can be gathered by analysis of the equations themselves. It is also a metric to ensure that the behavior of the solutions have biological meaning. In order to analyze our equations, we assume that we are in fact continuously suppressing the androgen, instead of intermittent suppression. This assumption will lead to the following change of equation governing the concentration of androgen:

$$\frac{dX_1}{dt} = r_1(A, X_1, X_2)X_1 - m_1(A)X_1 + m_2(A)X_2 - X_1f_1(X_1, X_2, T), \quad (12)$$

$$\frac{dX_2}{dt} = r_2(X_1, X_2)X_2 + m_1(A)X_1 - m_2(A)X_2 - X_2f_2(X_1, X_2, T), \quad (13)$$

$$\frac{dT}{dt} = \frac{eD}{g + D} - \mu T + Tf_3(I_L, T), \quad (14)$$

$$\frac{dI_L}{dt} = Tf_4(X_1, X_2) - \omega I_L, \quad (15)$$

$$\frac{dA}{dt} = -\gamma A, \quad (16)$$

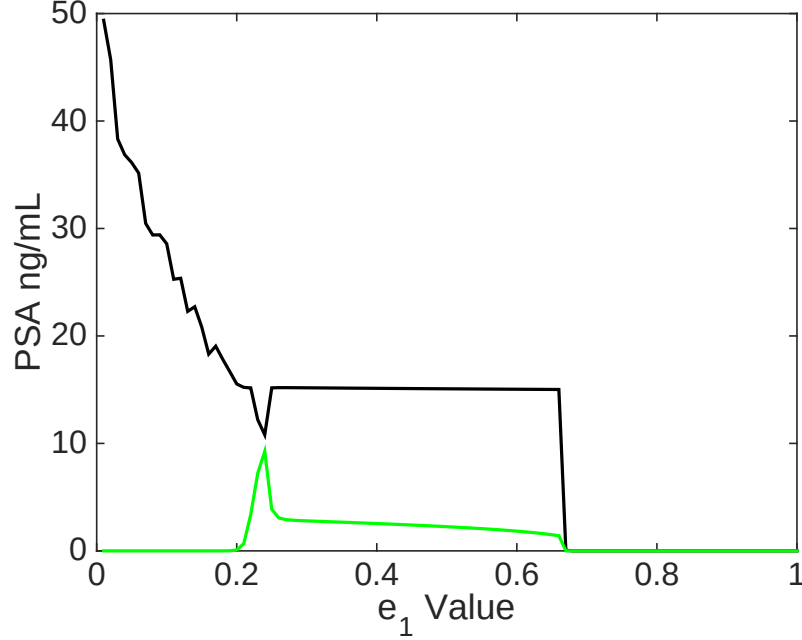


FIGURE 4. Bifurcation diagram for parameter e_1 , a measure of cytotoxicity of T-cells. Maximum PSA level in black, minimum PSA level in green. Carrying capacity is stable only for $e_1 = 0$. Immediately after, we have a Hopf bifurcation and limit cycles, until we reach $e_1 \approx 0.66$, after which the disease-free steady state is stable.

$$\frac{dD}{dt} = v - cD. \quad (17)$$

Proposition 1. *Solutions of (12)-(17) that start positive remain positive.*

Proof. Solutions for D and A are explicitly solvable, whose solutions are positive. If solutions do not remain positive there must be some time $t_1 > 0$ such that $X_1(t_1) = 0$, $X_2(t_1) = 0$, $T(t_1) = 0$ or $I_L(t_1) = 0$. We examine the first case where $X_1(t_1) = 0$. Then $X_1'(t) \geq -m_1(A)X_1$, $\forall t \in [0, t_1]$, or more specifically, $X_1(t_1) \geq X_1(0)e^{-m_1(A)t_1} > 0$, which is a contradiction. Similar arguments can be extended to the remaining variables, ensuring positivity of all solutions. $\square$

We now examine the equations in detail to determine equilibrium points and their respective stabilities. We hope that this will give us a sense of biological meaning. What values must biological parameters exhibit in order for prostate cancer to be eliminated?

