Treatment Options for Glioblastoma and other Gliomas

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New text added in 2017 is color-coded dark red
A listing of revisions for the 2017 edition is given in Appendix A

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Introduction

Since my own diagnosis of glioblastoma (GBM) in 1995 at age 50, I have spent considerable time researching treatment options, and the following discussion summarizes what I have learned. Most of the information is from medical journals and the proceedings of major cancer conferences. Some information has been contributed by others to various online brain tumor patient support groups, which I have followed up on, and some is from direct communications with various physicians conducting the treatments that are described. References are presented at the end for those who would like their physicians to take this information seriously. Although this discussion is intended to be primarily descriptive of the recent development of new treatment options, it is motivated by my belief that single-agent treatment protocols are unlikely to be successful, and patients are best served if they utilize multiple treatment modalities, and go beyond the "certified" treatments that too often are the only treatment options offered.

A more extensive account of my philosophy of treatment, and the reasons for it, are provided in my (2002) book, ***Surviving "Terminal" Cancer: Clinical Trials, Drug Cocktails, and Other Treatments Your Doctor Won't Tell You About***. Currently, it is available only at Amazon.com, where reviews of the book also are available.

When I began my search for effective treatments, the available options offered little chance for surviving my diagnosis. The standard treatment included surgery, radiation, and nitrosourea-based chemotherapy, either BCNU alone or CCNU combined with procarbazine and vincristine (known as the PCV combination). While this treatment has helped a small minority of people, its 5-year survival rate has been only 2-5%. Median survival has been about a year, which is 2-3 months longer than for patients receiving radiation alone without chemotherapy. Fortunately, as will be discussed in the next section, the past ten years has produced a new "gold standard" of treatment for newly diagnosed patients: the combination of radiation with a new chemotherapy agent, temozolomide (trade name Temodar in the USA and Temodal elsewhere in the world). While this new standard appears to produce a notable improvement over previous treatments, it still falls far short of being effective for the great majority of patients.

Also available now are three other treatments that have FDA approval for tumors that have recurred or have progressed after initial treatment: Gliadel, Avastin, and an electrical field therapy named Optune (formerly known as NovoTTF). All of these are considered standard of care for recurrent tumors (which is important for insurance reasons), and can legally also be used for newly diagnosed patients as well. Each will be discussed later in this article.

There are three general premises to the approach to treatment that will be described. The first is borrowed from the treatment approach that has evolved in the treatment of AIDS. Both viruses and cancer cells have unstable genetic structures susceptible to mutations. This implies that the dynamics of evolution will create new forms that are resistant to whatever the treatment may be. However, if several different treatments are used simultaneously (instead of sequentially, which is typically the case), any given mutation has a smaller chance of being successful. A mathematical model instantiating these assumptions has recently been developed and has been shown to describe the pattern of tumor growth for melanoma (1).

The second premise is that cancer treatments of all sorts are probabilistic in their effects. None work for everyone, in part because any given cancer diagnosis is an amalgam of different genetic defects that respond in different ways to any given treatment agent. This is especially true for glioblastomas, which have a multiplicity of genetic aberrations that vary widely across individuals and sometimes even within the same tumor of a given individual. As a result it is common that any given "effective" treatment agent will benefit only a minority of patients, often in the range of 10-35%, but do little if anything for the majority. The result is that the chances of finding an effective treatment increase the more different treatment agents that are utilized. Probabilistic effects can and do summate.

An important implication of the genetic diversity of GBM tumors is that tests of treatment agents presented individually will often fail, not because they lack effectiveness, but because they target only one or sometimes two growth pathways, leaving other growth pathways to be upregulated to maintain the growth of the tumor. Thus, even at the level of clinical trials, tests of individual treatment agents in isolation may be a misguided strategy. A drug that fails in isolation might in fact be effective when combined with other drugs that target the additional alternative growth pathways.

A third general principle is that any successful treatment needs to be systemic in nature because it is impossible to identify all of the extensions of the tumor into normal tissue. Moreover, cancer cells are typically evident in locations in the brain distant from the main tumor, indicating that metastases within the brain can occur, although the great majority of tumor recurrences are within or proximal to the original tumor site. Localized treatments such as radiosurgery may be beneficial in terms of buying time, but they are unlikely to provide a cure, except in cases when the tumor is detected early and is very small. Even if the localized treatment eradicates 99% of the tumor, the small amount of residual tumor will expand geometrically, eventually causing significant clinical problems.

Until the development of immunological treatments in just the last few years, which will be discussed in a later section, the only systemic treatment available has been cytotoxic chemotherapy, which historically has been ineffective except for a small percentage of

patients. An important issue, therefore, is whether chemotherapy can be made to work substantially better than it typically does. Agents that facilitate or augment its effects are critically important. As will be seen, a number of older drugs developed for other purposes have been shown in laboratory studies to be effective against cancer, often with minimal toxicity. The availability of these treatments raises the possibility that some combination of these new agents can be packaged that provide effective treatment based on several different independent principles. Thus, the AIDS-type of combination approach is now a genuine possibility whereas it would not have been fifteen years ago. Because many of these relatively nontoxic new agents were developed for purposes other than cancer, or for different kinds of cancer, their utilization in the treatment of glioblastomas is "off-label", with the result that many oncologists have been hesitant to prescribe them. Thus, patients themselves need to become familiar with these new agents and the evidence available regarding their clinical effectiveness. It is possible, although by no means proven, that some combination of these newly repurposed agents offers the best possibility for survival.

Patients may or may not learn about the treatments that will be described from their physicians. To appreciate why, it is important to understand how American medicine has been institutionalized. For most medical problems there is an accepted standard of what is the best available treatment. Ideally, such treatments are based on phase III clinical trials in which patients are randomly assigned to receive the new treatment or some type of control condition. Treatments that have been studied only in nonrandomized phase II trials will rarely be offered as a treatment option, even if the accepted "best available treatment" is generally ineffective. What happens instead is that patients are encouraged to participate in clinical trials. The problem with this approach is that most medical centers offer few options for an individual patient. Thus, even though a given trial for a new treatment may seem very promising, patients can participate only if that trial is offered by their medical facility. Yet more problematic is that clinical trials with new treatment agents almost always initially study that agent in isolation, usually with patients with recurrent tumors who have the worst prognoses. For newly diagnosed patients this is at best a last resort. What is needed instead is access to the most promising new treatments, in the optimum combinations, at the time of initial diagnosis.

In the discussion to follow, it is important to distinguish between treatment options at the time of initial diagnosis versus those when the tumor either did not respond to the initial treatment or responded for a period of time and then recurred. Different measures of treatment efficacy are often used for the two situations, which sometimes makes treatment information obtained in one setting difficult to apply to the other. The recurrent tumor situation is also complicated by the fact that resistance to the initial treatment may or may not generalize to new treatments given at recurrence.

When someone is diagnosed with a brain tumor they are faced with a situation about which they know very little, but nevertheless must develop a treatment plan very quickly, because GBMs grow very rapidly if left untreated. The first step, if possible, is to have as much of the tumor removed as possible, because various data show substantially increased survival times for those with complete resections, relative to those who have incomplete resections or only biopsies. Accordingly, it is best that patients seek treatment at a major brain tumor center because neurosurgeons there will have performed many more tumor removals than general neurosurgeons that typically work in the community setting. This is especially important in recent times, as surgical techniques have become increasingly more sophisticated and utilize procedures that community treatment centers do not have the resources to perform. I know of numerous cases in which a local neurosurgeon has told the patient the tumor is inoperable, only to have the same tumor completely removed at a major brain tumor center.

An additional advantage of utilizing a major brain tumor center is that they are better equipped to do genetic analyses of tumor tissue, which are increasingly important in guiding treatment decisions. Moreover, they provide a gateway into clinical trials.

1. The Standard of Care for Initial Treatment

Glioblastoma

Although chemotherapy has a long history of being ineffective as a treatment for glioblastoma, a large randomized European-Canadian clinical trial (EORTC trial 26981/22981) has shown clear benefits of adding the new chemotherapy agent, temozolomide (trade name Temodar in the USA, Temodal elsewhere in the world) to the standard radiation treatment (2). This treatment, followed by 6 or more monthly cycles of temozolomide, has become known as the "Stupp protocol" after Roger Stupp, the Swiss oncologist who led the trial. In this trial, one group of patients received radiation alone; the other group received radiation plus Temodar, first at low daily dosages during the six weeks of radiation, followed by the standard schedule of higher-dose Temodar for days 1-5 out of every 28-day cycle. Median survival was 14.6 months, compared to a median survival of 12 months for patients receiving radiation only, a difference that was statistically significant. More impressive was the difference in two-year survival rate, which was 27% for the patients receiving temodar but 10% for those receiving only radiation. Longer-term follow-up has indicated that the benefit of temozolomide (TMZ)

persists at least up to five years: The difference in survival rates between the two treatment conditions was 16.4% vs. 4.4% after three years, 12.1% vs. 3.0% after four years, and 9.8% vs. 1.9% after five years (3). As a result of these findings, the protocol of TMZ presented during radiation is now recognized as the "gold standard" of treatment. Note, however, that all of these numbers are somewhat inflated because patients over the age of 70 were excluded from the trial.

In July of 2016, the National Comprehensive Cancer Network (NCCN) recommended Optune as a category 2A treatment for newly diagnosed glioblastoma in combination with the standard temozolomide-based chemotherapy (see press release here). This rating indicates a uniform consensus by the NCCN that this treatment is appropriate. As the NCCN is recognized as setting the standards for cancer treatment in the USA and in other countries abroad which follow its guidelines, Optune in combination with standard chemotherapy following radiation could now be considered to be a new standard of care for newly diagnosed glioblastoma. See more detailed information on Optune in *Chapter 3*.

Anaplastic astrocytoma

Though the "Stupp protocol" of combined temoradiation (concomitant radiation and temozolomide chemotherapy) followed by monthly cycles of temozolomide has been routinely applied to anaplastic astrocytoma patients, prospective confirmation of this use in this patient population has awaited results of the "CATNON" randomized phase 3 trial for 1p/19q non-codeleted grade 3 gliomas. Results of the interim analysis for this trial were first released for the ASCO 2016 annual meeting. Between 2007 and 2015, 748 patients were randomized to receive either i) radiation alone, ii) radiation with concomitant temozolomide, iii) radiation followed by 12 adjuvant monthly cycles of temozolomide, or iv) radiation with temozolomide both concurrently and with follow-up monthly cycles. At the time of the interim analysis (October 2015), significant progression-free and overall survival benefit was found with adjuvant temozolomide treatment (arms iii and iv). Median progression-free survival was 19 months in arms i and ii (not receiving adjuvant temozolomide) versus 42.8 months (receiving adjuvant temozolomide). 5-year survival rate was 44.1% and 55.9% in arms i and ii versus iii and iv. Median survival was not yet reached for arms iii and iv.

This analysis did not address the benefit of temozolomide concurrent with radiation, a question that will be answered with further follow-up, and studies assessing the impact of IDH1 mutation and MGMT methylation were still ongoing.

Determining who will benefit

A two-year survival rate of less than 30% obviously cannot be considered an effective treatment, as the great majority of patients receiving the treatment obtain at best a minor benefit, accompanied with significant side effects (although Temodar is much better tolerated than previous chemotherapy treatments, especially with respect to the cumulative toxicity to the bone marrow). This raises the issues of how to determine who will benefit from the treatment, and, most importantly, how to improve the treatment outcomes.

One approach to determining whether an individual patient will benefit from chemotherapy is simply to try 1-2 rounds to see if there is any tumor regression. The debilitating effects of chemotherapy typically occur in later rounds, at which point there is a cumulative decline in blood counts. The extreme nausea and vomiting associated with chemotherapy in the mind of the lay public is now almost completely preventable by anti-nausea agents, including Zofran (ondansetron), Kytril (granisetron) and Emend (aprepitant). Marijuana also can be very effective in controlling such effects, and recent research has suggested that it has anti-cancer properties in its own right. Thus, for those patients who are relatively robust after surgery and radiation, some amount of chemotherapy experimentation should be possible without major difficulties.

An alternative way to ascertain the value of chemotherapy for an individual patient is the use of chemosensitivity testing for the various drugs that are possible treatments. Such testing typically requires a live sample of the tumor and *thus must be planned in advance of surgery*. Culturing the live cells is often problematic, but a number of private companies across the country offer this service. Costs range from $1000-$2500, depending on the scope of drugs that are tested. Such testing is controversial, in part because the cell population evolves during the process of culturing, which results in cells possibly different in important ways from the original tumor sample. Nevertheless, recent evidence has shown that chemosensitivity testing can enhance treatment effectiveness for a variety of different types of cancer, including a recent Japanese study using chemosensitivity testing with glioblastoma patients (4). However, this study did not involve cell culturing but direct tests of chemosensitivity for cells harvested at the time of surgery. In general, when chemosensitivity testing indicates an agent has no effect on a patient's tumor the drug is unlikely to have any clinical benefit. On the other hand, tests indicating that a tumor culture is sensitive to a particular agent do not guarantee clinical effectiveness, but increase the likelihood that the agent will be beneficial.

The role of MGMT

A significant advance in determining which patients will benefit from Temodar was reported by the same research group that reported the definitive trial combining Temodar with radiation. Tumor specimens from the patients in that trial were tested for the level of activation of a specific gene involved in resistance to alkylating chemotherapy (which includes temozolomide and the nitrosoureas, BCNU, CCNU, and ACNU). More specifically, there is an enzyme produced by the MGMT gene that allows the damaged tumor cells to repair themselves, with the result that chemotherapy is less effective. Patients whose tumors have an inactivated MGMT gene through gene promoter methylation (which occurs in 35-45% of patients) have a significantly greater chance of responding to Temodar than those for whom the gene is still functional (5). For patients receiving both radiation and temozolomide, those with methylated MGMT had a two-year survival rate of 46%, compared to 14% for those with unmethylated MGMT. This implies that patients should have tumor tissue taken at the time of surgery tested for the methylation status of the MGMT gene.

The use of genetic markers to predict treatment outcome is an important advance, but so far it has not been routinely incorporated into clinical practice. Considerable controversy exists about the predictive validity of the MGMT marker, as several studies have failed to show a relationship between that marker and clinical outcome. This appears to be due primarily to different measurement procedures. A recent paper (6) compared the degree of MGMT protein expression by using commercial anti-MGMT antibody and an assessment of the methylation status of the promoter region of the MGMT gene. The two measures correlated only weakly, and only the measure of gene promoter methylation correlated strongly with survival time. New methods for assessing methylation have recently been introduced (7) which may resolve the controversy.

The predictive validity of the methylation status of the MGMT gene promoter is an important issue to resolve because temozolomide appears to produce little survival improvement for those whose MGMT gene is active (i.e. the MGMT gene promoter is unmethylated). Thus, patients with unmethylated MGMT might be better served by use of a different chemotherapy agent. For example, a bi-functional alkyating agent known as VAL-083 or dianhydrogalactitol is currently being tested in phase 2 and 3 trials for recurrent glioblastoma. It damages DNA in a way that is not subject to repair by the MGMT enzyme and could therefore be more beneficial for patients with unmethylated MGMT status. Find more details on VAL-083 in *Chapter 13*.

In addition to changing the chemotherapy agent, there are other possible strategies for patients with unmethylated MGMT gene promoter. One involves the schedule of Temodar. An alternative to the standard 5 in 28 days schedule is a daily low-dose schedule. Previous studies using metronomic schedules have detected no effect of MGMT status on clinical outcome. The issue of the best schedule for Temodar will be discussed in a later section in *Chapter 2*. The second strategy is to utilize drugs that can inhibit MGMT

expression (in preclinical studies). Two such drugs are Antabuse (disulfiram) and Keppra (levetiracetam) (10, 206), discussed in *Chapter 6*.

Dexamethasone

Most glioma patients will be exposed to dexamethasone (Decadron) at some point, as this corticosteroid is the first-line treatment to control cerebral edema caused by the leaky tumor blood vessels. Many also require dexamethasone during radiotherapy, and perhaps beyond this time if substantial tumor remains post-resection. Dexamethasone is an analog to the body's own cortisol, but is about 25 times more potent. Though often necessary, dexamethasone comes with a long list of adverse potential side effects with prolonged use, including muscle weakness, bone loss, steroid-induced diabetes, immunosuppression, weight gain, and psychological effects.

New evidence also shows an association between dexamethasone use and reduced survival time in glioblastoma. This evidence has to be weighed against the fact that uncontrolled cerebral edema can be fatal in itself, and that dexamethasone is often required for its control. However, the attempt should always be made to use dexamethasone at the lowest effective dose, and to taper its use after control of edema is achieved, under a physician's guidance.

In a retrospective study of 622 glioblastoma patients treated at Memorial Sloan Kettering Cancer Center, multivariate regression analysis showed an independent negative association of steroid use at the start of radiotherapy with survival (324). A similar negative association with survival outcomes was found in patients in the pivotal phase 3 trial that led to temozolomide being approved for glioblastoma in 2005, and for a cohort of 832 glioblastoma patients enrolled in the German Glioma Network.

Follow up studies in mice helped elucidate these retrospective clinical observations. In a genetically engineered PDGFB-driven glioblastoma mouse model, dexamethasone alone had no effect on survival, but pretreatment with dexamethasone for 3 days prior to a single dose of 10 Gy radiation negatively impacted the efficacy of radiation. This negative impact of dexamethasone on radiation efficacy was even more dramatic with multiple doses of dexamethasone given before 5 treatments with 2 Gy radiation, which more closely mimics what GBM patients are exposed to. In contrast, an antibody against VEGF, which could be considered a murine surrogate for Avastin, did not interfere with the efficacy of radiation.

In vivo mechanistic examination revealed that dexamethasone may interfere with radiation by slowing proliferation, leading to a higher number of cells in the more radioresistant G1 phase of the cell cycle, and fewer cells in the more radiosensitive G2/M

phase. This finding has far-reaching implications about the potential interference by drugs with cytostatic mechanisms of action on the efficacy of radiation therapy.

The authors conclude by suggesting that antibodies against VEGF, most notably bevacizumab (Avastin), could be used as an alternative anti-edema drug during radiation in place of steroids. However, this use has to be weighed in importance against the exclusion from certain promising clinical trials due to prior use of Avastin being an exclusion criteria in some of these trials.

2. Strategies for improving the Standard of Care

Combatting chemoresistance

There are several ways that cancer cells evade being killed by cytotoxic chemotherapy. Already mentioned is that the damage inflicted by the chemotherapy is quickly repaired before actually killing the cell (due to the activity of the MGMT repair enzyme). A second source of resistance is that the chemo agent is extruded from the cancer before the next cell division (chemotherapy typically affects only those cells in the process of dividing). A third way is that the chemo agent doesn't penetrate the blood-brain barrier. While Temodar is generally believed to cross the blood-brain-barrier effectively, empirical studies of its concentration within the tumor tissue have shown that its penetration is incomplete.

A major source of chemo-resistance for many types of cancer comes from glycoprotein transport systems (technically called ABC transporters) that extrude the chemotherapy agent before it has the chance to kill the cell. This is important because chemotherapy is effective only when cells are dividing, and only a fraction of the cell population is dividing at any given time. The longer the chemotherapy remains in the cell, the more likely it will be there at the time of cell division. If extrusion of the chemotherapy drug could be inhibited, chemotherapy should in principle become more effective. Calcium channel blockers, which include commonly used medications for hypertension such as **verapamil**, have thus been studied for that purpose (11).

Unfortunately, these agents have potent effects on the cardiovascular system, so that dosages sufficiently high to produce clinical benefits usually have not been achievable. However, a recent study (12) did report a substantial clinical benefit for patients with breast cancer with a relatively low dosage (240 mg/day). An earlier randomized trial with advanced lung cancer (13) also demonstrated a significant benefit of verapamil, using a dose of 480 mg/day, both in terms of frequency of tumor regression and survival time. In

addition, the combination of verapamil with tamoxifen (which itself blocks the extrusion by a somewhat different mechanism) may possibly increase the clinical benefit (14). In laboratory studies, the calcium channel blockers nicardipine and nimodipine (15, 16) have also been shown to effectively increase chemotherapy effectiveness, and may have direct effects on tumor growth themselves. Quinine derivatives such as quinidine and chloroquine also inhibit the extrusion pump. Among the strongest inhibitors of the extrusion pump is a common drug used in the treatment of alcoholism, Antabuse, also known as disulfiram (17,18). Yet another class of drugs that keep the chemo inside for longer time periods are proton pump inhibitors used for acid reflux (e.g., Prilosec) (19). One approach to blocking the glycoprotein pump without the high toxic doses is to combine several agents together, using lower doses of each individual agent, as combining different agents has been shown to be synergistic in laboratory studies (20).

The most promising clinical results for combatting chemo-resistance has come from the addition of **chloroquine**, an old anti-malaria drug, to the traditional chemotherapy agent, BCNU. See *Chapter 5, Chloroquine* section for further details.

Disruption of the blood-brain-barrier (BBB) is also potentially very important and has been extensively investigated. The issue is complicated by the fact that tumor tissue already has a substantially disrupted BBB (which is the basis of using contrast agents to identify the tumor). However, this disruption is incomplete, so any chemotherapy agent that does not cross the intact BBB will not contact all portions of the tumor. Various ways of disrupting the BBB have been studied, but none has been generally successful, primarily because of their systemic side effects. Recently, however, the common erectile dysfunction drugs (Viagra, Levitra, Cialis) have been discovered to disrupt the BBB in laboratory animals. In a rat brain tumor model, the addition of Viagra or Levitra to a common chemotherapy agent, Adriamycin, substantially improved survival time (26).

Optimizing the Schedule of Chemotherapy

The standard schedule for using full-dose Temodar is days 1-5 out of every 28-day cycle. The large phase 3 EORTC-NCIC study (2005) also added daily Temodar during radiation at a lower dosage, followed by the standard five-day schedule after radiation was completed. But there has never been a persuasive rationale for why this standard schedule should be preferred over various alternatives.

In addition to the standard schedule, three other schedules have been studied: (1) a "metronomic" low-dose daily schedule; (2) an alternating week schedule; (3) a "dose-intense" schedule in which Temodar is used on days 1-21 of every 28-day cycle. While it is possible to compare the outcomes of these different studies across different clinical trials, only a few studies have compared the different schedules within the same clinical trial.

In one single-center randomized trial with newly diagnosed patients, the alternating week schedule of 150 mg/m2 on days 1-7 and 15-21 was compared with the metronomic schedule of 50 mg/m2 daily (29). Patients completing 6 cycles of adjuvant Temodar were switched to maintenace therapy with 13-cis retinoic acid (aka Accutane). One-year survival rates were 80% vs. 69%, and two-year survival rates 35% vs. 28%, both favoring the alternating week schedule. However, neither difference was statistically significant. Median survival times for the alternating week and metronomic schedules were 17.1 vs. 15.1 months.

A second very large randomized trial compared the standard 5-day schedule with a dose-intense schedule (75-100 mg/m2 on days 1-21). The rationale of the dose-intense schedule was that it would better deplete the MGMT enzyme (30). Median PFS favored the dose-dense arm (6.7 months vs. 5.5 months from the time of study randomization, p=0.06), while median overall survival favored the standard schedule (16.6 vs. 14.9 months from randomization). While neither difference was considered statistically significant, the dose-intense schedule had substantially more toxicity and hence cannot be recommended.

In a more recent retrospective study (313), 40 patients undergoing the standard 5-day temozolomide schedule and 30 patients undergoing a metronomic schedule (75 mg/m2) were included in the final analysis. The metronomic temozolomide schedule led to statistically significant increases in both progression-free survival and overall survival, and in both univariate and multivariate analysis. Even more importantly, this study found that the benefit of the metronomic schedule mainly occurs for those patients with EGFR overexpression (EGFR protein expression in over 30% of tumor cells), or EGFR gene amplification. Median overall survival for patients with EGFR overexpression treated with metronomic temozolomide was 34 months, compared to 12 months with standard schedule. EGFR overexpressing patients treated with metronomic temozolomide had highly statistically significantly improved progression-free survival and overall survival compared to all other groups (the other groups being EGFR overexpressing treated with standard schedule, and EGFR non-overexpressing treated with either schedule).

The investigators furthermore analysed tumor tissue samples from patients who underwent repeat resection at the time of recurrence. Interestingly, they found that samples from EGFR overexpressing tumors treated with metronomic temozolomide had significantly fewer cells positive for NF-kB/p65 (a promoter of cell proliferation and survival) compared with untreated tumors from the same patients at the time of diagnosis. No such change was observed between the primary and recurrent EGFR overexpressing tumors from patients treated with the standard schedule. Recurrent EGFR amplified tumors treated with the metronomic schedule showed fewer EGFR amplified cells and weaker EGFR staining at the time of recurrence compared with the primary tumor. No such difference was observed in EGFR amplified tumors treated with

the standard schedule. The authors draw the conclusion that this metronomic schedule impairs survival of EGFR expressing GBM cells more effectively than the standard schedule. These findings will hopefully lead to testing in prospective clinical trials. A major caveat when reviewing this study is that there is no explanation for why some of the patients were selected for the higher dose metronomic schedule rather than the standard schedule and it's possible that these patients were healthier at baseline and that selection bias contributed to the different outcomes.

