Mathematical Modeling of Brain Cancer to Identify Promising Combination Treatments

Background

For aggressive brain cancers such as glioblastoma multiforme (gbm), much of the discussion on this web site and others are on clinical trials for new agents. This is largely because the existing arsenal of treatments is so weak. The clinical trials take drugs that seem promising and test them for safety and efficacy. The trials have prevented widespread use of agents that were dangerous, and have led to some improvements in brain tumor treatments. Life has been marginally extended. Newer treatments generally have fewer side effects than older ones for a similar level of effectiveness.

However, taken in the broader context of the disease, the pace of progress through the conventional clinical trial approach has been arduously slow. The standard treatment (radiation with or without gross tumor resection) offers a median survival time for patients with gbm of only 8-12 months. With this prognosis, how valuable are trials which compare drug A that is not very effective with a newer drug B that may be slightly more effective, but still not very good? Consider that a highly effective new drug, which increases survival time by 50 percent, adds only 4-6 months to the median life of a gbm patient. The extra time is good, but hardly a cure. And, given the years it can take to get a trial approved, conducted, and reviewed, advances come excruciatingly slowly for those afflicted.

This paper demonstrates the use of mathematical modeling to design improved treatments for gbm. The approach may be able to accelerate the pace of innovation in brain cancer treatment. While clinical trials will always be needed, even relatively simple mathematical models can identify more complicated, multiple-agent clinical trials that can potentially come much closer to a cure. The modeling can also demonstrate important weaknesses in single agents that appear promising but in reality may have critical flaws that will always impede their efficacy. Identifying trials with a low chance of success is as important as structuring better multi-agent trials. This is because many brain cancer patients have an opportunity to be enrolled in only one or two trials during the course of their disease; choosing badly can be a serious mistake.

The use of mathematical modeling to accelerate medical breakthroughs is not fantasy. HIV was brought under control only through the use of multiple drugs at once. Which drugs to combine, and how to do so effectively, was done primarily through the use of mathematical models that did not require every single drug permutation to be tested in clinical trials first. The same types of breakthroughs may be possible with brain cancers such as glioblastoma, but only with some changes in the way things are done. There needs to be much better cooperation and coordination between mathematical modelers and neuro-oncologists, groups that currently seem to have little interaction. There needs to be a willingness on the part of the National Cancer Institute to initiate some more aggressive multi-agent trials, at least for people with recurrent, late stage gbm who have little to lose from trying a higher risk treatment protocol. And there needs to be an improved mechanism for centralized reporting of brain cancer treatments and outcomes.

Authorship and Caveats

The analytic portion of this paper was written by Lawrence Wein, a Professor at MIT's Sloan School of Management. (The introductory material was written by Doug Koplow, who is responsible for any remaining errors or omissions in those sections). Professor Wein is a mathematician who models complex systems. While his appointment is at a leading business school, and he does model complex systems at factories, much of his time over the past five years or so has been spent modeling disease. He worked with Alan Perelson of the Los Alamos National Laboratory on the multi-drug cocktails for HIV that have proven so successful. He is currently working on various aspects of improved treatment protocols for cancer, including fractionation regimens for radiotherapy; the scheduling of chemotherapy, radiation, and surgery for breast cancer; the phenomenon of prolonged dormancy after antiangiogenic treatment; and the design and administration of replication-competent viruses.

In his own words, some important caveats are important to keep in mind when using this paper:

"*I am a mathematician who has spent much of the last few years developing and analyzing mathematical models for cancer. However, I am not a clinical researcher and I have no clinical experience with cancer patients. Although I have read over 1,000 (clinical and mathematical) papers on many aspects of cancer, I do not pretend to have the depth of knowledge or the clinical experience that a medical researcher possesses. Also, I have not studied gliomas in my previous work. I have spent about two weeks preparing this report.*" Thus you should:

- **Treat this analysis as a first step, not a recipe**. Treat this paper as a preliminary demonstration of a promising new approach. The proposed treatments are ***suggestive***, based on their mechanism of action and likely efficacy. However, they are ***not exact*** and should not be treated as such.

- **Follow your own doctor.** Continue to rely on your own neuro-oncologists to design your individual treatment protocol. This is especially important because there may be debilitating side-effects from combining two or more treatments that are not evident in this first-stage analysis.

- **Recognize differences between your own case and the subject of this particular analysis.** The paper is focused on a 65-year old male patient with gbm who did not have any tumor removed by surgery. The suggested combinations may be less applicable to different patient profiles.

