A Comparative Analysis of Chemotherapeutic Administrations in Inhibiting Glioblastoma Multiforme Cellular Growth Utilizing an Integration of Differentiation-Based Growth Models and Pharmacokinetic Equations

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Glioblastoma multiforme, a disease of grade IV astrocytoma, accounts for 60-70% of malignant brain tumors.[1] Despite its low survival rate, little is known about GBM and its treatment. The purpose of this study was to assess the comparative effectiveness of the most common drugs (temozolomide, carmustine, bevacizumab) in reducing tumor growth within GBM chemotherapy. It was hypothesized that due to their properties as alkylating agents inhibiting growth genes within GBM, temozolomide and carmustine would provide the most reduction in cellular growth mathematically.[2] VisualPDE simulations were used to model administration of temozolomide, carmustine, bevacizumab, and none in chemotherapy and provide the cellular GBM counts at any given time within five months (60 time points were used). Results showed cell growth reduction by temozolomide and carmustine, but most drastically by bevacizumab, and upon further analysis, a One-Way ANOVA yielded that the difference between at least two chemotherapeutic administrations were statistically significant ($F(3, 236)=281.049$, $p<0.001$). A post-hoc Tukey HSD test revealed a significant difference between no administration and each chemotherapeutic administration (TMZ: $p<0.001$, BCNU: $p=0.046$, BEV: $p<0.001$), as well as significant differences compared to bevacizumab, which was drastically more effective than TMZ and BCNU ($p<0.001$, $p<0.001$). Thus, it was concluded that BEV provided the optimal treatment in slowing GBM growth with the mathematical model through its ability to regulate proteins and drug resistance, allowing for its integration in future chemotherapy.

Introduction

According to experts at the Technical University of Crete and the American Cancer Society, cancer (the disease primarily associated with the uncontrolled cell division into tumors) is the second cause of death around the world with a striking 19.3 million new cases each year. [3],[4] Divided into both metastatic and non-metastatic cancer, cancer is known to alter signaling and metabolism within the human body, and allow for the growth of malignant cells that eventually avert valuable nutrients away from necessary processes, leading to the deterioration of the nervous system.[5] However, due to a scarcity of knowledge in effective treatment, cancer continues to have a prevalent impact on the medical communities.

Of the carcinomic diseases, the most deadly are considered to be gliomas (brain tumors) such as glioblastoma multiforme (GBM), the brain cancer that accounts for 60-70% of glioma tumors, as stated by D'Alessio and her colleagues (2019) at the Institute of Histology and Embryology in Rome, Italy.[1] GBM is characterized as a grade IV astrocytoma, and the characteristic tumors are known to be rarely metastatic and often heterogeneous in nature. Furthermore, it was found by Taal and his fellow colleagues (2015) at the Erasmus Medical College Cancer Institute that the GBM and high grade glioma tumors are accompanied by the presence of necrosis (cell death) and endothelial proliferation (reproduction of cells responsible for blood vessel regulation) around the related astrocytoma (brain or spinal cord astrocyte-based tumor).[6] Due to the high defense system in the brain (including the regulatory blood brain barrier), the immune system is especially limited in inhibiting this complex tumor growth, which makes the human body relatively incapable of preventing the GBM progression.[1]

In order to compensate for a lack of immune response in GBM onset, numerous treatments have been used to help repress the further growth of these malignant tumors, including surgery, radiotherapy, and chemotherapy. However, these treatments continue to have low effectiveness and many side effects upon patients, and thus requires further analysis to be better utilized within treatment.

Surgery often serves as the first line of treatment to remove or reduce tumor mass within the brain, as outlined by Jain in her 2018 study.[7] While surgery is known to be successful in GBM tumors (with a progression free survival rate[PFS] of 10-11 months), there has been a particular inefficacy in utilizing surgery in recurrent GBM, which can highlight an absence of consistency in treatment. As a result, surgery is utilized as a supplementary treatment that allows for efficient tumor removal.[7],[8]

Radiotherapy works as a secondary treatment to surgery, utilizing high energy X-ray radiation and light rays to reduce tumor burden without direct interference.[7] This is relatively dangerous due to the fragility of neural mass in cognitive function. Recently, however, radiotherapy treatments have undergone scrutiny for their generalized impact on all neural cells rather than just carcinoma, which is stressed by Aiyappa-Maudsley and her fellow cancer experts in 2022.[9]

As stated in a 2017 scientific novel by Fernandes and others at the São João Hospital Center, chemotherapy is regarded as the most extensive method of treatment, which traditionally follows radiotherapy and surgery as a tertiary method of combating cancerous tumors.[8] Chemotherapeutic administration provides significant improvements in PFS rates and correlations to over-response rates within GBM.[6] However, this is not without the side effects of nausea, vomiting, hair loss, and weaker immunity cited by Rajaratnam and her fellow researchers (2020) at University of Wisconsin-Milwaukee.[10] In order to minimize these impacts cited by Rajaratnam et al., these treatments are administered in cycles, as stated by Hanif and her fellow biomedical science experts (2017), and drugs are taken by patients orally, intravenously, or both for a few days.[11] A period of chemotherapeutic drug administration is followed by weeks of rest, allowing the patient to regain a relatively stable condition for the next administration. While the length of administration and rest periods vary, each drug provides an effective treatment to the tumorous substances in GBM. Numerous drugs have been tested for use in GBM, including temozolomide (TMZ), carmustine (BCNU), and bevacizumab (BEV). However, there continues to be a significant problem with providing optimal doses of chemotherapeutic drugs to the brain because of the brain's complex entry system of multiple membranes and the blood brain barrier.[7]

The mental toll that the chemotherapy process exhibits on patients is severe and underreported, with patients seen to have significantly higher levels of depression during their treatment than any other form of cancer therapy, according to Baraki and others (2020) from the University of Gondar in Ethiopia.[12] As a result, many GBM patients are presented with difficulty passing through the weeks of treatment and the severely complex administration process of chemotherapy (as explained above).[6] Many patients continue to be unable to successfully pass through chemotherapeutic treatments. Thus, it has been well established that more research is needed into the efficiency of different chemotherapeutic

administrations over multiple chemotherapeutic cycles and into potential treatments that maximize effectivity without increasing the time necessary to deliver the impacts.

Much of this research into chemotherapeutic drugs regarding its capabilities in sensitive areas of the body (such as the brain) has been conducted by utilizing mathematical models. While each mathematical model serves a different purpose within the representation of chemotherapy within GBM, numerous studies, such as one 2015 study by Martirosyan and her colleagues from University of Arizona, move to differentiate models as being spheroid, vascular, morphological, or treatment-based.[13] Spheroid models utilize sphere shapes for the tumor growth of GBM, and highlight the extension of the tumors through proliferation in all three dimensions. Another type of model, vascular models, showcase the influence of nutrients and molecules within the brain on the GBM pathology and highlight how concentrations of nutrients are impacted by cancers. Furthermore, the morphological models assess the behavior of the GBM (and glioma) tumors over time, and how the proliferation and invasion of the tumor mass changes as the tumor grows. Finally, treatment models often model how different methods (in this case, of chemotherapy) of combatting GBM impact the brain and its associated processes. While each model serves a specific function that is crucial to understanding the behavior of GBM, current research trends indicate the inclination to utilize morphological and treatment based models to gain a deeper grasp of GBM.