Proposition 2. *The model system (12)-(17) has a disease-free equilibrium $E_0^* = (0, 0, \frac{ev}{\mu(cg+v)}, 0, 0, \frac{v}{c})$ with growth functions (9), mutation functions (10), and generic*

functions. E_0^* is unstable if $r_2 > f_2(0, 0, T_0^*)$, where $T_0^* = \frac{ev}{\mu(cg+v)}$, and locally asymptotically stable if $r_2 \leq f_2(0, 0, T_0^*)$. When $r_2 > f_2(0, 0, T_0^*)$, a positive endemic equilibrium $E^* = (X_1^*, X_2^*, T^*, I_L^*, A^*, D^*)$ emerges, stability unknown.

Proof. All variables except X_2 are easily solved and have only one steady state. For X_2 , we can either have $X_2^* = 0$, which exists always, or an X_2 that solves $g(X_2) := r_2 - \frac{r_2 X_2}{K} - f_2(0, X_2, T^*) = 0$. We examine this quantity in more detail by calculating its sign at $X_2 = 0$:

$$g(0) = r_2 - f_2(0, 0, T_0^*)$$

where $T_0^* = \frac{ev}{\mu(cg+v)}$ is the corresponding T^* value when $X_2^* = 0$. Next we calculate the sign as $X_2 \rightarrow K$, its maximum possible value:

$$g(K) = -f_2(0, K, T_K^*) < 0$$

Thus, if $r_2 - f_2(0, 0, T_0^*) < 0$, there is no biologically relevant X_2 value that solves $g(X_2^*) = 0$, since the function f_2 is monotonically decreasing in X_2 . Therefore, there is only the trivial, disease-free equilibrium $E_0^* = (0, 0, \frac{ev}{\mu(cg+v)}, 0, 0, \frac{v}{c})$ in our domain.

On the other hand, if $r_2 - f_2(0, 0, T_0^*) > 0$ then, by the Intermediate Value Theorem, there must be some $X_2^* \in (0, K)$ which solves $g(X_2^*) = 0$, giving us an endemic equilibrium $E_1^* = (0, X_2^*, T_1^*, I_{L1}^*, 0, \frac{v}{c})$, representing androgen-independent relapse.

Now that the equilibria have been found, we now turn to finding the eigenvalues of the Jacobian to determine stability. We examine the disease-free equilibrium E_0^* , which generates the following matrix:

$$\begin{pmatrix} a_{1,1} & 0 & 0 & 0 & 0 & 0 \\ m_1 & r_2 - f_2(0, 0, T_0^*) & 0 & 0 & 0 & 0 \\ 0 & 0 & -\mu & T_0^* \frac{\partial}{\partial I_L} f_3(0, T_0^*) & 0 & \frac{ceg}{cg+v} \\ T_0^* \frac{\partial}{\partial X_1} f_4(0, 0) & T_0^* \frac{\partial}{\partial X_2} f_4(0, 0) & 0 & -\omega & 0 & 0 \\ 0 & 0 & 0 & 0 & -\gamma & 0 \\ 0 & 0 & 0 & 0 & 0 & -c \end{pmatrix}$$

where $(a_{1,1} = -d_1 a_0 - m_1 - f_1(0, 0, T_0^*))$. This Jacobian results in the following set of eigenvalues, $-d_1 a_0 - m_1 - f_1(0, 0, T_0^*), r_2 - f_2(0, 0, T_0^*), -\mu, -\omega, -\gamma, -c$. Since we know that all parameters are non-negative, and assuming that all parameters are in fact positive, we can see that all eigenvalues except one are guaranteed to be negative. Only $r_2 - f_2(0, 0, T_0^*)$ has the possibility to be negative, positive, or zero. If $r_2 - f_2(0, 0, T_0^*) < 0$, we in fact have the cancer-free equilibrium to be stable. Otherwise if $r_2 > f_2(0, 0, T_0^*)$, the cancer-free equilibrium is unstable. $\square$

