A Mathematical Model of Angiogenesis in Glioblastoma Multiforme

Mary Alice Cameron,* Audrey Lucille Davis †
Department of Mathematics and Statistics
Arizona State University
P.O. Box 871804
Tempe, AZ 85287-1804

Advisors: Dr. Eric Kostelich ‡, Steffen Eikenberry §

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Abstract

This paper gives an introduction to the mechanisms involved in the development of aggressive brain tumors, specifically glioblastomas multiforme. The paper then introduces a mathematical model that predicts tumor growth, vascular development (angiogenesis), and the growth factors that affect them both. When angiogenesis is promoted, the model demonstrates more aggressive tumor growth, suggesting that anti-angiogenic therapy may slow tumor growth and provide an effective form of treatment.

*MaryACameron@gmail.com
†Audrey.Whitmer@asu.edu
‡Kostelich@asu.edu
§seikenbe@asu.edu

Contents

1 Introduction

Glioblastomas multiforme are stage four primary brain tumors that account for more than half of all malignant brain tumors. Even with optimal treatment, the two-year survival rate for recurrent glioblastoma is less than 30% [28]. Severe hypoxia, common among glioblastomas, results in sections of tumor death, or necrosis, and leads to the development of a tumor with a necrotic core, a band of quiescent (non-dividing) cells, and a proliferating rim. This hypoxia causes resistance to radiation therapy and inhibits cytotoxic drug delivery to cancerous cells [37]. Furthermore, glioblastomas are characterized by abundant, permeable blood vessels that lead to regions of edema and high interstitial pressure. These structural irregularities in glioblastomas result in the formation of a very deadly, heterogeneous tumor that is difficult to treat and impossible to cure. Consequently, it very important to understand the mechanisms involved in this tumor growth in order to better diagnose and treat patients with glioblastomas.

Rapidly dividing tumor cells require a steady supply of nutrients and oxygen from the circulatory system in order to grow beyond one to three millimeters in size [16]. We have developed a mathematical model that describes the interactions between tumor cells, vasculature, and the growth factors that affect them both. Angiogenesis, the formation of new blood vessels, is primarily dependent upon three growth factors: vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) [38]. The primary growth factor responsible for angiogenesis, VEGF, is over-expressed in glioblastomas and results in the formation of abundant, permeable vasculature [37]. Angiopoietin-1 is a growth factor that matures vasculature by reinforcing connections between endothelial cells and decreasing the permeability of newly-formed vessels. In normal vascular development, Ang-1 is expressed in order to improve the stability of newly-formed blood vessels. Conversely, angiopoietin-2 is a natural antagonist to Ang-1, in that it blocks the Tie-2 receptor that receives both growth factors. Consequently, Ang-2 destabilizes vasculature and makes it more permeable, a necessary condition for vascular remodeling. When coupled with

VEGF, Ang-2 causes angiogenic sprouting of new vessels. In the absence of VEGF, Ang-2 causes vascular regression, or pruning [38].

Because it is difficult to observe the early development of brain tumors in humans, there are multiple proposed theories of early tumor development. Previously it was believed that a tumor developed as an avascular mass of cells that would recruit its own vasculature [30]. However, recent studies in laboratory rats have shown that tumor growth may begin inside already mature host vasculature [16, 31, 38]. Our model of tumor growth assumes the latter theory of vascular development. When the tumor begins to form in a host vessel, it is supplied with adequate oxygen and nutrients necessary for survival and replication. As the rapidly growing tumor increases in size, the host releases Ang-2, potentially as a defense mechanism. Ang-2, normally unexpressed in healthy brain tissue, destabilizes the mature vasculature and causes vascular regression within the tumor [16]. The resulting lack of nutrients and oxygen causes massive cancer cell death in the tumor's core. The remaining cancer cells, deprived of oxygen, secrete VEGF in order to induce angiogenesis. The high concentration of VEGF, coinciding with the remaining Ang-2 results in angiogenic sprouting around the necrotic core of the tumor [38]. However, because tumor cells, especially glioblastoma, secrete abnormally high amounts of VEGF, the resulting vasculature is irregularly shaped, very permeable, and does not mature like normal vasculature. These vessels sustain new tumor growth, but also lead to regions of edema and swelling in the brain [18].

In this paper, we introduce a mathematical model that simulates the growth of glioblastomas multiforme. An anti-VEGF antibody is then introduced into the system as a form of cancer treatment.

2 Mathematical Model

Our mathematical model includes the following assumptions:

- VEGF is expressed by cancer cells at a constant rate and in response to hypoxia.
- Cancer cells do not express Ang-1 or Ang-2.

- Ang-2 is expressed by mature host vessels in response to tumor cell invasion.
- Hypoxia is expressed as a ratio proportional to cancer cells and vessel length.
- Ang-1 is produced by mature vessels at a constant rate.
- The concentration of Ang-2 must be at least 8 times greater than the concentration of Ang-1 in order to completely block the effects of Ang-1 [29].
- Immature endothelial cells are produced in response to VEGF only.
- Immature endothelial cells die when there is not enough VEGF to keep them alive [15].