Feedback

We want your feedback on this approach and any data you may have that can help make the paper more accurate and more useful to a range of patients. Feedback from clinicians, neuro oncologists, or other mathematicians is especially needed. The areas of feedback that would be most helpful include: tumor cell count, growth progression, tumor cell kill rates of various treatments, the variability in these values across the patient population, and other brain cancer modeling work you may be aware of. A structured feedback form can be found on Al Musella's http://www.virtualtrials.com website.

We are aware of a large multi-year modeling effort underway on gbm between Massachusetts General Hospital, the Barrows Neurologic Institute, Princeton University, Los Alamos National Laboratory, and others. While their anticipated results are years away, we hope to track the progress of this effort on the Virtual Trials website. We invite any information you may have about its status, sponsors, participants, or projected milestones.

Overview of Tumor Modeling

A brain tumor is a dynamic system in which bad cells grow and spread, eventually overwhelming good cells in the brain. Where in the brain they start, how quickly they grow, and how they spread will all affect how quickly the cancer spreads. Additional factors of import include the number of cells in the tumor at a given point in time and the *kill rate* of particular treatments (either singularly or combined). A brain tumor has been compared to a forest fire, because it spreads along the outer perimeter and often dies out in the center due to a lack of fuel (or, in the case of a tumor, oxygen and nutrients from the blood). Thus, tumor treatments must also be able to move in the brain more quickly than the tumor spreads if the treatment is to effectively destroy the tumor entirely.

Some basics of cancer and the terminology that follows will be helpful. First, brain cancer cells grow extremely fast. Second, at any point in time, only a portion of them are replicating, and many cancer treatments only kill cells during this active phase. Models must adjust for this constraint in determining the net tumor cell kill rates (the Wein paper does). Third, a small fraction of tumor cells (about one in a thousand) -- called *clonogenic* cells -- are capable of regrowing the entire tumor. All of the clonogenic cells must be killed if the tumor is not to grow back after treatment. Because a tumor such as gbm has so many billions of cells, no single treatment available is capable of such a high kill rate. Table 1 in Professor Wein's analysis demonstrates this problem. Finally, the ability of a treatment to seek out tumor cells rather than healthy cells, and to move through the brain to reach the outer perimeter of the tumor, all affect how well a treatment works.

Wein's paper presents treatments in terms of tumor cell "kill rates." A one log cell kill rate would kill 90 percent of the tumor cells. A two log treatment (such as radiation) would kill 99 percent of the tumor cells. A three-log kill rate would be 99.9%; four-log would be 99.99%, and so on. These are rough estimates; the exact efficacy of a particular treatment is largely

patient-specific. The combination of the expected kill rate, the ability of a new treatment to target tumor cells, and to migrate throughout the tumor all help to quickly identify proposed treatments that are not particularly effective when used singularly.

Another important thing that the model does is to combine multiple treatments with different mechanisms of action in order to greatly reduce the number of cells surviving. Combining treatments **at the same time** is important. As with HIV, the small fraction that survives any one of the drugs will tend to be resistant to that drug. If three drugs are used sequentially, the tumor will have a chance to become resistant to each one independently. If three drugs are used together, the likelihood of resistance developing is much, much smaller. As a result, three drugs used together will tend to yield much better kill rates than if they are used sequentially. This general conclusion must, of course, be tempered by the increased risk of cross-reactions among the agents.

Analysis of Treatment Options for a 65-year old Male with Glioblastoma Multiforme

Lawrence Wein, July 1999

I. Recommendation

I believe that the predicted median survival of 8 months is perhaps optimistic in this patient's case, given his age (65), lack of surgery (which probably would have only bought him a few months) and the diffuse nature of the disease at the time of presentation (unless his tumor burden at presentation was significantly smaller than normal). I think that the best -- and perhaps only -- hope for a cure in this case is an aggressive combination of novel therapies. More specifically, I recommend he initiate (as soon as possible!) a combination of several complementary angiogenesis inhibitors (e.g., thalidomide, SU101), an immune response stimulator (GM-CSF or Poly ICLC) and at least two of three complementary cytotoxins: IL-4 toxin fusion, HSV-tk plus ganciclovir, and a replicating virus (G207 or Onyx-015).

II. Rationale

In the case of glioblastoma multiforme (gbm), death is likely caused not just by the total tumor burden, but by the extent of penetration into the normal brain tissue (Burger *et al.* 1988, Concannon *et al.* 1960). There are three key challenges with gbm. First and foremost, the tumor cells that are the most invasive (i.e., penetrating the normal brain tissue) are less apt to be undergoing mitosis (i.e., cell division) (Chicoine and Silbergeld 1995). Tracqui *et al.* (1995) use the analogy of a spreading forest fire. Surgery, radiation, chemotherapy and other agents that are cell-cycle specific tend to kill the cells that are towards the interior of the forest fire, not at the advancing wave front. Not surprisingly, these traditional therapies, while they may generate promising MRIs, are unable to significantly slow the invasiveness of the disease, and have not put much of a dent into the grim survival statistics related to gbm. The second challenge is that

gbm is highly vascularized (Louis and Cavenee 1997); i.e., it has developed its own blood supply. Third, the tumor cells that remain after radiation are very likely to contain a number of mutations (e.g., the p53 mutation is very prevalent) that may confer chemoresistance.