We would like to understand biologically what this means. We write our stability condition as $r_2 < f_2(0, 0, T_0^*)$: this means the maximal growth rate of androgen independent cells is less than the death rate of androgen independent cells due

to T cells. We observe that since these functions are monotone, in the case of strictly monotone function, we can invert f_2 to find an equivalent condition that involves T_0^* . Recalling that $T_0^* = \frac{ev}{\mu(cg+v)}$, this means we can solve for an explicit solution involving v , our critical dosage parameter. Theoretically, if the remaining parameters could be measured for a patient, a critical dosage could be calculated. We note that if given patient specific parameters such as their individual T cell efficiencies, we are able to calculate a necessary dose level to stabilize the disease-free equilibrium. In the context of our proposed simulation functions f_i , given in (11), our condition for stability becomes $v > \frac{cgg_2r_2\mu}{e_2e - g_2r_2\mu}$, which is indeed a critical dosage value.

5. Global Analysis of Limiting System. As the full system is difficult to perform analysis on, we consider the limiting systems by performing a quasi-steady state approximation. This can give insight into the biology. We begin by assuming quasi-steady states for androgen, cytokines, and dendritic cells. This is a reasonable assumption, since the time scales at which these processes occur is much shorter than that of the populations of cancer cells growing. Then, we use the results of Thieme [42] to examine the asymptotic behavior of the limiting system. We consider the system when androgen deprivation therapy is continual ($A = 0$). We will examine the case where androgen deprivation therapy is continually on thoroughly and determine necessary conditions of $f_i(X_1, X_2, T)$ to obtain global stability of eradication of prostate cancer and conditions for global stability of the diseased steady state.

We examine the case where androgen deprivation therapy is turned on ($A = 0$). We let I_L, D , and A go to quasi-steady state. We end up with the following set of equations:

$$\frac{dX_1}{dt} = -d_1a_0X_1 - m_1X_1 - X_1f_1(X_1, X_2, T), \quad (18)$$

$$\frac{dX_2}{dt} = r_2X_2 \left(1 - \frac{X_1 + X_2}{K}\right) + m_1X_1 - X_2f_2(X_1, X_2, T), \quad (19)$$

$$\frac{dT}{dt} = \frac{eD}{g + D} - \mu T + Tf_3(I_L, T), \quad (20)$$

$$I_L = \frac{Tf_4(X_1, X_2)}{\omega}, \quad (21)$$

$$A = 0, \quad (22)$$

$$D = \frac{v}{c}. \quad (23)$$

Note that:

$$\begin{aligned} \frac{dX_1}{dt} &= X_1 [-d_1a_0 - m_1 - f_1(X_1, X_2, T)] \\ &\leq X_1 [-d_1a_0 - m_1] \\ &\leq -aX_1. \end{aligned} \quad (24)$$

It is apparent that $\lim_{t \rightarrow \infty} X_1(t) = 0$. Thus we can reduce the system to:

$$\begin{aligned} \frac{dX_2}{dt} &= r_2 X_2 \left(1 - \frac{X_2}{K}\right) - X_2 f_2(0, X_2, T), \\ \frac{dT}{dt} &= \frac{eD}{g+D} - \mu T + T f_3(I_L, T), \end{aligned} \quad (25)$$

which is defined on the subdomain $\bar{\Omega} = \{(X_2, T) : X_2 \geq 0, T \geq 0\}$ and is the limiting system of (18)-(23).

Before we begin the analysis of the system, we would like to ensure that the behavior of the quasi-steady state system is analogous to the full system. We must quantify what dynamics are preserved and eliminated by simplifying the system. We run the system with the same parameters for the full system and for the reduced system. Figure 5 shows the comparison. We can see that by examining the quasi-steady state system we do lose some of the dynamics in certain cases, like when $e_1 = 0.15$. We note that this falls into the regime of 'chaotic' behavior, so it is not surprising that we do not have identical dynamics. For all other values of e_1 , however, we note that the quasi steady-state system very closely resembles the full system, meaning that our results for the quasi-steady state system may be extended to the full system. We also note that our expected outcome – that X_1 goes to zero, is exhibited in all of the figures.

Theorem 5.1. *The disease-free steady state of (18)-(23) is globally asymptotically stable under the following conditions:*

- i) $\mu > f_3(I_L, T)$,
- ii) $r_2 < f_2(0, 0, T_0^*)$,
- iii) $\frac{\mu^2(cg+v)}{ev} > \frac{\partial}{\partial T} f_3(0, T_0^*)$.