Our derived model is a system of seven nonlinear ordinary differential equations dependent on time, t:

$a_1(t) =$ amount of angiopoietin-1 (Ang-1)
$a_2(t) =$ amount of angiopoietin-2 (Ang-2)
$v(t) =$ amount of vascular endothelial growth factor (VEGF in ng)
$c(t) =$ number of cancer cells
$y(t) =$ number of endothelial cells (ECs)
$b(t) =$ length of blood vessels (in μm)
$r(t) =$ amount of anti-VEGF treatment in the system

$$\frac{da_1}{dt} = \underbrace{\alpha_{a_1} b}_{\text{growth}} - \underbrace{\delta_{a_1} a_1}_{\text{degradation}}, \tag{1}$$

$$\frac{da_2}{dt} = \underbrace{\alpha_{a_2} b\left(\frac{c}{\theta_{a_2}+c}\right)}_{\text{growth}} - \underbrace{\delta_{a_2} a_2}_{\text{degradation}}, \tag{2}$$

$$\frac{dv}{dt} = \underbrace{\alpha_v c}_{\text{constant expression}} + \underbrace{\alpha_{v_2} c(\frac{h}{\theta_v+h})}_{\text{hypoxic growth}} - \underbrace{\delta_v v}_{\text{degradation}} - \underbrace{\tau r v}_{\text{antibody binding with VEGF}}, \tag{3}$$

$$\frac{dc}{dt} = \underbrace{\alpha_c c(1-\frac{c}{k})}_{\text{logistic growth}}, \tag{4}$$

$$\frac{dy}{dt} = \underbrace{\alpha_y y\left(\frac{g}{\theta_r+g}\right)}_{\text{EC proliferation}} - \underbrace{\omega y\left(\frac{n}{\theta_b+n}\right)\left(\frac{g}{\rho+g}\right)}_{\text{maturation to vasculature}} - \underbrace{\delta_y y\left(1-\frac{g}{\theta_y+g}\right)}_{\text{death}}, \tag{5}$$

$$\frac{db}{dt} = \underbrace{\frac{1}{s}\omega y\left(\frac{n}{\theta_b+n}\right)\left(\frac{g}{\rho+g}\right)}_{\text{maturation from ECs}} - \underbrace{\gamma b\left(\frac{m^4}{\theta_{EC}^4+m^4}\right)\left(1-\frac{g}{\rho+g}\right)}_{\text{vasculature breakdown}}, \tag{6}$$

$$\frac{dr}{dt} = \underbrace{u(t)}_{\text{Anti-VEGF dosage}} - \underbrace{\tau r v}_{\text{antibody binding with VEGF}} - \underbrace{\delta_r r}_{\text{degradation}} \tag{7}$$

where:

$$\begin{aligned} h &= c/b, \\ k &= b\lambda, \\ g &= v/y, \\ m &= a_2/a_1, \\ n &= a_1/a_2. \end{aligned}$$

2.1 Model Derivation

Equation (1) describes the behavior of Angiopoietin-1. Ang-1 is produced by vasculature at a constant rate, α_{a_1} and degrades at a rate δ_{a_1} relative to the concentration of angiopoietin-1 in the system.

Equation (2) describes the behavior of Angiopoietin-2. Angiopoietin-2 is produced by vascu-

lature at a maximum rate α_{a_2} in response to the presence of cancer cells. It degrades at a rate δ_{a_2} relative to the concentration of Ang-2 in the system.

Equation (3) describes the behavior of vascular endothelial growth factor. Vascular endothelial growth factor is produced by cancer cells both at a constant rate, α_v, and at a hypoxic rate, α_{v_2}, when there is not enough vasculature to support the growth of tumor cells (low VEGF per endothelial cell). VEGF degrades at a rate δ_v relative to the concentration of VEGF in the system. VEGF is also removed at a rate τ when it binds with the anti-VEGF antibody.

Equation (4) describes the behavior of cancerous tumor cells. Cancer cells grow logistically at a maximum rate α_c, limited by $k = b\lambda$, the amount of available vasculature that can support cellular growth.

Equation (5) describes the behavior of endothelial cells. Endothelial cells proliferate at a maximum rate α_y in response to the amount of VEGF per endothelial cell. Endothelial cells mature into vasculature at a maximum rate ω when there is a high enough Ang-1/Ang-2 ratio to mature vasculature, and when there is enough VEGF per endothelial cell to cause angiogenic sprouting. Endothelial cells die at a maximum rate δ_y when there is not enough VEGF per endothelial cell to continue survival.

Equation (6) describes the behavior of mature blood vessels. Vasculature is produced when endothelial cells mature into vasculature, but with a conversion factor s^{-1} for the amount of endothelial cells needed to make 1 μm of vasculature. Vasculature decays at a maximum rate γ when there is a high Ang-2/Ang-1 ratio and a low VEGF per endothelial cell ratio, causing vascular pruning.

Equation (7) describes the behavior of an anti-VEGF treatment. The anti-VEGF treatment is introduced as a function of time, $u(t)$ where 700 mg of anti-VEGF treatment are introduced every 14 days, consistent with clinical trials [35]. The treatment binds with VEGF at a rate τ and decays at a rate δ_r.