Consequently, my recommendation is based on three primary concerns: *mechanism of action, delivery* and *toxicity*. Any recommended treatment must be capable of either employing a killing mechanism that can overcome the remaining tumor cells' resistant and/or arrested nature, or attacking the angiogenic process. A successful treatment must also be able to deliver the appropriate agent to the wavefront of the forest fire; i.e., it must reach the tumor cells that have penetrated far into the brain tissue. For example, gbm cells are not restricted to one vertebral artery or internal carotoid, which makes gbm difficult to treat successfully by intra-arterial therapy (Burger *et al.* 1988). Finally, because therapy needs to be delivered throughout much of the brain, a successful treatment must be highly specific to avoid neurotoxicity.

Chemotherapy, including novel agents such as CPT-11, typically fails on all three counts. Hence, it is difficult to recommend chemotherapy in this specific case: given his indicators (age, no surgery, highly diffuse disease), he is likely to fall into the significant (30-40%) proportion of non-responders; if he does respond, chemotherapy will probably be only a (possibly toxic) Band-Aid, buying him several months.

In contrast, IL-4 toxin fusion, HSV-tk + ganciclovir, immunology and replicating viruses all have mechanisms of action that should be both effective against the remaining tumor cells and independent of one another. All these modes of therapy are based on solid science, and none of them appear to generate significant toxicities. My main concern is with the delivery: IL-4 toxin fusion appears capable of reaching most of the tumor cells in the brain; HSV-tk + ganciclovir probably will not distribute throughout the entire brain, but its bystander effect should help. According to my calculations (see §IV.d.), the replicating viruses travel slower than the invading gbm, and so they would need to be administered aggressively and smartly (e.g., on the periphery of the tumor). I do not foresee any of these drugs conferring resistance on the other, but my biological expertise is limited on this.

Ideally, you would want to use an angiogenic inhibitor that directly targets endothelial cells, such as endostatin. However, it is probably impossible to obtain any at this time. While resistance, and even cross-resistance, may develop against thalidomide and SU101, they do attack different targets, and should offer some help in slowing down the disease. However, I do not think these two inhibitors in combination are capable of significantly altering the outcome of gbm, but they would help support the three- or four-pronged attack of the combination in the last paragraph.

A crude mathematical analysis (see §III.a) suggests that about five or six logs of cell kill are required to get close to a cure. Because of the uncertainty involved in this estimate and in the efficacies of these novel therapies, using three or four of them in parallel greatly increases the probability of achieving a sufficient tumor cell kill to eradicate the gbm (see the computations in §III.c). Chemotherapy and radiation can easily achieve this amount of killing for some less

deadly forms of solid cancers. Hence, given the solid science behind these novel therapies, achieving eradication is not out of the realm of possibility. Moreover, as is the case with HIV, using these novel therapies in sequence is unlikely to be successful: none of these therapies in isolation is likely to produce a cure for this patient, and the sequential approach allows the tumor the time and opportunity to continue its partial growth and oncogenic changes, which may lead to further drug resistance.

The remainder of this report is organized as follows. In §III, we describe several mathematical models that frame the problem and aid in the recommendation. We assess the various classes of therapies under consideration in §IV, and provide some concluding remarks in §V.

III. Mathematical Models and Analyses

III.a. Temporal Model. A simple and time-tested (e.g., Skipper *et al.* 1970) approach to modeling tumors is to use differential equations for the total number of tumor cells. This model ignores the spatial aspects of the disease. Let n_t be the number of tumor cells at time t. Then the model states that the time derivative of the total tumor burden, denoted by $\dot{n}_t$, is given by

$$(1) \quad \dot{n}_t = pn_t - k_t n_{t,}$$

where p is the proliferation rate of the tumor and k_t is the (therapy-dependent) killing rate at time t. This simple model assumes that the tumor grows exponentially at rate p in the absence of treatment, and that treatment (such as radiation or chemotherapy) behaves according to the log cell kill hypothesis (treatment kills a fixed fraction of tumor cells, not a fixed number of tumor cells; see the classic works of Skipper *et al.* 1970, Coldman and Goldie 1983 and Norton and Simon 1977). Also, the tumor cure probability (TCP) is calculated using the so-called Poisson hypothesis (e.g., Travis and Tucker 1987),

$$(2) \quad TCP = e^{-fn_T},$$

where T is the length of treatment and f is the fraction of tumor cells that are clonogenic (capable of repopulating the tumor). So this model contains four parameters: the proliferation rate, p; the killing rate (for various treatments), k; the clonogenic fraction, f; and the initial tumor cell count, n_0.