The lowest Temodar dose in metronomic chemotherapy reported to date was presented to newly diagnosed glioblastoma patients (44). After completion of standard radiation treatment, continuous daily doses of temozolomide approximately 1/10 of the typically used full dose were used in combination with Vioxx (aka rofecoxib, a discontinued COX-2 inhibitor which was later replaced by celecoxib in studies by this group). Median progression-free survival was 8 months and overall survival for 13 patients was 16 months, with minimal toxicity. A second retrospective study (45) from the same medical group compared the very low-dose schedule (20 mg/m2) with a more typical metronomic dosage (50 mg/meter-squared), although only 17 patients and six patients were included in the former and latter groups. Also included were patients who received only radiation. Median survival was 17 months and 21 months, respectively, for the two metronomic chemotherapy groups vs. 9 months for the radiation-only patients.

Although clinicians will likely resist any alternative to the standard temozolomide schedule for newly diagnosed patients outside of clinical trials, a medium-dose metronomic schedule is worthy of consideration for patients with unmethylated MGMT status, and especially for those patients with unmethylated MGMT status and amplified EGFR. For patients unfit to receive the standard high dose temozolomide schedule, a very low dose metronomic schedule of TMZ may provide some benefit, perhaps through selective toxicity to immune suppressor cells, and in combination with COX-2 inhibition as in the German studies above.

How many cycles of TMZ?

An important question is how long the use of TMZ should be continued. The Stupp clinical trial continued it for only six cycles after radiation, but many patients have continued that protocol for longer period of times.

In what is perhaps the only randomized, prospective trial comparing different numbers of cycles of adjuvant temozolomide, 20 newly diagnosed glioblastoma patients were assigned to six cycles and another 20 patients were assigned to 12 cycles (355). Median progression-free survival outcomes were 12.8 months in the 6-month group and 16.8 months in the 12-month group, which was borderline statistically significant (p=0.069). Median overall survival was 15.4 versus 23.8 months, and achieved statistical significance

despite the low numbers of patients included in the trial (p=0.044). A serious limitation of this study is that no information on MGMT status of the patients was collected, and therefore it's possible that the proportion of patients with MGMT promoter methylated tumors was not equal in the two arms.

A retrospective study done in Canada (51) compared patients who received the standard six cycles of temozolomide with those who had more than six cycles (up to 12) Patients receiving six cycles had a median survival of 16.5 months, while those receiving more than six cycles had a median survival of 24.6 months.

The latest attempts to define the optimal length of monthly temozolomide (TMZ) therapy were published as abstracts for the SNO 2015 annual meeting. In the first of these studies (reference 325, abstract ATCT-08), a large team of investigators retrospectively analyzed data from four large randomized trials with the aim of comparing 6 cycles of monthly TMZ to >6 cycles. Only patients who had completed 6 cycles of TMZ and had not progressed within 28 days of completing cycle 6 were included. Important prognostic factors such as age, performance status, extent of resection and MGMT status were incorporated into the analysis. For these patients, treatment with more than 6 cycles of TMZ was associated with significantly improved progression-free survival [HR=0.77, p=0.03] independently of the examined prognostic factors, and was particularly beneficial for those with methylated MGMT status. Surprisingly, overall survival was not significantly different between the two groups (p=0.99).

In the second abstract (reference 326, abstract ATPS-38), a Japanese group attempted to clarify whether more than 12 cycles of TMZ was beneficial in terms of increased survival. Patients in this study were divided into four groups: a) 12 cycles, b) 24 cycles, c) more than 24 cycles until relapse, and d) beyond 12 cycles (this group includes the b) and c) groups). 12, 14, 12, and 40 patients were included in each of these groups. No significant progression-free survival difference was detected between groups a) and b), implying a lack of benefit of 24 versus 12 cycles. Importantly, patients who were able to continue TMZ treatment for at least 12 cycles (all the patients in this study) had a median progression-free survival of 4.3 years and median overall survival of 6.3 years. This study failed to show a benefit of continuing TMZ cycles beyond 12.

Combining the Standard Treatment with Additional Agents

Few oncologists believe that single-agent treatments are likely to be curative. The issue is finding the optimal combinations, based on toxicities and differences in the mechanisms of actions. Prior to the introduction of temozolomide, the PCV combination of procarbazine, CCNU, and vincristine had been the most widely used combination treatment for glioblastomas, but its use has never been shown to produce a better outcome than treatment with BCNU as a single agent. Nevertheless, there is now a large

amount of research studying the effects of combining temozolomide with other therapies, most of which supports the view that such combinations improve treatment outcome, sometimes substantially. A variety of additional therapies are discussed in the following chapters.

3. Optune (formerly NovoTTF-100A) by Novocure

In the spring of 2011, the FDA approved the fourth treatment ever for glioblastoma. Unlike the previous three (gliadel, temozolomide, and Avastin), the new treatment involves no drugs or surgery, but instead uses a "helmet" of electrodes that generates a low level of alternating electrical current. A biotech company called Novocure has developed the device, called Optune, based on experimental findings that electro-magnetic fields disrupt tumor growth by interfering with the mitosis stage of cell division, causing the cancer cells to die instead of proliferating (138). Healthy brain cells rarely divide and thus are unaffected. The treatment involves wearing a collection of electrodes for 18 or more hours per day, which allows the patient to live otherwise normally. This approval in 2011 was the outcome of a randomized phase 3 trial for recurrent glioblastoma, in which Novo-TTF (now called Optune) treatment was equally effective as physician's choice chemotherapy, but with reduced toxicity and better quality of life (139, 140).

Optune plus chemoradiation, the next standard of care?

EF-14 is a phase 3 randomized clinical trial for newly diagnosed glioblastoma which compared standard of care chemoradiation followed by **Optune (NovoTTF)** and monthly cycles of Temodar, versus chemoradiation followed by monthly cycles of Temodar alone.

In November 2014, at the annual SNO meeting in Miami Beach, Roger Stupp made a "late-breaking" presentation before a packed audience, describing interim survival outcomes from the EF-14 trial, essentially ushering in what may become the new standard of care for newly diagnosed glioblastoma. *This trial is the first major phase 3 trial since the "Stupp protocol" was established in 2005 to report a positive, statistically significant survival benefit for newly diagnosed glioblastoma.* In fact, the trial was so successful that it was terminated early and on December 2, Novocure announced that the FDA had approved an investigational device exemption (IDE) supplement allowing all the control patients in the EF-14 trial to begin receiving therapy with Optune tumor treating fields.

The interim results presented in Miami were based on the first 315 patients enrolled in the trial, who had at least 18 months of follow-up. Of these, 105 were randomized into the control arm and 210 were randomized to receive tumor treating fields. Survival and progression-free survival were measured from the time of randomization, which was a median of 3.8 months after diagnosis. Median progression-free survival was 7.1 months in the Optune arm versus 4 months in the control arm (hazard ratio 0.63, with a high degree of statistical significance, p=0.001). Median overall survival from randomization was 19.6 months in the Optune arm versus 16.6 months in the control arm (hazard ratio 0.75, statistically significant, p=0.034). 2-year survival was 43% in the Optune arm versus 29% in the control arm. It must be kept in mind that all these statistics are measured from randomization, roughly 4 months from diagnosis, meaning that median overall survival in the Optune arm approaches 24 months from diagnosis. The statistics above are for the intention-to-treat (ITT) population, which includes all patients randomized, as opposed to the as-treated (per-protocol) population, which excludes patients who did not start their second course of temozolomide or had major protocol violations.

An October 5, 2015 press release announced that the FDA had approved Optune in combination with temozolomide for newly diagnosed glioblastoma, not quite a year after the first survival data from the trial was publicized. This is the first approval of a therapy for newly diagnosed glioblastoma since temozolomide was approved for this indication in March 2005. Two months later, in December 2015, the preliminary results of the EF-14 trial were published in the Journal of the American Medical Association (327). This publication detailed results for the first 315 patients enrolled, the same patients reported upon at the SNO 2015 annual meeting. Additional survival analysis on the per-protocol population (as opposed to the intention-to-treat population) gave an overall survival from randomization of 20.5 months in the Optune group and 15.6 months for the control group (that is, about 24.3 months and 19.3 months from diagnosis) (HR=0.64, p=0.004).

Outcomes for the entire trial population of 695 patients were published for the 2016 SNO conference, with an updated press release from Novocure coming in April 2017. Confirming the results of the interim analysis, median progression-free survival was significantly improved in the Optune group by just under 3 months (6.7 versus 4 months from randomization). Median overall survival from randomization was improved by nearly five months (20.8 versus 16 months). Survival rate at 2 years from randomization was 42.5% in the Optune group versus 30% in the control group. The April 2017 update also reported on 5 year survival rate, which was 13% in the Optune arm versus 5% in the control arm.

As of July 2016, the National Comprehensive Cancer Network (NCCN) had given Optune a 2A listing for newly diagnosed glioblastoma, indicating uniform consensus amongst the NCCN panel that this treatment is appropriate. As the NCCN is known as setting the standard treatment guidelines for cancer in the USA, this formalizes the concept of Optune as part of a new standard of care for newly diagnosed glioblastoma. Although its

status as part of a new standard of care may still be disputed by some, Novocure has announced that Optune is now available at over 600 treatment centers in the USA (click here for a list of these US centers), as well as 350 additional institutions internationally, including locations in Germany, Switzerland, Austria, and Japan.

4. Other Chemotherapy and Cancer Drugs

CCNU (lomustine)

A report from Germany combined TMZ with CCNU (lomustine), the nitrosourea component of the PCV combination (52). Patients (N=39) received CCNU on day 1 of each 6-week cycle, and TMZ on days 2-6. Eight patients received intensified doses of both drugs, with somewhat better survival results (but with substantially increased toxicity). For present purposes, the results of all patients are aggregated. Median survival time was 23 months, and survival rates were 47%, 26%, 18%, and 16% at 2, 3, 4, and 5 years, respectively. Four of the 39 patients had no recurrence at the 5-year mark. Only 23 of the 39 patients were assessable for the status of the MGMT gene. Those with methylated MGMT had a median survival of 34 months, while those with unmethylated MGMT had a median survival of only 12.5 months.

These results, including a 5-year survival rate of 16%, are among the best yet reported, albeit with a relatively small number of patients. But it also should be appreciated that patients who suffered a recurrence received extensive salvage therapy of various types, which may have contributed substantially to survival time. The addition of CCNU to standard therapy for newly diagnosed glioblastoma is currently being tested in a phase 3 trial in Germany.

BCNU (carmustine) and Gliadel (carmustine wafers)

The combination of Temodar with BCNU, the traditional chemotherapy for glioblastomas, has also been studied, but has been complicated by issues of toxicity and the optimal schedule of dose administration for the two drugs. However, a recent published report involving patients with tumors recurring after radiation but no prior chemotherapy failed to show any benefit of combining BCNU with Temodar, compared to Temodar alone, as the PFS-6 for the combination was only 21%, accompanied by considerable toxicity (53).

An important variation in the use of BCNU has been the development of polymer wafers

known as gliadel. A number of such wafers are implanted throughout the tumor site at the time of surgery. BCNU then gradually diffuses from the wafers into the surrounding brain. A possible problem with the treatment is that the drug will diffuse only a small distance from the implant sites, and thus fail to contact significant portions of the tumor. However, a phase III clinical trial has demonstrated that survival time for recurrent high-grade gliomas is significantly increased by the gliadel wafers relative to control subjects receiving wafers without BCNU, although the increase in survival time, while statistically significant, was relatively modest (54). Probably the best estimate of the benefit of gliadel as an initial treatment comes from a randomized clinical trial, conducted in Europe (55), which reported a median survival of 13.9 months for patients receiving gliadel compared to a median survival of 11.6 months for patients implanted with placebo wafers. As with other forms of chemotherapy, larger differences were evident for long-term survival. After a follow-up period of 56 months, 9 of 120 patients who received gliadel were alive, compared to only 2 of 120 of those receiving the placebo. However, the results were not reported separately for glioblastomas vs. other high-grade gliomas, suggesting that the outcome results would have been more modest for the glioblastoma patients alone.

When gliadel has been combined with the standard TMZ + radiation protocol, survival time seems to be significantly improved, as assessed in three different retrospective clinical studies. In the first, from the Moffitt Cancer Center in Florida (56), the combination produced a median overall survival of 17 months, and a 2-year survival rate of 39%. In a second clinical trial reported by Johns Hopkins, where gliadel was developed (57), 35 patients receiving the combination had a median survival time of 20.7 months and a 2-year survival of 36%. In a third trial conducted at Duke University (58), 36 patients receiving gliadel in addition to the standard TMZ protocol had a median survival of 20.7 months and a 2-year survival of 47%. The Duke cohort also received rotational chemotherapy (which included TMZ) subsequent to radiation. It is important to keep in mind that patients eligible to receive gliadel must have operable tumors, which excludes patients who have received a biopsy only and have a generally poorer prognosis as a result. The effect of this selection bias is difficult to evaluate but it is likely to account for a significant fraction of the improvement in survival time when gliadel +TMZ is compared to TMZ alone.

A major advantage of gliadel is that it avoids the systemic side effects of intravenous BCNU, which can be considerable, not only in terms of low blood counts but also in terms of a significant risk of major pulmonary problems. But gliadel produces its own side effects, including an elevated risk of intracranial infections and seizures. However, the lack of systemic toxicity makes gliadel a candidate for various drug combinations. Especially noteworthy is a recent phase II trial with 50 patients with recurrent tumors that combined gliadel with 06-BG, a drug that depletes the MGMT enzyme involved in repair of chemotherapy-induced damage, but also causes unacceptable bone marrow toxicity when chemotherapy is given systemically. Survival rates at six months, one year

and two years were 82%, 47%, and 10%, respectively (59) which seems notably better than the earlier clinical trial with recurrent tumors using gliadel without the 06-BG, in which the corresponding survival rates were 56%, 20%, and 10%. Median survivals were also notably improved by the addition of 06-BG (50.3 weeks versus 28 weeks).

Platinum compounds

An improvement in results relative to those obtained with Temodar alone has also been reported when Temodar has been combined with cisplatin. In a pair of clinical studies performed in Italy (61, 62) with patients with recurrent tumors, the PFS-6 was 34% and 35%. A treatment protocol with newly diagnosed patients that also seems to have produced better results than Temodar as a single agent combined Temodar with both cisplatin and etoposide (VP-16), given through the carotid artery (63). Cisplatin and etoposide were given after surgery and continued for three cycles spaced every 3 weeks apart, followed by the standard protocol of radiation plus low-dose Temodar, then high-dose Temodar on the schedule of days 1-5 of every month. For 15 patients studied, median survival was 25 months.

Procarbazine

Temodar has also been combined with procarbazine (64). While the report of that study did not include the PFS-6 statistic, it did report an unusually high percentage of tumor regressions, suggesting that this combination might be effective.

Bevacizumab (Avastin)

The most notable development in drug combinations has been the addition of the anti-angiogenic drug, Avastin (also known as bevacizumab), to the standard Stupp protocol. As will be discussed later, Avastin has FDA approval for the treatment of glioblastomas that have recurred or progressed after initial treatment. Several clinical trials have now investigated its combination with the gold standard Temodar protocol.

Recently, there have been two large randomized phase III clinical trials comparing the Stupp protocol and the Stupp protocol + Avastin, for newly diagnosed patients. In the first of these (70), known as the AVAglio trial, median PFS was 10.6 months for those receiving Avastin versus 6.2 months for those receiving only the Stupp protocol, a statistically significant difference. However, median overall survival was not different (16.8 months vs. 16.7 months). It should be noted that patients in the control group

typically received Avastin after tumor progression occurred, so that the comparison was really between Avastin given early versus Avastin given only after recurrence. Additional results were that 72% of the Avastin group was alive at one year, compared to 66% of the control group, while two year survival was 34% vs. 30%.

In the second of these large trials (71), conducted by the RTOG consortium, the design was essentially similar to the AVAglio trial, as were the results. Median PFS was 10 months for those receiving Avastin vs. 7.3 months for the control group (again statistically significant), while median overall survival was 15.7 months for the Avastin group compared to 16.1 months for the control, a nonsignificant difference.

The best interpretation of these results is that patients have a longer time without tumor progression, and presumably a better quality of life, when Avastin is used as part of the initial treatment. However, there is no benefit for overall survival, when compared to withholding Avastin until recurrence is detected. An additional feature of the results, not emphasized by the authors of the reports, is that the overall survival times were not notably better, and in many cases worse, than those obtained when the Stupp protocol is combined with various other treatment agents.

EGFR inhibitors: Iressa, Tarceva, and Erbitux (gefitinib, erlotinib, and cetuximab)

These three drugs, which have FDA approval for several different types of cancer, have the common feature that they target a growth-signaling channel known as the epidermal growth factor. Overexpression or mutation of EGF receptors is involved in the growth many different kinds of cancer, including more than half of glioblastomas. In general, use of these drugs as single agents has produced disappointing results, although occasional long-term survivors have occurred. More promising results have occurred when EGFR inhibitors have been used in combination with the Stupp protocol.

When Tarceva has been added to the standard Temodar protocol for newly diagnosed patients, median survival was 15.3 months (N=97) in one study (72) and 19.3 months (N=65) in a second study (73). The results of the second study were compared to two previous phase II trials involving a similar patient population, in which Temodar was combined with either thalidomide or accutane. Median survival for those trials was 14.1 months.

The moderately positive results of the just described trial are in conflict with a very similar trial (N=27) conducted at the Cleveland Clinic (74). In that trial median survival was only 8.6 months, notably worse than the outcomes obtained when temodar has been used without tarceva. How the conflicting results can be reconciled is unclear.

Erbitux (also known as cetuximab) is a monoclonal antibody, which differs from Iressa and Tarceva, which are small molecules, Because monoclonal antibodies are not believed to cross the blood-brain barrier, the natural expectation is that Erbitux would be ineffective against brain tumors. As a single agent, this seems to be true, as PFS-6 was only 10% for patients with recurrent high-grade gliomas (75). But when Erbitux was added during the radiation phase of the standard temozolomide protocol for 17 newly diagnosed patients (76), 87% of patients were alive at the end of one year and 37% were progression free. The median survival time had not been reached at the time of the report (an abstract at a meeting). It is possibly important to note that some investigators believe that radiation temporarily disrupts the blood-brain-barrier, which would allow a monoclonal antibody such as erbitux to reach the tumor.

An important development for identifying patients likely to respond to Tarceva has come from a study (77) of glioma patients whose tumor pathologies were also assessed for their levels of a second protein called PKB/AKT. This is a signaling channel that results from inactivation of the PTEN gene, a tumor suppressor gene commonly mutated in glioblastomas. None of the tumors with high levels of PKB/AKT responded to treatment with Tarceva, whereas 8 of 18 tumors with low levels did respond to the treatment. A refinement of this approach tested for three different proteins: expression of PTEN, expression of EGFR, and of a mutation of the EGFR protein known as EGFR variant III (78). The level of EGFR was not related to clinical outcome, whereas the co-expression of EGFR variant III and PTEN strongly predicted clinical outcome.

Because the inhibition of PKB/AKT should plausibly increase the effectiveness of EGFR inhibitors, a treatment strategy now being tested is the combination of EGFR inhibitors with rapamycin (trade name rapamune, generic name sirolimus), an existing drug used for organ transplants to suppress the immune system and prevent organ rejection, but which also inhibits mTOR complex 1, a tumor growth promoter downstream of AKT. A phase I trial (79) combined Iressa with rapamycin for 34 patients (25 GBM) with recurrent tumors; two patients had a partial tumor regression and 13 patients achieved stable disease. PFS-6 was 24%. A second clinical trial (80) with 28 heavily pretreated patients with low performance status (median Karnofsky score of 60) received either Iressa or Tarceva in combination with rapamycin, with the result that 19% of patients had tumor regression while 50% had stable disease, with a PFS-6 value of 25%. Yet a third clinical trial (81) that combined tarceva and sirolimus for recurrent GBM had much worse results, with PFS-6 value of only 3%.

The foregoing results of the use of EGFR inhibitors for GBM treatment range from moderately positive to minimal efficacy. The reasons for this variability are not obvious, although treatment efficacy is likely dependent on numerous genetic markers. Thus, without a genetic analysis of individual tumors, it is hard to see a basis for recommending their use.

One recent paper (83) of potential major importance has noted that tumors may not respond to anti-EGFR drugs because of activation of the gene for a second growth factor known as the insulin-like growth factor receptor I (IGF1R). IGF1R has also been implicated as a source of resistance to tamoxifen and various other treatment agents. It is noteworthy, therefore, that two of the supplements to be discussed, silibinin and lycopene, are known to inhibit IGF-I. This suggests that silibinin and lycopene might substantially increase the effectiveness of any treatment that relies on EGFR inhibition. Metformin, a widely used diabetes drug, is also known to reduce the level of IGF-1, currently is under investigation as a treatment for several different kinds of cancer.

An important issue is how the effectiveness of EGFR inhibitors are related to the findings discussed earlier that metronomic schedules of Temodar produce a large survival improvement for GBMs that have EGFR overexpression. All of the clinical trials discussed in this section used the standard Temodar schedule, so it is unclear whether a metronomic schedule might produce different outcomes.

Gleevec (imatinib)

Gleevec (also known as imatinib), a small molecule which targets a specific gene involved in the growth of a form of leukemia, received a great deal of publicity because of its unprecedented effectiveness. As will be discussed later, this general strategy of identifying the growth signals for tumor growth and then targeting those signals, or their receptors, is one of the major new areas in cancer research. Such growth signaling channels often are involved in several different types of cancer. Although Gleevec was developed specifically for chronic myelogenous leukemia, it also has been shown to inhibit a more general type of growth signal, platelet-derived growth factor (PDGF), which is also involved in the growth of gliomas and other forms of cancer (e.g., small-cell lung cancer). Laboratory research has supported the importance of this similarity in that gleevec has been shown to strongly inhibit glioma growth, with the result that there now have been a number of studies reporting its use with high-grade gliomas.

The generally disappointing results using gleevec for brain tumors may have occurred for several different reasons. It may not readily cross the blood-brain-barrier, and it may engender different mechanisms of resistance than other treatment agents. In the study of gleevec for leukemia, for example, high levels of autophagy have been observed, which can be inhibited by the concurrent use of chloroquine or other autophagy inhibitors.

An important variation in the use of gleevec was to restrict its usage to patients with recurrent tumors who tested positive for overexpression of the platelet-derived growth factor receptor (90). PDGFR is overexpressed in 50-65% of tumors, especially tumors

labeled secondary glioblastomas, which are believed to have evolved from lower-grade tumors (in contrast to de novo glioblastomas that occur without such evolution). For this restricted patient population, the PFS-6 value was 53%.

5. Hormones and Cancer Therapy

Unlike traditional cancer chemotherapy, which kills cancer cells through directly cytotoxic mechanisms, a different approach may also prove to be effective: manipulation of the body's balance of circulating hormones to achieve the most unwelcoming environment for the growth of tumors.

Angiotensin-II Receptor Blockers (ARB)

Angiotensin-II is a peptide hormone produced from angiotensin-I by the action of angiotensin converting enzyme (ACE). The main effect of angiotensin-II is vasoconstriction and a resulting increase in blood pressure. Therefore ACE inhibitors and angiotensin-II receptor blockers are used as anti-hypertensive drugs, especially in heart disease. More recently these drugs have been repurposed for use in cancer studies.

A retrospective study published in 2012 (328) examined the steroid-sparing effects of angiotensin-II inhibitors, incuding ACE inhibitors and angiotensin-II receptor blockers (ARBs). Of a total cohort of 87 newly diagnosed glioblastoma patients, 29 patients were identified who were treated prior to radiation for high blood pressure. 18 of these were treated with either ACE inhibitors (n=3) or angiotensin-II receptor blockers (n=15). Although no survival benefit with angiotensin-II inhibitors was observed in this study, the 18 patients treated with an angiotensin-II inhibitor required half the steroid dose compared to all other patients in the study (mean prednisolone dose of 29 mg per day versus 60 mg per day), and this difference remained significant in multivariate analysis (p=0.003).

A later retrospective study by the same group published in January 2016 (329) focused specifically on the angiotensin-II receptor blocker class of drugs and their effects on vasogenic edema in glioblastoma patients. In this study, 11 patients taking angiotensin-II receptor blockers (ARBs) for hypertension were compared with 11 matched patients with similar age, tumor size, and tumor location, but not taking medication for hypertension. There was a significant 66% reduction in the FLAIR ratio in the patients taking ARBs compared to the matched patients not taking ARBs. As FLAIR signal can represent either tumor infiltration or vasogenic edema, the nature of the peri-tumoral FLAIR signal was assessed with apparent diffusion coefficient (ADC) mapping. Nine evaluable patients taking

ARBs had a 34% reduction in ADC ratios compared to their matched controls not taking ARBs, confirming the ability of this class of drugs to reduce peri-tumoral edema.