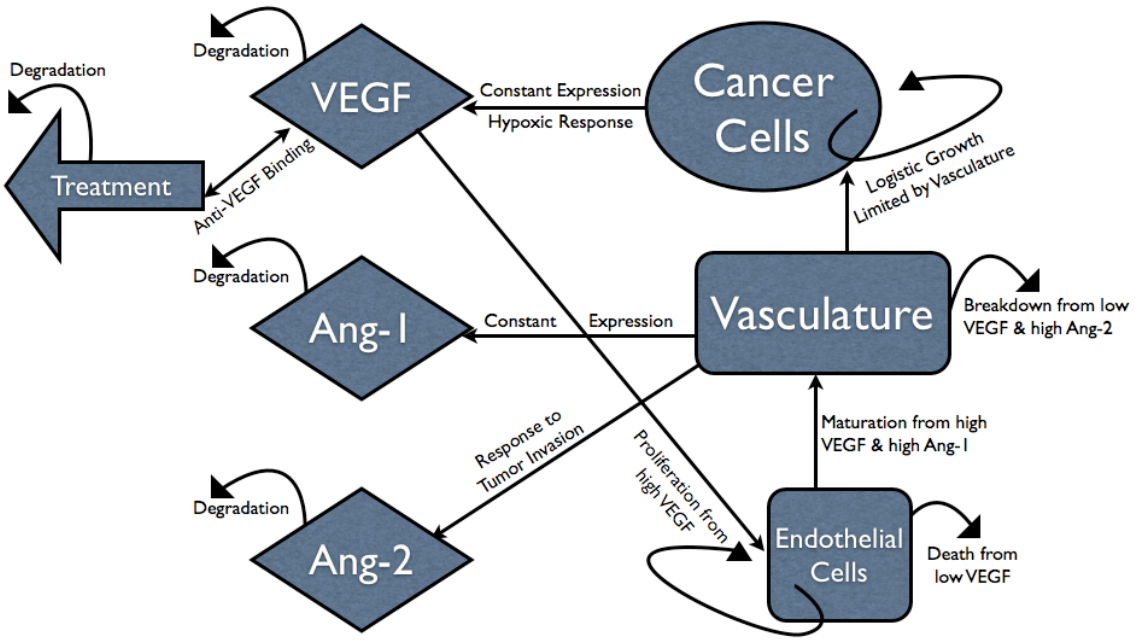

Figure 1: Schematic Diagram of the Mathematical Model

2.2 Parameterization

Most of our model parameters were extracted from biological literature (see Table 1). Gevertz and Torquato estimate maximum growth and death rates for angiopoietin-1 and angiopoietin-2 in their model [11]. We used their estimates for $\alpha_{a_1} = 0.24$, and $\alpha_{a_2} = 1.92$, coinciding with an Ang-2/Ang-1 ratio of 8 [23]. Gevertz and Torquato also estimate the degradation rate of Ang-1 to be 0.072. We assume this degradation rate for Ang-2 as well, so $\delta_{a_1} = \delta_{a_2} = 0.072$. The units for these parameters are unimportant because Ang-1 and Ang-2 only appear as ratios in our model.

In a study where gliomas were injected into rats, high levels of Ang-2 were expressed at two weeks when the tumor was approximately 2 millimeters in diameter [16]. This converts to a 2,000μm tumor diameter. We assume a cancer cell is at least 20μm in diameter [7]. Assuming a spherical tumor and spherical cancer cell, $\theta_{a_2} = 10^6$ cancer cells.

Meister, *et al.* measured the constant expression of VEGF by glioma cells to be $\alpha_v = 3 * 10^{-6} ng\, cell^{-1} day^{-1}$[25]. Ikeda, *et al.* measured the VEGF secretion rate to be approximately 8

to 10 times higher under hypoxic conditions[17]. In order to model a more aggressive tumor that displays qualitatively realistic results, we increased the constant to $\alpha_{v_2} = 3 * 10^{-2}\, ng\, cell^{-1} day^{-1}$.

Chen, *et al.* measured the degradation rate of VEGF to be $2.31 * 10^{-4}\, sec^{-1}$[8]. This converts to $\delta_v = 19.96$ day^{-1}.

Tumor cells are able to receive nutrients and survive at a distance between $90 - 165\mu$m from a blood vessel [33, 36]. Taking a tumor cord of length 1μm, and assuming the diameter of a cancer cell as 20μm [7], the maximum number of non-hypoxic cancer cells per μm of blood vessel is between 6.1 and 20.4 cancer cells. Biological literature supports this estimate. Data shows that one endothelial cell is able to support 50-100 tumor cells [10]. John Nagy cites between 17 and 22 vascular endothelial cells per 100μm of microvessel length in his model [26], so $.17\, EC\, \mu m^{-1} <$s$< .22\, EC\, \mu m^{-1}$. This data results in a maximum density between 8.5 and 22 tumor cells per μm of microvessel length. In this model we assume tumor cells become hypoxic beyond 100μm from a vessel, so $\lambda = 7.5\,$cancer cells$\,\mu m^{-1}$.

θ_v is the half-max of the ratio of cancer cells per μm of microvessel where hypoxia occurs and VEGF is produced. We assume $\theta_v = \lambda = 7.5\,$cancer cells$\,\mu m^{-1}$.

The doubling time for a glioblastoma cell is 24 hours [22], corresponding to $\alpha_c = .69$ day^{-1}.

The doubling time for an endothelial cell in the presence of growth factors is 3.5 days [6], so therefore $\alpha_y = 0.198$ day^{-1}. We can assume an endothelial cell will have an apoptotic rate similar to its growth rate, so $\delta_y = \alpha_y = 0.198$ day^{-1}.

Bian *et al.* measured approximately 90% of glioma-derived endothelial cells to mature to tubule-like structures after 96 hours [6]. We assume these tubule-like structures to be similar to microvessels in their formation. Thus, $\omega = 0.576$ day^{-1}.

Gupta, *et al.* noticed endothelial cell replication at a VEGF concentration of $1\, ng\, VEGF\, ml^{-1}$[14]. Their methods used $5 * 10^4$ endothelial cells per well in a 6-well tissue culture. Similar culture wells cite volumes of 2.7 to 3.2 milliliters per well [5]. Taking the average, this converts into $5.9 * 10^{-5}\, ng\, VEGF\, EC^{-1}$ needed to cause EC proliferation. Thus, $\theta_r = 5.9 * 10^{-5}\, ng\, VEGF\, EC^{-1}$.