The doubling time is estimated to be about two months for gbm (Alvord 1992, Tracqui *et al.* 1995), and so we set $p = \frac{\ln 2}{60} = 0.012 day^{-1}$.

The number of tumor cells at the point of initial diagnosis is difficult to estimate. There were two 1 x 0.2 x 0.2 cm cores, which is 0.08 cm^3. Another possible estimate of the volume is a sphere of 1 cm diameter, which gives a volume of about 0.13 cm^3. Burgess *et al.* (1997) state that

the average tumor size at presentation is 27 cm^3, which is several hundred times larger than my estimate! This leads me to be highly suspect of my estimate. To convert the volume to cells, we need an estimate of cell density. The classic estimate of cell density for solid tumors is 10^9 cells per cm^3. However, because gbm is so diffuse, it is possible that the cell density is considerably less. Chicoine and Silbergeld (1995) state that a 3 cm diameter sphere of astrocytoma contains approximately 10^{11} cells, which corresponds to a cell density of 7.1 x 10^9 cells per cm^3. Burgess *et al.* (1997) claims that a 3 cm diameter tumor has only 3.5 x 10^7 cells, or a cell density of 2.5 x 10^6 cells per cm^3. Thus, there is more than a three order-of-magnitude difference between these two estimates for cell density! However, the mathematical model of Burgess *et al.* (1997) assumes that tumors become (self-)diagnosed when the diameter of the portion of the tumor that is visually detectable (defined as having a cell density above the threshold value of 8 x 10^6 cells) is 3 cm. Hence, ignoring the cells outside of this radius and the increased concentration inside this radius, we see that the total number of cells in their model at the time of diagnosis must be at least $(\frac{4}{3}\pi 1.5^3)(8\times 10^6) = 1.1\times 10^8$ cells, which is much more than the reported value of 3.5×10^7. I am not sure what to make of this discrepancy.

In addition to the tumor core of gbm, there is considerable tumor burden in other parts of the brain. Chicoine and Silbergeld (1995) claim that the "undetectable" number of tumor cells is typically of the same order of magnitude as the detectable number. Hence, our estimate for n_0 ranges from $2\times 0.1\times 2.5\times 10^6 = 5\times 10^5$ cells, to $2\times 27\times 7.1\times 10^9 = 3.8\times 10^{11}$ cells, a six order-of-magnitude difference! For now, I will use an intermediate value of 10^9 cells, but you should ask your doctor(s) about their best estimate of the total number of tumor cells and the cell density.

Chicoine and Silbergeld (1995) claim that conventional gbm therapy on the bulk disease (e.g., radiation or surgery or chemotherapy) only achieves a two log cell kill, at best (i.e., 99% of the cells are killed by treatment); I was unable to find an independent estimate of the cell killing from the radiation literature (e.g., Thames and Hendry 1987, Hall 1994). In addition, the tumor has probably attempted to double in size during the last few months. So the current tumor burden after radiation might be about 10^7, and could range from 10^4 to 10^{10}.

The clonogenic fraction, *f*, is almost impossible to measure, but is often taken to be in the range of 0.001 to 0.01. To get a reasonable (i.e., e^{-1}=36.8%) chance of a cure, it would be desirable to have the final tumor burden equal to f^{-1}, which is about 10^2 to 10^3 cells. So we need somewhere between one and eight logs of cell kill by subsequent therapy. Here, we can see our parameter uncertainties compounding to the point where we are getting unrealistic results: clearly, more than one log cell kill is needed to eradicate gbm. Let us suppose that we need five or six logs of cell kill to achieve a cure.

III.b. Spatio-temporal Model. A more detailed, but still relatively simple, model is to add a killing term to equation (1) of Burgess *et al.* (1997):

$$(3) \qquad \frac{\partial n(r,t)}{\partial t} = D\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial n(r,t)}{\partial r}\right) + pn(r,t) - k_t n(r,t).$$

In this partial differential equation, *n(r,t)* is the concentration of tumor cells at location *r* at time *t*, and *D* is the diffusion coefficient (estimated to be 0.0013 cm^2 per day for gbm), which captures the invasiveness of the gbm cells. The tumor spread is assumed to be spherically symmetric in this model, and *r* measures the distance from the center (i.e., the origin of the gbm). This type of model has been used by Jim Murray and his colleagues to investigate the effects of chemotherapy (Tracqui *et al.* 1995) and surgery (Woodward *et al.* 1996) on survival. They fit their model to clinical date by assuming that a tumor is (self-)diagnosed when the diameter of the portion of the tumor that is visually detectable (defined as having a cell density above the threshold value of 8 x 10^6 cells) reaches 3 cm and that a patient dies when this diameter reaches 6 cm. They report that the actual median tumor volume at death is between 58 and 84 cm^3. They also estimate that the tumor wave front travels at a rate of 0.1 cm per week.