To prove this theorem, we break down the result into several propositions for simplicity. We will begin with positivity and boundedness, move to local asymptotic stability, and end with conditions necessary for global asymptotic stability.

Proposition 3. *If $\mu > f_3(I_L, T)$ then solutions of (18)-(23) remain positive and bounded.*

Proof. We begin with the proof for positivity, examining T first. We note that since we are assuming that $T(t_0) \geq 0$, in order for $T(t) < 0$ for some t , we would require that $\frac{dT}{dt} < 0$ when $T = 0$. However, $\frac{dT}{dt}|_{T=0} = \frac{eD}{g+D} > 0$ since all parameters are positive. For X_2 , since we are assuming that $X_2(t_0) \geq 0$, in order for $X_2(t) < 0$ for some t , we would require that $\frac{dX_2}{dt} < 0$ when $X_2 = 0$. However, $\frac{dX_2}{dt}|_{X_2=0} = m_1 X_1 > 0$ since we have already proved that X_1 stays positive and all parameters are positive.

We have already proven that solutions remain nonnegative, so we now look to boundedness. We begin with T :

$$\begin{aligned} \frac{dT}{dt} &= \frac{eD}{g+D} + T(f_3(I_L, T) - \mu) \\ &\leq c - \bar{g}T \end{aligned} \quad (26)$$

where $\bar{g} = \min[\mu - f_3(I_L, T)] > 0$ by assumption. This implies that T is bounded.

We look towards the boundedness of X_2 now. We immediately see that X_2 is bounded above by K . □

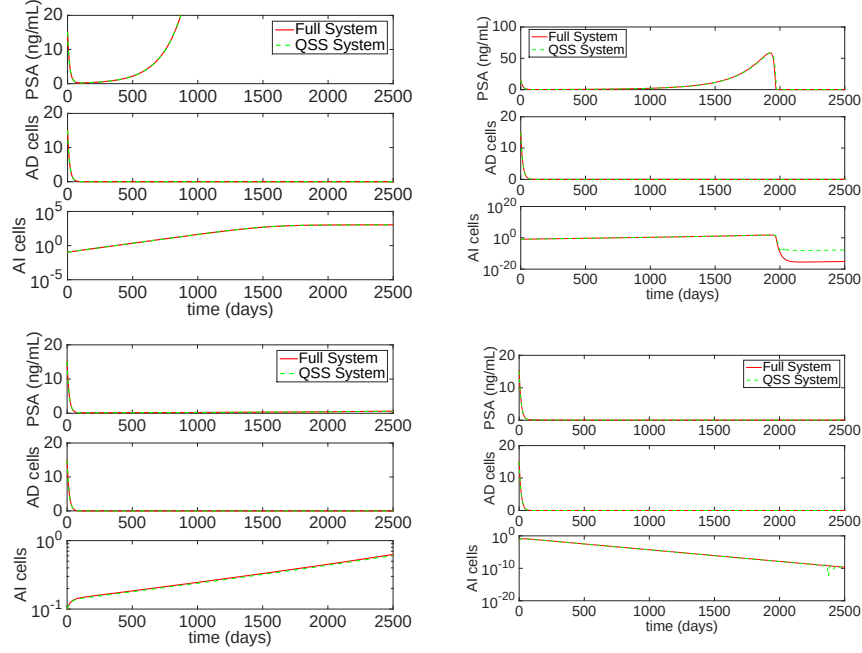


FIGURE 5. Comparisons of the full system and quasi-steady state system for various values of e_1 : 0, 0.15, 0.25, and 0.65, assuming androgen deprivation therapy is constantly on. These values of e_1 show many differing dynamics. The quasi-steady state system closely approximates the full system in every case, but shows slight differences in the case of $e_1 = 0.15$

Proposition 4. *The limiting system (25) contains two equilibria: the disease-free equilibrium and a negative equilibrium, under assumption ii): $r_2 < f_2(0, 0, T_0^*)$. The disease-free equilibrium is locally asymptotically stable under assumption ii) and iii): $\frac{g+D}{\epsilon D} > \frac{\partial}{\partial T} f_3(0, T_0^*)$.*