Ang-2 expression must be approximately eight times as much as the expression of Ang-1 in order to completely block the effects of Ang-1 [23]. With this we assume our half-max Ang-2/Ang-1 ratio where Ang-2 blocks the tie-2 receptor as $\theta_{EC} = 4$. We estimated the half-max Ang-1/Ang-2 ratio where Ang-1 matures vessels to be $\theta_b = 1$.

Addison, *et al.* report adding $50\,ngVEGF\,ml^{-1}$ to 12-well plates, each well containing 15,000 endothelial cells in order to optimally stimulate endothelial cell replication [1]. Since no specific well brand was described, we assumed wells to be 1 milliliter in size. Therefore, the amount of $VEGF\,EC^{-1}$ needed to stimulate angiogenesis or vascular regression is $\rho = 0.0033\,ngVEGF\,EC^{-1}$.

For the minimum amount of VEGF needed to keep endothelial cells alive, Gupta, *et al.* measured $10\,ngVEGF\,EC^{-1}$ for $5 * 10^6$ endothelial cells in 3.4 milliliter wells [15, 9]. This converts into $6.8 * 10^{-6}\,ngVEGF\,EC^{-1}$ required to keep endothelial cells alive. Thus, $\theta_y = 6.8 * 10^{-6}\,ngVEGF\,EC^{-1}$.

We assume the breakdown of microvessels to endothelial cells to be faster than the conversion of endothelial cells into microvessels. Hence, we estimate $\gamma = 0.8$ day^{-1}.

We estimated the binding rate of the anti-VEGF antibody with VEGF to be $\tau = 5 * 10^{-8}$ day^{-1}.

Yang, *et al.* cite the half-life of bevacizumab as 21 days. This converts into a degradation rate of $\delta_r = 0.033\,day^{-1}$[39].

3 Simulations

All simulations were modeled and displayed in MATLAB [24]. The numerical solutions to the fully-parameterized model yielded interesting results. Figure 2 shows a graph of the number of cancer cells versus time. For each line, all parameter values are the same except for α_{v_2}, the max rate of VEGF secretion by cancer cells under hypoxic conditions. This parameter ranges from 10^{-6} to $3 * 10^{-2}\,ngVEGF\,cell^{-1}day^{-1}$. When α_{v_2} was small (less than $6.7 * 10^{-3}\,ngVEGF\,cell^{-1}day^{-1}$), the tumor peaked at approximately 1 million cells then regressed or oscillated before reaching a steady

state of less than 1 million cells. However, when α_{v_2} was greater than $6.7 * 10^{-3}\, ng\, VEGF\, cell^{-1} day^{-1}$, the tumor displayed exponential growth after approximately 100 days.

In all cases, the tumor cells displayed similar behavior for the first 15 days of growth. The tumor cells grow until they peak at approximately 1 million cells, corresponding to a tumor that is about 2 millimeters in diameter. Multiple studies agree that this is the limit for avascular tumor growth, and that tumors must initiate angiogenesis in order to continue to grow[16, 34, 11]. It has also been shown that a VEGF blockade will inhibit this angiogenic growth [11, 3, 13, 32]. Therefore, our simulations suggest that all tumors will reach 1 to 2 millimeters in size before they initiate angiogenesis. However, tumors that have a higher VEGF secretion rate will continue to grow because they secrete enough VEGF to promote angiogenesis. The tumors that do not display exponential growth do not have high enough VEGF secretion rates to cause angiogenic sprouting.

In a study by Holash, *et al.*, Ang-2 is detectable 2 weeks after rats are implanted with glioma cells [16]. In our model, Ang-2 is secreted as the tumor grows larger. After 15 days, the Ang-2 levels in the system are high enough to cause either vascular pruning or angiogenic sprouting, depending on the availability of VEGF. In the simulations with high VEGF, angiogenic sprouting occurs, whereas in the simulations with low VEGF, vascular regression occurs.

Our model shows that VEGF is highly influential in determining the aggressive behavior of glioblastomas, agreeing with previous studies on VEGF rates [12]. If the glioblastoma cells are not secreting enough VEGF after Ang-2 is introduced into the system, they will not produce a viable tumor. However, if the VEGF production rate is high enough, the tumor will grow exponentially. Even after 300 days, our simulations show that low VEGF secretion rates will not lead to a viable tumor, whereas higher VEGF secretion rates lead to the formation of a much larger tumor (see Figure 3). This suggests the need for a form of glioblastoma treatment that blocks VEGF secretion.

4 Treatment

The results of our model indicate that anti-VEGF treatments might be an effective form of clinical glioblastoma treatment. Currently, the only processes of treatment commonly used on glioblastoma patients are surgical resection, radiotherapy, and chemotherapy. However, severe hypoxia and permeable vasculature inhibit the delivery and effectiveness of the latter two therapies [4]. An emerging concept in tumor therapy is the idea of vascular normalization. In vascular normalization, a VEGF blockade is introduced into the tumor vasculature, preventing the continual overexpression and regulating the concentration of VEGF. In the absence of overexpressed VEGF, Ang-1 is able to mature the vasculature, and Ang-2 is able to prune the unstable and unnecessary vasculature [37]. This process of vascular normalization makes drug delivery more effective by decreasing permeability in blood vessels, thereby increasing the oxygen levels and drug distribution in the tumor [19]. Unfortunately, the time period in which vascular normalization occurs is not clearly understood. In order to deliver cytotoxic drugs with maximum efficiency, the normalization window, during which VEGF inhibition allows for the most effective drug delivery, must be identified.