Despite the array of models utilized, there continues to be a lack of concrete synthesis of these mathematical models to provide a more holistic approach to how the growth (morphological models) of the GBM can actively be counteracted by chemotherapeutic treatment methods (treatment models). Without a comprehensive model, little can be concluded about the impact of different chemotherapeutic drugs (the basis of differences in chemotherapeutic treatments) on the active growth and behavioral trends of GBM. When coupled with the previously mentioned absence of understanding regarding the efficiency of different drugs within the brain over a set time period, a research question was generated stating: How do the administrations of different chemotherapeutic agents vary in reducing the cell growth within a glioblastoma multiforme tumor over a set chemotherapeutic period as modeled by differential growth and pharmacokinetic mathematical simulations?

This research question was sought to be answered by utilizing various mathematical modeling techniques from both morphological and treatment models to assess the effectiveness of each chemotherapeutic drug over a given time period. By utilizing VisualPDE to visibly simulate the behavior of tumor cells, the Proliferation-Invasion model created by Jackson et al. (2015) was used to model the growth of the GBM tumor over a given period of treatment.[14] In addition, the chemotherapeutic treatments utilizing TMZ, BCNU, and BEV in their given treatments were implemented into the growth functions on VisualPDE using the concentration equations.[15],[16] It was hypothesized that carmustine and temozolomide would reduce the tumor growth more successfully as compared to untreated GBM growth based on differentiation-based models, because both chemotherapeutic agents directly target the genetic cell division pathways of GBM as alkylating agents in order to regulate cell division.[2]

Literature Review

Temozolomide as a Chemotherapeutic Agent

The standard of treatment for GBM chemotherapy is known as temozolomide (TMZ), established and tested on human patients in 1992.[2] TMZ works as an alkylating agent, according to Thomas and her fellow researchers (2013) at the Department of Neurological Sciences at Stanford University, which means it helps regulate and mutate GBM tumor DNA to eventually inhibit growth and induce apoptosis.[2] TMZ has been widely recognized as the most effective in patients. Specifically, a dosage of 40 mg/m^2 daily of temozolomide saw a significant increase in the PFS rate.[17] Not only this, but as indicated by Hotchkiss and Sampson (2021), professors at Duke University, TMZ was observed to directly reduce tumor burden of GBM tumors within the brain.[17] Due to these various benefits, TMZ is currently administered as an oral drug on an alternating weekly schedule, which doctors have deemed to be an efficient administration schedule.[2],[17] But, as stated by Thomas et al. (2013), there continues to be problems with TMZ-resistance in O6-methylguanine-DNA-methyltransferase (MGMT), a tumor protective protein in the brain, which serves to make TMZ significantly less optimal.[2] This problem has remained over the past decade despite claims that higher dosage can alleviate such resistance.[17] As a result, TMZ's effectiveness as a drug in the chemotherapy industry has been called into question, and must be assessed thoroughly in comparison to other drugs.

Carmustine as a Chemotherapeutic Agent

Another prevalent drug utilized in modern chemotherapy is carmustine (BCNU). BCNU, as studied by Jungk and his fellow researchers (2016) at University Hospital Heidelberg, is considered relatively effective with a PFS rate of 7.53 months and 46% of administrations being handled well.[18] BCNU is in many ways comparable to TMZ; for instance, it is known to interact with and regulate MGMT similar to TMZ. While it is a very potent option for chemotherapy in GBM, there have been a multiplicity of disadvantages associated with the administration of BCNU. According to Reithmeier and his counterparts (2010) at University Medical Center Freiburg, BCNU was seen to be significantly more toxic and was associated with numerous side effects such as lung fibrosis or pulmonary embolism.[19] Furthermore, BCNU is also associated with being worse for patients if another chemotherapeutic drug (including TMZ) is administered beforehand.[18] This directly contrasts with a point made by Reithmeier et al. in 2010, in which BCNU is established as a potent secondary drug to temozolomide.[19] The lack of a consensus within previous studies regarding the use of BCNU in GBM chemotherapy, and how exactly BCNU can be maximized in efficiency as compared to the drugs once again contribute to a gap in the knowledge that should be addressed.

Bevacizumab as a Chemotherapeutic Agent

The final chemotherapeutic drug of interest is bevacizumab (BEV), which was the first drug approved to combat GBM through use of blood vessels, according to Garcia and her oncology experts (2023).[20] BEV is one of the primary chemotherapeutic drugs for GBM that involves modulating vascular endothelial growth factors (VEGFs), which are crucial to the process of angiogenesis. BEV is seen to have a multitude of benefits (as with all chemotherapeutic drugs studied) with a 51% reduction in GBM progression, significant improvements of PFS rates, and impacts on tumor size.[20],[21] BEV side effects are also noted to be relatively milder compared to other drugs, including hypertension, bowel perforation, and abdominal pain.[20],[21] However, BEV has been predominantly utilized as a complementary drug to TMZ, in order to strengthen the response of TMZ to the resistance of MGMT in regulation of tumor growth, as highlighted by Funakoshi and her fellow researchers (2020) at the Graduate School of Medical Sciences of Kyushu University.[21] Thus, BEV has not been studied individually to assess its uses in chemotherapy as prevalently as TMZ and BCNU. As a result, BEV still needs to be compared individually to the current chemotherapeutic drugs on the market in order to thoroughly assess the effectiveness of each drug over a specific period of time.

Synthesizing a Standpoint on Chemotherapeutic Drugs in Glioblastoma Multiforme

As stated previously, there is a gap in current knowledge regarding the comparison of chemotherapeutic agents, solely due to their different administrations and times of approval. For instance, the three drugs considered in the current research, TMZ, BCNU, and BEV, have each been studied individually for their PFS rates and consequent impacts. They have also been compared generally to the standard of chemotherapy (TMZ). However, the primary lack of knowledge arises from the fact that most of these chemotherapeutic drugs have never been comparatively assessed against each other because of their unique properties and administration methods. While Hotchkiss & Sampson (2021) was able to provide a general overview of TMV, as Jungk et al. (2016) did for BCNU, and Funakoshi et al. (2020) did for BEV, none of these studies are able to directly compare these chemotherapeutic agents using data that was procured in the same manner.[17],[18],[21] With each study relying on different methods of research (for example, while Funakoshi et al. in 2020 takes on a more statistical approach for BEV, Hotchkiss & Sampson in 2021 complete a literature review for TMZ), it is impossible to compare these chemotherapeutic agents thoroughly and accurately.[17],[21] Furthermore, the approval dates and differing uses of each drug have resulted in different starting points or uses within chemotherapy, which has in turn caused an absence of current research or foundation for multiple of the drugs. A lack of current research or foundation directly corresponds to a lack of comparison. As a result, such an absence of research on chemotherapeutic drugs in GBM has constituted through the inability to compare each chemotherapeutic agent in an equal and consistent manner.