While I think the simple temporal model in equation (1) can be used to estimate TCP, equation (3) is much more appropriate for survival time and for assessing the effects of regrowth after therapy. For example, equation (3) has been used to show why surgery does not add very much time to survival: the wave front of the "forest fire" is not greatly affected by cutting out its core. This is why the tumor burden at death is only two or three times as large as the tumor burden at presentation. In contrast, equation (1), which ignores the spatial aspects, predicts that it would take the tumor about 13 months to grow back to its pre-treatment size (radiation reduces the tumor to 1/100th of its size, and so it takes 6.65 tumor doublings -- or about 13 months if the tumor doubles every two months -- to grow back, because $2^{6.65} = 100$). The traditional way (e.g., for breast cancer) to estimate time until death with the temporal model is to assume a lethal tumor burden of 10^{12} cells. But this temporal model would overestimate the survival time because it fails to capture the fact that the most invasive tumor cells are unaffected by radiation. Hence, the spatial model emphasizes the fact that an effective therapy must attack the invading wave front as soon as possible.

There is also a separate literature that models the spatial dynamics of a macromolecule infused into the brain (Morrison *et al.* 1994). I am currently working with an oncologist to combine the pharmacodynamic model of Morrison *et al.* with the Burgess *et al.* model. This effort will use Morrison *et al.*'s model to compute *c(r,t)*, which is the concentration of the macromolecule (such as IL-4 fusion toxin) at location *r* at time *t*, and then multiply the last term in equation (3) by *c(r,t)*. The analysis of such a model allows one to explicitly compute the tumor cure probability in terms of the primitive parameters (diffusion, clearance and killing rate of the toxic agent, the amount of drug infused, the growth and diffusion rates of the tumor, and the location and size of the tumor). However, the mathematical and empirical work will require at least several more months of analysis.

III.c. A Simple Probabilistic Analysis. A key question we would like to answer is: how many drugs in the combination do we need to achieve a tumor cure? Unfortunately, there is not sufficient data to answer this question in a reliable manner. However, I will analyze a simple hypothetical example in order to introduce the thought process required to address this question, and to gain some intuition. In our hypothetical example, suppose we had up to four agents (e.g., an angiogenesis inhibitor, IL-4 toxin fusion, a cytotoxic virus and an immunotherapy) that we could simultaneously employ, and that -- independently of each other -- they each achieved a one log cell kill (i.e., 90% of cells killed) with probability 0.25, two logs of cell kill (i.e., 99% of cells killed) with probability 0.5, and three logs of cell kill (i.e., 99.9% of cells killed) with probability 0.25. Hence, we are assuming there are no cytotoxic (antagonistic or synergistic) interactions among the drugs; while naive, this assumption is not unreasonable given the independent mechanisms of the agents. We are also assuming for simplicity that each drug is equally efficacious (although the same thought process holds if we relax this assumption). Recall that conventional gbm therapy achieves about a two log cell kill, and so each of our drugs is assumed to be as powerful as a conventional therapy on average, but their efficacies are uncertain.

In §III.a, we concluded that roughly five or six logs of cell kill were required (after radiation) to achieve a reasonable shot at a tumor cure. In Table 1, we display the probability of achieving x logs of cell kill (for x=5, 6, 7, 8) by simultaneously using y agents in combination (for y=2, 3, 4) for our hypothetical example.

For this hypothetical example, the table shows that a three-drug combination suffices to reliably (89.1%) achieve a five-log reduction and a four-drug combination is required to reliably (85.5%) achieve a seven-log reduction. In contrast, a two-drug combination has very little chance of achieving a reduction greater than or equal to six logs, and a three-drug combination has very little chance of achieving a reduction greater than or equal to eight logs. If our efficacies for novel therapies (i.e., between one and three logs) are of the right order-of-magnitude (note that HSV-tk achieved a three-log reduction in mice in Rubsam *et al.* 1999, which seems unusually high in gbm research), then Table 1 argues for the testing of three- and four-drug combinations for curative purposes.