Bevacizumab (Avastin^{TM}) is a monoclonal antibody that is currently used to treat certain solid tumors, not including glioblastomas [27]. Bevacizumab is an angiogenic inhibitor that binds with VEGF molecules. We introduced bevacizumab into the system in order to model the effects of an anti-VEGF treatment on the growth of glioblastomas and investigate vascular normalization. To simulate the growth of a viable tumor, a tumor with a high VEGF secretion rate ($3 * 10^{-2} ng VEGF\, cell^{-1} day^{-1}$) grew for 170 days, until it was 10.7 millimeters in radius. At this time, 700 milligrams of anti-VEGF treatment were introduced into the system every 2 weeks for 6 cycles, consistent with clinical trials [35] (see Figure 4). The introduction of an anti-VEGF treatment showed immediate tumor regression (see Figure 5). Even when treatment ceased after 240 days, the tumor took over 200 days to return to its pre-treatment size. Therefore, our model

results indicate that a VEGF blockade is a very effective form of glioblastoma treatment and may be of use in a clinical setting.

5 Discussion

Our model shows that vascular endothelial growth factor (VEGF) is highly influential in determining the level of aggressive behavior in glioblastomas. A high VEGF production rate causes exponential tumor growth, while a low VEGF production rate does not allow the tumor to grow beyond 2mm in diameter. The results of our model indicate that anti-VEGF treatments might be an effective form of clinical glioblastoma treatment. However, a 2007 clinical study by Vredenburgh, *et al.* concluded that treatment with bevacizumab is most effective when used in conjunction with chemotherapy; bevacizumab normalizes vasculature, allowing for more efficient cytotoxic drug delivery [35]. To simulate more comprehensive treatment, further modifications to the mathematical model will aim to incorporate cytotoxic drug delivery.

Further modifications will also aim to improve the efficacy of the anti-VEGF treatment. We estimated that treatment binds with VEGF at a rate of $\tau = 5 * 10^{-8}\ ng\ day^{-1}$. Since this parameter is estimated, it may not give accurate results. In fact, our model may work too well, indicating that an anti-VEGF treatment will effectively treat glioblastomas for an indefinite period of time. However, in the same study on bevacizumab, Vredenburgh, *et al.* showed that an anti-VEGF treatment slows tumor growth, but can also lead to sometimes fatal hemorrhaging, pulmonary embolus, or stroke [35]. Thus, bevacizumab must not be seen as a "miracle drug" that cures glioblastoma. Like most cancer treatments, there are significant side-effects that must also be taken into consideration when administering treatment. However, our results indicate that the new drug bevacizumab displays promising results that may help improve the quality of life for glioblastoma patients.

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[1] For more information contact the program coordinators; Eric Kostelich (Kostelich@asu.edu) and Bruno Welfert (Welfert@asu.edu), or visit the Web page
`http://math.asu.edu/CSUMS`

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Appendices

A Parameters

Table 1: Parameters, their symbols and default values used in model (1) – (7).

Parameter	Meaning	Value	References
α_{a_1}	Max growth rate of Ang-1	.24	[11]
δ_{a_1}	Degradation rate of Ang-1	.072	[11]
α_{a_2}	Max growth rate of Ang-2	1.92	[11]
δ_{a_2}	Degradation rate of Ang-2	.072	[11]
θ_{a_2}	1/2 max cancer cells needed to trigger a_2 production	10^6 cells	[7, 16]
α_c	Max growth rate of glioblastoma cancer cells	.69 day^{-1}	[22]
λ	Max $\frac{cancer\ cells}{\mu m}$ of microvessel length	6.1-22$\frac{cancer\ cells}{\mu m}$	[7, 10, 33, 36]
α_v	Rate VEGF is constantly expressed by glioma cells	$3*10^{-6}\frac{ng}{cell*day}$	[25]
α_{v_2}	Max growth rate of VEGF production	$3*10^{-2}\frac{ng}{cell*day}$	[25, 17]
δ_v	Degradation rate of VEGF	19.96 day^{-1}	[8]
θ_v	1/2 max $\frac{cancer\ cell}{\mu m}$ hypoxic ratio where VEGF is produced	6.1-22$\frac{cancer\ cells}{\mu m}$	[7, 10, 33, 36]
α_y	Proliferation rate of endothelial cells	.198 day^{-1}	[6]
δ_y	Apototic rate of endothelial cells	.198 day^{-1}	[6]
s	Conversion factor from microvessels to ECs	.17-.22$\frac{EC}{\mu m}$	[26]
γ	Max rate microvessels break down to ECs	.8 day^{-1}	—
ρ	1/2 max $\frac{VEGF}{EC}$ needed to cause regression, growth, etc.	.0033$\frac{ng\ VEGF}{EC}$	[1]
ω	Max rate ECs mature to microvessels	.576 day^{-1}	[6]
θ_y	1/2 max $\frac{VEGF}{EC}$ needed to keep ECs alive	$6.8*10^{-6}\frac{ng\ VEGF}{EC}$	[15, 9]
θ_r	1/2 max $\frac{VEGF}{EC}$ needed to induce EC cell cycle	$5.9*10^{-5}\frac{ng\ VEGF}{EC}$	[14, 5]
θ_{EC}	1/2 max $\frac{a_2}{a_1}$ ratio where a_2 blocks tie-2 receptor from a_1	4	[23]
θ_b	1/2 max $\frac{a_1}{a_2}$ ratio where a_1 matures vessels	1	—
τ	Binding rate of anti-VEGF antibody with VEGF	$5*10^{-8}$ day^{-1}	—
δ_r	Degradation rate of anti-VEGF antibody	.033 day^{-1}	[39]