Mathematical Models: Morphological Models

Morphological models utilize the spheroid entities developed in simpler models and different growth pathways, such as proliferation, diffusion, and invasion rates to help complexify and more accurately model GBM tumors.[13] Morphological cancer models often differ in regards to how they are created; some contain numerous equations and modeling platforms, while others are more simplistic. However, these models still utilize very similar mathematical concepts to frame their model creation process - the difference is that many choose to be more specific by using more input values.[13] For instance, the Proliferation-Invasion Model developed by Jackson, Rockne, and their fellow colleagues (2015) at Northwestern University helps to showcase behavior of GBM tumors over time, as measured by changes in cell density. It is pictured below:[14]

Equation 1:

$$\overbrace{\frac{\partial c}{\partial t}}^{\substack{\text{Rate of change of} \\ \text{glioma cell density}}} = \overbrace{\nabla \cdot (D \nabla c)}^{\substack{\text{Net dispersal} \\ \text{of giloma cells}}} + \overbrace{\rho c\left(1 - \frac{c}{K}\right)}^{\substack{\text{Net prolif eration} \\ \text{of giloma cells}}}$$

As can be seen, the Proliferation-Invasion model (Equation 1) utilized a partial-differential equation to model the two most basic parameters that make up the growth of the GBM tumors: invasion (net dispersal) and proliferation (net proliferation).[14] Despite being a relatively simple mathematical morphological model for the mathematical neuro-oncology field, the Proliferation-Invasion model is still able to provide a very accurate representation of GBM tumors through modeling changes in cell density, and therefore cell growth. Equation 1 provides a working model of the GBM growth that can be easily implemented with the use of multiple parameters into VisualPDE, thus justifying its primary use within this study. However, it may not be as accurate as another model which utilizes more parameters to derive the same quantity (cell density changes). One of these models is Martirosyan et al.'s (2015) invasive behavior model, which provides a specified outlook onto the behavior of GBM tumor (Equation 2):[13]

Equation 2:

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

In essence, Martirosyan and her colleagues utilize a similar partial-differential structure for the modeling of brain tumor growth in Equation 2, however, there are numerous variances in the model developed that allow for it to be more specific and accurate.[13] First and foremost, the authors utilized more specific parameters, including diffusion, logistic growth, cells leaving the tumor, and shed cells from the core, which reduce the abstraction of generalized values that may be rounded off or estimated (this allows for more accurate outputs).[13] Secondly, there are more portions of this equation specified as compared to Jackson et al.'s Proliferation-Invasion model, where the items within the equation are more generalized.[14] Essentially, the invasive behavior model chooses to derive more of the quantities within these equations to provide exact values as opposed to estimating like Equation 1, which causes it to be more accurate through detail. This is not to say that the Proliferation-Invasion Model cannot be extremely useful - many times, a simplistic equation to model a process is utilized to build larger, more synthesized models as is the case with this study (since much complicated model such as Martirosyan et al.'s (2015) Equation 1 would be extremely difficult to utilize in a synthesized model).[13] In the end, it is important to understand that these models, despite a plethora of differences in the representation of the tumors (through differences in parameters and variables), aim to showcase the same information: the growth of a glioma tumor within the brain - one is just more specific than the other. However, no integration of morphological models and treatment models has yet allowed for a model that provides representation of active and growing GBM as impacted by chemotherapy. Without such knowledge, the use of the morphological models can only be accurate for an unrestricted, unintercepted growth of GBM, which, many times, is not likely.

Mathematical Models: Treatment Models of Interest

Treatment models showcase various treatments (in this case, chemotherapy within the brain) and how they could consequently interact with the glioma tumors in the brain.[13] Typically, these models are used to measure the effectiveness of different treatments by utilizing the concentration of the treatment (the chemotherapy drug) and the net loss of tumor cells. These models may approach the process of modeling chemotherapy within the brain in different ways, but are very similar in their end product. For example, Vendel and his colleagues at the Mathematical Institute of Leiden University in 2020 utilized basic pharmacokinetic equation to model oral GBM chemotherapeutic drug concentration:[15]

Equation 3:

$$C_{\text{pl}} = \frac{F k_{\text{a}} Dose}{V_{\text{d}}\,(k_{\text{a}} - k_{\text{e}})}\left(e^{-k_{\text{e}}t} - e^{-k_{\text{a}}t}\right)$$

Within this treatment model, concentration is utilized to highlight how well a chemotherapeutic drug diffuses into the brain, which can contribute to the calculation of the number of tumor cells remaining and the effectiveness of the drug. This model was created utilizing a restricted input value and exponential factors within a larger product. However, it is extremely important to understand that this model provides

concentrations of drugs for oral administration only. Many times, treatment models rely upon administration techniques to provide accurate insight into the drug concentration within the body, or more specifically the brain. Along the same lines, two other models to consider would be the basic intravenous bolus (Equation 4) and intravenous infusion (Equation 5) concentration models explained by Ahmed (2015), a pharmaceutical expert in Uganda, which utilize time and initial dose similar to Equation 1 to find the chemotherapy concentration within the brain:[16]

Equation 4: $$C = \frac{D}{V} e^{-k_e t}$$

Equation 5: $$C = \frac{D}{V k_e T} \cdot \left(e^{k_e T} - 1\right) \cdot e^{-k_e t}$$

Ahmed in 2015 showcased an exceptionally similar model to Vendel and colleagues in 2020, especially in terms of utilizing a constant coefficient and exponential terms with base *e*.[15],[16] However, there are definitely numerous significant differences, particularly in complexity. Equation 3 by Vendel et al. (2020) shows a significantly more complex constant coefficient in comparison to Ahmed's (2015) Equations 4 and 5, and the use of an absorption coefficient (k_a).[15],[16] In addition, Vendel et al. (2020) shows numerous *e* exponential terms acting in an additive relationship in Equation 3, while Ahmed (2015) takes on a more multiplicative approach with multiple *e* terms in Equation 5.[15],[16] However, similar to the occurrences with the morphological models, the treatment models utilize and aim to produce very similar products despite structural differences: drug concentration within the brain to predict efficiency. While the similarities between drug treatments and concentration (despite administration differences) like Ahmed and Vendel et al.'s works allow for these models to be successfully integrated, there is little applicability for these models in assessing the effectiveness of the chemotherapeutic agents when the tumor is assumed to have stopped growing the moment the chemotherapeutic agent is administered.[15],[16] As a result, integration of treatment models with morphological models would allow for increased applicability for treatment models to represent the chemotherapeutic behavior within the brain as the tumor is actively behaving the way it should. Without integration with morphological models, the treatment models virtually serve as estimates because the concentrations predicted to be effective in a static tumor environment will not likely be the same as that of an environment with an active tumor.

Synthesizing the Standpoint on Mathematical High Grade Glioma Models

In terms of a secondary gap in neuro-oncology knowledge, many mathematical papers look into one type of model or the other, but never fully integrate models in order to show the treatment of GBM as a whole with consideration of GBM growth during treatment. For example, while Jackson et al. (2015) and Martirosyan et al. (2015) looks in to the growth of GBM without consideration of treatment (like radiotherapy or chemotherapy), studies by Vendel et al. (2020) and Ahmed (2015) focus solely on chemotherapy and how to model its concentrational impact on GBM, with no consideration for the growth of these tumors prior to direct interception of the drug to the tumor site.[13],[14],[15],[16] This absence of integration could result in inconsistent results when utilizing different methods as well as a lack of complete understanding of the relationships between both the chemotherapeutic models and the tumor growth models. Such understanding of the relationships between treatment (chemotherapy) and disease (GBM) is crucial to understanding how to most effectively combat the impacts of GBM. As a result, there is a large gap in the literature in addressing the integration of models to provide insight into both the growth and treatment of GBM.