A 2015 study, also by the group in France, suggests angiotensin-II inhibitors (including ACE inhibitors and ARBs) may also lead to superior survival outcomes (330). In this study, 81 GBM patients were included. Seven of these patients were taking ACE inhibitors and 19 were taking ARBs for hypertension. The 26 patients using angiotensin-II inhibitors had increased progression-free and overall survival (8.7 and 16.7 months) compared to the patients not taking these drugs (7.2 and 12.9 months). Use of angiotensin-II inhibitors was a significant positive prognostic factor for both PFS and OS in multivariate analysis.

A randomized phase 3 trial in France (NCT01805453) has recently completed recruitment, and is testing the influence of losartan (an ARB) versus placebo on the steroid dose required to control edema on the last day of radiotherapy. Another drug in this class, telmisartan, has superior penetration into the central nervous system (331) and may therefore be a better choice.

See Chapter 13 for a discussion of *Angiotensin system inhibitors plus Avastin*

Beta-blockers (especially propranolol) and the role of the sympathetic nervous system

Recenty the role of the sympathetic nervous system in cancer progression, and the potential role of beta-adrenergic antagonists (beta-blockers) have come into focus in some corners of the cancer research community. Early studies linking stress to increased rates of cancer progression led to epidemiological studies showing lower rates of cancer in subjects taking beta-blockers. Beta-blockers such as propranolol have more recently entered controlled clinical cancer trials.

The sympathetic nervous system is a division of the autonomic nervous system, most often associated with "fight or flight" responses. The sympathetic nervous system depends upon catecholamines, mainly epinephrine (adrenaline) and norepinephrine (noradrenaline), which activate two classes of adrenergic receptors in target tissues throughout the body: alpha and beta adrenergic receptors (which are further subdivided into alpha-1, alpha-2, beta-1, beta-2 and beta-3 receptors).

The research and evidence concerning the link between the sympathetic nervous system and cancer progression has narrowed in more specifically on beta-adrenergic receptors and signaling. Animal studies in various cancer models demonstrated that stress contributed to tumor progression, and these effects could be blocked with beta-blockers

(333). Investigated mechanisms are manifold, and include the following downstream effects of beta-adrenergic signaling: stimulation of pro-inflammatory cytokines such as interleukin 6 and 8; increased recruitment of macrophages into tumors and increased macrophage expression of genes such as TGFB, VEGF, IL6, MMP9, and PTGS2 (encoding the COX-2 enzyme), which together promote angiogenesis, invasion, and immunosuppression; inhibition of type 1 and 2 interferons, dampening down cell-mediated anti-cancer immunity, and decreased function of T-lymphocytes and natural killer cells; activation of transcription factors that promote epithelial-mesenchymal transition, leading to tumor metastasis and invasion; and increased production of pro-angiogenic growth factors and cytokines, such as IL-6 and VEGF. A 2015 review summarizes the current evidence for the sympathetic immune system's influence on cancer progression and the tumor microenvironment (334).

Clinical evidence supports the importance of beta blockers in cancer treatment. An epidemiological study in Taiwan (335) reported that the incidence of cancer was greatly reduced (30-50%) in subjects using propranolol for at least six months, including incidence of head and neck cancer and cancers of the esophagus, stomach, colon, and prostate. Incidence of brain cancer was too low in both the propranolol and no-propranolol groups to achieve a statistically significant reduction, although the risk of brain cancer was also lower in the propranolol group. Confirming these findings is a recent clinical study in the USA of ovarian cancer in which patients were divided into those who used no beta blockers, those that used older non-specific beta blockers (such as propranolol), and those that used the newer selective beta blockers specific to beta-1 adrenergic receptors. Ovarian cancer patients not using beta blockers had median survival of 42 months, those using the beta-1 selective agents had a median survival of 38 months, and those using non-selective beta blockers (eg propranolol) had a superior median survival of 95 months (336).

Vicus Therapeutics, headquartered in Morristown New Jersey, is a company developing a combination treatment they call VT-122, which consists of a "chrono-modulated" formulation of propranolol (a beta-blocker first approved by the FDA in 1967) and etodolac (a non-steroidal anti-inflammatory first approved by the FDA in 1991). Both drugs are off-patent and available as generics. Vicus has three clinical trials listed at clinicaltrials.gov: one, starting in 2007, tested VT-122 as a treatment for cachexia in non-small cell lung cancer patients (NCT00527319); another, starting in 2010, is testing VT-122 in combination with sorafenib for hepatocellular carcinoma (NCT01265576); a third, starting in 2013, is testing VT-122 for progressive prostate cancer (NCT01857817).

Not listed on clinicaltrials.gov is a trial presented in abstract form for the 2015 ASCO meeting, comparing low dose daily temozolomide (20 mg twice daily) with or without VT-122 for recurrent glioblastoma. 20 patients were assigned to low-dose temozolomide alone, and another 21 patients were assigned low-dose temozolomide plus VT-122. Patient characteristics are not given in the abstract apart from Karnofsky score, which

was over 60 (median) in both groups. The most remarkable outcome was a median overall survival of 17.6 months in the low-dose TMZ + VT-122 group versus only 9.2 months in the low-dose TMZ alone group. In the VT-122 group there were 5 complete responses (24%) and 12 responses altogether (57%), compared to the corresponding figures of 5% and 35% in the group receiving TMZ alone. One-year survival rate was 67% in the VT-122 group, and 30% with TMZ alone. Rates of thrombocytopenia, neutropenia, and anemia were higher in the VT-122 group. Statistical tests for significance were not reported in the abstract. Although this abstract leaves out vital information (enrollment criteria, patient characteristics, progression-free survival data, statistical significance, etc), a median survival of 17.6 months for recurrent glioblastoma is intriguing, while the 9.2 months median survival in the low-dose TMZ alone group is closer to the average for recurrent glioblastoma trials.

Thyroid Hormone T4 (Thyroxine) Suppression

Based on observations of the relationship between hypothyroid status (depressed thyroid function) and improved outcomes in cancer patients dating at least back to 1988, Aleck Hercbergs and colleagues at the Cleveland Clinic conducted a clinical trial, published in 2003, in which 22 high grade glioma patients were treated with propylthiouracil to induce chemical hypothyroidism, and high dose tamoxifen (349). 15 of the patients had the diagnosis of glioblastoma and the remainder were grade 3 gliomas. Half of the patients (11 of 22) attained hypothyroid status, although no clinical symptoms of hypothyroidism were observed. A survival analysis determined that median survival in the 11 hypothyroid patients was 10.1 months, while median survival in the non-hypothyroid group was only 3.1 months. After adjusting for the younger age of the hypothyroid patients, survival was still longer in the hypothyroid group, with borderline statistical significance (p=0.08).

Later, in 2005, the discovery of cell surface receptors for thyroid hormones on $\alpha v\beta 3$ (alphaVbeta3) integrins, provided a mechanism for their cancer-promoting effects (350). This particular integrin tends to be overexpressed on cancer cells, and stimulation of this integrin by thyroid hormones leads to increased angiogenesis, tumor cell proliferation, and resistance to apoptosis (351).

Following publication of the 2003 trial, many cancer physicians and cancer patients reached out to Hercbergs, resulting in a cohort of 23 advanced cancer patients treated informally with thyroid suppression therapy in addition to standard treatments (351). Patients who were taking synthetic T4 for pre-existing hypothyroidism were abruptly switched to synthetic T3 (Cytomel) and in three of these patients there was a rapid and durable tumor remission observed in conjuction with standard treatments. In the remaining patients, methimazole was used to depress T4 levels to below the reference range, and patients again received synthetic T3 hormone (Cytomel). The rationale for this

is that even though T3 is the active form of thyroid hormone, the affinity for T4 at the thyroid hormone receptor on the integrin is greater than for T3, and T4 is a stronger inducer of cancer cell proliferation. The suppression of T4 and supplementation with T3 (Cytomel) is therefore thought to reduce the major cancer-promoting effect of thyroid hormones while avoiding the clinical symptoms of hypothyroidism, such as fatigue.

Four patients with glioblastoma were included in this study, including a 67 year old male with a KPS of 70 and a partial resection who survived 36 months (3 years), and a 64 year old male with a KPS of 60 who had undergone a biopsy only and lived for 48 months (4 years). Both of these patients had an expected survival of 10 months. A third female glioblastoma patient, aged 68, had a low KPS of 40 and survived for 8 months.

Several patients were excluded from the study who had either failed to achieve free T4 depletion, or who voluntary discontinued treatment (perhaps due to a perception of lack of benefit or an actual lack of benefit). Therefore the 100% response rate observed in this study is perhaps an exaggeration, although the long survival of two out of four advanced GBM patients certainly suggests an effect of the treatment, as standard treatments alone rarely lead to such positive outcomes. Additionally, Hercbergs et al. published a case report of a 64 year old patient with optic pathway glioma, progressive after standard treatments, who responded to T4 depletion with propylthiouracil followed by carboplatin chemotherapy with a remission period of 2.5 years and overall survival of 4.5 years (352).

A phase 2 clinical trial testing T4 suppression with methimazole and Cytomel (synthetic T3) in addition to standard treatment for newly diagnosed glioblastoma has started recruiting in Tel-Aviv, Israel early in 2016 (NCT02654041).

Melatonin

This is a naturally occurring hormone secreted by the pineal gland that regulates the body's diurnal rhythm. It is commonly used for the treatment of jet lag and for insomnia. It is readily available in any health food store and most drug stores. Its role in cancer treatment has been based on the assumption that it boosts the immune system, with the current hypothesis being that it augments the activity of T-helper cells. It recently also has been shown to inhibit angiogenesis (225). It may also have direct cytotoxic effects on some types of cancer cells, notably melanoma cells. It has no known toxic side effects.

Clinical research on the use of melatonin for cancer treatment has been done primarily in Italy, where it has been used either as a single agent after radiation treatments, or in combination with various chemotherapy or immunotherapy regimens, most frequently interleukin-2. Part of the rationale for such combinations is that it decreases the side effects of the chemotherapy, especially with respect to blood counts. One of the clinical

studies (226) randomly assigned 30 GBM patients either to radiation alone (n=16) or to radiation concomitant with 20 mg/day of melatonin (n=14). Melatonin was continued after completion of the radiation. Survival was significantly greater for subjects receiving the melatonin. In terms of one-year survival rates, 6/14 patients receiving melatonin were alive, while only 1/16 patients without melatonin was alive.

This GBM study involved a relatively small number of patients, so that the effects should be considered tentative until a larger study is conducted. However, comparable effects have been reported in a similar design for the use of melatonin with advanced lung cancer (227). Like the GBM study, a substantial increase in survival rate occurred for the patients receiving melatonin.

To date there have been at least a dozen phase-2 clinical trials using melatonin either alone or in combination with other agents and five phase-3 trials involving random assignment of subjects to melatonin versus some type of control group. The majority of these has been relatively small and has involved patients in the terminal stages of their disease, which is perhaps why American oncologists have largely ignored them. However, some trials have been much larger and seem to leave little doubt that melatonin significantly increases the efficacy of chemotherapy. One of the most extensive randomized clinical trials involved 250 patients with advanced metastatic cancer of various types (228). Patients were randomly assigned to chemotherapy alone (using different chemotherapies for different types of cancer) or chemotherapy plus 20 mg of melatonin per day. Objective tumor regression occurred in 42 (including 6 complete regressions) of 124 patients receiving melatonin but in only 19/126 (with zero complete regressions) of the control patients. A comparable difference occurred for survival rate: 63/124 of those receiving melatonin were alive after one year while only 29/126 were alive of those receiving chemotherapy alone. A different trial, involving 100 patients with metastatic non small-cell lung cancer (229), compared chemotherapy alone with chemotherapy in combination with melatonin. With chemotherapy alone, 9 of 51 patients had a partial tumor regression, while 17 of 49 chemo + melatonin patients had either a complete (n=2) or partial (n=15) regression. Twenty percent of the chemo-alone patients survived for one year and zero for two years, while the corresponding numbers for chemo + melatonin were 40% and 30%. Melatonin not only increased the efficacy of chemotherapy, but also significantly reduced its toxicity.

The most extensive report included 370 patients, subdivided into three different types of cancer: lung cancer (non-small cell), colorectal cancer, and gastric cancer (230). Aggregated over all three types, the response rate (percentage of patients with tumor regression) was 36% for those treated with chemotherapy and melatonin, versus 20% for those treated with chemotherapy alone. The corresponding two-year survival rates were 25% vs. 13%. Melatonin's benefits occurred for all three cancer types that were included. Moreover, patients receiving melatonin had fewer side effects.

These trials leave little doubt that the effects of melatonin are of clinical significance. Moreover, a recent study has shown that using multiple components of the pineal gland secretions instead of melatonin alone enhances clinical effectiveness still further (231). One caveat about the use of melatonin is that a recent randomized trial compared radiation treatment for metastatic brain cancer with and without melatonin and found no benefit of the melatonin (232). Given that almost all of the supporting evidence for the use of melatonin has come from its addition to chemotherapy, it is possible that it offers no benefit when added to radiation, perhaps because of its strong antioxidant properties.

Vitamin D

Numerous laboratory studies have shown that Vitamin D is highly cytotoxic to cancer cells, due to several different mechanisms (although labeled as a vitamin it more properly should be considered a hormone). While most research has focused on its ability to activate genes that cause cancer cells to differentiate into mature cells, other effects have also been identified, including cell cycle regulation, inhibition of the insulin-like growth factor, and the inhibition of angiogenesis (246). However, the calcitriol form of Vitamin D is not readily usable for cancer treatments because the dosages producing anti-cancer effects also cause hypercalcemia, which can be life threatening (the major function of Vitamin D is to regulate calcium absorption and resorption from the bones and teeth). But like many vitamins/hormones, the generic designation refers not to a specific chemical structure but to a family of related molecules that may have different properties of various sorts. For Vitamin D several of these variants (commonly referred to as analogues) have been shown to effectively inhibit cancer cell growth but without the same degree of toxic hypercalcemia. In a 2002 paper in the Journal of Neuro-oncology (247), 10 patients with glioblastoma and one with a grade III AA tumor received a form of Vitamin D called alfacalcidol in a dosage of .04 micrograms/kg each day, a dosage that produced no significant hypercalcemia. The median survival was 21 months, and three of the eleven were long-term survivors (greater than 5 years). Although the percentage of patients who responded to the treatment was not high, the fact that any relatively non-toxic treatment can produce any number of long-term survivors is remarkable. There is also strong reason to believe that Vitamin D is synergistic with retinoids such as accutane (248). Its effectiveness is also increased in the presence of dexamethasone (249) and a variety of anti-oxidants, notably carnosic acid, but also lycopene, curcumin, silibinin, and selenium (250).

Alfacalcidol is not available in the USA, but is available in Europe and Canada. For those in the USA it is possible obtain it from various online marketers. It also should be noted that several other Vitamin D analogues are available, which also have much reduced hypercalcemic effects. One of these, paricalcitol, was developed for treatment of a disorder of the parathyroid gland, and recently has been the subject of several experimental studies (251, 252, 253) that have shown it to be highly cytotoxic to

many different types of cancer. Given that other forms of Vitamin D have been shown to be highly cytotoxic to for glioblastoma cells, and that glioma cells are known to have receptors for Vitamin D, it seems likely that paricalcitol should have efficacy for glioblastoma as well. Unfortunately, its routine use is complicated by the fact it is available only in a form that requires intravenous injection.

The most common version of Vitamin D3 found in health food stores is cholecalciferol, which is the precursor of calcitriol, the form of Vitamin D utilized by the body. A recent study of cholecalciferol with prostate cancer patients who had progressed after standard therapy (254) suggests that this common form of Vitamin D3 may be clinically beneficial. Fifteen patients who had failed standard treatments were given 2000 I.U. daily. PSA levels were reduced or stayed the same for nine patients, and there was a reliable decrease in the rate of PSA increase for the remainder. No side effects of the treatment were reported by any of the patients.

Because serum Vitamin D levels have recently been shown to be inversely related to cancer incidence, there recently has been considerable discussion about the dosage that is toxic. Doses as high as 5000-10,000 I.U. per day appear to be safe. Recently, it has become common for women suffering from osteoporosis with low Vitamin D levels to be given as much as 50,000, I.U./day for short time periods. Nevertheless, it is important to note that all forms of Vitamin D can occasionally produce dangerous serum calcium levels, in part because there is a great deal of variability in their effects across individuals. It is thus important that blood calcium levels be monitored, especially while a nontoxic dosage is being established.

6. Repurposed Drugs

There are a large number of drugs that were developed initially for various different purposes that subsequent laboratory research demonstrated to have significant anti-cancer properties. Given these old drugs have been used for years, have well-defined toxicity profiles, and are generally cheaper due to being off-patent, they offer the possibility of augmenting the benefits of the current standard treatment without significant additional toxicity. However, because their FDA approval is for different purposes, many if not most neuro-oncologists have been reluctant to take advantage of their possible benefits as components of a treatment cocktail. Some of these drugs have been investigated as single agents for brain cancer treatment and some have also been combined with the now standard Stupp protocol.

Accutane (isotretinoin, 13-cis retinoic acid)

When Temodar has been combined with accutane, a retinoid used for acne treatment (also known as 13-cis-retinoic acid, or isotretinoin), the PFS-6 (for recurrent tumors improved from the 21% historical value of Temodar alone, to 32% (96).

In contrast to the improvement in clinical outcome when accutane was combined with Temodar for recurrent tumors, a clinical trial with newly diagnosed patients that combined Temodar with accutane produced less impressive results (97). Fifty-five evaluable patients used both accutane and low-dosage Temodar during radiation, followed by full-dose Temodar + accutane, and produced a median survival time of only 57 weeks and a two-year survival of 20%, both below the survival rates from the large clinical trial with the same protocol that used Temodar without accutane. A second, retrospective clinical trial in Canada (98) that combined accutane with Temodar with newly diagnosed patients produced a median survival of 15.1 months and a two-year survival of 26.7%, both comparable to when Temodar has been used alone.

Although accutane appears not to improve outcome when added to the standard Temodar protocol, it does seem to have activity as a single agent. A phase II clinical trial evaluating accutane for recurrent gliomas was conducted at the M. D. Anderson Brain Tumor Center (99). The median survival time was 58 weeks for glioblastoma patients and 34 weeks for grade III gliomas. Aggregated over both tumor types (43 evaluable patients) 3 achieved a partial tumor regression, 7 had minor regressions, and 13 had tumor stabilization. A more complete report, using accutane with 86 glioblastoma patients with recurrent tumors was less impressive (100). Median survival time from the onset of treatment was 25 weeks and PFS-6 was 19%. Accutane now is used at M. D. Anderson as a "maintenance therapy" for patients after initial treatment with radiation or traditional chemotherapy. It also has been used in Germany for patients who have had a complete response to other treatment modalities as a maintenance therapy (101). The major side effects have been dry skin, cracked lips, and headaches, although occasional liver toxicity has also occurred. Increases in blood lipid levels frequently occur, often requiring anti-cholesterol medication such as Lipitor. Accutane also may produce severe birth defects if taken during pregnancy.

Although various data now suggest that accutane should not be combined with chemotherapy (for example, see the discussion below in this chapter entitled *A trial of 3 repurposed drugs plus Temodar*), a series of studies with various types of cancer, including pancreatic, ovarian, colorectal, and melanoma (although not yet with brain tumors), suggest it can be very effective for patients who get a good response from their initial treatment protocol. This is especially relevant to GBM patients who have clean MRIs either after surgery or after treatment with radiation and chemotherapy. An example of the protocol with ovarian cancer involved 65 patients who received the

standard treatment of a taxane and a platinum drug (316). After one year of the standard treatment those receiving a benefit were moved to a maintenance treatment using subcutaneous low-dose IL-2 plus oral 13 cRA at a dose of 0.5 mg/kg. This plan was continued for one year after which frequency of dosing was gradually reduced. Patients receiving this treatment plan had a median PFS of 23 months and a median survival of 53 months. Concomitantly, various measures of immune function (lymphocyte count, NK cell count) were substantially improved and there was a substantial reduction in the level of VEGF, reflecting a reduction in angiogenesis.

Celebrex (and other NSAIDs)

Carcinogenesis of several types involves an inflammatory process. When anti-inflammatory drugs such as aspirin or ibuprofen are taken on a regular basis the incidence of colon cancer is reduced as much as 50%. This substantial effectiveness has motivated investigation of the mechanisms of these benefits. One component of the inflammatory process is angiogenesis, which is now believed to be a critical component of cancer growth. COX-2 enzymes play an important role in inflammation, so that COX-2 inhibitors should reduce angiogenesis and inhibit tumor growth. Many nonsteroidal anti-inflammatory drugs (NSAIDs) are known to be COX-2 inhibitors, but most (e.g., ibuprofen) also inhibit COX-1 enzymes, which are necessary for healthy maintenance of the stomach lining, which is why many users of NSAIDs eventually develop intolerance to them. Thus, much recent attention has been given to the new COX-2 inhibitors such as Celebrex that were developed to avoid COX-1 inhibition for the purposes of arthritis treatment. Because inhibition of angiogenesis is one of the major new approaches to the treatment of cancer, some oncologists have begun adding Celebrex to their regular treatment protocols, based on laboratory findings that COX-2 inhibitors inhibit tumor growth. In recent meetings of American Society for Clinical Oncology (ASCO), there have been various clinical trials reported that combined one or another COX-2 inhibitor with conventional radiation, chemotherapy, and new targeted treatments. The great majority of these were phase 2 clinical trials which had only historical controls with the conventional treatment alone to assess the value of the added COX-2 inhibitors, but most concluded there appeared to be a significant benefit, Some larger randomized clinical trials (115, 116) have shown substantial outcome improvements when celebrex has been added to standard chemotherapy protocols, but others have failed to find a benefit.

Two clinical trials have been reported that have used celebrex in the treatment of gliomas In a clinical trial conducted jointly by several hospitals in New York, Temodar was combined with celebrex (117). For the 46 patients in the study (37 with GBM), the PFS-6 was 35%. However, an unusual schedule of Temodar was also used, so whether the results were due to the new schedule or the celebrex is uncertain. Celebrex has also been combined with CPT-11 (118), a chemotherapy agent used widely for colon cancer, with

patients with recurrent tumors, and produced a PFS-6 value of 25%.

Because of the mild toxicity of NSAIDS, considerable recent research has investigated the mechanisms of their clinical benefit. Whereas initial research focused on the anti-angiogenic properties of this class of drugs, several other mechanisms have been identified, including the enhancement of various aspects of the immune system, and inhibition of the genes that prevent damaged cells from undergoing apoptosis (119). It is critical to note that many of the mechanisms by which NSAIDS work are strongly involved in the growth of high-grade gliomas, and that the expression of the cyclooxygenase enzyme that is the target of COX-2 inhibitors correlates strongly with the proliferation rate of glioblastoma tumors and correlates inversely with survival time (120, 121).

Chloroquine and Hydroxychloroquine

 In a series of studies conducted in Mexico City (23, 24, 25) patients received the traditional chemotherapy agent BCNU, with or without a 150 mg daily dose of chloroquine (the equivalent of 250 mg chloroquine phosphate). The results were that patients receiving chloroquine had a median survival time of 25-33 months, while those receiving BCNU alone had a median survival time of 11 months. Chloroquine at the dose used had no detectable toxicity. Because the cytotoxic mechanism of BCNU is similar to that of Temodar, it seems likely that chloroquine should increase the efficacy of Temodar, although this has yet to be demonstrated. One of several mechanisms by which chloroquine makes chemotherapy more effective is that it inhibits autophagy, an intracellular process that involves the cell digesting some of its internal parts to allow repair of the damage caused by the chemotherapy.

Disappointingly, a multi-center phase I/II trial testing the addition of7hydroxychloroquine (which differs from chloroquine only by a single hydroxyl group) to standard radiochemotherapy for newly diagnosed glioblastoma failed to show any improvement in survival over historical averages. In the phase I safety and toxicity study, all 3 subjects given 800 mg/d hydroxychloroquine along with chemoradiation experienced grade 3 or 4 neutropenia or thrombocytopenia, and 600 mg/d was determined to be the maximum tolerated dose. 76 patients were then treated at this dose in the phase 2 cohort. Autophagy inhibition (the proposed mechanism of action) was not consistently achieved at that dose, and patient survival (median OS 15.6 months, 2-year survival of 25%) was not improved relative to historical control groups. The study concluded that hydroxychloroquine was ineffective in this context at the maximum tolerated dose (304).

Recent preclinical work (305) has shown increased reliance on autophagy and sensitivity to chloroquine treatment in EGFR-overexpressing glioma cells, and any future trials with chloroquine for high-grade gliomas may benefit from a subgroup analysis based on EGFR status.