Proof. There are two possible equilibria of the system. Following the proof from Proposition 2: if $r_2 < f_2(0, 0, T_0^*)$, only the disease-free equilibrium exists, $E_0^* = (0, T_0^*)$. The local stability of the disease-free steady state $E_0^* = (0, \frac{ev}{\mu(cg+v)})$ is exhibited in the Jacobian:

$$\begin{pmatrix} r_2 - f_2(0, T_0^*) & 0 \\ T_0^* \frac{\partial}{\partial X_2} f_3(0, T_0^*) & -\mu + T_0^* \frac{\partial}{\partial T} f_3(0, T_0^*) \end{pmatrix}$$

and the eigenvalues are given by $\lambda_1 = r_2 - f_2(0, 0, T_0^*) < 0$ as given by the assumptions, and $\lambda_2 = -\mu + T_0^* \frac{\partial}{\partial T} f_3(0, T_0^*) < 0$ (by condition iii), so we have local asymptotic stability. $\square$

We are now ready to prove the main result for global asymptotic stability.

Proof of Theorem 5.1. Since solutions to the system are positive and bounded, we immediately see that there can be no limit cycles around our non-negative equilibrium, since our only equilibrium is on the boundary. By Poincare-Bendixson, all solutions tend towards the disease-free steady state, so E_0^* is globally asymptotically stable. $\square$

We can interpret two of these conditions for the global stability in terms of biology. We can see that the condition $r_2 < f_2(0, T_0^*)$ can be interpreted as the maximal intrinsic growth rate of the cancer cells must be less than the death rate due to the T cells. Recall that this was the condition for local stability of the full system, so we are unsurprised to see the same condition again. Similarly, $\mu > f_3(I_L, T)$ means that we want the death rate of the T cells to be greater than the production of T cells due to I_L . Now that we have examined the conditions for global stability for the disease-free equilibrium, we also want to explore the dynamics for the equilibrium that is not disease-free.

Theorem 5.2. *The diseased steady state of (18)-(23) is globally asymptotically stable under the following conditions:*

- i) $\mu > f_3(I_L, T)$,
- ii) $r_2 > f_2(0, T_0^*)$,
- iii) $\mu - f_3(I_L, T) > -X_2 \frac{\partial}{\partial X_2} f_2(X_1, X_2, T) + T \frac{\partial}{\partial T} f_3(I_L, T) - \frac{r_2 X_2}{K} \forall X_2, T \geq 0$.

To prove this theorem, we break down the result into several propositions for simplicity. We will begin with positivity and boundedness, move to local asymptotic stability, followed by eliminating limit cycles, and end with conditions necessary for global asymptotic stability.

Proposition 5. *If condition i) is satisfied, then solutions of (18)-(23) remain positive and bounded.*

Proof. Positivity and boundedness have already been proven in Proposition 3, and the results hold for current conditions, as long as we assume condition i). $\square$

Proposition 6. *The limiting system (25) contains two equilibria: the disease-free equilibrium, E_0^* , and a secondary equilibrium, E_1^* . The secondary equilibrium is positive (assuming condition ii)). The disease-free equilibrium is a saddle point (under conditions ii) and iii)).*

Proof. The existence of the equilibria follow from Proposition 2, so we know that under condition ii), if $r_2 > f_2(0, T_0^*)$, we will have two biologically relevant equilibria: $E_0^* = (0, T_0^*) = (0, \frac{ev}{\mu(cg+v)})$ and $E_1^* = (X_2^*, T_1^*)$.

The local stability of the disease-free steady state E_0^* is exhibited in the Jacobian:

$$\begin{pmatrix} r_2 - f_2(0, T_0^*) & 0 \\ T_0^* \frac{\partial}{\partial X_2} f_3(0, T_0^*) & -\mu + T_0^* \frac{\partial}{\partial T} f_3(0, T_0^*) \end{pmatrix}$$

and the eigenvalues are given by $\lambda_1 = r_2 - f_2(0, T_0^*) > 0$ by condition ii) and $\lambda_2 = -\mu + T_0^* \frac{\partial}{\partial T} f_3(0, T_0^*) < 0$, by condition iii). Thus, the disease-free equilibrium is a saddle point.