Methods

Utilizing the Apple MacBook, the VisualPDE Simulation (a mathematical modeling website that is free to users) was loaded onto a webpage through visualpde.com, selected to showcase the representation upon the recommendation of Dr. Russell Rockne. The Mathematical Biology tab was selected, followed by the Logistic Travelling Waves tab, chosen for its ability to model basic outward growth within biology (effective for tumor modeling). Next, the one-dimensional simulation was opened on the MacBook, which was chosen for not only its simplicity but due to the ability to model cell concentration by area under the curve (easily quantified as part of the simulation) . The settings of the model were changed under "timestepping" to show "elapsed time" and turn on "autopause," and under "miscellaneous" to show the area under the curve (by selecting "integrate"). This was set up in order to provide quantifiable results and data for each simulation, and was repeated for four different VisualPDE simulations (four different tabs). This setup of the VisualPDE simulation is shown in Figure 1.

The model was also changed based on the function ("edit") and "parameters", which were modeled to the chemotherapeutic administration technique (oral, intravenous bolus, and intravenous infusion) associated with each of the drugs being studied. The existing growth equation was retained for all four simulations, since the equation models the Proliferation-Invasion model minimalistically.[14] Jackson et al. 's (2015) Equation 1 was utilized within this study as a result of its similarity to the starting equation within the VisualPDE simulation and its simplistic take on a complex 3-dimensional cellular growth.[14] The parameters for the model were changed to model the growth of GBM cell concentration by equating each of the parameters of the PI model to the parameters of the given growth equation. The values utilized for each parameter of the PI model is shown as part of Table 1A, and were derived from Swanson and her fellow neuroscience experts' research in 2002 into the behavioral aspects of GBM growth.[22]

These values were entered into the model for all four simulations to showcase the growth of GBM in cell concentration over a given number of days, as showcased in Figure 2. This was the final setup for one of the simulations of the four and was utilized as an indication of untreated GBM growth, serving as the control group within this study.

In order to effectively model the treatment techniques within the models to provide the three different drug groups, the non-differential function ($\square_u$) was subtracted by u*(given administration technique equation), once again upon the feedback of Dr. Russell Rocke. This allowed for the concentration to serve as a proportion of the given concentration of cells that are removed from the growth (which provides a simple but direct correlation between increases in concentration and increases in the number of cells inhibited from growth). In essence, this multiplied relationship between the chemotherapeutic term and growth term provided a simplistic proportional relationship that related the growth and treatment terms, which better modeled glioblastoma chemotherapy by showing a direct relationship. The chemotherapeutic technique that was utilized as the method of chemotherapy for each drug was decided based on how the chemotherapeutic drug is most commonly administered. The equations for each chemotherapeutic administration technique and the drug it was paired with is listed as part of Table 1, as utilized in the findings of Vendel et al. (2020) and Ahmed (2015).[15],[16]

In addition, the variables were defined within the "parameters" section for each of the three drug-based simulations. The variables utilized were assigned values based upon the qualities of the drug, which allowed for the simulation to most effectively model the use of the drug within its

typical administration technique. The properties of temozolomide were clearly delineated as shown in Table 2A by Ostermann and fellow researchers at Multidisciplinary Oncology Center at Switzerland (2004).[23] Carmustine was also well researched prior to its use in Table 2A by both Henner and fellow pharmacology and cancer experts (1986) and Garside and other scientists at the Peninsula Technology Assessment Group in the United Kingdom (2007).[24,25] This was also the case for bevacizumab as represented in Table 2A through the investigations of Han and colleagues in the Clinical Pharmacology Department of Genentech Inc. in 2016 and Wu and other pharmaceutical scientists in 2012.[26,27] The values garnered and supported by these studies were utilized in defining the variables in the "parameters" section of the three treatment sections as shown in Table 2A.

As a result, four simulations were created: one uninhibited growth simulation (no chemotherapeutic term in f_u), one oral administration simulation for TMZ, one intravenous bolus administration for BCNU, and one intravenous infusion administration for BEV. Each one was first set up by changing the settings in the top-right corner, the f_u based on administration technique, and the given parameters based on which variables were utilized in f_u. The final setup of each of the four simulations is shown as part of Figure 1A.

After setting up the simulations, a random generator was utilized to randomly generate 60 points from zero to five (decimal points) to represent different points in time from zero to five months (five months being the total time for the chemotherapeutic treatment). Time points were selected as the most effective method of assessing each drug's impact on the cellular growth of glioblastoma due to the ability to assess the impacts of the chemotherapeutic agent at different points within the treatment and create a synthesized perspective on the entirety of its use. Furthermore, these methods were based upon the findings of Fernandes et al. (2017) in assessing chemotherapy use in treating glioblastoma, which allows for a degree of certainty over the quality of such methods.[8] These t values were each entered into each of the drug simulations through the "autopause" features and the simulation was reset and played. The integrated values for each of these times was recorded for each drug simulation. Finally, once this data was collected, it was analyzed utilizing a One-Way ANOVA, and in the case of its significance, be analyzed using a post-hoc Tukey test. A One-Way ANOVA was chosen for its analysis of numerical data through comparison of the groups to one another (in terms of their means and sum of squares), and the Tukey was considered a viable statistical analysis of where the significance lies due to its analysis of groups with the same number of trials (in this case, 60 each). The final experimental design used to show the set up of this study is shown in Figure 2A.

Results

The data collected from the VisualPDE simulations yielded quite different results per group. Each chemotherapeutic agent showed a generalized trend of an initial cell count decrease, followed by a reduction in the impact of the chemotherapeutic agent on the GBM cell growth, which resulted in a renewed increase in the GBM cell count (Table 3A). In terms of the control group (with no chemotherapeutic administration), there appeared to be a steady increase in the GBM cell count per five initial cells (Table 3A). The administration of TMZ showed an initial growth of GBM cells, though at a reduced rate compared to no chemotherapeutic administration, followed by a prevalent decrease in the number of GBM cells between 0.13 and 1.03 months (Table 3A). After this decrease, the TMZ administration indicated no decrease in the GBM cells, but rather a reduced rate of growth as compared to the control (Table 3A). The BCNU administration, similar to the TMZ administration, highlighted an initial decrease in the number of GBM cells for up to 0.56 months, after which the cell count began to similarly to TMZ increase at a reduced rate as compared to the control (Table 3A). However, the BCNU administration reduced the number of GBM cells much less than the TMZ administration did (Table 3A). Finally, the BEV group shows similar trends of an initial decrease, followed by the reduction of the increase in GBM cells per five initial cells, however, the general increase of cellular GBM count did not occur within the five months time frame (Table 3A). Thus, the time points indicated a decrease in the GBM cell count over the five month period, however, this decrease in cell count became less and less prominent over time (Table 3A).

A descriptive analysis of this data indicated similar trends to the raw data. The means and medians of the various administrations computed to be very similar values, which highlighted minimal skew of the results (Table 2). The control group, as expected, has the highest mean and median (μ=5.797, M=5.730), followed relatively closely by the BCNU administration (μ=5.511, M=5.401) (Table 2). The TMZ administration showed an admittedly lower mean and median compared to BCNU (μ=5.285, M=5.139) (Table 2). However, it is very clear from the descriptive analysis that BEV is extremely low in the mean and median as compared to their other administrations (μ=3.128, M=2.949) (Table 2). In terms of measures of spreader, the interquartile ranges across groups remained relatively similar, however, the TMZ administration varied the most from the other groups with a low interquartile range of 1.057 (Table 2). The range of no administration and BCNU administration were extremely similar at a middle point of around 1.6, with the TMZ administration having the lowest range and BEV having the highest range (by a significant margin) (Table 2).