Hydroxychloroquine plus Rapamycin (Sirolimus)

Both rapamycin (sirolimus), an inhibitor of mTOR complex 1, as well as hydroxychloroquine and chloroquine have been tested in early stage clinical trials for glioblastoma. In 2016 a group based in Taiwan published a case series of three newly diagnosed glioblastoma patients who were treated with a combination of rapamycin and hyroxychloroquine in addition to standard radiation and temozolomide chemotherapy (356). Maintenance rapamycin and hydroxychloroquine treatment was also given after the completion of adjuvant temozolomide cycles. The three patients were aged 62, 69, and 71, making it likely that all three were wild-type (non-mutant) for IDH1.

Patient 1 (age 71) was treated with the rapamycin plus hydroxychloroquine combination for an additional 18 months after the completion of chemotherapy cycles. She had remained free of recurrence for over 3 years at the time of publication. Similarly, the second patient (age 62) continued rapamycin plus hydroxychloroquine for one year beyond the completion of temozolomide chemotherapy. She remained free of recurrence at the time of publication, 30 months from initial diagnosis. In the third case (age 69), grade 2 fatigue necessitated dose reductions in rapamycin and hydroxychloroquine after 2 weeks, and he remained on half doses for the duration of treatment. In this case treatment continued for only one month beyond completion of chemotherapy. Recurrent disease appeared 18 months from initial surgery and overall survival was 28 months.

As two of the three patients were progression-free at the time of publication, median PFS and survival for these patients was not yet reached, but was at least 30 months (median follow up). The shortest survival was in a patient who required early dose reductions and early discontinuation of therapy. In contrast to these three patients, the entire cohort of 20 patients treated at this institution during the same time period, mainly with radiation and temozolomide alone post-resection, had a median survival of only 13.7 months.

Unfortunately there was no information given on EGFR or PTEN status or other genetic alterations for these three patients, which might haven given clues as to biomarkers of response to the rapamycin plus hydroxychloroquine treatment.

Cimetidine (Tagamet)

A strong candidate for a nontoxic addition to standard therapy is the old stomach acid drug, cimetidine (trade name Tagamet). While no clinical studies have yet been reported using it with brain cancer, very impressive results have been reported from its use with colon cancer (132), the rationale being that it decreases cell migration (and hence the spread of the tumor beyond the original site) by affecting the critical genes controlling cellular adhesion. Support for its use comes from a recent experimental study using mice with implanted glioblastoma tumors that received either temozolomide or temozolomide + cimetidine (133). Survival was substantially longer in the latter group. One important caveat about cimetidine is that it has the potential to interact with numerous other drugs in terms of their metabolism in the liver, thus affecting their effective concentration.

Clomipramine (chlorimipramine)

This old FDA-approved drug was first used for the treatment of depression, and now also for treatment of obsessive-compulsive neuroses. Its rationale as a treatment for gliomas is that it selectively depresses mitochondrial function in glioma cells while leaving normal cells unaffected, causing the glioma cells to undergo apoptosis (programmed cell death). Reported at the 2005 ASCO meeting (122) was a clinical trial evaluating the outcome of its use with 27 patients with high-grade gliomas (the distribution of GBMs vs. grade 3 tumors was not reported in the abstract, nor was the clinical history of the patients). Chlorimipramine was added to their conventional treatment with doses from 25 mg daily escalated to 150 mg daily. Median survival was 27 months; 20 of the 27 patients showed partial tumor regressions. This appears to be a promising new treatment, although additional testing with more detailed reporting of the results is clearly needed. An interesting sidelight on chlorimipramine is that laboratory research has shown that it strongly potentiates the toxicity of gleevec for glioma cells (123).

Dichloroacetate (DCA)

This simple chemical compound has been used for the treatment of childhood lactic acidosis, a disorder of the mitochondria that control a cell's energy production. Its use as a cancer treatment is based on the Warburg Effect, the finding that cancer cells are much more likely to utilize anaerobic metabolism, a very inefficient process, even in the presence of sufficient oxygen. DCA affects the membrane of the mitochondria, thus inhibiting the anaerobic metabolism, which results in changes in the cell's microenvironment that can cause the cancer cells to die.

Because DCA is a simple chemical, it can be easily manufactured, which caused early experimental reports of its effectiveness against cancer to motivate many cancer patients

to take it on their own. Only recently has there been a report from a clinical trial that seems to corroborate the earlier laboratory results (124). A group in Alberta, Canada reported the results for five GBM patients, three with recurrent tumors even after multiple forms of therapy, and two who were newly diagnosed, who received DCA in combination with the standard temozolomide protocol. One of the three recurrent tumor patients died after three months, due to massive edema from his very large tumor present prior to DCA treatment. All of the others were alive as of the follow-up period of 18 months from the start of therapy. Patients were treated with an oral starting dose of 12.5 mg/kg twice per day, escalated to 25 mg/kg twice per day. The only apparent significant toxicity was peripheral neuropathy, which was reversible. Doses of 6.25 mg/kg twice per day produced no neuropathy. The authors noted that the serum concentration required 2-3 months to reach therapeutic concentrations. A notable recent laboratory finding using implanted GBM cells in a mouse xenograft model showed a dramatic synergy between DCA and Avastin with a coherent rationale for why such synergy should occur (125).

Disulfiram (Antabuse)

This old drug has been used for decades for the purpose of preventing alcohol consumption. A great deal of research in Germany has shown it also has several anti-cancer properties. With regard to GBM treatment, one of its mechanisms is to block the glycoprotein pumps that extrude the chemotherapy agents from the cell body before they have had a chance to be effective. It also inhibits the MGMT enzyme that allows the cell to repair treatment damage before the cell undergoes apoptosis (programmed cell death), and metalloproteinase activity, which is a primary mechanism by which GBM cells invade adjacent tissue. Perhaps most important it also inhibits the growth of stem cells, which are now believed to be the major source of treatment failures. When alcohol is not consumed, it has minimal toxicity. There is also evidence that its anti-cancer effects are potentiated by the concurrent use of copper gluconate, a common nutritional supplement.

Disulfiram is currently being tested in a phase I pharmacodynamics trial at Washington University, St. Louis, Missouri. This trial consists of two arms: in one arm patients are given one of two disulfiram doses (500 mg or 1000 mg) daily beginning in conjunction with monthly cycles of temozolomide; in the second arm 6 mg of copper gluconate is given in combination with disulfiram plus temozolomide. Results for the first arm (disulfiram and temozolomide without copper) were published in the Journal of Neuro-Oncology in early 2016 (332). Twelve patients were evaluated: seven on a dose of 500 mg disulfiram per day, and five patients on 1000 mg per day. Two of seven patients in the 500 mg cohort discontinued disulfiram after 55 and 80 days due to delirium and peripheral motor neuropathy. Two of five patients in the 1000 mg per day cohort suffered from grade 3 delirium after 15 days on disulfiram, and the maximum tolerated dose of

disulfiram in combination with adjuvant temozolomide was determined as 500 mg per day. The pharmacodynamic endpoint of the trial was proteasome inhibition and minor decreases in proteasome activity were detected in the whole blood of patients at week 4 (average 5% inhibition for 500 mg dose and average 11% inhibition for 1000 mg dose). At the time of the analysis, 9 of the 12 patients had experienced disease progression. Results for the arm of the trial receiving copper gluconate in addition to disulfiram have not yet been reported.

Keppra (levetiracetam)

Keppra (levetiracetam) was approved by the FDA in 1999 as an anti-seizure medication, and the drug has since become perhaps the most commonly prescribed agent for seizure prevention in brain tumor patients. Laboratory studies have shown that Keppra can inhibit the activity of the DNA-repair enzyme MGMT and sensitize glioblastoma cells to temozolomide chemotherapy (206). Furthermore, retrospective studies in newly diagnosed glioblastoma patients show that the use of Keppra during chemotherapy can lead to significantly increased progression-free and overall survival. In one such study by Korean investigators (323), 58 glioblastoma patients who received Keppra for at least three months during temozolomide chemotherapy were compared with 45 patients who received standard treatments without extended Keppra use. Patients receiving Keppra during chemotherapy had a median progression-free survival of 9.4 months versus 6.7 months in the group not taking Keppra, a highly significant difference (HR=0.42, p=0.004 in multivariate analysis). Likewise, overall survival was also extended in the patients receiving Keppra: median OS was 25.7 months versus 16.7 months in the patients not taking Keppra (HR=0.31, p=<0.001). Whether the apparent survival advantage for patients taking Keppra during standard chemotherapy is restricted to patients with unmethylated MGMT status remains to be determined.

Methadone

A paper published in 2014 by Claudia Friesen et al. of the University of Ulm, Germany showed a chemosensitizing effect of methadone on glioma cells in vitro and in a subcutaneous glioma mouse model (365). This paper built off earlier preclinical work on the use of methadone plus doxorubicin in acute lymphoblastic leukemia models. In these papers, methadone stimulation of mu-opioid receptors expressed on the cancer cells led to the blocking of adenyl cyclase and consequent downregulation of cyclic adenosine monophosphate (cAMP). As cAMP has protective effects against apoptosis, the overall outcome was a decreased expression of anti-apoptotic proteins, and greatly increased apoptosis when methadone was applied in combination with doxorubicin, a chemotherapeutic agent. In the previous publication (366), the combination of methadone with doxorubicin led to significantly reduced ALL tumor volume in the mice.

In the glioma study, methadone alone led to a slightly reduced tumor growth rate in the mice, but inexplicably methadone was not combined with chemotherapy in this mouse experiment.

Methadone is an opioid drug indicated for severe pain management and as a replacement therapy for addiction to heroin or other morphine-like drugs. Because of the potential for addiction and abuse, methadone is a Schedule II controlled substance in the USA. In most countries, methadone is most commonly used in the form of a racemic mix, meaning that it contains the (R)- and (S)- enantiomers of the molecule in equal proportion. These two forms of methadone are also known as levomethadone and dextromethadone, and are mirror images of each other. Racemic methadone is therefore called (R,S)-methadone or D,L-methadone (for dextro- and levomethadone). The use of (R)-methadone for heroin addiction has become common in Germany, though not approved in the United States. Although only the (R)-enantiomer has opioid receptor agonist activity, the racemic form containing both enantiomers in equal proportion is preferred by the authors of the cancer studies as "This effect is most pronounced with D,L-methadone since D-methadone stabilizes the opioid receptors and thus facilitates more binding of L-methadone." Furthermore, it has also been shown that the (S)-enantiomer, dextromethadone, has inhibitory activity against NMDA glutamate receptors, which are implicated in seizure activity.

In 2017 a retrospective safety and tolerability study was published which gave details on 27 newly diagnosed and recurrent glioma patients treated with D,L methadone (367). Of these, 12 patients with newly diagnosed glioblastoma were given methadone alongside the standard of care Stupp regimen. The dose was started at 5 drops twice daily (a total of 5 mg per day), and increased by steps to a final dose of 15-35 mg per day (ie. a maximum of 35 drops twice daily).

During the dose escalation period, nearly half the patients experienced side-effects, the most common being nausea. Side-effects resolved in the majority of cases after one month of methadone therapy. In three cases obstipation (constipation), a well-known side-effect of opioid therapy, was a problem beyond week 4.

The 12 patients with newly diagnosed GBM given methadone in combination with standard treatments were considered in an efficacy analysis. Progression-free survival at 6 months for this group was 91%. These outcomes appear to be an improvement versus historical controls, although longer follow-up will be required to really determine improved efficacy with methadone.

In addition to this safety and tolerability study with very preliminary efficacy outcomes are the reports of some impressive tumor responses in refractory cancer patients with combinations of chemotherapy plus methadone, as mentioned in articles on the topic (here and here).

Proton Pump Inhibitors

Cancer cells of all varieties thrive in an acidic environment. They also produce large amounts of lactic acid due to their reliance on anaerobic metabolism. Proton pumps are critically involved in extruding the intracellular acid to the extracellular microenvironment. Proton pump inhibitors, which were developed for heartburn due to excess stomach acid, can disrupt this extrusion, and hence suppress tumor growth. A variety of recent evidence indicates that pretreatment of cancer cells with PPIs causes the cells to become much more sensitive to cytotoxic drugs (19), and also to DCA (126). Importantly, the effect occurs only when the PPI is begun prior to treatment, because it takes 1-3 days to fully suppress the proton pump. Evidence for the clinical benefit of PPIs (in vivo) comes from a study of pet dogs and cats with various kinds of cancer. Thirty-four cats and dogs given lansoprazole (Prevacid) prior to their normal chemotherapy were compared to 17 dogs and cats receiving only the chemotherapy (127). Twenty-three of the patients receiving the PPI had a complete or partial response, and the remainder had stable disease and improved quality of life. Of patients that received only the chemotherapy, only 3 (17%) had a partial response (of short duration) and the remainder died of progressive disease within two months.

The clinical efficacy of proton pump inhibitors for human patients is supported by a Chinese study of metastatic breast cancer (128) that compared conventional chemotherapy alone with chemotherapy in combination with 100 mg of nexium twice per day, or in combination with 80 mg of nexium twice per day. The median PFS values were 7.5 months for those receiving only chemotherapy, 9.5 months for those with the 100 mg dose and 10.9 months for the 80 mg dose. The greater PFS value with the lower nexium dose suggests that even lower doses might also be efficacious.

Tamoxifen

This drug is well known for its usage in the treatment of breast cancer. Its mode of action is to compete with estrogen for attachment to the estrogen receptors of breast cells, thus reducing estrogen's ability to serve as a growth factor for carcinogenesis. This mode of action has little to do with tamoxifen's ability to serve as a therapeutic agent for gliomas. Effects on glioma are instead due to tamoxifen being an inhibitor of protein kinase C activity - an intracellular enzyme that is involved in glioma cell proliferation. Protein kinase C is now also known to play a significant role in stimulating angiogenesis. To obtain inhibition of PKC activity, and thus slow or stop the growth of the cancer cells, very high doses of tamoxifen are used, in contrast to its usage for breast cancer. The

typical dosage for breast cancer is 10-20 mg daily, while for gliomas the dosage used has ranged from 160-240 mg per day. This high dosage is potentially problematic and does indeed have side effects. The most important is an increased risk of blood clots. For women, there is also an increase in the risk for uterine cancer, and for men, impotence and loss of libido are frequent problems. Weight gain is another significant side effect. Overall, however, such side effects are mild in comparison to traditional chemotherapy.

A phase II clinical trial (102) evaluating the effects of tamoxifen for patients with recurrent gliomas produced tumor regression in 25% of patients and stabilization of tumor growth for an additional 20% of patients. The percentage of patients with responses to treatment was greater with Grade III Astrocytomas than for patients with GBMs. The median survival time from the initiation of tamoxifen treatment was 16 months for Grade III tumors and 7.2 months for glioblastomas. This perhaps seems to be a minimal benefit (survival time for recurrent glioblastomas typically ranges from 3-7 months when second-line chemotherapy is used) but it should also be noted that a percentage of those who had either regression or stabilization had survival times greater than two years. Thus, for those "responders" tamoxifen produced a major benefit.

Tamoxifen has been studied as a single agent, in combination with radiation, in a clinical trial with 77 newly diagnosed GBM at a dose of 80 mg/m-sq. (103). Median survival was 11.3 months, not notably better than studies with radiation alone. Here long–term survival was not evident, as only 9% of patients lived longer than two years.

Tamoxifen has also been used in combination with traditional chemotherapy, because it should in principle reduce the level of chemo-resistance in addition to having its own direct effects on tumor growth. A European clinical trial combined tamoxifen with carboplatin as the initial treatment after radiation (104). Dosages of tamoxifen ranged from 40 to 120 mg/day, all of which were smaller than that used when tamoxifen has been used alone (160-240 mg/day). Combined over all dosages, the 12-month and 24-month survival rates were 52 and 32 %, respectively. For the patients receiving the highest dosage of tamoxifen, 12-month survival rate was 78%. In comparison, a matched set of subjects who received carboplatin alone after radiation had 12- and 24-month survival rates of 30% and 0%. However, a second similar study combining tamoxifen with carboplatin (105) reported a median survival time of only 55 weeks, which was only slightly superior to historical controls using carboplatin alone (48 weeks). However, the latter study noted that a minority of patients did have unusually long survival times, which was not reflected in the median survival times. The combination of carboplatin and tamoxifen has also been studied with patients with recurrent tumors. Here the median survival time was 14 months, but only 6 months for the subset of 16 patients with GBM (106).

Tamoxifen with a dosage of 240 mg/day has also been studied in combination with BCNU as the initial treatment after radiation (107). Median survival time was 69 weeks,

while the 1-year, 2-year, and 3-year survival rates 65%, 45% and 24%, respectively. It should be noted that while the 1-year survival rate and median survival time are only marginally greater than those obtained with BCNU alone, the 2-year and 3-year survival times are substantially greater. Note, however, that these numbers are based on a small number of patients (N=23). This benefit in terms of the number of longer-term survivors again reflects the fact that tamoxifen is effective only for a minority of patients, but for those its benefits can be very substantial. That only a minority of patients benefit from tamoxifen is relevant to the negative results of a phase III trial conducted in France (108). Patients received BCNU alone or BCNU in combination with 40-100 mg/day of tamoxifen (note that these dosages are substantially below that used in the other studies). No increase in median survival time was found, whereas the addition of tamoxifen did significantly increase the frequency of serious blood clots.

Several clinical trials have studied tamoxifen in combination with Temodar. In one preliminary report with sketchy details (109), the combination treatment, presented as the initial treatment after standard radiation, resulted in all of the patients being alive at 12 months after diagnosis. More details are clearly needed, but the results as described are unusually promising. However, a second published trial combining Temodar and tamoxifen (110) produced especially negative results and was in fact terminated early because of the low response rate and frequency of toxicity. However, this toxicity most likely resulted from the daily schedule of TMZ used, which involved a dose apparently too high for patients that were heavily pretreated. One important feature of tamoxifen is that its toxicity to glioma cells is due primarily to its first metabolite, which takes 2-8 weeks to reach asymptotic levels. Thus, short-term usage, even with high dosages, is not likely to be effective.

A third study (111) combining tamoxifen with the standard Stupp protocol (N=17) used a dose of 100 mg/m-sq., and reported a median survival of 17 months and a 2-year survival of 35%, slightly better than the Stupp protocol alone.

The most recent report (112) of using the combination of tamoxifen with temozolomide was with recurrent tumors (N=32) and used an alternating week schedule of temozolomide. Patients had previously received temozolomide according to the usual schedule. After start of the new schedule combined with tamoxifen, median time to tumor progression was 7 months and median survival time was 17.5 months, unusually high for recurrent tumors. The tamoxifen dose was 80 mg/sq. meter. In addition, the authors reported no difference in outcome as a function of the MGMT status of the tumors.

An important development with respect to tamoxifen has been the report (113) that it may be possible to predict which patients will be among the minority that benefits from tamoxifen. This Canadian study compared patients who responded to tamoxifen with those who did not and reported that there was a systematic difference in the metabolites from tamoxifen. This potentially allows a decision very early in treatment about whether

tamoxifen is worth continuing.

Tamoxifen's efficacy can be increased by suppressing thyroid function (114). Thyroid hormones maintain the level of the insulin-like growth factor (IGF), which is now known to play an important role in causing resistance to several different kinds of cancer treatments. Eleven of 22 patients with recurrent tumors became hypothyroid as a result of a drug treatment. Their median survival time was 10.1 months, versus 3.1 months for patients whose thyroid function was not effectively suppressed. However, no information is available for how thyroid suppression affects survival time, independently of whether tamoxifen is used.

Thalidomide

This drug became infamous during the 1950s and 1960s because it produced a large number of birth defects involving abnormal or completely missing limbs. It is now believed that this was due to its effects on inhibiting new blood vessels because limb buds are especially dependent on the growth of new blood vessels for normal development. Thalidomide was initially approved by the FDA for the treatment of leprosy, but now also is approved for multiple myeloma. It also has several common off-label uses, including melanoma, Kaposi's sarcoma, and prostate cancer. Unfortunately, a considerable amount of paperwork is necessary, both by the pharmacist and the prescribing physician, so obtaining it for off-label uses is not as simple as having your physician write a prescription. These bureaucratic restrictions have been imposed despite the fact that the majority of potential users of the drug, males, and females past the age of menopause, are unaffected by the drug's teratological potential.

Thalidomide's utility as a cancer treatment comes from it being the first anti-angiogenic drug that has been FDA approved, although it is now believed to have other mechanisms of action as well. The major side effects are somnolence (thalidomide was originally introduced for its sedative purposes), constipation, and neuropathy with long-term use.

The best results using thalidomide as a single agent comes from a small study performed in Switzerland (91). Nineteen glioblastoma patients received 200 mg/day of thalidomide, starting after radiation, escalating to 600 mg/day if tolerated. The actual median dose used was 200 mg/day. Median survival time was 63 weeks (14.5 months). Median progression-free survival was 17 weeks (3.9 months). Some patients had surgery for recurrent tumors so it is difficult to know how much of the survival time was due to the additional surgery. The same study also reported the results of 25 patients who received the same regimen of thalidomide but in combination with temozolomide. Here the median survival time was 103 weeks (23.7 months) and the median progression-free survival was 36 weeks (8.3 months) .

A subsequent study produced a more conservative estimate of the benefits of the Temodar + thalidomide combination. In contrast to the median survival time of 103 weeks from the clinical trial just described, this second trial using the combination of Temodar + thalidomide with newly diagnosed patients produced a median survival time of 73 weeks (16.8 months), marginally better than the 61 weeks from the now standard treatment of Temodar alone (92). Two differences in their protocols are evident: first, the latter study used Temodar and thalidomide during radiation which was then continued after radiation was finished; the earlier study began the Temodar and thalidomide only after the standard radiation treatment was completed. Secondly, the dosage of thalidomide was considerably less in the earlier study. This latter difference is interesting because clinical trials using thalidomide as a single agent seem to have better results with lower dosages of the drug. It is possible, but not proven, that the dose-effect curve for thalidomide is non-monotonic just as it appears to be for some other agents that have angiogenesis as their target. However, the most likely difference in the results for the two studies is that the earlier study included many patients who had re-operations for their tumors when they recurred, while there is no mention of re-operations in the latter study.

A subsequent study failed to find an improvement in outcome from adding thalidomide. (92). When the combination of temodar + thalidomide has been used with patients with recurrent GBM (93), PFS-6 was 24%. While the reports of thalidomide's efficacy have been inconsistent, the weight of the evidence suggests it adds to treatment efficacy, although probably not a large amount.

A major exception to the generalization that thalidomide has limited benefit comes from an Austrian study (317) in which apparent survival benefits were restricted to patients with "secondary" GBM, i.e. those that evolved from initially lower-grade tumors. Twenty-three patients whose tumors had advanced after both radiation and chemotherapy received 100 mg of thalidomide nightly, in part to help with their sleep. The median survival time after the start of thalidomide was 18 months, substantially longer than typically obtained with recurrent GBM. It should be noted that the 100 mg dosage is much lower than the studies in which thalidomide had limited benefits.

Valganciclovir (Valcyte)

Since 2002, Charles Cobbs and others have demonstrated a role of human cytomegalovirus in promoting the progression of glioblastoma tumors, the majority of which are positive for CMV proteins. This has led to the conjecture that treatment of brain tumors with anti-CMV drugs such as valganciclovir (Valcyte) could have therapeutic benefit. A small clinical trial using this approach has been conducted at the Karolinska Institute in Sweden. Forty-two patients were randomly assigned to the standard Stupp

protocol versus the Stupp protocol combined with Valcyte (173). Although there were some differences in tumor volume, these did not reach statistical significance, nor did the median survival time (17.9 vs. 17.4 months). However the design of the study allowed patients to receive Valcyte when their tumors progressed or after six months, thus confounding the determinants of the outcome.

Accordingly, the authors did a post-hoc analysis of patients who had at least six months use of Valcyte. For those patients, median survival was 24 months and 4-year survival of 27%. A subsequent report analyzed the trial patients with at six months exposure to Valcyte, along with others receiving the treatment outside of the trial (174). For these patients, 2-year survival was 70% and median survival was 30 months.

The benefits of Valcyte seem partly dependent on the degree of CMV infection (175). For patients with low-grade infection, median survival was 33 months, while those with high-grade infection had a median survival of 14 months.

The retrospective analysis described above has generated a great deal of controversy, mostly centred around the built-in bias inherent in such a time-dependent analysis (technically termed "immortal time bias"). Properly designed trials will be necessary to prove the efficacy of Valcyte for glioblastoma. In the meantime, many patients impressed with the results of the retrospective analysis have included Valcyte in their treatment regimens, with or without their oncologist's blessing.

Valproic acid/sodium valproate (Depakote)

A common anti-epileptic drug, valproic acid (trade name Depakote), is an inhibitor of histone deacetylase (discussed in the section on epigenetics). It also has the advantage of not inducing liver enzymes that reduce the concentration of chemotherapy agents in the serum, which does occur when using many other anti-epileptic drugs (in fact valproic acid may increase concentration of chemotherapy, so that the standard dosages need to be monitored for toxicity.