Now we examine the Jacobian of the diseased equilibrium:

$$\begin{pmatrix} -\frac{r_2 X_2^*}{K} - X_2^* \frac{\partial}{\partial X_2} f_2(X_2^*, T_1^*) & -X_2^* \frac{\partial}{\partial T} f_2(X_2^*, T_1^*) \\ T_1^* \frac{\partial}{\partial X_2} f_3(I_L^*, T_1^*) & -\mu + T_1^* \frac{\partial}{\partial T} f_3(I_L^*, T_1^*) + f_3(I_L^*, T_1^*) \end{pmatrix}.$$

Thus, the trace is given by

$$\tau = -\frac{r_2 X_2^*}{K} - X_2^* \frac{\partial}{\partial X_2} f_2(X_2^*, T_1^*) - \mu + T_1^* \frac{\partial}{\partial T} f_3(I_L^*, T_1^*) + f_3(I_L^*, T_1^*) \quad (27)$$

and the determinant is given by

$$\begin{aligned} \Delta = & \left(-\frac{r_2 X_2^*}{K} - X_2^* \frac{\partial}{\partial X_2} f_2(X_2^*, T_1^*) \right) \left(-\mu + f_3(I_L^*, T_1^*) + T_1^* \frac{\partial}{\partial T} f_3(I_L^*, T_1^*) \right) \\ & + \left(X_2^* \frac{\partial}{\partial T} f_2(X_2^*, T_1^*) T_1^* \frac{\partial}{\partial X_2} f_3(I_L^*, T_1^*) \right). \end{aligned} \quad (28)$$

In order for E_1^* to be stable we require $\tau < 0, \Delta > 0$. Notice that $\tau < 0$ is given by assuming condition iii). However, we have no idea about the sign of Δ . Given our information of $\tau < 0$, we know that E_1^* is either a saddle point or a stable node/spiral. $\square$

Proposition 7. *The limiting system (25) has no limit cycles as long as condition iii) is satisfied.*

Proof. We will be using the Dulac criterion to establish that there are no periodic orbits within. Using $h(X_2, T) = \frac{1}{X_2}$, we can see that

$$\begin{aligned} \Delta &= \frac{\partial}{\partial X_2} \left[\frac{1}{X_2} \left(r_2 X_2 \left(1 - \frac{X_2}{K} \right) - X_2 f_2(X_2, T) \right) \right] \\ &\quad + \frac{\partial}{\partial T} \left[\frac{1}{X_2} \left(\frac{eD}{g+D} - \mu T + T f_3(I_L, T) \right) \right] \\ &= \frac{\partial}{\partial X_2} \left[r_2 - \frac{r_2 X_2}{K} - f_2(X_2, T) \right] + \frac{\partial}{\partial T} \left[\frac{eD}{X_2(g+D)} - \frac{\mu T}{X_2} + \frac{T(f_3(I_L, T))}{X_2} \right] \\ &= -\frac{r_2}{K} - \frac{\partial}{\partial X_2} f_2(X_2, T) - \frac{\mu}{X_2} + \frac{f_3(I_L, T)}{X_2} + \frac{T}{X_2} \frac{\partial}{\partial T} f_3(I_L, T) \end{aligned}$$

To ensure that there are no periodic orbits, we must prove that this quantity Δ does not change sign. We begin by re-writing this condition:

$$X_2 \Delta = -\frac{r_2 X_2}{K} - X_2 \frac{\partial}{\partial X_2} f_2(0, X_2, T) - \mu + f_3(I_L, T) + T \frac{\partial}{\partial T} f_3(I_L, T). \quad (29)$$

We know that for $X_2, T \geq 0$, $\Delta < 0$ by condition iii). Thus, the Dulac criterion has ensured that we will have no periodic orbits in our domain. $\square$

We are now ready to prove the main result for global asymptotic stability.