The over-time analysis of the various chemotherapeutic administrations highlighted similar trends to the data collected visually. As can be seen, the control group showed consistently higher GBM cell counts as compared to the chemotherapeutic administrations, which is generally predicted as a measure of the model's accuracy (Figure 3). The BCNU and TMZ administrations both indicated a small dip in the GBM cell count initially, followed by a reduced rate of GBM cell count growth following the initial administration (Figure 3). It is important to note that the TMZ administration showed a later initial decrease in GBM cell count, but compensated by reducing the following cell count growth rate significantly more than the BCNU administration (Figure 3). Finally, the BEV administration showed a drastic decrease in the GBM cell count as compared to other administrations, however this decrease started to level off near the end of the administration period (Figure 3).

The analysis of variance was conducted as part of analyzing the data through a One-Way ANOVA. The analysis resulted in an extremely high mean sum of squares between the administration groups and a very low mean sum of squares within each administration group (Table 3). This yielded an extremely high F value of 281.049, which surpassed the critical threshold F value of 2.605 by a large margin (Table 3). This was equated to the $p<0.001$, deeming the data collected from the VisualPDE simulations regarding the effect of different chemotherapeutic administrations on the glioblastoma cell count over a chemotherapeutic period of five months to be statistically significant (Table 3).

In order to assess where the significance lies within the data set, a post-hoc Tukey test for significance was conducted. The honestly significant difference (HSD) derived from this test was computed to be 0.265, establishing that the means of the administration groups must have a difference of 0.265 or greater to be considered statistically significant (Table 4A). Based upon the means of the TMZ, BCNU, BEV, and no administration groups derived, each chemotherapeutic administration was significantly different from the no administration group, with the TMZ administration and BEV being astoundingly significant ($p<0.001$, $p<0.001$) (Table 4A, Table 5A). The BEV administration was significantly different from both the TMZ and BCNU administration, with p values of virtually zero (Table 5A). However, the difference between the BCNU and TMZ administrations were not significant (p=0.091), meaning neither lowered GBM cell count more effectively (Table 5A). Overall, the mean of the groups indicated that BEV decreased the GBM cell count the most, followed by TMZ and BCNU (in no particular order, with no administration showing the least reduction of the cell count (Table 5A).

Discussion

The purpose of this study was to assess the comparative impact of different chemotherapeutic drug administrations on the tumor growth in glioblastoma multiforme over a chemotherapeutic period. To fulfill this purpose, simulations on the website VisualPDE were utilized to run and analyze mathematical models of different drug chemotherapy in relation to existing growth of glioblastoma multiforme, yielding GBM cellular counts at different time points within a five month chemotherapeutic period for each chemotherapeutic drug. It was hypothesized that the TMZ and BCNU administration would most effectively reduce the GBM cellular growth due to correlations of the administrations with inhibiting genetic cell division pathways of GBM as alkylating agents to slow tumor growth. In the end, the study yielded through the conducted One-Way ANOVA that there was a significant difference between at least two of the chemotherapeutic administrations in regulating GBM tumor growth ($F(3, 236) = 281.049$, $p<0.001$). Upon further examination of the results using a post-hoc Tukey HSD test of multiple comparisons, it was concluded that each chemotherapeutic administration of TMZ, BCNU, and BEV was significantly different from the lack of an administration ($p<0.001$, $p=0.046$, $p<0.001$, respectively), and that all administrations were statistically different from the BEV administration ($p<0.001$, $p<0.001$). Thus, the null hypothesis was rejected in addition to the alternative hypothesis, and it was concluded that each chemotherapeutic administration is able to effectively slow glioblastoma tumor growth, and that BEV administration was most effectively able to reduce GBM cell count.

The TMZ, BCNU, and BEV chemotherapeutic administrations were statistically different from the lack of an administration ($p<0.001$, $p=0.046$, $p<0.001$ respectively). As successfully administered drugs on the market, TMZ, BCNU, and BEV have been known to increase PFS significantly through the inhibition of cell growth pathways, as seen by researchers like Fernandes et al. (2017).[8] The findings of Fernandes et al. and others help significantly supplement the results of the present study in that they provided specific insight into the effectiveness of the chemotherapeutic drugs (TMZ, BCNU, and BEV) as compared to the control.[8] By indicating the increase in PFS, the Fernandes et al. study shows significant reduction in the growth of the tumor within the brain, thus citing the significant decrease in cellular growth by TMZ, BEV, and BCNU on a tumor as compared to an actively growing glioblastoma (which was a finding within the present study).[8] This effectiveness of each drug is corroborated by Ohka and his fellow researchers at Nagoya University School of Medicine (2012), who highlights that the use of alkylating agents (such as TMZ and BCNU) and growth factor inhibition (BEV) in chemotherapy of GBM is able to significantly reduce the impacts of the pathology.[28] Ohka et al.'s findings highlight an ability for each of these drugs to significantly reduce symptoms of GBM, including the cellular growth that accompanies the given astrocytoma, which is further supported by findings of the study for control versus TMZ, control versus BCNU, and control versus BEV.[28] It can therefore be understood based upon previous literature that the approval of these drugs by the Food and Drug Administration for administration relies on their abilities to adequately inhibit the pathology of GBM, which includes cellular growth within the tumors. This supports the present study since there was a significant difference between each of the drug administrations and the lack of an administration. By justifying the effectiveness of the drugs in treating growth when compared to an untreated tumor and keeping in mind the Food and Drug Administration (FDA) approval of the drugs, the significance of the difference between the control and each chemotherapeutic administration serves to validate and prove the reliability of the glioblastoma chemotherapy modeling methods utilized.

The present study also established an insignificant difference between BCNU and TMZ ($p=0.091$). Vinjamuri and her fellow experts at Mary Babb Randolph Cancer Center (2009) found that TMZ was unable to provide significant differences in PFS when compared to this previous standard of treatment.[29] Once again, the present study is corroborated due to the inability for Vinjamuri et al. to find a significant difference, since the similarity of BCNU and TMZ substitutes to the insignificance of their difference.[29] In adjunct to Vinjamuri et al. (2009), Garside and his fellow researchers at Peninsula Medical School in the Universities of Exeter and Plymouth (2007) found that there was no significant advantage to BCNU administration as compared to the TMZ administration.[25] This sufficiently supports the current findings since the use of BCNU and TMZ in chemotherapeutic administrations have been established to be very similar in nature and impact of GBM pathogenesis. Though more recently, TMZ has replaced BCNU as the standard of treatment, TMZ shows little to no significance in improving the treatment of GBM as compared to BCNU.