B Figures

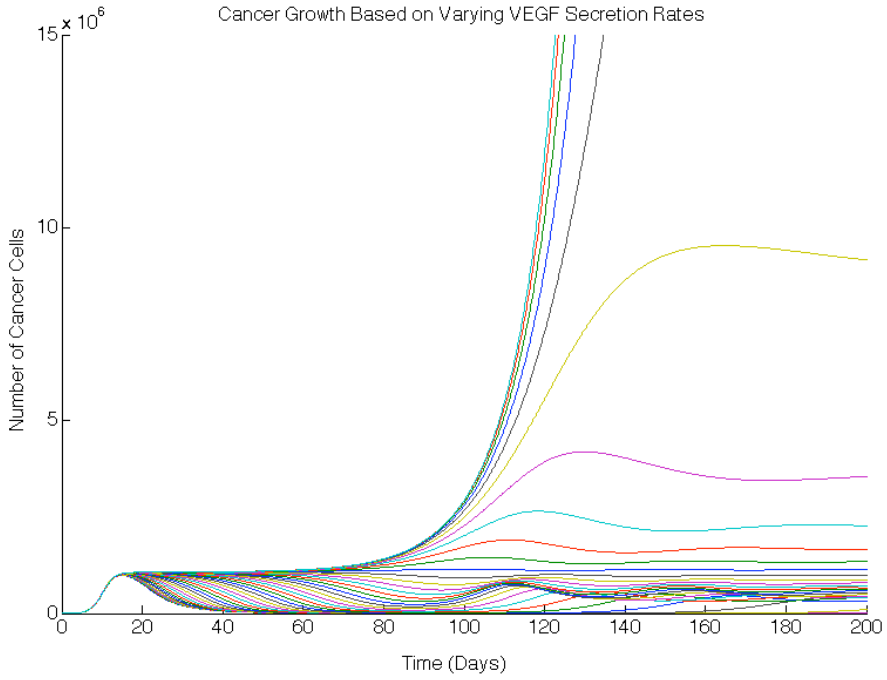

Figure (2a)

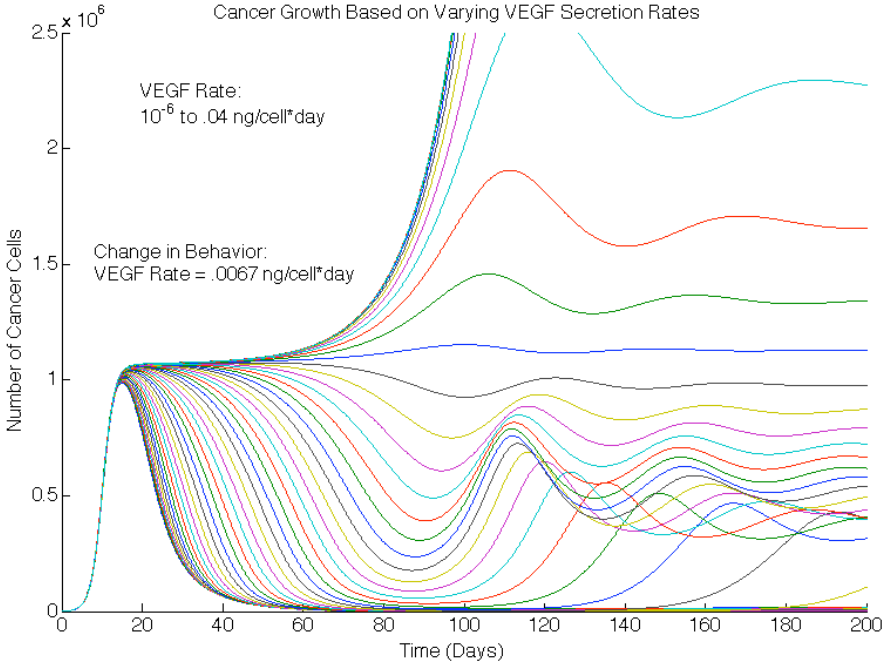

Figure (2b)

Figure 2: These figures display the change in tumor behavior when varying the VEGF secretion rate. As the VEGF secretion rate increases, the tumor begins to grow exponentially. The behavior diverges after 15 days when the tumor has grown to 1 million cells (1 to 2 mm diameter), coinciding with experimental data on rat gliomas [16]. Figure (2b) is a zoomed-in version of Figure (2a) that highlights early behavior.