Retrospective data

That its use rather than other anti-epileptic drugs might improve clinical outcome is supported by a retrospective clinical study comparing enzyme-inducing anti-convulsants with valproic acid. Median survival for the former (n=43) was 11 months, while median survival for those receiving non-enzyme inducing antiepileptics (n=37) was 14 months (203). Valproic acid was the main antiepileptic drug used in this latter group (85%). Similar results were obtained in a post-hoc analysis of the Stupp trial that definitively showed the effectiveness of temozolomide (204). For patients receiving the combined

temozolomide + radiation protocol, median survival was 14 months for those not using any anti-convulsant drugs, 14.4 months for those using a drug other than valproic acid, and 17. 4 months for those using valproic acid. A similar pattern occurred for the rate of 2-year survival: 25%, 26% and 30.6%.

A retrospective study from the Sloan-Kettering dataset produced a similar result. Patients using valproic acid had a median survival of 16.9 months versus 13.6 months for those using other antiseizure medications. When the analysis was restricted to patients receiving valproic acid during radiation, the corresponding median survivals were 23.9 months vs. 15.2 months (318).

Although the foregoing results support the use of valproic acid because of its ability to inhibit HDAC, a recent Korean study directly compared 38 patients prospectively enrolled to receive Keppra with 42 patients taking valproic acid as a control group. Median progression free interval was 9.3 months for Keppra vs. 6.5 month for valproic acid. Overall survival was 26 months vs. 16 months (205). This abstract does not include full details of drug dosing or scheduling, and it is possible that valproic acid is more effective as an adjuvant during the radiation phase of treatment, while Keppra may be more effective during monthly temozolomide cycles, especially for those tumors with unmethylated MGMT. *See the Keppra (levetiracetam) section of this chapter (above), for a discussion of Keppra as a chemosensitizer in glioblastoma therapy.*

Prospective data

The most impressive results with valproic acid were reported by the brain tumor center at the National Cancer Institute at the 2014 SNO meeting. In a prospective study of 37 newly diagnosed patients, valproic acid at a dose of 25 mg/kg per day was used during the six weeks of combined chemoradiation. Median survival was a very impressive 29.6 months and median PFS was 10.5 months. The study was published in full in July 2015 (319).

Continuing Debate

In 2016, a very large retrospective analysis (337) based on data from four large randomized clinical trials was carried out with the intention of substantiating the need for a phase 3 trial of valproic acid as an addition to the standard of care for glioblastoma. This combined dataset was drawn from the control arms of the AVAglio and RTOG0825 trials, and both arms of the CENTRIC and CORE clinical trials testing cilengitide in combination with standard of care. This total validation cohort consisted of 1582 patients. In the first analysis of this large validation cohort, no significant difference was found in progression-free (PFS) or overall survival (OS) between users (whether alone or in combination with other anti-epileptic drugs) and non-users of valproic acid at study entry.

In a further analysis, the trend towards improved PFS and OS in users of valproic acid as anti-epileptic monotherapy (versus those receiving no anti-epileptic therapy or those receiving only enzyme-inducing anti-epileptic drug) was lost upon adjustment for other prognostic factors. When the analysis was focused on those patients receiving valproic acid at both the time of study entry before radiochemotherapy as well as at the time of the first follow-up visit after radiochemotherapy, there was no significant difference found in PFS or OS versus patients who received no anti-epileptic drug at both time points. This latter analysis did not include patients from the RTOG 0825 trial for lack of data.

Similar analyses found no PFS or OS advantage for patients with any use of levetiracetam (Keppra) at baseline, nor for patients using levetiracetam at both study entry and at the first follow-up visit after radiochemotherapy (versus patients not using any anti-epileptic drug at both time points). However, as one of the primary mechanisms proposed for a survival benefit of levetiracetam is the inhibition of MGMT, it could be argued that sustained use of this drug during monthly adjuvant cycles of temozolomide is the time-frame of most concern, rather than time points immediately before or after chemoradiation.

Notably, the corresponding author of this study is Michael Weller, who was also the first author of the 2011 retrospective analysis that found a survival advantage of valproic acid on the basis of data from the pivotal 2005 EORTC-NCIC trial. In contrast to that earlier study, the negative findings in the present study lead the authors to conclude that even if a survival advantage of valproic acid could be proven by a prospective randomized phase III trial, the advantage is likely to be so slight that a sample size of 5000 patients would be necessary to confirm this. The authors also admit that a major limitation of the study is that patients were grouped into categories of anti-epileptic drug use on the basis of use at the single time point of study entry (and at a second time point after radiochemotherapy for a subset of patients), and also that doses of both drugs (valproic acid and levetiracetam) and length of exposure would have varied significantly among patients.

In response to critics of this study (338), the authors again acknowledge that the major weakness in their study is the "lack of solid data on the dose and duration of valproic acid exposure". They go on to write that, "It is conceivable that for a beneficial effect in glioblastoma, early and high-dose treatment with valproic acid would be required, although no categorical data truly support this contention. Thus, we contend that analyses such as those reported here are not suitable to completely rule out an effect of valproic acid on outcome, especially on minuscule subsets with unique biologic characteristics. However, our data are robust enough to exclude any major effect of valproic acid, especially in significant proportions of patients with glioblastoma".

In this context, it's interesting to recall that the phase II trial of valproic acid in addition to standard radiochemotherapy mentioned above (319) used rather high doses of Depakote (25 mg/kg/day), and that the majority of patients using the drug for seizure

control may not be taking the drug at such high doses. Although the EORTC Brain Tumor Group and the authors of this latest large retrospective study (Happold, Weller, et al.) conclude that valproic acid has little if any survival benefit as an anti-tumor therapy for unselected cohorts of glioblastoma, phase II trial data suggests that higher doses of valproic acid (25 mg/kg per day) during the six weeks of radiochemotherapy may provide a survival benefit, although prospective randomized trials remain to be carried out. The issue may be that typical anti-seizure doses of valproic acid may not be sufficient for consistent HDAC inhibition. As Happold et al. ask, "is valproic acid truly the best histone deacetylase inhibitor to study in this context?". Novel HDAC inhibitors such as panobinostat are being studied in clinical trials for glioblastoma, and while new HDAC inhibitors may be found more effective than valproic acid, the latter has the advantages of a long history of clinical use in glioma patients, and is both off-patent and inexpensive, and therefore accessible for off-label use.

A trial of 3 repurposed drugs plus Temodar

The above list of drugs do not exhaust the list of older drugs that have the potential to improve treatment outcome when added to standard treatment. The critical issue is whether using combinations of these drugs actually does improve outcome in the clinic.

The most disappointing outcome has been for a treatment combination involving Temodar, thalidomide, and celebrex for newly diagnosed patients (134). Fifty GBM patients received the standard radiation therapy followed by the standard monthly schedule of high-dose Temodar in combination with celebrex and thalidomide. Median survival from the time of diagnosis was 16.1 months and 2–year survival was 21%, seemingly not an improvement over the current gold standard of treatment.

More positive results were obtained in a phase 1 study (135) of different combinations of Temodar, thalidomide, accutane, and celebrex. Although the goal of the study was a factorial design of different 2 –and 3-way combinations, not enough patients were recruited into the various arms of the study to conduct the planned comparisons at the time of the initial report. Forty-two patients were assigned to receive Temodar alone (with an alternating week schedule), or Temodar in combination with one or more additional drugs. For unclear reasons 19 of the 42 patients received Temodar alone and 23 patients received some combination. Unfortunately, results were reported in aggregate without any distinction between patients receiving the different combinations, nor any distinction between those receiving only Temodar versus Temodar + additional therapy. Nevertheless, median survival was 20 months and two-year survival rate was 40%, despite the inclusion of 12 patients who never received any of the combinations due to early progression. The authors also noted that ten patients were alive 4.8 to 6.9 years from entry into the study.

A follow-up report on the phase 2 portion of this trial consisting of 155 was presented at the 2012 ASCO meeting (136), and published in full in Neuro-Oncology in September 2014 (advance online access) (307). Patients were randomized into one of eight arms, with approximately 20 patients in each arm:

- Temodar alone
- Temodar+isotretinoin (Accutane)
- Temodar+celecoxib
- Temodar+thalidomide
- Temodar+isotretinoin+celecoxib
- Temodar+isotretinoin+thalidomide
- Temodar+celecoxib+thalidomide
- Temodar+isotretinoin+celecoxib+thalidomide

Thus, for each of the three additional drugs, there were four arms that included that drug, and four arms not including that drug. The primary objective of the study was to judge the efficacy of the three additional drugs by comparing the four arms including a drug versus the four arms not including that drug, in terms of progression-free survival measured from the time of randomization. The four arms including celecoxib showed a trend toward improved progression-free survival compared to the four arms not including celecoxib (hazard ratio=0.8), though the effect did not reach formal statistical significance. The four arms including isotretinoin and the four arms including thalidomide had worse outcomes than the arms not including these agents (hazard ratios of 1.3 and 1.2 respectively), though again the differences did not reach formal statistical significance.

When each of the 8 treatment arms were compared individually, Temodar plus isotretinoin led to significantly worse outcomes than Temodar alone (hazard ratios of 2 and 2.2 for progression-free survival and overall survival compared with Temodar alone). The combination of Temodar+celecoxib had an outcome equivalent to Temodar alone (hazard ratio=1). All other combinations had inferior outcomes to Temodar alone, though as only approximately 20 patients were included in each arm, only the Temodar+isotretinoin combination reached statistical significance (worse survival compared with Temodar alone). Thus, with the dosages and schedules used in this study, both isotretinoin and thalidomide appeared to be antagonistic when combined with Temodar, with the antagonistic effect of isotretinoin on Temodar efficacy appearing especially significant. Other results with thalidomide cited above (91-95, 317) argue that at least with some usage parameters thalidomide can be effective, with some indication that lower dosages are more effective.

CUSP9 (Co-ordinated Undermining of Survival Paths) with 9 repurposed drugs

A paper appearing in April 2013 introduced a "conceptually new" approach for recurrent glioblastoma (10). In this paper, various repurposed drugs in addition to metronomic temozolomide are proposed as part of an extensive treatment cocktail, including aprepitant (an anti-nausea drug), artesunate (a malaria drug), disulfiram (discussed above), sertraline (an anti-depressant), captopril (an ACE inhibitor used for hypertension), auranofin (a gold compound used for arthritis), nelfinavir (an HIV drug), and ketoconazole (an anti-fungal drug). In the updated version of this combination, called CUSP9* (306), ritonavir has been substituted for nelfinavir, itraconazole replaced ketoconazole, copper gluconate was removed, and celecoxib was added. All of these have extensive in vitro evidence for inhibiting various biochemical processes underlying glioblastoma growth, but none as yet has traditional evidence from human clinical trials. However, the main argument of the authors of the article is that tests of individual treatment agents in isolation are doomed to failure, because there are multiple growth pathways that must be inhibited simultaneously.

7. Over-the-Counter Drugs and Supplements

The treatments discussed above generally require a physician's cooperation in prescribing them. However, there are a number of agents available over-the-counter that have promising anti-cancer properties, and it is reasonable to believe that these can increase the chances of surviving. Some of these with supporting clinical evidence (e.g., proton-pump inhibitors such as Prilosec) have been discussed above. A frequent conflict between patients and their oncologists is that patients, often desperate to find treatment agents that will improve their chances of survival, are eager to use such adjunctive treatment while their oncologists generally oppose using such supplementary agents, on the ground that they might interfere with the standard treatment. While negative interactions are possible, to date there have been very few if any documented cases. Given the bleak prognosis of a glioblastoma diagnosis, my belief is that concerns about negative interference are misplaced and get in the way of potentially useful treatment adjuncts. However, it is important to attend to the evidence supporting the use of any specific agent under consideration, as there are many products on the market that are hyped, supported only by testimonials of dubious validity, and some have the potential for harm.

PSK and other polysaccharides

PSK is the abbreviation for polysaccharide krestin (sometimes known simply as krestin),

which is an extract from the mushroom, Coriolus versicolor. It has become a standard component of cancer treatment protocols in Japan (a Chinese version of the same extract is known as PSP) for many different kinds of cancer, predicated on the assumption that it is an immune-system enhancer. Among the effects on the immune system that have been identified are gamma-interferon production, interleukin-2 production, and in increase in T-cell activity. Other effects include inhibition of matrix-degrading enzymes that underlie tumor invasion of adjacent tissue, and the inhibition of angiogenesis. Numerous clinical trials have been conducted in Japan comparing chemotherapy regimens with the same regimens with PSK added, for a variety of different cancers, most frequently stomach and colon cancer.

In one representative study, with non-small cell lung cancer (233), stage I patients receiving PSK (3 g/day) had a five-year survival rate of 39% compared to 22% for patients not receiving PSK. For stage III patients, the 5-year survival rate with PSK was 16% versus only 5% for those not receiving PSK. Both differences were statistically significant. A meta-analysis of several different clinical trials with colorectal cancer (over 1000 patients) who were randomized to receive either the standard chemotherapy or the standard chemotherapy in combination with 3.0 g/day of PSK showed that the addition of PSK increased both the survival rate and the duration of disease-free survival, with relative risks of .71, and .72, respectively (234). The three-year disease-free survival rate was 81% for patients receiving PSK, compared to 69% for those receiving only chemotherapy. I have found only one study that used PSK in the treatment of glioma, in combination with ACNU (a chemical cousin of BCNU) and vincristine (235). The survival rate for 25 GBM patients after one, two, and three years was 56%, 37%, and 12%, respectively. No control condition was studied that did not receive PSK, so exactly what its effect was is unclear. Note, however, that the two-year and three-year survival rates are substantially greater than that typically seen for GBM following traditional treatment with chemotherapy alone.

The source for PSK that I have used is JHS Natural Products in Eugene, Oregon, now known as Mushroom Science. Other sources undoubtedly can be found through a web search. Other mushroom extracts that also have the long-chain polysaccharides (beta-glucans) that appear to be the active ingredient in PSK are more readily available. These include maitake, reishi, and shiitake mushrooms. However, none of these has the same level of scientific evidence for treatment efficacy in human clinical trials. Maitake D-fraction seems an especially promising mushroom extract based on a laboratory study of chemically induced tumors in mice (236). Tumor growth was inhibited 90% when the mushroom extract was combined with chemotherapy versus an inhibition of only 50% when chemotherapy was used alone for control subjects.

Gamma-Linolenic Acid (GLA)

We currently recommend fish oil containing omega-3 fatty acids, rather than GLA oil, as rodent studies suggest that GLA does not cross into the central nervous system or brain tumors after oral administration (353). GLA is also not detectable in the cerebrospinal fluid of humans (without supplementation), while the long-chain omega-3 fatty acids EPA and DHA are detected (354). Omega-3 fatty acids from fish oil are less expensive, and far more likely to enter the central nervous system following oral administration. Their mechanisms of action are similar.

Perillyl Alcohol/ Limonene

These closely related chemical compounds are derived from citrus oils, and have been extensively investigated as anti-cancer agents, including several early-stage clinical trials. Unfortunately, the gastro-intestinal side effects of these compounds have retarded their clinical development. A recent clinical trial with recurrent glioma patients, conducted in Brazil, circumvented this problem by administering perillyl alcohol intranasally four times daily. In the initial report, of 29 GBM patients with recurrent tumors receiving the treatment, one had a partial response and 13 had stable disease, for a PFS-6 value of 48% (255). In a later study of 89 GBM patients, who had failed a minimum of three prior treatments (and thus had especially poor prognoses), patients were separated into those that had primary GBM vs. secondary GBM (tumors that evolved from lower-grade tumors), median survival for primary GBM was 5.9 months, while that for secondary GBM was 11.2 months. Median survival for a set of matched control patients who received only supportive care was 2.3 months (256). It was also noted that patients with tumors in their midbrain area benefited more from the treatment than did patients with tumors in their cerebral lobes.

Metabolic therapy with Sodium R lipoate plus hydroxycitrate

Following up on in vivo work and earlier reports with mixed groups of cancer patients, a research group based in France published results of 11 brain tumor patients (8 primary, 4 metastatic) treated with a metabolic therapy consisting of sodium R lipoate (a form of alpha-lipoic acid) and hydroxycitrate (357). In newly diagnosed patients this treatment was combined with standard radiation and chemotherapy, and in some patients, low dose naltrexone or ketogenic diet were combined with the lipoate and hydroxycitrate. Previous publications referred to this metabolic therapy as METABLOC.

In this study, sodium R lipoate was the form of alpha-lipoic acid used. Unless otherwise stated, alpha-lipoic acid typically consists of a racemic (50/50) mix of R and S forms of lipoic acid. These forms are mirror images of each other, but have different properties. Only the R form occurs in nature. Alpha-lipoic acid is a cofactor of pyruvate dehydrogenase, and similarly to dichloroacetate, can increase the flow of pyruvate into mitochondria, thwarting the Warburg effect whereby pyruvate is fermented to lactate outside the mitochondria. Hydroxycitrate is derived from the tropical plant Garcinia cambogia, and is an inhibitor of ATP citrate lyase, an enzyme involved in the preparatory stages of fatty acid synthesis.

In this study, of the eight primary glioma patients, six were treated with R-lipoate and hydroxycitrate before tumor recurrence, alongside standard up-front treatment (radiation and temozolomide chemotherapy).

One 59 year old GBM patient died 11 months after starting conventional therapy and 4 months after starting metabolic therapy, the only short term survivor of the six newly diagnosed patients. The remaining five patients in the newly diagnosed group were followed up for 23, 24, 25, 36 and 87 months from diagnosis and were all still alive at the time of publication, all of them without disease progression except for the 87 month survivor. This longer-term survivor was 38 years old, and we cannot exclude that mutant IDH1 status is responsible for the long survival as no information on IDH status is given. Four of these patients were over the age of 50, and apart from the one short-term survivor, these patients survived for 2-3 years from diagnosis without disease progression, including one patient who had an unresectable glioblastoma (3 years without progression, treated with temozolomide, lipoate, hydroxycitrate, and low dose naltrexone). Another glioblastoma patient had a 60% tumor shrinkage following initiation of lipoate, hydroxycitrate and ketogenic diet.

While the authors acknowledge that a small case series does not provide definite proof of efficacy, the longer than expected progression-free survival in the majority of these patients, including one with an unresectable glioblastoma, provides justification for a "well conducted trial".

Nutraceuticals and Herbals

Oncologists routinely warn their patients not to use supplements, usually based on the belief that supplements that are anti-oxidants will interfere with both radiation and chemotherapy. While this issue is extremely complex, my own evaluation of the relevant evidence strongly disagrees with this opinion. Accordingly, I have posted my own analysis of the clinical evidence as an accompanying article on this website. Here I list the supplements that seem most likely to be efficacious, based on extensive laboratory

data. Unfortunately, few clinical results are available to corroborate the experimental data, primary because the supplements cannot be patented; hence there is no financial incentive to develop their clinical usage. The result is that little information is available about the best dosage and about bioavailability, which is often a problem. However, a great deal is known about the mechanisms of action of the various supplements, which often overlap those of conventional drug therapy. A detailed consideration of such mechanisms is not possible here, as it would require a great deal of molecular biology. A special issue (2009, Vol. 269, Issue #2) of the journal, Cancer Letters, was devoted to the molecular targets of many of the individual agents to be considered. A more general review is provided in Reference 257.

The list of supplements to be considered is necessarily selective. Undoubtedly, there are numerous other agents that could be useful that are omitted.

Berberine

This is an alkaloid extract from *Coptides rhizoma* commonly used in China as an herbal medicine. It is also found in high concentration in the widely used supplement, goldenseal. In one laboratory study of using various kinds of glioma cell cultures and implanted tumors in rodents (289), the cytotoxic effects of berberine were compared to those of BCNU and to the combination of berberine and BCNU. Berberine alone produced a 91% kill rate in cell cultures, compared to 43% for BCNU. The combination produced a kill rate of 97%. Comparable results were obtained with the in vivo implanted tumors. Such results suggest that berberine is among the most promising treatment agents, but to date very little research using it has been reported. Part of the reason may be that berberine is poorly absorbed from the GI tract. It appears that the structure of berberine is closely related to Ukrain, a drug that combines an alkaloid from a plant named celandine with an old chemotherapy agent named thiotepa. After years of Ukrain's use only in alternative medicine, it recently has been licensed for commercial development. A recent clinical trial using it for pancreatic cancer has produced impressive results. (290).

Boswellic Acids

This is a collection of aromatic acids related to the biblical spice, frankincense. Its relevance to cancer treatment is that is a potent inhibitor of the lipoxygenase inflammatory pathway, one of the two major sources of inflammation associated with cancer progression. Cyclooxgenenase is the other pathway, which can be inhibited by celebrex. Both pathways should be suppressed to maximally inhibit inflammation. Of

more immediate interest to glioma patients is that Boswellic acid is a powerful inhibitor of the edema caused by tumor growth, which is the major reason many brain tumor patients require steroids to suppress the swelling. In a randomized, double-blind study conducted in Germany, 44 brain tumor patients received either boswellia serrata (one of the several forms of boswellia) or a placebo (298). Both groups also received radiation. Compared to baseline, patients receiving boswellia had a 75% reduction in edema, while placebo patients had a reduction of 26%. There were no significant side effects of the boswellia. Given the many side effects of steroids, boswellia offers the promise of substantially improving the quality of life. However, the dose of boswellia used in this study was 4200 mg/day, far greater than can be readily obtained by the usual sources of boswellia that can be obtained from health food stores.

Cannabis

After years of governmental discouragement of research on Cannabis (the plant from which marijuana is derived), the last few years has seen a proliferation of research on its mechanisms of action. One result of this research is the finding that cannabis inhibits the growth of various kinds of cancer cells, including gliomas (294). In one recent paper (295), cannabinoids were shown to significantly inhibit angiogenesis in gliomas implanted in mice, which was accompanied by significant inhibition of glioma growth. A subsequent paper with a mouse model combined cannabis with temozolomide and reported a strong synergy between them (296).

The direct anti-cancer effect of cannabis is noteworthy because it is also one of the most effective anti-nausea agents, without many of the side effects of those drugs routinely used (Zofran and Kytril). Moreover, a liquid form of cannabis (Sativex) has been government approved in both Canada and various European countries (for neuropathic pain), and can be used as an aerosol much like an asthma inhaler.

Preliminary efficacy data from the first trial of Sativex for glioblastoma was released by GW Pharmaceuticals in February 2017. Find the press release here, and abstract from the 2017 ASCO conference here. In this clinical trial, 12 recurrent GBM patients were randomized to receive Sativex plus dose-intense temozolomide, and 9 patients were randomized to receive dose-intense temozolomide plus placebo. Median survival from trial start was 369 days (12.1 months) in the placebo + TMZ group and over 550 days (18 months) in the Sativex + TMZ group. Survival at one year was 56% for the placebo group and 83% in the Sativex group, a difference that reached statistical significance (p=0.042) despite the small numbers of patients in the trial. *This trial provides the first prospective clinical trial data showing efficacy of cannabis products in the treatment of gliomas.*

Curcumin

This is an ingredient in the Indian cooking spice, turmeric. It has been shown to inhibit the growth of cancer cells of various types in laboratory studies via numerous different mechanisms (272). Like genistein, it inhibits the tyrosine kinase signaling and also inhibits angiogenesis. Perhaps most importantly, it inhibits proteins that prevent damaged cells from undergoing apoptosis, a family of genes known as nuclear factor kappa B. Of all of the supplements on this list it is the most potent anti-cancer agent in laboratory studies. However, it also should be noted that its bioavailability from oral intake is limited, although bioavailability supposedly is increased when curcumin is combined with piperine (the main ingredient in black pepper). The Life Extension Foundation sells a version of curcumin that they claim has much greater bioavailability than anything else on the market. Despite the limited bioavailability, there is some evidence of clinical effectiveness. In a study of dermatitis induced by radiotherapy for breast cancer, a double-blind placebo controlled trial compared a placebo with curcumin (2 grams three times/day), both of which were taken throughout radiation treatment. Significantly less dermatitis occurred in patients receiving curcumin (273).

Curcumin has also been used in combination with a second supplement, quercetin, (see below) for the treatment of an inherited disorder of the colon in which hundreds of adenomas develop and eventually colon cancer (274). Five patients with the disorder received 480 mg of curcumin and 20 mg of Quercetin three times daily. Polyp number and size were assessed at baseline and then six months after starting the supplements. For all patients there was a decrease in polyp size and number, which was statistically significant.

Ellagic Acid

This is a family of phenolic compounds present in fruits and nuts, including raspberries, blueberries, strawberries, pomegranate juice, and walnuts. In laboratory experiments it has been shown to potently inhibit the growth of various chemical-induced cancers, with the basis of the effect being an arrest of cell division in the G stage, thus inducing the programmed cell death known as apoptosis. While there have been no trials to assess its clinical effects with brain cancer, a recent clinical trial, performed at UCLA with prostate cancer demonstrate its potential (288). Patients with prostate cancer, whose PSA levels were rising after initial treatment with either surgery or radiation, drank pomegranate juice (8 oz/ daily), which contains high levels of eligitannnins (precursors to ellagic acid). The dependent measure was the rate of increase in the PSA level, which typically rises at a steady rate for this category of patients. Pomegranate juice produced

an increase in PSA doubling time, from 15 months at baseline to 54 months after consuming the juice. Of the 46 patients in the trial, 85% exhibited a notable increase in the doubling time, and 16% had decreases in their PSA.