The reason for the lack of difference between BCNU and TMZ in its impact on GBM can be concluded to be derived from the similarity of their chemical properties and history within the chemotherapy industry. Both TMZ and BCNU have been characterized as DNA alkylating agents, which illustrates that their means of regulating GBM cellular growth comes from the ability to transfer methyl groups to the cancerous DNA, according to both Thomas et al. (2013) and Reithmeier et al. (2010).[2,19] As a result, the growth pathways that they play a role in regulating are quite similar in nature, highlighting their similarity and lack of significant difference in GBM chemotherapy.[2]

Arguably most important is that the present study shows the most significant difference to be that between BEV and the other administration types (TMZ and BCNU) ($p<0.001$, $p<0.001$). Numerous studies, such as Funakoshi et al. (2020), cited BEV to work only as a complementary drug and note that it provides less of an impact on GBM progression when compared to drugs such as TMZ and BCNU.[21] This directly contrasts with the present findings in that BEV showed a strong correlation with increased inhibition of GBM growth, while TMZ and BCNU provided less inhibitory effects in the long term (despite initial increases). However, as stated previously, the dosing of BEV has been smaller for use in chemotherapy as compared to TMZ and BCNU, which could cause such differences in findings, according to Foss and his counterparts at Queen's Medical Centre in England (2015).[30] While the present study eliminated such dosing barriers, studies such as Funakoshi et al. (2015) considered the drugs with their current dosing, leading to the differences mentioned above.[21] This argument is further supported by Yu and colleagues at the Jilin University Hospital in China (2016), who note that BEV shows extremely promising characteristics in comparison to the standards of chemotherapy (particularly due to its ability to modulate angiogenesis through its inhibition of VEGFs), and that a lack of research on higher dosing inhibits its primary usage.[31] This most directly aligns with the findings of the study, because it highlights that in an equal dosing circumstance between TMZ, BCNU, and BEV (as was the case in this study), BEV could prove to be exceptionally more effective than the standards of treatment, thus resulting in the lower cell counts in this study.

The primary explanation that could justify the effectiveness of BEV as compared to TMZ and BCNU reverts back to their similarities as DNA alkylating agents and the characteristics of BEV as an inhibitor of VEGF. As both TMZ and BCNU revolve around the activation and deactivation of growth genes with the GBM cells through their role as alkylating agents, both have been associated with drug resistance in the tumor cells. TMZ, for instance, has faced resistance due to the presence of MGMT, a factor that can reverse the cytotoxicity of TMZ as a drug, according to Chien and her counterparts at the National Institute of Cancer Research in Taiwan (2021).[32] Similarly, in a study Wang and colleagues at Yantashan Hospital in China in 2017, BCNU has seen resistance within brain carcinoma cells through the regulation of miR-21.[33] Thus, the DNA alkylating agents such as TMZ and BCNU could be correlated with an increased risk of future resistance. This is shown within the present study in that TMZ and BCNU are able to provide a significant initial reduction in cellular growth, but are not able to maintain this as the treatment progresses, which is shown by an eventual increase in cellular counts. On the other hand, as a VEGF inhibitor, BEV is able to provide a significantly larger reduction in the GBM cellular growth as compared to the DNA alkylating agents, and are able to maintain this reduction past the treatment period. This ability could be attributed to its properties of regulating proteins as opposed to activating genes.

However, this significant reduction in GBM cellular growth by BEV is accompanied by raised concerns about increased toxicity at higher doses. While the present study fails to adequately address research regarding toxicity, the use of BEV has been seen to have varying impacts on treatment toxicity, some highlighting negative impacts and others highlighting the positives. While high-dose BEV is noted to decrease in PFS, according to Ajilan and her colleagues at Stanford University (2017), Fleischmann and cancer experts in Germany (2019) highlight specific BEV capabilities in reducing toxicity reirradiation treatments.[34],[35] Thus, understanding the side effects and toxicity of BEV as a potent drug requires increased research by the FDA.

In conclusion, the use of TMZ, BCNU, and BEV for the chemotherapy of GBM is extremely well justified, due to their capabilities in decreasing rates of replication. TMZ and BCNU, as DNA alkylating agents, show a pronounced increase in the risk of drug resistance in the GBM cells due to their activation of genes that face interference. However, BEV shows a significantly more effective reduction of GBM cellular growth compared to TMZ and BCNU and a definitive capability to maintain this reduction for an extended period of time. Its properties as a VEGF inhibitor allow it to slow angiogenesis, which accounts for its effectiveness. However BEV toxicity, which continues to be a very conflicted area of research, should be adequately noted and further researched. It can therefore be understood that the consideration of approval of BEV for high dosing in GBM can only be achieved after prompt research by the FDA into the toxicity and side effect management of BEV.

This research contained few sources of error due to its simulation-based nature, which controlled the accuracy of the data. However, one source of error included the use of a 1D as opposed to a 3D VisualPDE simulation. Due to the three-dimensional nature of tumor growth, such a simulation would have resulted in more accurate modeling to visualize the behavior of the GBM tumor cells. As a result, one-dimensional model data (which reduced the behavioral complexity) may have been slightly skewed due to the reduction in growth dimensions. However, the “integrate” feature would not have been able to provide the GBM cellular count within the 3D model as in the 1D simulation, due to the difference between the scalar and vector integration. As the GBM cellular count was essential to the quantification of the results within this study, the use of the one-dimensional simulation was justified. However, to improve this procedure in the future, a simulation outside of VisualPDE could be utilized to model 3D tumor growth while showing the changes in cell count, allowing for accurate tumor growth modeling and quantifiable GBM cell counts.

In addition, another source of error within this study was that the data collected modeled continuous chemotherapy with a large initial dose over five months through the use of pharmacokinetic concentration equations. Since chemotherapy is typically administered in cycles of a short period of administration followed by weeks of rest, the modeling of continuous chemotherapy was not entirely accurate. However, the administration of multiple doses of the chemotherapeutic agent in different cycles would cause the concentration of the drug within the brain to rely on multiple uses of the concentration equations simultaneously, which VisualPDE does not have the ability to manage. As a result, the decision to utilize continuous chemotherapy (with a larger dose to compensate for the lack of readministration) was once again justified. However, to improve this aspect in the future, the calculations could be run outside of VisualPDE in order to account for the total chemotherapeutic concentration including the multiple doses of chemotherapeutic agents through cycles. This would allow for a cyclic rather than continuous administration to be accurately utilized.

A final source of error was that the impact of the chemotherapeutic administrations was only measured for the first five months of administration, as opposed to a longer period of time (which could have yielded more insight into long term impacts of chemotherapeutic administrations). This could be improved by measuring a longer period of time following the chemotherapeutic administration to better understand the impacts of the chemotherapeutic agents.

Future research could focus on analyzing the effectiveness of various combinations of TMZ, BCNU, and BEV in inhibiting GBM cell growth within a designated tumor, providing fundamental knowledge into the use of combination therapy within cancer treatments, especially GBM. Also, more research into the impacts of different administration techniques as opposed to different drugs within the chemotherapeutic treatments of GBM could allow for insight into the effectiveness of treatment methods and the streamlining of chemotherapeutic administration to optimize GBM cell reduction. Finally, following research could look into the impacts of changing parameters values on the ability of the chemotherapeutic treatment to stop GBM tumor growth. The results of such research could be instrumental in finding the properties of chemotherapeutic drugs that contribute to an effective treatment.

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Tables and Figures

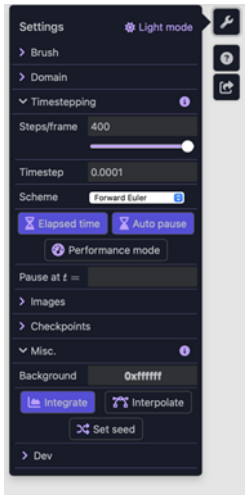

Figure 1. *Customization of VisualPDE one-dimensional logistic traveling waves settings to provide necessary data on various glioblastoma multiforme treatments.* This figure highlights the right-hand settings changes that were utilized to set up for the use of VisualPDE simulations to aid in experimentation.