Table 1:
Cell Kill Rates for Use of Multiple Agents
in Combination: A Hypothetical Example, Post Radiation
[Probability of achieving 'x' logs of cell kill (x=5,6,7,8) using a 'y'-drug combination (y=2,3,4)

Number of Agents in Combination	5 logs	6 logs	7 logs	8 logs
2	31.2%	6.2%	0.0%	0.0%
3	89.1%	65.6%	34.4%	10.9%
4	99.6%	96.5%	85.5%	63.7%

IV. Alternative Therapies

IV.a. Chemotherapy. I will not survey the FDA-approved chemotherapeutics. They offer several months of median survival for gbm patients. But the distribution is skewed, with nearly all of the benefits going to a minority of patients. Chances are small that this patient would be among the beneficiaries. Of the newer chemotherapeutics, temozolomide and irinotecan (CPT-11) appear to be the most promising. As reviewed by Williams (1998), temozolomide achieved a total response rate in 58% of 103 patients, although the duration was for only 4.6 months. Similarly, 7 of 17 patients responded in a phase I study (Brock *et al.* 1998); comparable phase II results are reported in Spagnolli *et al.* (1999). Moreover, *in vitro* studies display cross-resistance between temozolomide and CCNU (Sankar *et al.* 1999) and suggest that temozolomide is less effective against p53 mutant tumor cells (Tentori 1998).

In a phase II study (Friedman *et al.* 1999), CPT-11, which appears to have complementary resistance profiles to other chemotherapeutics, achieved slightly better results (9/60 had a partial response and 33/60 were stable for at least 12 weeks, for patients with recurrent or progressive malignant glioma); typical chemotoxicities (nausea, etc.) were reported. An attempt at an every-three-week regimen led to less impressive results (median time to progression for recurrent gbm was seven weeks) (Cloughesy *et al.* 1999).

IV.b. Angiogenesis Inhibitors. Angiogenesis is a very complex process, and has many promoters and inhibitors. One might think of this process as a complex network consisting of different environmental (e.g., tumor oxygen level), oncogenic (existence of mutations) and angiogenic factors that can be up- and down-regulated. Tumor endothelial cells are the key target in anti-angiogenesis therapy. A landmark study (Boehm *et al.*, 1997) has shown that endothelial cells, unlike tumor cells, do not develop acquired drug resistance. Hence, ideally one would want to use an angiogenic inhibitor that directly attacks tumor endothelial cells, such as the much-touted endostatin. However, at this point in time, it is probably impossible to obtain this (or a similar) agent. Unfortunately, the agents that inhibit the various upstream targets (e.g., VEGF, bFGF) in the angiogenic process are susceptible to resistance (because of the multiple pathways through the network), and may lose their potency over time; e.g., Yoshiji *et al.* (1997) has shown that vascular endothelial growth factor (VEGF) is essential for initial but not continued growth of breast cancer. However, combinations of angiogenesis inhibitors aimed at different targets work better than any inhibitor alone (e.g., Brem *et al.* 1993).

Thalidomide, which blocks basic fibroblast growth factor (bFGF)-induced angiogenesis (bFGF is over-expressed in gbm), is the only FDA-approved angiogenesis inhibitor. The studies by Fine (cited in Williams 1998) (50% response rate, including 4/32 regressions) and Glass *et al.* (1999) (carboplatin plus thalidomide achieve a median response of 24 weeks and median survival of 40 weeks for patients with recurrent gbm) suggest that it can be helpful, but only as a small part of a larger regimen. It also can cause fatigue and hematologic toxicity.

Marimastat is a matrix metalloproteinase inhibitor. It can cause (reversible) severe joint and muscle pain if given in doses greater than 50 mg bid in Steward (1999), but was well tolerated at these doses in Wojtowicz-Prage *et al.* (1998). It achieved a 58% response rate for doses greater than 50 mg bid for other cancer types (colorectal, ovarian and prostate).

SU101 inhibits platelet-derived growth factor, which is over-expressed in gbm. Malkin (ASCO abstract, 1998) reported prolonged stability in 13/57 and regression in 5/57. Adamson *et al.* (1999) reported no toxicity in 19 of 20 pediatric patients.

SU5416 (Fong *et al.* 1999) blocks a VEGF receptor and inhibits tumor growth in mice. Rosen *et al.* (1999) reported results from the first phase I trial on humans: it had mild-to-moderate toxicities and appeared effective, but was not tested specifically on gbm patients.

Based on this information, I would recommend the use of Thalidomide and SU101 as the anti-angiogenic portion of a treatment regimen. Of course, if direct endothelial cell inhibitors such as endostatin or angiostatin become available, these would be preferable. Moreover, the second generation inhibitors (e.g., SU5416) are likely to be more efficacious than the first-generation ones, but also more difficult to procure. I cannot recommend the use of marimastat at this point, due to its side effects and the lack of data on gbm.