Fish oil (source of omega-3 fatty acids)

The major omega-3 fatty acids found in cold-water fish oil, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have also been demonstrated to have potent cytotoxic effects on cancer cells in various laboratory experiments. Part of their mechanism of action is similar to that of GLA, in that the metabolism of these fatty acids creates high levels of free radicals. In addition, a recent laboratory study has shown that EPA-treated tumors showed a significant arrest of cell division due to inhibition of cyclins at the G1 phase of cell division, which resulted in an increased rate of programmed cell death known as apoptosis (241).

A clinical trial comparing fish-oil supplements versus a placebo has also been reported, involving patients with several different types of advanced cancer (242). Thirty malnourished patients suffering from cachexia were randomly assigned to receive 18 g of fish oil per day or a placebo sugar pill. An additional thirty subjects, adequately nourished, received a similar random assignment. For both groups the fish oil significantly increased survival. For the malnourished patients the median survival times, as estimated from their survivor functions, were 110 days for the patients receiving placebo and 210 days for patients in the fish oil group. For the adequately nourished patients, the corresponding numbers were 350 versus 500 days.

In laboratory studies (243) fish oil has also been shown to increase the effectiveness of chemotherapy and radiation. A phase II trial involving 25 heavily pretreated metastatic breast cancer patients, used 1.8 g/day of DHA, one of the two major fatty acids in fish oil, in combination with standard anthracycline-based chemotherapy (244). Patients previously had failed both chemotherapy and hormone treatments and many had multiple metastases, including many liver metastases. Because this was a phase II trial, there was no control group that received chemotherapy alone, but patients were subdivided according to their level of plasma DHA. The two groups were approximately equal with respect to all major prognostic variables. Median survival for the high DHA patients was 34 months, vs. 18 months for the low-DHA patients.

A second clinical trial presented 2200 mg of EPA plus 240 mg of DHA to patients with advanced non small cell lung cancer (245). Patients either received only the standard of care of chemotherapy, or the same treatment in combination with daily fish oil. Response rate (tumor regressions) was 60% in the fish oil group and 26% in those receiving only the standard of care. One-year survival was 60% in the fish oil group versus 39% in those

receiving only chemotherapy. Chemotherapy toxicity was also decreased in those using fish oil.

Garlic

Garlic, like green tea, has been used hundreds of years for its medicinal purposes. A recent cell culture study with glioblastoma cell lines demonstrated its potent cytotoxic effects that were mediated by its ability to induce apoptosis (293). It is also a potent inhibitor of histone de-acetylase (HDAC).

Genistein

This is an isoflavone derived from soy products (it is also found in red clover extract) that has been shown in the laboratory to inhibit the growth of many different types of cancer, including glioma cells. In addition to the laboratory evidence, there is substantial epidemiological evidence that high dietary intakes of soy products decrease cancer mortality by at approximately 50%. There is also evidence from scattered clinical trials, mainly for prostate cancer.

Soy extracts containing genistein are available in most health-food stores. The concentration of genistein is often not well specified. Most importantly, the listed amounts of genistein are so low that they are unlikely to provide much clinical benefit. The highest concentration (about 10 times greater than the others that I have found) is marketed by the [Life Extension Foundation](#) (phone: 800-841-5433). It may also be possible to purchase it wholesale in the form of a product named NovaSoy, manufactured by the Archer-Daniels-Midland Corporation.

Recent experimental studies have examined the mechanisms whereby genistein produces its anti-cancer effects (261). The consensus is that this results from its ability to inhibit tyrosine kinase activity. This is a general class of intra-cellular signals that strongly stimulate cell division. Genistein also appears to produce inhibition of protein kinase C (discussed earlier with respect to the mechanisms of tamoxifen). This in turn suggests that a combination of genistein and tamoxifen might be especially effective. Finally there is increasing evidence that genistein is an inhibitor of angiogenesis.

Of special interest to brain cancer patients is a laboratory study in which glioblastoma cells were treated with a combination of genistein and BCNU (262). The result was a highly synergistic suppression of the rate of growth. It has also been shown to increase the effectiveness of other chemotherapy agents (e.g., carboplatin, tamoxifen) and other

supplements (263).

Green Tea

Green tea has been consumed in both China and Japan for 5000 years based on its medicinal properties A recent review has summarized its anti-cancer effects in several different animal models using both mice and rats (including major inhibition of glioblastoma cell lines), both when human tumors have been implanted and when they have been induced by various chemical carcinogens (264). In a representative study of chemically induced tumors in mice (265), green tea was provided as the sole source of fluid, at a concentration of 6% (6 g of tea per liter of water), the incidence of lung tumors was reduced by 30%. The same study identified several different mechanisms of action, the most prominent of which was the inhibition of angiogenesis.

The major active ingredient in green tea is epigallocatechin gallate (EGCG), one of a family of molecules known as catechins. Not only has this molecule been shown to be cytotoxic to glioma cells in vitro, it also substantially increases the effectiveness of both cisplatin and tamoxifen (266).

Of special interest is a recent in vivo study in which glioblastoma cells were implanted into mouse brains, after which the mouse were treated with either temozolomide alone, EGCG alone, or their combination. EGCG alone did not increase survival time, but its combination with temozolomide greatly increased its efficacy, relative to temozolomide alone (267).

A recent review by the new Division of Alternative Medicine of the National Institutes of Health identified green tea as the most promising of treatments advocated by proponents of alternative medicine. Accordingly, several clinical trials investigating its efficacy are ongoing. The only one reported to date used green tea in the treatment of patients with androgen independent metastatic prostate cancer (268). Dosage was 6 g of green tea per day. Only limited clinical benefit was reported. It is important to recognize that anti-angiogenic agents generally take a long time to produce clinical regressions, work better with less advanced stages of disease, and also work better in combination with other treatment agents.

A second clinical trial used a green tea extract at a dose of 2000 mg twice daily with patients diagnosed with chronic lymphocytic leukemia (269). Significant reductions in the absolute lymphocyte count were observed along with substantial reductions in the size of the lymph nodes reflecting the extent of disease. However no survival data were reported.

Green tea also has been used with patients who have had polyps excised from their colons, or who had tumors previously removed, known high-risk factors for the development of colon cancer (270). Patients received a combination of apigenin (20 mg), a flavonoid most commonly found in celery, and 20 mg of EGCG; the remaining patients received no supplements. Both groups had surveillance colonoscopies. In the supplemented group (n=-31), only one patient developed an adenoma (7%), while in the matched controls (n=56), 47% of the patients had cancer recurrence or the development of adenomas.

One counter-indication for the use of green tea is in combination with Velcade (Bortezomib). Green tea combines with the boron component of the drug, thus inactivating it (271). However, this interference effect appears to be unique to Velcade due to its chemical structure.

Lycopene

This is a carotenoid that is found most abundantly in tomatoes but occurs in various other red-colored vegetables as well (including watermelon). Unlike the most well known carotenoid, beta-carotene, it is not transformed into Vitamin A, and thus has no hepatic toxicity. In a small clinical trial involving prostate cancer patients about to undergo surgery (281), those who consumed lycopene for several weeks before surgery had a reduction in both the size and malignancy of their tumors relative to control patients not receiving lycopene. In a study of 54 patients with advanced prostate cancer (282), patients were randomized to receive castration or castration plus 2 mg of lycopene daily. At two years after treatment inception both groups had reductions in PSA level with 40% of the castration-only group having a complete PSA response, while 78% had a complete PSA response for those also receiving lycopene. Bone scans also showed a greater clinical benefit for those receiving lycopene.

In an experimental study involving both cell cultures and implanted glioma tumors in rats (283) lycopene (and beta-carotene) were found to substantially inhibit tumor growth in both experimental preparations, and in fact had a greater inhibitory effect than did a collection of retinoids commonly used clinically. Of further relevance to gliomas is that one of lycopene's mechanisms of action is to inhibit the insulin-like growth factor, which as noted above is involved in the development of resistance to a variety of different treatment agents (284). Also of interest is evidence that it synergizes with Vitamin D (285).

The only report of lycopene's clinical use with gliomas is from a meeting abstract of a randomized clinical trial conducted in India with 50 high-grade (32 GBM) glioma patients receiving a treatment protocol of radiation + taxol. Patients also received

lycopene (8 mg/day) or a placebo (286). Eighty percent of patients receiving lycopene had either complete or partial tumor regressions, while this was true for only 44% of those receiving a placebo. Progression-free survival was also greater for those receiving lycopene (40.8 weeks vs. 26.7 weeks). However, neither difference was statistically significant using the $p < .05$ probability criterion.

Resveratrol

This is a naturally occurring polyphenol found most abundantly in grapes and mulberries. Red wine is among the sources. Numerous experimental studies have shown that it inhibits proliferation of various kinds of cancer, including glioma, leukemia, prostate, breast, and colon cancer. It has also been shown to be synergistic with temozolomide in in vivo rodent models (291). Among its mechanisms of action are activation of the P53 gene, inhibition of protein kinase C, and the inhibition of new blood vessel growth. In the one recent study of its use with implanted glioma tumors (292), rats received either sub-cutaneous injections or intra-cerebral injections of tumor cells, which in control animals rapidly grew and became fatal. With sub-cutaneous tumors a dose of resveratrol of 40mg/kg produced major growth inhibition with 70% of the rats becoming long-term survivors. A higher dosage (100 mg/kg) was necessary to inhibit the growth of the intracranial tumors, and even then it was only marginally effective. The difference in outcome for the two preparations suggests that resveratrol may be impeded by the blood-brain barrier. However, the authors note that it had significant anti-angiogenic effects, which may be independent of the blood-brain barrier. Whether resveratrol has clinical utility for brain cancer is unclear, although it is known that anti-angiogenic agents of various sorts synergize with various kinds of conventional treatment.

Silibinin (an ingredient of Milk Thistle)

Silymarin is an extract from the milk thistle plant that has been used extensively in Europe as an antidote for liver toxicity, due to mushroom poisoning and overdoses of tylenol. Its active ingredient is a molecule called silibinin. Recently a great deal of laboratory research has shown it to have anti-cancer effects, which recently have been reviewed (275). Like genistein and quercetin it is a tyrosine kinase inhibitor, but it appears to have multiple other effects, including the inhibition of the insulin-like growth factor (IGF) that contributes to the development of chemoresistance (276) (see the section on tamoxifen), and the inhibition of angiogenesis (277). It also inhibits the 5-lipoxygenase inflammatory pathway and suppresses nuclear factor kappa B, which is a primary antagonist to apoptosis (278). It also appears to protect against common chemotherapy toxicities (279), while at the same time increasing the effectiveness of

chemotherapy (280).

Sulforaphane

Brassica vegetables, which include broccoli, cauliflower, brussel sprouts, and cabbage, have long been believed to have anti-cancer properties. A major source of these effects is a substance known as sulforaphane. Recently it has been discovered that the 3-4 day-old broccoli sprouts contain 10-100 times the concentration of sulforaphane as that of the full-grown vegetables. To test whether the oral ingestion of sprouts has anti-cancer effects, dried broccoli sprouts were included in the diet of rats with chemically induced cancers, with the result that considerable regression of the tumors were observed (287). Broccoli sprouts are also very tasty additions to salads. Subsequent research has shown that sulforaphane is a powerful inhibitor of histone de-acetylation, the target of several new drugs, including vorinostat (discussed in a previous section)

The Importance of Synergy

There is also evidence that supplements may be synergistic when combined.
An experimental demonstration of synergy between supplements with glioma cells studied the combination of resveratrol and sulforaphane (299). Low doses of either in isolation produced moderate inhibition of cell growth but the combination of the same low doses produced major growth inhibition by a variety of different mechanisms.

The most systematic analysis of synergy between various supplements targeted two different pancreatic cancer cell lines, known to be highly resistant to treatment. In the first set of experiments, dose-effect functions were established independently for curcumin and soy isoflavones (containing a high level of genistein). As expected, the tumor cells were highly resistant to treatment. Then the combination of agents was tested, using dosages that were ineffective in isolation. The combination produced strong inhibition of cell growth (300). In the second set of experiments the same strategy was used, but now with four different agents: curcumin, soy isoflavones, resveratrol, and EGCG (the active ingredient in green tea). Once again the combination produced inhibition of cell growth at even lower dosages than used with the two-way combinations. The interpretation of the synergy was that the use of several supplements caused the suppression of multiple different growth pathways, which seems necessary given the multiplicity of the signals controlling tumor growth.

Skeptics of supplements/dietary components such as those discussed above have argued that the laboratory studies providing evidence for their anti-cancer effects have used

dosages that can never be achieved in human patients, and therefore the supplements are unlikely to be useful clinically. Without a study of the dose-effect relations in clinical settings there is no easy way to evaluate this concern. However, in several cases investigators of the various substances have noted that their effects in the laboratory were obtained with dosages comparable to what easily can be realized by dietary supplementation, and in several cases there is direct clinical evidence supporting its use. In any event, for most of what has been discussed there is little if any risk to using the supplements, with the only cost being financial in nature. Contrary to the concern expressed by many oncologists, the addition of supplements to standard treatment protocols generally do not interfere with the standard treatment, but make the treatment more effective (301).

Promising New Treatments

The above discussion focuses on ways to improve the efficacy of the Stupp protocol, the gold standard of treatment for newly diagnosed glioblastoma patients. While a variety of changes and/or additions to the protocol seem promising, none has obtained general acceptance. An alternative strategy for newly diagnosed patients is to enroll in clinical trials. While new treatment agents studied for the first time in clinical trials are unknown quantities, some have preliminary outcome data that can help the patient's decision. Many of the clinical trials also test the new treatment in combination with the gold standard rather than as single agents alone. When I was diagnosed 20 years ago, few clinical trials seemed promising. Now, however, many more seem likely to be an improvement over the current gold standard.

8. Immunological Approaches

Because cancer cells have a genetic structure different from normal cells they generate foreign proteins that in principle should be detected by the immune system and evoke the same type of immune reaction as any foreign virus or bacteria. This basic fact suggests that augmenting one's immune system might be an effective approach to cancer treatment. Such an approach has an immediate appeal because it is surely preferable to reinforce the immune system than to poison the entire body in the hope the cancer cells will be killed before the body is depleted of vital resources. However attractive this philosophy may be, translating it into an effective cancer treatment has proven to be extraordinarily difficult. Contrary to general belief, immunological treatments are not

benign to implement. Interferon treatment has very definite debilitating effects, as do cytokines such as interleukin-2 and tumor necrosis factor, because their modus operandi are essentially to create an inflammatory immune reaction not unlike a severe allergic reaction. When this inflammatory process is too severe, it can in fact be fatal.

Vaccines

The holy grail of immunological approaches to cancer treatment is the development of effective vaccines. In principle this should be possible because of the differences in the protein structure of cancer cells and normal cells. But, two general problems must be overcome. The first is that different individuals have tumors with different collections of antigens (proteins), so that generic vaccines are unlikely to be effective; thus patient-specific vaccines are required. The second problem is that the immune system is not an efficient detector of the tumor's foreign antigens. In part this is due to the tumor secreting enzymes that in effect provide a protective cloak preventing such detection. The larger the tumor the stronger is its defense mechanisms to counteract immune-system detection. This is one reason that most vaccines work best when there is a minimum of tumor burden.

Personalized vaccines

DCVax and other lysate-pulsed dendritic cell vaccines

Methods to enhance the detection of tumor antigens are now the subject of intensive research, for various types of cancer. The most successful approach to date involves the use of dendritic cells, which have been characterized as "professional antigen-presenting cells". Dendritic cells are extracted from the blood, then co-cultured with a lysate prepared from cells from the patient's tumor, and stimulated with granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (GM-CSF is the growth factor used to counteract the decrease in white-cell blood counts due to chemotherapy). This growth factor causes the mixture of tumor and dendritic cells to be expanded as well. This mixture is then injected into the patient, evoking an increased reaction from the immune system.

This use of dendritic cells has been applied to several different types of cancers. Its use with brain cancer was pioneered by Dr. Keith Black and his team at UCLA, then continued at Cedars Sinai when Dr. Black's team moved to that institution. A separate program at UCLA was continued by Dr. Linda Liau. Other centers using this approach are in Belgium, China, and Japan. In one of the first small clinical trials (149) nine newly

diagnosed high-grade glioma patients received three separate vaccinations spaced two weeks apart. Robust infiltration of T cells was detected in tumor specimens, and median survival was 455 days (compared to 257 days for a control population). A subsequent report (150) involving 8 GBM patients produced a median survival time of 133 weeks, compared to a median survival of 30 weeks of a comparable set of patients receiving other treatment protocols. At two years 44% of patients were progression free, compared to only 11% of patients treated with the gold standard of Temodar during radiation and thereafter. An excellent review of the clinical outcomes and technical issues associated with the vaccine trials is provided by Wheeler and Black (151).

In the largest of the initial clinical trials (152), 34 GBM patients (23 with recurrent tumors, 11 newly diagnosed) were assessed for their immunological response to the vaccine using interferon production as the measure, with the result that only 50% of patients exhibited a response. The degree of response was moderately correlated with survival time: 642 days for responders, 430 days for nonresponders. Five of the 34 patients were alive at the time of the report, with survival times ranging from 910 to 1216 days, all of whom were classified as immunological responders. It should be noted that the average age of patients in this trial was 52 years, only slightly lower than the typical GBM population, whereas many of the other vaccine trials have included mainly younger patients.

Among the most promising results using lysate-pulsed dendritic cell vaccines has come from the UCLA research program led by Dr. Liau. In the most detailed report of the results (153) 15 newly diagnosed GBM patients and 8 patients with recurrent tumors (average age =51), received the initial dendritic vaccine (followed by three booster vaccines in combination with either POLY ICLC or imiquimod (applied locally to the injection site). For all patients, median time to progression was 15.9 months. Median survival time for newly diagnosed patients was 35.9 months, and 2- and 3-year survival rates were 77% and 58%. For recurrent patients, mean survival from the time of initial enrollment in the trial was 17.9 months. Subsequent reports have come from press releases from Northwest Biotherapeutics, the biotech company sponsoring the DCVax trials. Survival at four years has been 33 %, and 27% have exceeded six years (154). Currently underway is a large multi-center phase III trial.

As of July 2015, no outcomes from the phase 3 DCVax-L trial have yet been made public, though patient outcomes from an "informational arm" receiving DCVax-L were published by Northwest Biotherapeutics in March (see press release here). This informational arm consisted of 51 patients who had enrolled into the phase 3 trial, but were excluded from the trial due to early disease progression prior to the first vaccination. The patients received the DCVax injections and were followed up on a Compassionate Use basis. Survival outcomes in this group are summarized on a youtube video featuring Marnix Bosch, the company's Chief Technical Officer. Within this group of 51 patients was a

subgroup of 25 patients considered to be "indeterminate", meaning that they had evidence of disease progression at the baseline visit (rendering them ineligible for the trial), but subsequently had either stable disease, modest progression, or modest regression. This group of patients is reported to have a median survival of 21.5 months (the report does not make clear whether this is from surgery or from randomization post-radiation). As of March 2015, nine of these patients were still alive after 24 months of follow-up, six of these nine were alive after 30 months of follow-up, and four of these nine are alive at 35 to over 40 months. Therefore we can expect that median survival in the phase 3 trial (patients without disease progression at the baseline visit) will be at least greater than 21.5 months.

Agenus Prophage (heat-shock protein peptide complex-96) vaccine

A variation in the use of dendritic cells first subjected tumor tissue to a heat-shock treatment to elevate the expression of heat-shock proteins, which were extracted from the blood and incubated with dendritic cells from individual patients. In a clinical trial (163) conducted at UCSF and Columbia for patients with recurrent heavily pretreated tumors, the vaccine produced a median survival of 42.6 weeks (about 9.8 months), which compares favorably to the 6-month survival time for historical controls, and is comparable to the 9-11 months when Avastin is used with patients with recurrent tumors.

A subsequent news release from Agenus, Inc, a biotech company sponsoring the research, reported the results of phase II clinical trial in which the heat-shock dendritic vaccine was combined with the standard Stupp protocol (164). Median progression-free survival was 17.8 months and median survival was 23.8 months. ***This median progression-free survival of 17.8 months is perhaps the longest PFS yet seen in any substantially sized phase 2 trial for newly diagnosed glioblastoma.***

Follow-up data (reference 339, abstract 2011) presented at the ASCO 2015 conference revealed that patients with high PD-L1 expression (the ligand for the PD-1 immune checkpoint on the surface of immune cells which is the target for the therapeutic antibodies nivolumab and pembrolizumab) had a median survival of 18 months, while those with low expression of PD-L1 had a median survival of 44.7 months. This finding suggests that efficacy of the heat shock protein peptide vaccine could be greatly improved by co-administration of PD-1 antibodies such as nivolumab or pembrolizumab.

Tumor-associated antigen vaccines

ICT-107

One disadvantage of the DCVax approach is that it requires that brain tissue be extracted from individual patients in order to make the vaccine. An alternative approach has been used by Dr. Black's team at Cedars Sinai. Dendritic cells are still drawn from the peripheral blood of individual patients, but instead of tumor tissue lysate being mixed with those cells, a collection of six proteins typical of of GBMs is mixed with the dendritic cells, creating an immune response to those antigens, with the mixture then returned to the patient via vaccinations. In a phase I trial (158), 20 GBM patients (17 newly diagnosed, 3 with recurrent tumors) received three vaccinations two weeks apart. Median PFS was 16.9 months, and median overall survival was 38 months. At the time of the clinical trial report, six of the patients had shown no sign of tumor recurrence. A later follow-up was reported in a Press release from ImmunoCellular Therapeutics (159), the biotech company sponsoring the vaccine (now called ICT-107). Survival rate at three years was 55%, with 38% of patients showing no evidence of recurrence, The most recent update of the clinical trial (160), presented at the 2013 meeting of the World Federation of Neuro-oncology, reported that 7 of the original 16 patients in the trial were still alive, with survivals ranging from 60 to 83 months. One additional patient who was still tumor free after five years died from leukemia.

Currently ongoing is a randomized phase II trial, the interim results of which have recently reported by ImmunoCellular Therapeutics (161). Despite the impressive results described above, there was no statistically significant difference in median survival between the vaccine group and those treated with a placebo, although there was a numerical 2-3 month advantage for the vaccine group. However there was a similar difference in progression-free survival, which was statistically significant. The company emphasized that the results were preliminary and that they expected the difference in progression-free survival to translate into differences in overall survival with longer follow-up. However, the results also suggest that median survival and percentage of long-term survivors may be only weakly correlated due to the possibility that only a minority of patients benefit from the treatment, but those who do benefit a great deal.

Updated data from the phase II ICT-107 trial were presented on June 1, 2014 at the annual ASCO meeting (309). An important conclusion to be drawn from the new data is that mainly patients positive for HLA-A2 (a variant of the Human Leukocyte Antigen-A gene) seem to derive significant benefit from the vaccine. HLA are antigen-presenting proteins found at the cell surface. HLA-A2 is the most common variant in North America and Europe according to the press release and this group comprised 62% of patients randomized in this trial. The updated results are presented only for HLA-A2 positive patients, with results further subgrouped according to MGMT methylation status. Survival results in this trial are measured from the time of randomization after

chemoradiation, and average time from initial surgery to randomization was 83 days (2.7 months).

For HLA-A2 positive patients with *unmethylated MGMT*, the ICT-107-vaccinated group had a median 4-month survival advantage compared with the placebo-vaccinated group. The ICT-107 group also had a median 4.5 month advantage in progression-free survival. These advantages in the vaccine-treated group did not reach statistical significance, though that is perhaps due to the small numbers of patients within these subgroups. 21% of ICT-107 treated patients were still alive at the time of the analysis, compared with only 7% of the placebo-treated patients.

Median survival has not yet been reached in the HLA-A2 positive, *MGMT methylated* group, though in this subgroup, ICT-107 treatment led to a dramatic and statistically significant increase in median progression-free survival: 24.1 months versus 8.5 months in the placebo-treated group. It is likely that this huge improvement in median progression-free survival in this subgroup will translate into significant median overall survival improvement.

Sadly, in June 2017, Immunocellular Therapeutics announced that their phase 3 ICT-107 trial was suspending recruitment due to insufficient funding. In the press release it was stated the company was looking for a partner for collaboration or acquisition of its ICT-107 program, and they were also taking steps to ensure the continued follow up of patients already being treated in the trial.