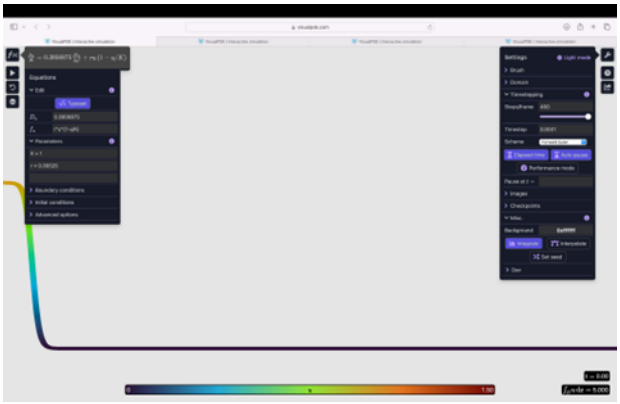

Figure 2. *Preparation of the growth function of glioblastoma multiforme on VisualPDE.* This figure shows the implementation of the GBM values into the VisualPDE Simulation to indicate the cellular growth of GBM.

Table 1. *Chemotherapeutic concentration equations and f_u based on administration technique*

Administration Technique	Equation	f_u
Oral (TMZ) [15]	$C = \frac{F \cdot D \cdot k_a}{V \cdot (k_a - k_e)} \left(e^{-k_e t} - e^{-k_a t}\right)$	r*u*(1-u/K)-u*((F*D*ka)/(V*(ka-ke)))*(exp(-ke*t)-exp(-ka*t))
Intravenous Bolus (BCNU) [16]	$C = \frac{D}{V} e^{-k_e t}$	r*u*(1-u/K)-u*(D/V)*(exp(-ke*t))
Intravenous Infusion (BEV) [16]	$C = \frac{D}{V k_e T} \cdot \left(e^{k_e T} - 1\right) \cdot e^{-k_e t}$	r*u*(1-u/K)-u*(D/(V*ke*T))*(exp(k 1)*(exp(-ke*t))

This table shows both the equations utilized for the concentrations of the chemotherapeutic administration based on different administrations and the □u term utilized in VisualPDE.

Table 1A. *Proliferation-Invasion Model implementation into the VisualPDE simulation*

PI Model Term	VisualPDE Term	Value	Units	Source
c	*u*	No value.	cells	No source.
D(x)	D_u	0.3956875	1/month	[22]
p	*r*	0.36525	mm²/month	[22]
K	*K*	1	None.	No source.

This table provides a list of terms which were equated from the PI model to the VisualPDE simulation, as well as the values that were assigned to them in order to effectively model the growth of GBM over time

Table 2A. *Parameter Values for the Three Treatment Technique Simulations on VisualPDE*					
Parameter	Term	Units	TMZ	BCNU	BEV
Concentration	C	mol/liter	No value.	No value.	No value.
Bioavailability	F	%	1.00	1.00	1.00
Dose	D	Molar	1.00	1.00	1.00
Volume of Distribution	V	L	10.5	5.1	2.88
Elimination Constant	k_e	1/h	0.33	1.86	0.00312
Absorption Constant	k_a	1/h	5.8	No value.	No value.
Duration of Treatment	T	months	No value.	No value.	5.00
Time	t	months	Variable.	Variable.	Variable.
Sources	[15]	[15]	[23]	[24,25]	[26,27]
This table indicates the given values that were assigned to each of the variables of the chemotherapeutic treatment equations (as differentiated by technique and drug). Each variable that was utilized within the equation of f_u for a given treatment was defined as the above value in the "parameters" section.					

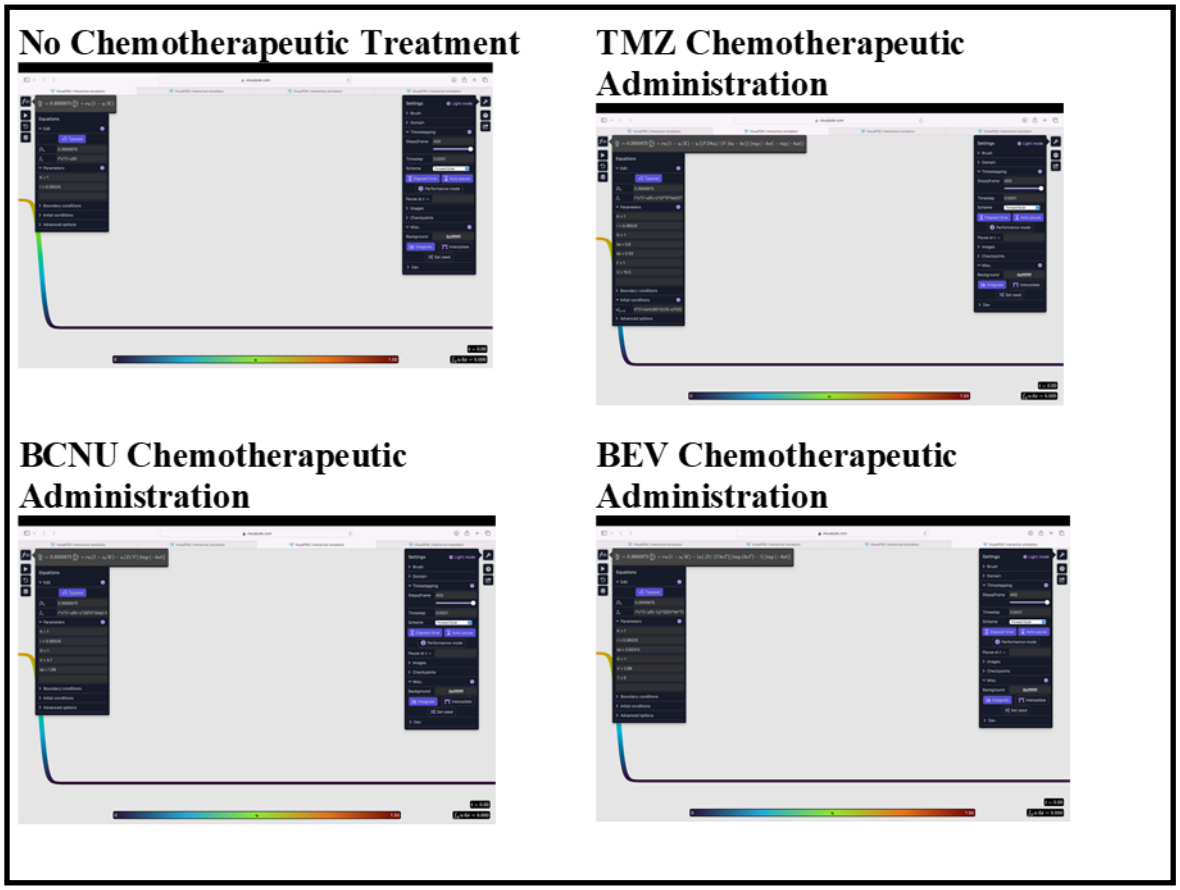

Figure 1A. *Final VisualPDE Setup of the Chemotherapeutic Administration Techniques within Their Respective Simulations.* This figure highlights the complete set up of the four simulations prior to testing.