Proof of Theorem 5.2. By Proposition 7, we can see that there are no limit cycles present. Solutions are positive and bounded, and the model system has two fixed points, one of which is a saddle point. By the Poincare-Bendixson theorem, we know that there are three possibilities: all solutions tends to a fixed point, all solutions

tend to a periodic orbit, or there is a homoclinic or heteroclinic orbit which connects our two fixed points. We have ruled out periodic orbits by Dulac criterion using condition iii).

Now we must show there is no homoclinic or heteroclinic orbits connecting our fixed points.

In order to show there is no heteroclinic orbit connecting the two fixed points, we look towards the stable manifold of the saddle point. Looking back towards our Jacobian, we can clearly see that the stable manifold is given by the T -axis

$$\begin{pmatrix} X_2 \\ T \end{pmatrix} = \begin{pmatrix} 0 \\ 1 \end{pmatrix}. \quad (30)$$

Due to assumption ii), we notice that near the disease free steady state E_0^* , we have $dX_2(t)/dt > 0$. These observations preclude the existence of a heteroclinic orbit connecting E_0^* and E_1^* .

The fact that $dX_2(t)/dt > 0$ near E_0^* also implies that there is no homoclinic orbit originating from E_0^* . There can not be any homoclinic orbits originating from E_1^* if it is stable. The Dulac criteria also rules out homoclinic orbits originating from E_1^* if it is a saddle.

By Poincare-Bendixson Theorem, the only option remaining is that all solutions of (25) converge to E_1^* . Thus, E_1^* is globally asymptotically stable. $\square$

6. Conclusion. Current treatment options for late-stage prostate cancer are sub-optimal in terms of survival and quality of life. By examining a model of intermittent hormone therapy coupled with dendritic cell vaccines, we are able to prolong both the life and quality of life of the patient. We found that, keeping total yearly dosages the same, more frequent injections are conducive to managing prostate cancer for a longer period of time. We extrapolate this idea to the extreme by modifying the model to include a 'continual' dosage, as if administered through an intravenous fluid.

In our model, there are several parameters which are patient specific, or do not have accepted literature values. We examined the effect of varying values of e_1 , the killing T-cell efficiency. Predictably, increasing e_1 led from androgen-independent relapse, to stable limit cyclical behavior, and when increased enough, total eradication of the disease. This parameter measures how effective dendritic cell vaccine therapy will be - if e_1 is small, the therapy will be negligible. e_1 also acts to elongate the cycle times for a stable cyclical disease, which means increased time for the period when the patient is not undergoing androgen deprivation therapy. We performed bifurcation analysis on parameter e_1 to examine the various behaviors that exist in the system. As our simulations only considered the case where $e_1 = e_2$, we could also investigate how the dynamics change when we allow these values to be different.

Additionally, for mathematical analysis, we simplify the model to have continuous androgen suppression, in order to determine the effect of continual dosages. We notice there are two possible equilibria - cancer-free equilibrium and an androgen-independent (fatal) cancer equilibrium. If our continual dosage is less than a determined critical value, the cancer-free equilibrium exists, but it is unstable. If our continual dosage is higher than that critical value, the cancer-free equilibrium is stable and a cancerous equilibrium is born (stability unknown). These findings have biological significance. In previous papers it has been determined that for a

30-day vaccine, it is necessary for a large values of e_1 to ensure cancer-free progression [36]. In this analysis, we could have smaller values of e_1 that still result in stable disease-free equilibrium. For patients with less effective immune systems (lower e_1 values), it is possible to eradicate cancer with higher presence of dendritic cells. As dendritic cell vaccines are not known to have an adverse effect on the human body, it is possible that for patients with weakened immune systems, a tailored dose could be administered.

We further examined the limiting cases of behavior for this system, by allowing several parameters to go to quasi-steady state. We were able to determine requirements for global stability – or the guarantee of elimination of prostate cancer in some cases. Additionally, we were able to determine further conditions for the global stability of the endemic equilibrium.

Despite the insights that this model has afforded us, there is still much to be done. Future work may include comparing the model with available patient data. This will allow us to determine the efficacy of this model at predicting behavior of the prostate cancer. We will also be able to explore the patient-specific parameters and the effect these have on final outcome. Additionally, we would like to be able to compare our model with other patient-data validated models. Mathematically, the local stability of the endemic equilibrium of the full system should also be studied in detail.

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