Finally, Mauceri *et al.* (1998) have shown that VEGF levels increase for two weeks after radiation in mice. It is likely that other pro-angiogenic factors are also increased in response to the insult from radiotherapy. Hence, it may be beneficial to start angiogenesis inhibitors as soon as possible after radiotherapy.

IV.c. IL-4 Fusion Toxin. IL-4 toxin, IL-4(38-37)-PE38KDEL binds specifically to IL-4R and is highly cytotoxic to glioblastoma cells. It has exhibited impressive results in human glioblastoma xenografts and animals, including some complete regressions and no sign of toxicity (Puri *et al.* 1996, Husain *et al.* 1998). According to its developer, Dr. Puri, it should not cause any toxicity problems for this particular patient, although patients at risk for brain stem swelling would not be able to use it. As far as I know, the IL-4 receptor is independent of radioresistance and life cycle effects. My main concern is the delivery. Apparently, it is delivered via convection caused by the natural pressure within the skull, and it follows the tumor migration path. For some types of binding drugs, there is a *binding site barrier* (e.g., van Osdol *et al.* 1991): if the binding is too strong, the drug does not penetrate far enough into the tissue. Overall, this looks to be one of the most promising approaches available.

IV.d. Replicating Viruses. One of the main reasons that conventional gene therapy has not lived up to its potential is *delivery*: it has been difficult to deliver sufficient quantities of the vehicle (e.g., monoclonal antibody, adenovirus) into a tightly compressed solid tumor. One way around this is to use a replicating virus: such a virus does to cancer cells what HIV does to white blood cells: it attaches to the cell surface, infects the cell, replicates exponentially inside the cell,

and kills the cell (i.e., causes lysis). When lysis occurs, all of the new virus particles (hundreds or even thousands) are released from the cell and are free to undergo new rounds of infection and lysis. These viruses are designed to be highly selective to tumor cells, so that they do not run amok in the normal tissue.

In conjunction with Onyx pharmaceuticals (they have developed ONYX-015 (Heise *et al.* 1997), which is the lead drug in this field and is supposed to be starting Phase III trials soon), I have performed a mathematical analysis (paper is in preparation) of replicating viruses. Each injection of this virus into a solid tumor generates a traveling wave of infection in the tumor (not unlike the traveling wave of the brain tumor into the normal tissue). These viruses have shown impressive and non-toxic results in head and neck tumors, and are beginning to be tested on brain tumors. The two main options are G207, (Martuza 1999, who pioneered this field), which is used in trial #00554, and Onyx-015, which I believe is the drug being used in Trial #00397 (www.virtualtrials.com). Neurovir, makers of G207, announced their phase I results for G207. Five of 21 very advanced patients were stable for 3-9 months. This is not that impressive, but 18 out of 21 patients received only a single injection, and phase II clinical trial patients will receive five injections into the tumor at the time of surgery.

I still have some concerns about delivery with replicating viruses. My analysis of replicating viruses suggests that the wave of infection travels at a speed of about three cell radii divided by the infected cell lifetime, which is roughly 30μm/48 hours=0.01 cm/week; the herpes virus G207 may travel at about twice this speed. This is still 5-10 times slower than the tumor wave front velocity reported in §III.b. Hence, the replicating virus needs to be injected liberally and peripherally to be effective (I know that Onyx delivers its virus around the periphery of the tumor, but I do not know about G207). Moreover, the wave needs a sufficient tumor cell density in order to propogate, and so the wave could die out at the very edges of the tumor invasion. Consequently, a replicating virus is not likely to be able to cure a gbm by itself.

IV.e. Nonreplicating Viruses. There are several interesting nonreplicating viruses. CRM107 had reasonably impressive results (median survival of 46 weeks, 2/18 patients alive after 140 weeks) despite using a nonreplicating virus that had mediocre specifity (Laske *et al.* 1997). Hence, I suspect that G207, which is a replicating virus, could be considerably more efficacious (see Martuza's letter that accompanies Laske *et al.'s* 1997 paper).

The herpes simplex virus thymidine kinase (HSV-tk) plus ganciclovir works impressively on rats: e.g., it kills tumor endothelial cells (Tanaka *et al.* 1999) and achieves a greater than three log cell kill (Rubsam *et al.* 1999). Its mechanism (see the first paragraph of LeMay *et al.* 1998) causes a "bystander effect" that allows the effective killing of invasive gbm cells. However, hematopoietic toxicity is a serious side effect (Davey 1990), but the blood-brain tumor barrier permeabilizer, RMP-7, may lessen this effect (LeMay *et al.* 1998), and at least this toxicity is different than the neurotoxicities caused by most other gbm therapies. In summary, this is a very promising approach, and several trials are ongoing. But low gene transfer and insufficient delivery of ganciclovir will probably prevent this approach from providing a cure in humans (Di Meco *et al.* 1997).