Title of the Experiment

A Comparative Analysis of Chemotherapeutic Agents in Inhibiting Glioblastoma Multiforme

Hypothesis

It was hypothesized that carmustine and temozolomide would reduce the tumor growth the most as compared to untreated GBM growth based on mathematical differentiation-based models, because both chemotherapeutic agents directly target the genetic cell division pathways of GBM in order to slow and regulate cell division by acting as alkylating agents within DNA to slow tumor progression.[2]

Independent Variable

Type of chemotherapeutic agent that is being used within the model (for characteristic coefficients and values)

Levels of Independent Variable	TMZ	BCNU	BEV	NONE
Number of Repeated Trials	60	60	60	60

Dependent Variable

The number of cells based on the integrated value computed that have grown in the given glioblastoma

Constants

Control Group

The group with no chemotherapeutic agent (no coefficient or value input)

Figure 2A. *Experimental Design Diagram*

Table 3A. *The glioblastoma cell count per every five initial cells at 60 different time points*

Time Points	None	TMZ	BCNU	BEV
0.02	5.004	5.004	4.985	4.969
0.06	5.012	5.008	4.957	4.909
0.13	5.027	5.009	4.917	4.808
0.19	5.040	5.006	4.889	4.725
0.29	5.062	4.995	4.855	4.594
0.32	5.069	4.992	4.847	4.557
0.35	5.076	4.988	4.841	4.520
0.56	5.126	4.962	4.819	4.280
0.74	5.171	4.946	4.828	4.098
0.94	5.224	4.936	4.857	3.916
1.03	5.249	4.935	4.876	3.840
1.17	5.288	4.937	4.910	3.729
1.26	5.314	4.940	4.934	3.662
1.28	5.320	4.941	4.940	3.648
1.35	5.340	4.945	4.960	3.598
1.41	5.358	4.949	4.979	3.557
1.68	5.440	4.976	5.068	3.384
1.74	5.459	4.984	5.089	3.349
1.81	5.481	4.994	5.114	3.308
1.83	5.488	4.997	5.122	3.297
1.87	5.501	5.003	5.136	3.275
1.87	5.501	5.003	5.136	3.275
1.96	5.530	5.017	5.170	3.226
1.99	5.540	5.022	5.181	3.210
2.11	5.579	5.044	5.227	3.149
2.15	5.593	5.052	5.243	3.129
2.19	5.606	5.060	5.258	3.110
2.41	5.682	5.107	5.346	3.008
2.48	5.706	5.123	5.374	2.977
2.54	5.728	5.138	5.399	2.951
2.55	5.731	5.140	5.403	2.947
2.60	5.749	5.153	5.423	2.926
2.75	5.803	5.192	5.486	2.866
2.87	5.847	5.225	5.536	2.820
3.16	5.956	5.311	5.660	2.717
3.33	6.022	5.365	6.734	2.661
3.34	6.025	5.369	5.739	2.657
3.44	6.065	5.402	5.783	2.626
3.45	6.068	5.405	5.787	2.623
3.57	6.116	5.447	5.840	2.587
3.63	6.140	5.468	5.867	2.569
3.66	6.152	5.478	5.880	2.560
3.77	6.196	5.518	5.930	2.529
3.87	6.237	5.555	5.975	2.502

Table 3A. Continued. *The glioblastoma cell count per every five initial cells at 60 different time points*

Time Points	None	TMZ	BCNU	BEV
4.00	6.291	5.604	6.034	2.467
4.02	6.299	5.612	6.043	2.462
4.03	6.303	5.615	6.048	2.459
4.16	6.357	5.666	6.108	2.426
4.19	6.370	5.678	6.121	2.419
4.24	6.391	5.698	6.144	2.407
4.31	6.421	5.726	6.177	2.390
4.34	6.433	5.738	6.191	2.383
4.41	6.463	5.767	6.223	2.366
4.42	6.468	5.771	6.228	2.364
4.46	6.485	5.787	6.247	2.355
4.57	6.532	5.833	6.298	2.330
4.58	6.537	5.838	6.303	2.328
4.60	6.545	5.846	6.313	2.323
4.78	6.624	5.923	6.398	2.285
4.92	6.686	5.985	6.465	2.256

This table highlights the growth of GBM cells under different chemotherapeutic treatments at 60 different time points within a five month chemotherapy period.

Table 2. *Descriptive statistics by chemotherapeutic drug administered in a period of five month chemotherapy as modeled by partial-differential VisualPDE Simulations*

	Control	TMZ	BCNU	BEV
Mean	5.797	5.285	5.511	3.128
Median	5.730	5.139	5.401	2.949
IQR	0.946	0.612	1.057	1.113
Range	1.682	1.050	1.646	2.713

This table shows the mean, median, interquartile range (IQR), and range of the number of GBM cells per five initial cells that had grown at randomized points of time in five months of chemotherapy, as differentiated by the chemotherapeutic agent administered.

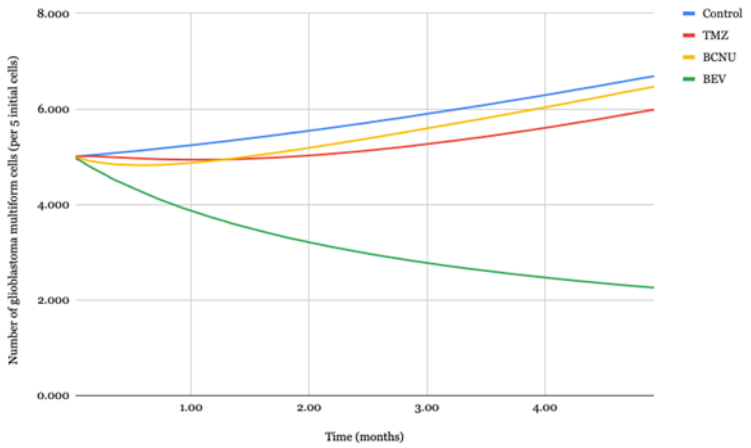

Figure 3. *The number of cells per five initial cells within a given glioblastoma multiforme tumor as impacted by various chemotherapeutic drug treatments over a period of five months.* This graph highlights the over-time trends of the chemotherapeutic agents (as well as the lack of one) on the growth of the GBM cells over a five month period.

Table 3. *Analysis of variance for One-Way ANOVA on the effect of different chemotherapeutic drugs on the cellular growth of a glioblastoma multiforme tumor per every five initial cells*

	SS	**df**	**MS**	**F**
Between	268.974	3	89.658	281.049
Within	75.287	236	0.319	
Total	344.260	239		
			Significant.	$p<0.001$

This table highlights the sum of squares (SS), degrees of freedom (df), mean sum of squares (MS), and final F and *p* value as computed for the data set regarding the effect of different chemotherapeutic agents on GBM cell growth.

Table 4A & 5A. *Post-Hoc Tukey test and mean summary table for test of significance between the different chemotherapeutic agents utilized in a partial-differential model to assess comparative impact on cellular growth*

Post-Hoc Tukey	q	3.63
	HSD	0.265

T1 : T2	$\mu_1 : \mu_2$	**Difference**	***p* value**
Control : TMZ	5.797 : 5.285	0.512	<0.001
Control : BCNU	5.797 : 5.527	0.27	0.046
Control : BEV	5.797 : 3.128	2.669	<0.001
TMZ : BCNU	5.285 : 5.527	0.242	0.091
BCNU : BEV	5.527 : 3.128	2.399	<0.001
TMZ : BEV	5.285 : 3.128	2.157	<0.001

These tables highlight the significant difference between means as computed by the post-hoc Tukey test, which is 0.265. The differences between each of the means for the four groups were greater than this value, indicating that each group was significantly different from the others, except for the TMZ administration compared to the BCNU administration.