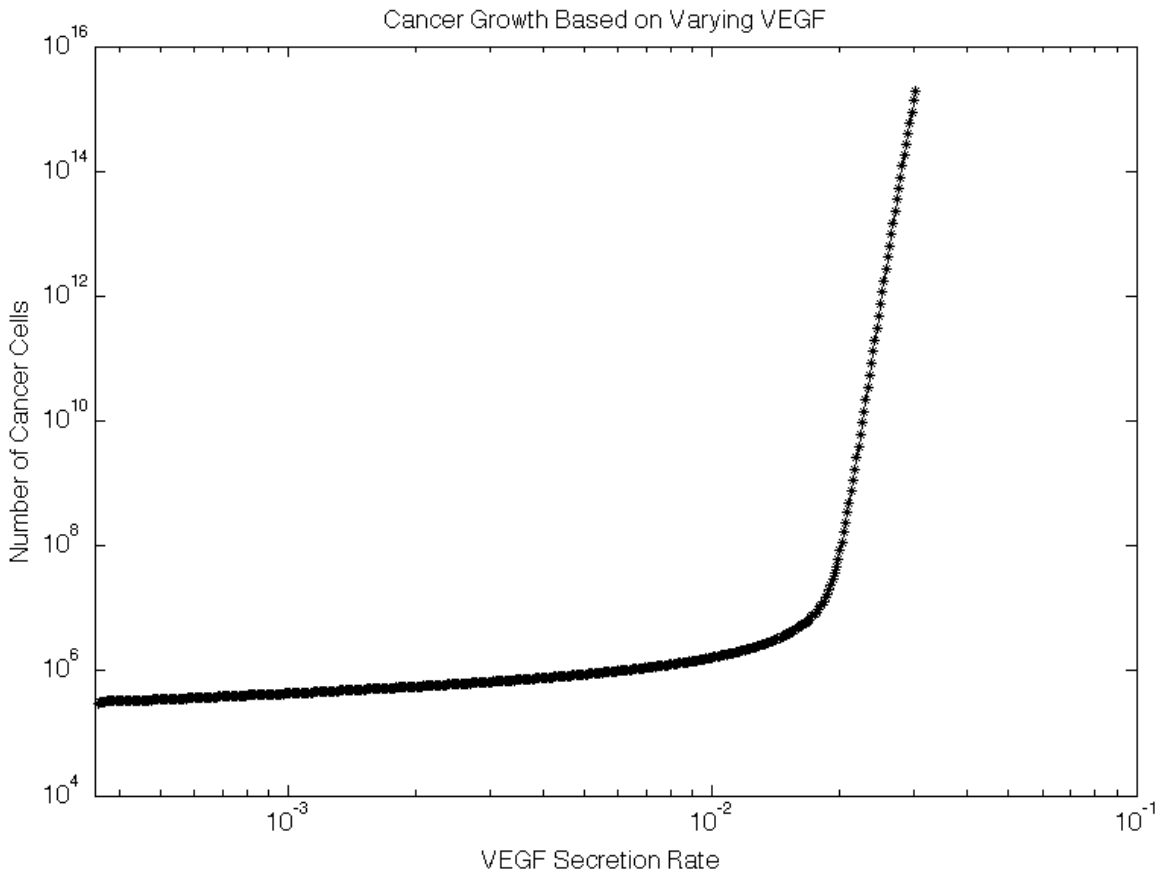

Figure 3: Log plot of Number of Tumor Cells vs VEGF Secretion Rate after 300 Days. A higher VEGF secretion rate leads to exponentially larger tumor growth.