Then there is the provocative Newcastle disease virus vaccine (MTH-68/H). Csatary *et al.* (1999) present a case study of a 14-year old boy with a 95% tumor shrinkage of a gbm, and mention two other children with two-year responses. These are the only reports for gbm. In a phase II double-blind placebo trial (Csatary *et al.* 1993), it achieved significant regressions in only 3 of 20 tumors (none of them brain tumors). Yet it performed very well (17 out of 18 complete regressions) on neuroblastoma xenografts on mice (Lorence *et al.* 1994). Unfortunately, the researchers do not seem to know the mechanism of action. In summary, it is difficult to know what to make of this. I think G207 or ONYX-015 or HSV-tk + ganciclovir would be preferable, but the Newcastle virus may be more accessible (if you are willing to fly to Europe and get it).

IV.f. Immunology. Intracavitary interleukin-2 and lymphokine-activated killer cells (IL-2/LAK) showed some response in humans, but generated significant neurotoxicity (Hayes *et al.* 1995). The two patients in their 60s in this study did not fare well. Liau *et al.* (1999) show that dendritic cell immunotherapy (Trial #00487 on www.virtualtrials.com) works on rats. However, this requires another biopsy because close to 3 gm of tumor are needed to derive the necessary antigens. Results on the first humans (reported in Biotechnology Newswatch, 2/1/1999) have shown an increase in the immune response, but only minimal tumor shrinkage. Researchers are also planning a GM-CSF trial that is not patient specific. GM-CSF has impressive results (40% alive after 300 days vs. controls dying by day 59) in rats (Wallenfriedman *et al.* 1999). It takes a while to kick in: the tumor volume peaked at day 23 and then regressed completely. GM-CSF is probably more practical than the patient-specific approach, and may prove effective. I recommend Poly ICLC, which achieved 19 month survivals and no toxicity in humans, as reported by Williams (1998), or GM-CSF.

IV.g. Antineoplastons. The FDA-approved drug, Tamoxifen, is the most popular compound in this therapeutic area. Very high doses (160-320 mg/day) of this protein kinase C inhibitor are needed for gbm patients, which can cause blood clots. A stage II clinical trial (Couldwell *et al.* 1996) shows that tamoxifen achieves tumor regression in about 20% of recurrent gbm cases. Chang *et al.* (1998) report neurotoxicity when tamoxifen is administered with interferon α-2a. Hypericin (Zhang *et al.* 1997) is another protein kinase C inhibitor, and Burzynski's (1990) A10 + As2-1 combination has low toxicity and may work better, but only early results are available. Overall, the risk of toxicity combined with their modest benefit do not make this class of drugs very attractive as part of a potentially curative arsenal.

IV.h. Boron Neutron Capture Theory. According to Diaz *et al.* (1998), low doses of this approach gives comparable results to conventional radiation. Higher doses are likely to be more efficacious, but may have some toxicity. I cannot recommend this option at this point in time.

V. Concluding Remarks. The recommendation in this report assumes that the "utility function" of the patient and his family is to maximize the probability of tumor cure. In many decision problems under uncertainty (e.g., investing in the stock market), there is a tradeoff of risk and return: you can either play it safe (e.g., invest in bonds) and receive a moderate reward with a high probability, or you can attempt to get a big reward by exposing yourself to the risk of a devastating outcome (e.g., bankruptcy in the stock market example).

For this particular disease (gbm) at this particular point in time (summer 1999), I honestly do not see much of a tradeoff. The chances of the patient surviving for another year using only FDA-approved drugs appears to be slim; the chances of long-term survival are essentially nil. (In contrast, the decision for HIV patients is more difficult, due to the existence of some long-term nonprogressors.) On the other hand, there is an unusually large differential in potential efficacies between the FDA-approved drugs and novel, largely untested drugs. It is conceivable that an aggressive combination of these drugs could either cure the tumor, or at least buy the patient several years, at which point more effective treatments may be available. Because these novel therapies have a high uncertainty of success, the more approaches that are tried in parallel, the more likely is success (at least for the first 3 or 4 modes of therapy; after that, decreasing returns are possible, as shown in Table 1). Of course, the likelihood of serious toxicity also goes up as the number of modes of therapy is increased. Nonetheless, the biggest downside risk appears to be fatal toxicities due to an untested drug combination, which would result in death perhaps a handful of months earlier than if the patient followed the "business as usual" route.

If this was me or my loved one, and we had the financial resources, and the patient had the will and the physical strength to undergo an enormously demanding (put perhaps not highly toxic) regimen, I would not only (in David Ho's words) "hit early, hit hard", but would "hit everywhere with everything".

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