SL-701

A similar approach has been used by Dr. Hideho Okada and colleagues at the University of Pittsburgh. In a pilot study using this approach with patients with recurrent tumors (162) several major tumor responses were observed. Median survival for the 13 GBM patients in the trial was 12 months, with several of the patients still progression-free at the time of the report. A later version of this therapy, called SL-701, consists of three shortened peptides corresponding to glioma-associated antigens and is now being tested in a phase I/II trial for HLA-A2 positive recurrent glioblastoma. NCT02078648

Dendritic cell vaccine targeting Cytomegalovirus (CMV)

This approach relies on the finding that most GBM tumors are infected with the cytomegalovirus, a common herpes virus. GBMs have a high incidence of the virus being

present (by some estimates over 90%) whereas normal brain cells do not. The new treatment approach involves targeting a specific protein component of the CMV virus, which then kills the virus and the cell harboring it.

Results of a small trial for Duke's anti-CMV dendritic cell vaccine with or without preconditioning with an injection of tetanus/diptheria toxoid was published in Nature in March 2015 (320). There were 6 newly diagnosed glioblastoma patients in each arm. In the 6 patients treated with the vaccine but without tetanus/diptheria preconditioning, median progression-free and overall survival freom diagnosis was 10.8 and 18.5, not significantly better than historical controls. In the group of patients receiving preconditioning of the injection site with tetanus/diptheria, three of the patients were alive without disease progression at 44-47 months from diagnosis. A Wall Street Journal article published at the same time as the Nature study gave more up-to-date information, revealing that two of these longer-term survivors had died at nearly 5 and 6 years from diagnosis, while the remaining patient was still alive over 8 years from diagnosis. An update from the 2016 AANS conference revealed that this patient was still alive without tumor regrowth at 120 months (10 years). The purpose of the tetanus/diptheria booster is to improve migration of the dendritic cells to lymph nodes. Despite the striking success of the anti-CMV dendritic cell vaccine combined with a tetanus/diptheria booster injection, a *randomized* phase 2 trial is scheduled to open in 2015 with one arm randomized to receive the tetanus/diptheria toxoid preconditioning, and the other arm randomized to receive saline (essentially placebo). Both arms receive the anti-CMV dendritic cell vaccine (trial NCT02366728).

A second single-arm phase II trial (ATTAC-GM) combined dose-intense temozolomide (100 mg/m2 for 21 days of a 28 day cycle) with anti-CMV dendritic cell vaccine and tetanus preconditioning. Median progression-free and overall survival for the 11 patients was a remarkable 25.3 and 41.1 months. This data was presented at the 2016 annual AANS meeting by Kristen Batich.

A separate trial (NCT00626483) at Duke for newly diagnosed glioblastoma is testing the CMV-targeted dendritic cell vaccine in combination with basiliximab, a CD25 antibody intended to inhibit the regulatory T-cell (Treg) population. In an abstract published for the ASCO 2015 meeting, we can read that in a pilot study of seven patients treated with this combination therapy, median progression-free and overall survival was an impressive 23.5 and 30.3 months respectively.

Currently recruiting clinical trials testing CMV pp65 vaccines with or without tetanus/diptheria preconditioning or basiliximab include the ELEVATE trial at Duke University (NCT02366728), the PERFORMANCE trial also at Duke (NCT02864368), the ATTAC-II trial at the University of Florida (NCT02465268), and the AVERT trial for recurrent grade III glioma and GBM at Duke University (NCT02529072).

Rindopepimut: anti-EGFR variant III (EGFRvIII) vaccine

A very different approach to developing a treatment vaccine, which has the virtue of being usable "off-the-shelf", without modification for individual patients, targets a mutation of the epidermal growth factor receptor, known as variant III, which occurs in 25-40% of GBMs. One reason that EGFR inhibitors such as Iressa have not been more effective is that they target the normal EGFR receptor, not this mutated receptor. EGFR variant III is also rarely seen in anything other than GBM tumors. To be eligible for the trial, patients must first be tested whether they possess the mutation.

Disappointing news was delivered by Celldex in a press release dated March 7, 2016, when the company announced that the phase III ACT IV clinical trial of rindopepimut for newly diagnosed glioblastoma patients with minimal disease would be discontinued, after an independent review board found that the trial was unlikely to meet its primary endpoint (improved overall survival). Although survival results were consistent with previous phase II trials, the control arm in this trial had survival outcomes that were better than expected (median overall survival was 20.4 months in the rindopepimut arm and 21.1 months in the control arm, hazard ratio = 0.99).

Rindopepimut is also being tested in a randomized phase II trial for recurrent glioblastoma called ReACT, in combination with Avastin. Data presented at the ASCO 2015 meeting showed that the primary endpoint of the trial (six month progression-free survival) was met. PFS-6 was 30% in the rindopepimut + Avastin arm, versus 12% in the control arm (per protocol). Additional data (reference 340, abstract IMCT-08) was presented later in 2015 at the SNO meeting, where it was reported that overall survival was also significantly improved and 2-year survival was 25% for the rindopepimut arm versus 0% in the control arm. Patients receiving rindopepimut had also reduced dependency on steroids, as 33% of patients were able to cease steroid treatment for six months or longer, versus none in the control group.

While the development of Rintega (rindopepimut) as a first-line treatment for GBM is unlikely to continue given these trial results, the therapy still holds promise combined with Avastin in the recurrent setting, according to the outcomes of the ReACT trial.

Wilms Tumor 1 (WT1) peptide vaccine

In March of 2015, a Japanese group published results of a trial that tested a Wilms tumor 1 (WT1) peptide vaccine in addition to radiation and chemotherapy for newly diagnosed glioblastoma (341). Wilms Tumor 1 (WT1) is a protein overexpressed in various types of solid and liquid cancers, not to be confused with the pediatric cancer it is named after (Wilms' Tumor). Seven patients were included in the analysis, with four having undergone total tumor resection, two having partial resection, and one having biopsy only. None of the tumors were positive for IDH1 mutation. Patients received up to 24 monthly temozolomide cycles, the standard at this institution. Remarkably, five of these seven patients (71%) were still disease-free at three years or more. Only one patient had experienced disease progression at the time of analysis and all were still alive. Median progression-free and overall survival were at least 43.5 months (about 3 and a half years), which was the median follow-up time at the time of the analysis. These very impressive results were not likely simply due to the prolonged cycles of temozolomide: at the same institution, median PFS and OS with up to 24 cycles of TMZ (but no vaccine) is 10.7 and 21 months.

An abstract (reference 342, <u>abstract IMCT-09</u>) for the SNO 2015 meeting tells us that median progression-free survival for the seven patients is now over 48 months (4 years) as five of seven patients were still without progression at that time point.

Vaccine adjuvants: Poly-ICLC

A generalized immunostimulant with minimal toxicity is Poly-ICLC, a double-stranded RNA, which initially was developed to induce the body to produce its own interferon, but is now believed to have a variety of immune-system enhancement effects, including de-activating an as yet unknown tumor suppressor mechanism of the immune system. These latter effects apparently only occur at low doses and are suppressed by high doses of Poly-ICLC. Its initial results for AA-III tumors were exceptional: the initial clinical trial with Poly-ICLC (in combination with CCNU for about 1/2 of the patients) reported that all but one patient with AA-III tumors were alive with a median follow-up time of 54 months (145). It was less effective for glioblastomas, with a median survival time of 19 months (but note that this too is greater than the standard treatment). There were minimal side effects except for a mild fever early in treatment (145). However, a more recent multi-center clinical trial with recurrent AA-III tumors produced less impressive results (146), as the initial cohort of patients had a PFS-6 value of only 23%. Note, however, that the latter study involved patients with recurrent tumors while that of the earlier study involved patients after initial diagnosis.

Two trials using Poly-ICLC with newly diagnosed glioblastoma patients recently have been reported. In the first, Poly-ICLC was given in combination with standard radiation, followed by its use as a single agent (147). No chemotherapy was given. One-

year survival was 69% and median survival was 65 weeks (about 15 months). Both values are superior to historical studies using only radiation without chemotherapy. In the second study with 83 newly diagnosed glioblastoma patients (148), Poly-ICLC was combined with the standard temozolomide + radiation protocol. For 97 patients median survival was 18.3 months with a 2-year survival rate of 32%. Thus, the addition of Poly-ICLC increases survival by several months, relative to the standard protocol, notably with minimal additional toxicity.

The fact that immunological treatments have produced at least some degree of success is encouraging, and highlights the need to strengthen the patient's immune function as much as possible. The effects of melatonin and mushroom extracts such as PSK presumably are due at least partly to such strengthening, and therefore should be generally useful.

Immune checkpoint inhibitors (drugs targeting CTLA-4, PD-1, PD-L1 etc.)

Another immunotherapy approach involves the combination of two new immunological agents, ipilimubab (Yervoy) and nivolumab (Opdivo), which have produced unprecedented clinical efficacy in the treatment of metastatic melanoma, one of the most intractable of all malignancies. For patients using the combination at the highest dose, 53% had tumor regression, all with a reduction of 80% or more (176). This treatment protocol is now being tested with multiple different forms of cancer, including glioblastoma.

At the 2015 annual meeting of ASCO, outcomes of 20 recurrent GBM patients treated with either nivolumab (3 mg/kg) or nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg) were reported (download poster here). In the nivolumab arm, no patients discontinued treatment due to toxicity, while in the combination arm, 3 out of 10 patients discontinued treatment due to drug toxicity.

Further information on this trial was given in conjunction with the ASCO 2016 annual meeting. Three treatment groups are now comparable: nivolumab alone (n=10), nivolumab 1 mg/kg plus ipilimumab 3 mg/kg (n=10), and a third previously unreported group receiving nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (n=20). Stable disease or response was achieved in 6/10 (60%) of patients in the group receiving nivolumab alone, 4/10 (40%) of patients receiving nivolumab plus ipilimumab (1 and 3 mg/kg), and in 9/20 (45%) of patients receiving nivolumab plus ipilimumab (3 and 1 mg/kg). 12-month overall survival rate for these same 3 groups was 40%, 30% and 25%. According to a news release, median survival for the three groups was 10.5, 9.3 and 7.3 months. On the

basis of these results, adding ipilimumab to nivolumab therapy does not improve the response, and adds to patient toxicity.

In early April 2017 Bristol-Myers Squibb, the maker of nivolumab, announced in a press release that their phase 3 Checkmate-143 trial failed to meet its primary endpoint, which was improved overall survival with nivolumab versus bevacizumab (Avastin) monotherapy. An abstract published for the May 2017 World Federation of Neuro-Oncology Societies provided further details. 182 recurrent glioblastoma patients received nivolumab treatment and 165 received Avastin. Median overall survival from trial entry was 9.8 months with nivolumab versus 10 months with Avastin, and 12 month survival rate was 42% in both arms. Median progression-free survival was 1.5 months with nivolumab versus 3.5 months with Avastin. Response rate was 8% for nivolumab versus 23% for Avastin. However, for the responding patients, responses were more durable with nivolumab - 11.1 months median versus 5.3 months for Avastin. Though disappointing, these results are perhaps not surprising given previous reports of low response rates (1 out of 10 patients responding to nivoluamb alone in an earlier phase of the same trial). Hopefully future trials involving checkpoint inhibitors such as nivolumab will enroll specific patient groups deemed more likely to respond to checkpoint inhibitors, such as those with hypermutated recurrences (NCT02658279), or give these therapies in combinations with other agents, rather than as monotherapies.

Hyperprogression following anti PD-1/PD-L1 therapy

In late 2016, a somewhat disturbing study (358) appeared showing that in a minority of patients treated with antibodies targeting the immune checkpoints PD-1 or PD-L1, these treatments can lead to accelerated disease progression, defined as an increase in tumor growth rate of at least 2-fold that of the pre-treatment period. This study included all patients treated at Gustave Roussy Institute in phase 1 trials testing anti PD-1 or PD-L1 antibody monotherapy, between December 2011 and January 2014. Of 131 patients evaluated, 12 (9%) were found to have hyperprogressive disease following therapy. For the twelve patients that responded to PD-1 or PD-LI antibodies with hyperprogressive disease, tumor growth rate increased by a median of 20.7-fold. Although no biomarkers were indentified that could predict which patients might respond with hyperprogression to these treatments, it was observed that hyperprogressors were older on average than non-hyperprogressors (average age of 66 versus 55, p=0.007).

Several months after the Gustave Roussy Institute publication, another group published a report attempting to identify the genomic alterations associated with the hyperprogression reponse to immune checkpoint inhibitors (antibodies against CTLA-4, PD-1, or PD-L1). This study included 155 patients with stage IV cancers treated with checkpoint inhibitors. Significantly, four out of five patients (80%) with MDM2 amplified tumors had hyperprogression following anti PD-1 or PD-L1 monotherapy. Two out of ten

patients (20%) with EGFR alterations (presumably amplification or mutation) had hyperprogression, and EGFR alterations were independently associated with short time to treatment failure on checkpoint inhibitors. Eight out of 10 patients with EGFR alterations had time to treatment failure (TTF) less than 2 months.

The authors concluded with the warning "In summary, our observations suggest that patients for whom anti-PD1/PDL1 monotherapy is planned may require genomic testing to determine whether their tumors harbor specific alterations associated with hyperprogression." Specifically, those found to have MDM2 amplification in their tumors will do well to approach these therapies with great caution.

T-cell therapies

Chimeric Antigen Receptor T-cell therapy

Chimeric antigen receptor (CAR) T-cells are T-cells that have been genetically engineered, commonly by the use of a retroviral vector, to express artificial receptors specifically targeted to a chosen tumor-associated or tumor-specific antigen. CAR T-cells directed to CD19 have been used with impressive success for B-cell acute lymphoblastic leukemia (ALL), and tisagenlecleucel, an anti-CD19 CAR T-cell therapy was approved for this indication on August 30, 2017. For glioblastoma, phase 1 CAR T-cell trials are currently active, with preliminary outcomes now published for two of these trials, as discussed below.

EGFRvIII-directed CAR T-cells

In 2014, a phase 1 safety and feasibility study began recruiting at the University of Pennsylvania testing treatment with autologous CAR T-cells redirected to the mutant EGFRvIII protein for patients with EGFRvIII-positive recurrent glioblastoma. Of 369 glioblastoma patients, 21% tested positive for the EGFRvIII mutation. Fourteen patients underwent leukapheresis to obtain T-cells and 10 were finally infused with EGFRvIII CAR T-cells. The outcomes and observations based on these 10 cases are described in a publication appearing in July 2017 (359).

Due to the uncertainty of interpreting MRI images in the context of immunotherapy, the study focused on CAR T-cell trafficking into tumors and their effects there, rather than response rates based on MRI imaging. Without the ability to directly image CAR T-cells in the brain, the study relied on the examination of resected tumor tissue from seven of the ten patients. Four of these patients had early progression before T-cell infusion, and

underwent surgical tumor resection within 14 days after T-cell infusion. Another three patients underwent "late" surgeries 34 to 104 days after T-cell infusion, due to suspected recurrences based on MRI imaging. Therefore early and late time points were available for analysis of CAR T-cell trafficking into the tumor.

The trial met the primary endpoint of safety and feasibility, as successful manufacture of the target dose of CAR T-cells and successful engraftment in peripheral blood was achieved for all patients, despite their being heavily pretreated with prior chemotherapy. No dose-limiting toxities were observed, although two patients were treated with the anti-IL-6 antibody siltuximab due to suspected intracranial cytokine release.

In the four patients who had early resection within two weeks after CAR T-cell infusion, CAR T-cells were detected in tumor tissue, with greatly increased prevalence in the tumor compared to the blood in two of the patients (3 and 100x higher concentrations in the brain versus peripheral blood). In the three patients with late resections (one to three months) following T-cell infusion, CAR T-cells were only detected in one case, at lower levels than in the blood. Five of seven post-infusion resected tumor samples showed a mean decrease in EGFRvIII expression compared to the paired pre-infusion samples. Some tumor samples showed robust infiltration of T-cells and CAR T-cells post infusion, with more activated CD8+ cytotoxic T-cells, although in all tumors with detectable CAR T-cells, the infiltration was patchy and inconsistent.

Unsurprisingly, tumors adapted to the CAR T-cell therapy by causing upregulation of immunosuppressive mechanisms and proteins such as IDO1, PD-L1 and infiltration of immunosuppressive T-regulatory cells.

In terms of clinical outcomes, three of the ten patients were still alive at approximately 100 days, 200 days, and > 18 months from infusion. This last patient was without progression and still on-study. By Kaplan-Meier estimate, median survival of the 10 patients from infusion was 251 days (just over 8 months), which is perhaps better than expected considering that eight of the 10 patients were on their 3rd or 4th line of treatment (at second or third recurrence), and nine of the 10 patients had multifocal disease, with the remaning patient having a deep-seated thalamic/midbrain tumor. This cohort thus consisted of patients with rather poor prognosis at baseline. Especially impressive is the one case with >18 month progression-free survival.

The authors rightly concluded that due to the heterogenous expression of EGFRvIII by tumors, and the increasingly immunosuppressive microenvironment created by tumors post-CAR T infusion, future therapies and trials will require targeting of multiple antigens to prevent antigen escape and combination therapies to counteract the increased expression of immunosuppressive molecules. Combination therapies could include the use of drugs targeting IDO1 and/or PD-1/PD-L1 antibodies.

IL13Rα2-targeted Chimeric Antigen Receptor T-cells (CAR T-cells)

A remarkable case study of a 50 year old recurrent glioblastoma patient treated with chimeric antigen receptor (CAR) T-cells targeted to the interleukin 13 receptor alpha 2 (IL13Rα2) was published in late 2016 by the City of Hope trial investigators (360). This patient suffered from an aggressively recurrent GBM, with multifocal leptomeningeal metastasis, indicating a poor prognosis. The patient was at this time enrolled into the IL13Rα2 CAR T-cell study and received 6 infusions of T-cells into the resection cavity following resection of three of five intracranial tumors. With these weekly intracavitary infusions, the primary tumor site remained stable, while several new tumors appeared, including two in the spine, and the unresected tumors continued to progress. At this point the patient was enrolled onto a compassionate-use protocol in order to receive intraventricular infusions of the CAR T-cells, with the reasoning that such infusions would improve T-cell trafficking to the distant sites of multifocal disease. Upon treament with five intraventricular infusions, all tumors had decreased by 77% to 100%, and after 10 infusions no tumors were detectable by MRI or PET imaging, including the metastatic spinal tumors.

After 7.5 months and 16 infusions of CAR T-cells, the patient unfortunately suffered a relapse of disease with four tumors appearing at new locations. These new tumors had decreased expression of IL13Rα2, perhaps allowing their escape from the T-cell therapy, and this points out the need for immune therapies that target multiple antigens rather than a single antigen alone.

9. Antibody-Drug Conjugates and other protein-drug conjugates

ABT-414

ABT-414 is an antibody-drug conjugate targeting EGFR overexpressing cells. ABT-414 consists of an antibody (ABT-806) linked to a microtubule-targeting cytotoxin, called monomethyl auristatin F. The antibody binds to EGFR only when it is in the active conformation, thus selectively targeting EGFR expressing tumor cells and minimizing effects on healthy tissue.

Safety, dose-finding, and efficacy results of a multicenter phase 1 study were published late in 2016 (361). This phase 1 trial did not require patients to have EGFR amplification,

and only 39% of 38 patients were found to have EGFR amplification in their tumors. Toxicities of the eye, especially blurred vision, were the most common side-effects, occuring in 89% of the 45 patients.

In exploratory analysis for antitumor activity, median progression-free survival (PFS) for all patients (n=45), or for patients in the expansion cohort only (n=16) was 6.1 months for both. Median PFS for all EGFR amplified patients (n=15) was similar, at 5.9 months. Median survival was not yet reached at a median follow-up of only 5.8 months.

The median progression-free survival observed in this study does not reflect an improvement upon standard treatments, though the authors warn that "*Caution should be used in evaluating the efficacy parameters in this phase I study, given the variable doses administered, number of discontinuations of study drug due to toxicities, small sample size, and numerous confounding prognostic factors that can contribute to PFS and OS measurements in this disease.*"

MDNA55

Medicenna's investigational drug MDNA55 is a fusion of a truncated form of interleukin 4 (IL-4) with the bacterial Pseudomonas exotoxin A. The toxin is only active within the cell following uptake via interleukin 4 receptors (IL-4R). Therefore this therapy targets cells with high IL-4R expression, which is common in tumor tissue and myeloid-derived suppressor cells, but rare in normal brain tissue, thus sparing healthy cells. The drug is infused directly into the tumor by Convection Enhanced Delivery.

Preliminary data released by Medicenna on their website shows the drug having surprising efficacy in early phase trials for recurrent malignant gliomas. In one trial, in which 25 recurrent glioblastoma patients were treated with a single infusion of MDNA55 and no resection, response rate was 56%, complete response rate was 20% and response + disease stabilization rate was 68%. These response and complete response rates are among the highest reported for recurrent glioblastoma, where response rates are typically under 20% and complete response rates under 5%.

10. Oncolytic virotherapy

Genetically modified Poliovirus (PVS-RIPO)

In 2015, this phase I trial for recurrent glioblastoma at Duke University received a boost in public interest when an episode of the television show 60 minutes was devoted to it. Most exceptionally, the first two patients treated in this study were complete responders. As of March 2015 (when the 60 minutes special aired) these two complete responders were still alive and progression-free at 33 and 34 months from treatment. 11 of 22 patients in the trial were still alive, though six of these patients were less than 6 months from treatment. Importantly, dose escalation of PVS-RIPO failed to improve efficacy, and the more recent patients in the trial are being treated with a smaller dose than the trial originally started with. Read an interview with Darrel Bigner discussing this trial here.

DNX-2401 adenovirus

Another viral therapy in phase 1 has had impressive results, comparable to the PVS-RIPO trial. DNX-2401 is a modified adenovirus that is directly injected into the tumor. Preliminary results of a phase 1 trial at MD Anderson in Houston, Texas were presented at the November 2014 SNO conference in Miami. 37 recurrent high-grade glioma patients had been treated, with no adverse events attributable to the virus being reported. 3 of 25 patients responded to the treatment with complete, durable responses of 42, 32, and 29 months so far. These three complete responders had vigorous immune responses, with 10-1000 fold increased levels of interleukin-12p70, a cytokine with great importance for type-1 anti-tumor immune responses.

Newcastle Disease Virus

An alternative approach to vaccine treatment utilizes viruses. Newcastle disease is a lethal chicken disease, which is caused by a virus that is innocuous to humans, causing only transitory mild flu-like symptoms. It was developed as a cancer treatment in Hungary but has largely been ignored in this country until only recently. Newcastle Disease Virus is currently being utilized in combination with autologous dendritic cell vaccines by the IOZK clinic in Köln (Cologne) Germany.

Herpes Virus

Another virus used in cancer therapy is a modified form of the herpes virus. Initial trials used a retrovirus version, which infects only those cells dividing when the virus was infused. Subsequent trials have used an adenovirus version, which infects both dividing and non-dividing cells. Because the herpes virus can be lethal to the brain if allowed to proliferate, soon after the virus infusion patients receive ganciclovir, an effective anti-herpes agent. In one study using this technique performed at Mt. Sinai Hospital in

New York (170), median survival of 12 patients with recurrent GBM tumors was 59 weeks from the point of treatment, with 50% of the patients alive 12 months after the treatment. The authors also reported the absence of toxicity from the treatment, which was a major concern due to significant brain damage when the procedure was tested with monkeys. Why the difference from the monkey study's results is unclear.

More recent research with the herpes virus has been focused on forms of the virus that have been engineered to retain the anti-cancer effects of the virus but without its property of producing neurological inflammation. The first use of this modified virus in a clinical trial was in Glasgow, Scotland. Nine patients with recurrent glioblastomas received the virus injected directly into the tumor. Four were alive at the time of the report of the study, 14-24 months after the treatment (171).

Parvovirus (with bevacizumab)

An abstract presented at the SNO 2015 annual meeting (reference 343, abstract ATNT-07) gave some very encouraging preliminary results from the German phase I/II trial of parvovirus H-1 (ParvOryx) for recurrent glioblastoma (NCT01301430). The abstract reports on six patients in this trial who receieved a second dose of parvovirus combined with Avastin following recurrence after initial treatment on trial. This second injection of parvovirus was based on a compassionate use agreement. These six patients had tumor recurrence between 4 and 12.6 (mean 7.8) months after the first parvovirus injection. They underwent re-resection and a second injection of parvovirus, followed by chemotherapy (n=1), Avastin (n=5), or no additional treatment (n=1). Mean survival of these patients after second injection of parvovirus was 14.7 months (range 9-26 months). Three of the patients were still alive at 9, 11, and 26 months after this second injection of parvovirus. For the five patients treated with Avastin following second parvovirus injection, results were even better: 3 of 5 showed "striking remissions" with a censored mean survival of 15.4 months, very impressive for patients at second or more recurrence of glioblastoma.