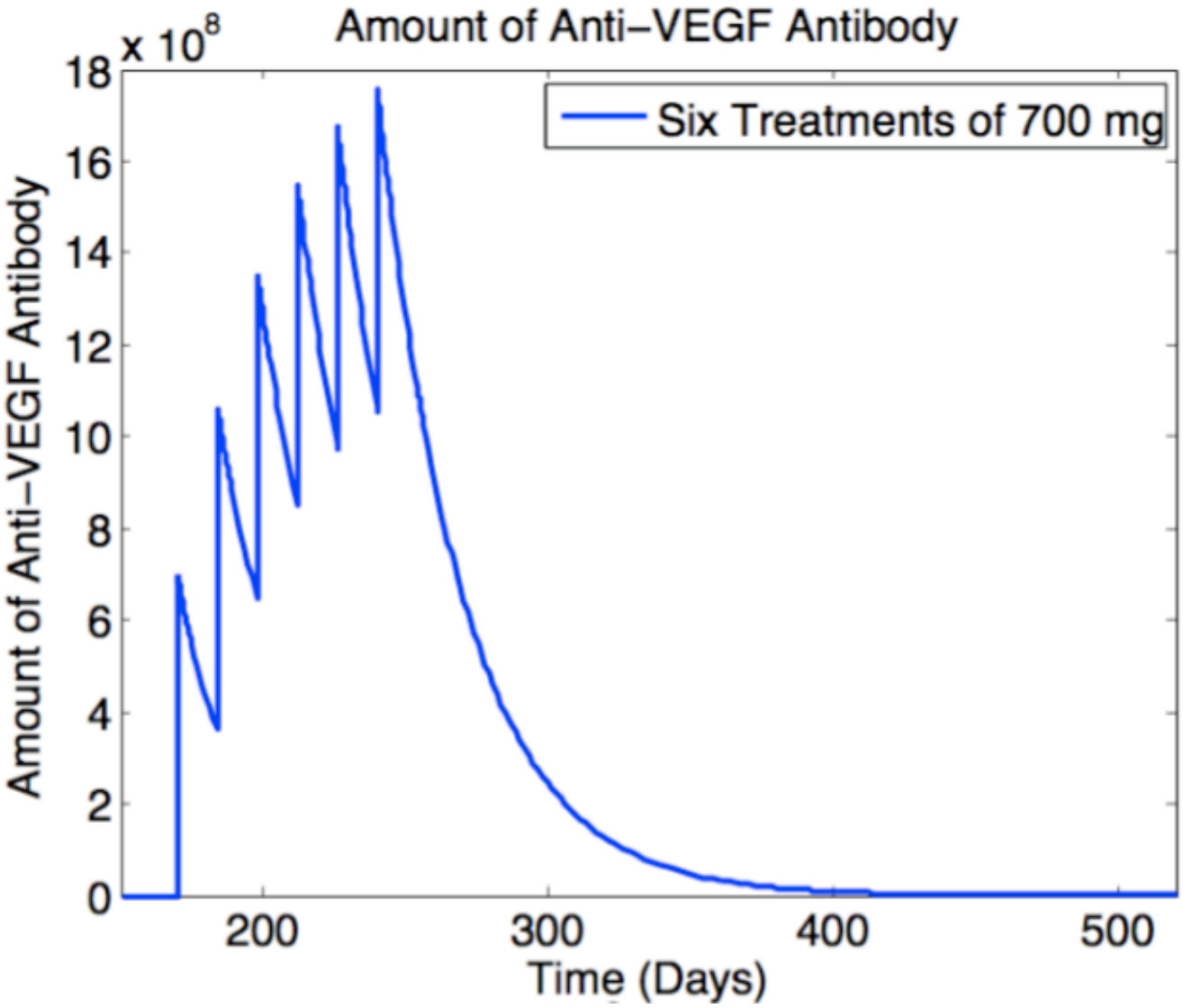

Figure 4: The amount of anti-VEGF treatment in the system versus time. Every 2 weeks, 700 milligrams of treatment are introduced into the system after the tumor has grown to a reasonable size. The treatment binds to VEGF or degrades, causing the anti-VEGF levels to drop between treatments.

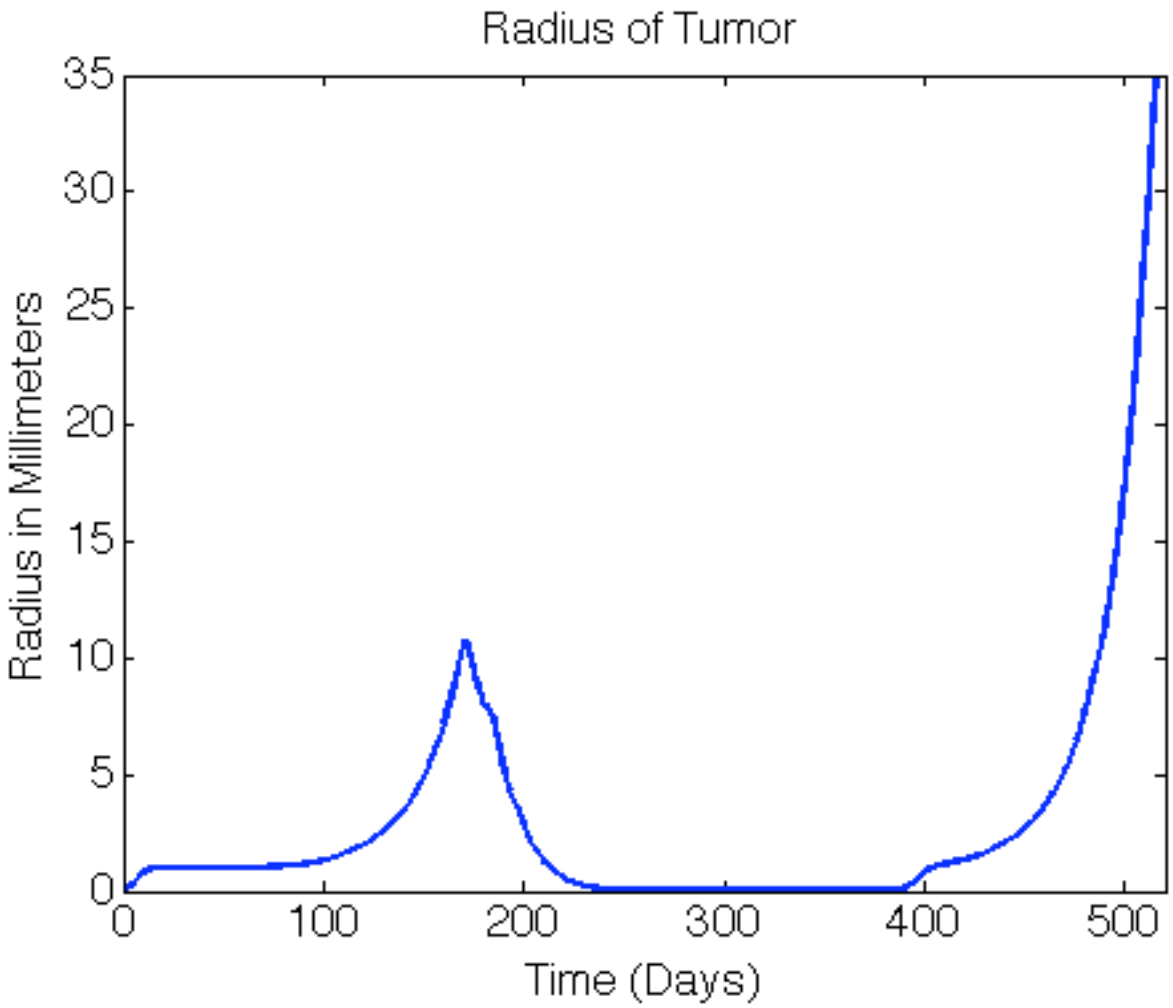

Figure 5: Plot of tumor radius versus time. An anti-VEGF treatment is introduced after 170 days, following the schedule in Figure 4. The treatment is immediately effective in reducing tumor size.