11. Gene therapy

Toca 511 / TocaFC

Toca 511 is an engineered form of murine leukemia virus which delivers a specific gene to tumor cells, which then induces the tumor cells to make an enzyme named cytosine

deaminase (CD). After the vector spreads throughout the tumor, patients receive a course of oral 5-FC, a prodrug of the common chemotherapy agent, 5-FU. The CD gene converts the 5-FC to 5-FU, thus killing the cancer cell. Rodent model data with this approach have been extremely impressive. The first human trials of the drug have begun enrolling patients in multiple treatment centers. Data from two phase 1 ongoing clinical trials have been published by the company (view press release here). Several partial responses have been seen, though the dramatic efficacy seen in the rodent studies has not scaled up to human patients thus far. A randomized phase II/III trial of this therapy for recurrent GBM or anaplastic astrocytoma began in late 2015 (NCT02414165).

In June of 2016, results of the phase I trial of Toca 511/TocaFC for recurrent high grade glioma patients undergoing surgery (NCT01470794), with Toca 511 directly injected into the resection cavity, were published in Science Translational Medicine (344). A total of 43 patients were evaluable for efficacy, including 35 with glioblastoma, and the remaining eight were anaplastic astrocytoma patients. For the entire group of 43, there were 2 complete responses (both in anaplastic astrocytoma patients), 2 partial responses (both in glioblastoma patients), for a response rate of 9.3%. Disease stabilization rate was 18.6%, and clinical benefit rate (responses plus stabilizations) was 27.9%. It must be kept in mind that this was a dose escalation trial, and that many of the patients were likely treated at sub-optimal doses of Toca 511. A clinical benefit rate of 36.7% was observed in the high dose Toca 511 cohort. 13 patients treated with lower doses of Toca 511 had a median survival of 11.8 months, while 30 patients treated with higher doses of Toca 511 had a median survival of 14.4 months from the trial start.

The trial was open to patients with any number of prior recurrences. Median survival for the 27 glioblastoma patients at first or second recurrence was the same as for the entire group of 43 (including anaplastic astrocytoma patients): 13.6 months. Results for the 27 glioblastoma patients at first or second recurrence were compared with results for a matched contemporary control group receiving lomustine (CCNU) as part of a phase III trial. Median survival from the start of treatment for recurrence was 7.1 months for this contemporary control group, and the longer survival (median 13.6 months) for the patients in the Toca 511 trial was statistically significant (HR=0.45, p=0.003). There were also fewer serious adverse events and an absence of hematologic toxicity in the Toca 511 group compared to the contemporary controls receiving lomustine.

All phase I trials of Toca 511/TocaFC for glioma are now closed to further recruitment, a new randomized phase II/III trial for recurrent high grade glioma is now recruiting (NCT02414165), and a phase I trial for newly diagnosed high grade glioma is not yet recruiting (NCT02598011).

12. Photodynamic Therapy

When brain tumor cells absorb a molecule named hematoporphyrin (and other photo-sensitizers), exposure to high-intensity laser light will kill the cells. A treatment based on this rationale has been developed in Australia, used there and in some places in Europe, but not to my knowledge in the United States. Early results with this approach were not impressive but the most recent report of clinical trial results with patients with newly diagnosed high-grade gliomas indicates greater success. For patients with AA- III tumors median survival was 77 months while that for glioblastoma patients was 14 months (222). More impressive were long-term survival rates, as 73% of grade III patients survived longer than 3 years, as did 25% of glioblastoma patients. Also impressive were the results for patients with recurrent tumors. Median survival was 67 months for AA-III patients and 14.9 months for GBM. Forty-one percent of patients with recurrent GBM survived beyond 24 months, and 37% beyond 36 month. However, a review (223) of six different clinical trials using the procedure indicated wide variability in outcomes, with an aggregate median survival for newly diagnosed GBM of 14.3 months and for recurrent GBM tumors of 10 months. The treatment was reported to have minimal toxicity.

More positive results have come from a Japanese study using a new photo-sensitizer named talaporfin sodium (224), followed by the standard Stupp protocol. For 13 patients with newly diagnosed GBM, the median PFS was 12 months and the median overall survival was 25 months, a substantial improvement over the result obtained with the Stupp protocol used alone.

An abstract presented at the SNO 2015 annual conference by a Japanese team provided further details of this phase II trial of photodynamic therapy for malignant glioma carried out between 2009 and 2012 (reference 345, abstract ATCT-24). The trial included 27 patients, including 13 with glioblastoma. Median survival of 31.5 months and median progression-free survival of 19.6 months was reported, though in fairness, results for the grade 3 glioma patients should be given separately from the GBM results. More significantly, tumor resection and photodynamic therapy for 16 recurrent glioblastoma patients led to a one-year survival rate of 77.1% and median survival of 13.8 months, comparing very favorably with other trials for recurrent glioblastoma. However, only patients with resectable surface tumors would be eligible for such treatment.

13. Treatments for Recurrent Glioblastoma

The unfortunate nature of glioblastoma tumors is that they typically recur. When the gold

standard Stupp protocol is used as the initial treatment, the median progression-free interval before recurrence is detected is about 8 months from diagnosis. This means that the median patient will need to seek additional treatment sometime in the first year after his/her diagnosis.

As noted above, there are three treatments that have FDA approval for the treatment of recurrent GBM: Avastin, gliadel, and the Novocure TTF device. However these do not exhaust the possibilities, as additional chemotherapy, including a rechallenge using Temodar itself, are also used. Indeed, all of the treatments discussed above for newly diagnosed patients can be used in the recurrent setting as well. The question for the patient is which to choose to optimize the chances of survival.

Avastin (bevacizumab)

Currently, the most frequently used treatment for recurrent GBM is Avastin (bevacizumab), the anti-angiogenic drug that is widely used in many different forms of cancer. In the earlier section on additions to the Stupp protocol for initial treatment, Avastin was considered as one possible addition, but two different clinical trials failed to show any improvement in survival outcome relative to the Stupp protocol alone followed by Avastin used only after recurrence has been detected. In this section we discuss the results of Avastin as a treatment for recurrent tumors. Its first use with brain tumors was reported at a 2005 European Neuro-oncology conference (177). Avastin at a dose of 5 mg/kg was given every two weeks to 29 patients with recurrent tumors (apparently including both glioblastomas and grade III tumors), followed by weekly infusions thereafter. Patients also received CPT-11 (irinotecan) concurrently with Avastin. Tumor regressions occurred for a high percentage of patients, with 19 patients having either complete or partial regressions, some of which were evident after the first course of treatment. Long-term survival data were not mature at the time of the report. Avastin does increase the risk of intracranial bleeding, but in the aforementioned clinical trial, this occurred for only 1 of the 29 patients.

Since the initial study just described, additional studies has been reported. The largest of these, performed at Duke University (178), involved 68 patients with recurrent tumors, 35 of whom had glioblastomas. For those, the PFS-6 was 46% and median survival was 40 weeks. The latter number is disappointing given that a high percentage of patients had tumor regressions early in treatment, although the 10-month survival for GBM patients after recurrence compares favorably to the typical value of 5-7 months, as shown by a retrospective analysis (179). From the other reports a similar pattern emerged: high response rates in terms of tumor regression, but then often a rapid regrowth of the tumor thereafter. A longer-term follow-up of the Duke study reported a two-year survival rate of 17 % (180), not impressive in absolute terms but much better than the 0-5% 2-year

survival typical for recurrent tumors.

One concern about the use of Avastin is that several investigators have observed that its use results in a higher likelihood of the tumor spreading to brain locations distant from the original tumor site. This issue remains controversial, in part because distant tumor spread may occur for many different treatments, not just those that rely upon the inhibition of angiogenesis.

Avastin, like other drugs, typically is given until tumor progression. However, a report at the 2012 meeting of ASCO suggests this may not be optimal (182). Patients receiving Avastin for recurrent tumors until treatment failure (N=72) were compared to those who began Avastin but stopped for reasons other than tumor progression (N=18), either because they had completed a planned schedule, or due to toxicity. In the latter group, progression--free survival at 1 year was 83%, and the median progression-free interval was 27.6 months, much better than patients receiving Avastin until treatment failure (PFS-12 = 25% and Median PFS 9.7 months. Moreover, the former group was less likely to show an infiltrative pattern of recurrence.

An important issue is the efficacy of Avastin as a single agent without concomitant chemotherapy. In a large (N=167) randomized trial (183), Avastin alone was compared with Avastin + CPT-11 (irinotecan) in patients with recurrent glioblastoma. PFS-6 values were 43% for Avastin alone and 50% for Avastin + CPT-11; corresponding numbers for the percentage of tumor regressions were 28% and 38%. However, this outcome advantage for the combination group was offset by its higher rate of adverse events (46% vs. 66%). Moreover, median survival times were slightly in favor of Avastin as a single agent (9.3 vs. 8.9 months). A longer-term follow-up was reported at the 2010 ASCO meeting (184). Two-year survival rates were 16% and 17%, respectively. Overall, therefore, adding CPT-11 to Avastin appears to provide a marginal improvement in survival outcome, a benefit that must be weighed against the added toxicity.

The best results yet reported when Avastin has been used for recurrent tumors has come from its combination with hypofractionated stereotactic irradiation, based on the idea that Avastin prevents the re-vascularization that is required to repair the damage caused by radiation. Twenty patients with recurrent GBM received the standard bi-weekly Avastin infusions in combination with radiation during the first five cycles (187). Fifty percent of patients had tumor regressions, including five with a complete response. The PFS-6 value was 65% and median survival time was 12.5 months. Positive results were obtained in a second study (188) combining Avastin and stereotactic radiosurgery with heavily pretreated patients. The median PFS was 5.2 months for those receiving the combination versus 2.1 months for those receiving stereotactic radiosurgery alone. The corresponding results for overall survival were 11.2 months vs. 3.9 months.

The important question of whether to administer Avastin at first or later recurrences was addressed in a large retrospective study (310) published in the June 2014 edition of Neuro-Oncology (corresponding author Albert Lai, Department of Neurology, UCLA). A large cohort of 468 glioblastoma patients treated with Avastin was examined retrospectively, which included 80 patients treated with Avastin at diagnosis, 264 at first recurrence, 88 at second recurrence, and 36 at third or higher recurrence. Between all three recurrence groups (first, second, or third and higher), no significant difference was found in progression free survival on Avastin, overall survival from the start of Avastin treatment, or post-Avastin survival. In other words, treatment with Avastin led to "fixed" median progression-free and overall survival times from the start of Avastin, regardless of whether Avastin was started at early or later recurrences. The implication of this finding is that delayed use of Avastin may be preferable and lead to longer overall survival (from diagnosis) when administered at later rather than earlier recurrences. This idea remains to be tested in a prospective clinical trial.

This study also identified risk factors for the inability to receive further treatments at recurrence, thereby identifying patients who may benefit from earlier therapy with Avastin. The risk factors for inability to receive further treatment at first recurrence were: age over 60, and biopsy only. The one risk factor for inability to receive further treatment at second recurrence was age over 60. The conclusion of this study is that delayed use of Avastin is not associated with diminished efficacy and may even be preferable for those patients who can afford to delay such treatment until later progressions. On the other hand, older patients and patients with inoperable tumors, at risk of being unable to receive treatment at later recurrences, may benefit from earlier use of Avastin.

A study done at the MD Anderson Cancer Center reaching similar conclusions was published in the August 2014 edition of the Journal of Neuro-Oncology (311). This was a retrospective study including recurrent glioblastoma patients treated with Avastin between 2005 and 2011. 298 patient records were included in total, and divided into 112 patients treated with Avastin at first recurrence, and 133 patients treated with Avastin at second or higher recurrence. There was no significant difference in progression-free survival on Avastin between the two groups (PFS, 5.2 vs 4.3 months, p=0.2). In contrast, median overall survival from diagnosis was significantly shorter in the group treated with Avastin at first recurrence (OS, 20.8 vs 25.9 months, p=0.005). There was no significant difference in the time from diagnosis to first recurrence between the two groups. The authors conclude that delayed use of Avastin is not inferior to use of Avastin at first recurrence. The apparent improvement in overall survival in the patients receiving delayed, rather than early, Avastin requires testing in a prospective clinical trial.

In a post-hoc analysis based on results of the phase 3 AVAglio trial of upfront Avastin for newly diagnosed glioblastoma, patients who did not end up receiving further therapy at recurrence did have significant prolongation of both overall and progression-free survival with upfront Avastin (346). This finding suggests that the failure of upfront Avastin to

improve overall survival outcomes versus the placebo arm in the phase 3 trial was due to patients in the placebo arm receiving Avastin at recurrence. This finding corroborates the two studies mentioned above, in which delayed Avastin did not lead to inferior outcomes versus upfront Avastin, and also stresses that patients who will likely be unable to tolerate further therapy at recurrence (due to advanced age or general weakness etc.) may benefit from upfront Avastin in terms of both progression-free and overall survival. In this post-hoc analysis, considering only patients who did not receive further therapy at disease recurrence, upfront Avastin improved both median survival and progression-free survival by 3.6 months, and this improvement was statistically significant for both PFS (HR=0.62, p=0.0016) and OS (HR=0.67, p=0.01).

Lower dose Avastin

Except for the initial study by Dr. Stark-Vance, which used a dosage of 5 mg/kg, almost all other studies have used a dosage of 10 mg/kg every two weeks. A paper presented at the 2013 meeting of the Society of Neuro-oncology (181) suggests that the lower dosage may have better outcomes. Forty-eight patients who had received the 5 mg/kg dose were compared retrospectively to all of the remaining patients receiving the standard dose at the same institution. Median survival for the standard dose was 8.6 months, similar to the typical outcome. Median survival for the 5 mg/kg patients was 14 months, a notable improvement.

A second retrospective analysis concerned with different dosing regimens of Avastin included patients treated at Northern California Kaiser Permanente hospitals between 2008 and 2013 (347). During this time, the first author of the study (Victor A. Levin) was treating patients with Avastin doses of 7.5 mg/kg or lower every three weeks, less than the standard dose of 10 mg/kg every two weeks. 181 patients were included in this study and the median dose of Avastin for these recurrent patients was found to be 3.6 mg/kg per week. When patients were divided into those receiving less than or more than 3.6 mg/kg per week, patients receiving lower doses had longer median survival from the start of Avastin treatment than those receiving higher doses, 60 weeks versus 45 weeks, a significant difference (p=0.029). To control for the possibility of pseudo-progression, separate analyses were done for patients who progressed more than one month after the completion of radiotherapy, and more than three months after completion of radiotherapy. In all analyses, patients receiving less than the median Avastin dose of 3.6 mg/kg per week survived longer than patients receiving more than 3.6 mg/kg per week. The standard dose of 10 mg/kg every two weeks amounts to 5 mg/kg per week. This study therefore supports the use of Avastin doses less than the standard dose.

A third retrospective analysis published later in 2015 (348) by investigators in Tel Aviv, Israel compared outcomes of 87 patients given 5 mg/kg Avastin every two weeks with 75 patients given the standard dose of 10 mg/kg every two weeks. All patients were

diagnosed with recurrent glioblastoma. The majority of patients in the 5 mg/kg group (65.5%) were treated with a combination of chemotherapy (mostly irinotecan) and Avastin. A minority of patients in the 10 mg/kg group (20%) were treated with a combination of chemotherapy (mostly TMZ) and Avastin. No statistically significant difference in PFS or OS was found in the 5 mg/kg versus 10 mg/kg cohorts. There was also no significant difference in PFS or OS for patients treated with 5 mg/kg or 10 mg/kg Avastin as monotherapy (without additional chemotherapy). The small subgroup of 15 patients treated with a combination of 10 mg/kg Avastin plus chemotherapy had improved survival compared to the other groups (median survival from start of Avastin treatment 14.5 months, p=0.007). The improved survival in this small subgroup may have been partially due to this group having a significantly higher median KPS at baseline, as suggested by the authors, and may also be partially due to the superiority of temozolomide chemotherapy compared to irinotecan. This study agrees with previous retrospective studies that lower doses of Avastin are not inferior to the higher standard dose and can be administered with substantially reduced Avastin-related toxicities (proteinurea and hypertension).

One important effect of Avastin, and of other drugs that target VEGF, is that they reduce the edema common to brain tumors that is a major cause of the need for steroids. VEGF causes a large number of tiny leaky capillaries, which are pruned away when VEGF effects are blocked. Some have argued that the initial stage of blocking VEGF increases blood flow to the tumor, and hence makes it easier for chemotherapy agents to reach the tumor and be effective.

Baseline blood neutrophil counts predict efficacy of bevacizumab in recurrent glioblastoma

Based on prior preclinical reports that neutrophils in the blood may promote tumor angiogenesis, a French research group hypothesized that a high neutrophil count may predict better response to antiangiogenic therapy such as Avastin (bevacizumab) (362). To test this hypothesis, the records of 265 glioblastoma patients treated at their institute were reviewed. 159 of these patients received Avastin, mainly at recurrence, and the remaining 106 patients received no Avastin. Using the cutoff neutrophil count of 6000/mm3 (which may also be expressed as $6 \times 10^3/\mu L$ or $6 \times 10^9/L$), patients were divided into high and low baseline neutrophil count groups. In the high neutrophil count group, Avastin use was associated with significantly improved survival versus no Avastin, while in the low neutrophil group, Avastin use did not significatly improve survival. Within the group not receiving Avastin, high neutrophil count was associated with worse survival, whereas in the group receiving Avastin, this association was lost and patients with high neutrophil count had similar survival as the low neutrophil group. The neutrophil count at recurrence (as opposed to at baseline) was likewise found to predict the efficacy of Avastin in these same patients. Similar trends were seen in a validation

cohort of 120 patients with unresectable glioblastoma taking part in the TEMAVIR clinical trial.

In a series of 12 glioblastoma cases, expression of the gene CSF3 (which encodes granulocyte colony-stimulating factor, or G-CSF, a neutrophil growth factor) in the tumor bed was strongly correlated with neutrophil count. Glioblastoma data from The Cancer Genome Atlas also showed that CSF3 expression was linked to VEGFA-dependent angiogenesis. In the BELOB randomized clinical trial, high CSF3 expression was linked to better survival with lomustine + Avastin versus lomustine alone. In contrast, for those with low CSF3 expression, there was a non-signficant trend toward worse survival in those receiving lomustine + Avastin versus lomustine alone. This leads the authors to comment that high production of G-CSF (encoded by the CSF3 gene) by the tumors, leading to increased VEGFA-dependent angiogenesis, may be the cause for the observed association between high neutrophil count and response to Avastin, with neutrophil counts being a surrogate marker of high CSF3 expression.

Avastin combined with CCNU (lomustine)

A 3-arm randomized phase 2 trial called BELOB, conducted at 14 centers in the Netherlands, tested Avastin alone, or CCNU (lomustine) alone, or Avastin + lomustine combined for glioblastoma at first recurrence or progression following standard radiochemotherapy (312). Overall, the patient group receiving combined Avastin and lomustine had better median PFS, 6-month PFS rate, median overall survival and 12-month survival rate than either of the single-agent groups. When the data was separated out on the basis of MGMT methylation status, the patient group with methylated MGMT receiving combination therapy had a 6-month progression free survival rate that was about twice as high (62%) as the MGMT methylated groups given Avastin alone (33%) or lomustine alone (26%). Predictably, patients with unmethylated MGMT did poorly on lomustine alone (0% were progression-free at 6 months), and also did better with combined therapy (6-month PFS rate of 23% versus 8% with Avastin alone). The methylated group had an equal 9-month overall survival rate with combined therapy or Avastin alone (67%), while the unmethylated group had a better 9-month overall survival rate with combination therapy (58%). The combination treatment was well tolerated after an early dose reduction from 110 mg/m2 to 90 mg/m2. This study concluded with the interpretation that combined treatment with Avastin and lomustine was superior to single agent therapy and the combination is now being studied in a phase 3 EORTC trial (NCT01290939).

Angiotensin system inhibitors plus Avastin

The term angiotensin system inhibitor (ASI) refers to angiotensin-converting enzyme (ACE) inhibitors, such as captopril and lisinopril, and angiotensin II receptor blockers (ARBs), such as telmisartan and losartan.

Prompted by studies such as those reviewed above, Levin et al. performed a retrospective analysis of 1,186 diffuse glioma (grades 2-4) patients in the Northern California Kaiser Permanente Tumor Registry treated between 2007 and 2015 (363). Approximately 318 of these patients had also been treated with an ASI drug during treatment with chemotherapy and/or Avastin. In a multivariate analysis (adjusted for covariables such as age, GBM versus lower grade, extent of resection, and exposure to Avastin), the use of an ASI drug during chemotherapy and or Avastin was significantly associated with increased survival (hazard ratio 0.82 for ASI use compared to no use; p=0.003). This apparent benefit of ASI drug use was even more pronounced in patients also receiving Avastin (HR 0.75, p=0.002).

A smaller cohort of 181 recurrent GBM patients treated with Avastin (with outcomes previously published in a study concerned with low-dose Avastin and reviewed elsewhere in this document) was examined with attention to concurrent use of an ASI drug for hypertension. In the previous study, patients receiving less than the Avastin dose of 3.6 mg/kg/week had significantly better survival than those receiving more than 3.6 mg/kg/week (median 60 weeks versus 45 weeks, or 13.8 versus 10.4 months). The standard Avastin dose of 10 mg/kg every two weeks equates to 5 mg/kg per week, so patients in the lower dose Avastin group were receiving less than 72% of the standard weekly dose equivalent. In the follow-up study, lower dose Avastin, as well as use of an ASI drug were both associated with better survival (hazard ratio of 0.649 for those receiving an ASI drug). Most of the patients in the ASI exposure group were on ACE inhibitors (n=78), versus ARBs only (n=9), or both classes of drugs combined (n=15). Strikingly, for all those receiving low dose Avastin (n=89), those also receiving ASI drug treatment (n=47) had a median survival of 99 weeks (22.8 months) versus 55.6 weeks (12.8 months) for those receiving low dose Avastin but no ASI drug. *Survival is presumably measured from the first dose of Avastin for recurrence, as was the case in the prior publication on this same cohort. If so, this sizeable group of 47 patients receiving lower doses of Avastin while simultaneously on angiotensin system inhibitors for hypertension, suriving for a median of 22.8 months post-recurrence, is the most impressive statistic this writer has seen for recurrent glioblastoma.* All the same caveats apply to this study as for any retrospective study, but clearly low dose Avastin combined with angiotensin system inhibitors requires further investigation and prospective clinical trials.

Rechallenging with Temodar

When a treatment drug fails to be effective, or becomes ineffective with continued use, standard practice in oncology is to stop using the drug for that specific patient. However, a major exception to this general rule is to continue to use the drug but with a different schedule of presentation, usually with lower doses but given on a daily or more frequent basis. Part of the rationale for this approach is that continuous chemotherapy, even at low doses, will inhibit the growth of new blood vessels feeding the tumor (37, 38). The optimal dosage for this metronomic schedule of chemotherapy remains to be established because dividing blood vessel cells are more sensitive to chemotherapy than are dividing tumor cells, but they are also much quicker to recover when chemotherapy is removed, which implies that any recess from using chemotherapy will allow the blood vessels feeding the tumor to quickly regrow.

A German medical group administered very low-dose metronomic schedules of Temodar to 28 patients with recurrent tumors after initial treatment with the standard Temodar protocol (four had prior treatment with CCNU or PCV instead) (46). A twice-daily dose of 10 mg/square meter twice daily was given in combination with 200 mg of Celebrex. PFS-6 was 43% and median survival was 16.8 months from recurrence. Unlike the standard Temodar protocol, toxicity was virtually absent except for one patient who developed lymphopenia. An important feature of the metronomic schedule was that even after tumor progression was detected, patients could continue on the schedule for several months before the progression produced significant clinical problems. It also should be noted that a high percentage of patients (68%) had surgery for their recurrent tumors prior to starting the metronomic schedule of temozolomide. How much this, as well as the use of Avastin following progression on metronomic TMZ, contributed to the positive overall survival outcome is impossible to assess.

The positive results of the just-described clinical trial appears to be in conflict with a prior study that also used a metronomic schedule for 28 GBM patients with recurrent tumors after nitrosourea prior treatment; here the PFS-6 value was only 19%, and the median survival was 8.7 months (47). However, there were several important differences between the two studies. Most obvious was the use of Celebrex in combination with metronomic Temodar in the German study, and its use of a much lower dose of Temodar. In the second study, the daily dose was 75 mg/meter-squared, almost twice that of the German study. Patients in the second study were also given a hiatus from chemotherapy after 7 weeks of treatment. A critical feature of the metronomic schedule approach is that the chemotherapy agent be constantly present until the tumor finally regresses from starvation, as regrowth of the blood vessels feeding the tumor can occur very rapidly. Also important is that patients in the second study had different treatment histories.

The alternating weekly schedule has also been studied in patients who have recurred after the standard protocol. In a study done in Germany (35), patients with high-grade gliomas who had failed the standard protocol were given 150 mg/sq. meter on days 1-7 and 15-21

of a 28-day cycle. The PFS-6 value was 43% and the median time to progression was 18 weeks (4.1 months).

Somewhat less positive results with the alternating week schedule were obtained in a Dutch study of 24 GBM patients (36), where the PFS-6 value was only 29%. Given the small number of patients, however, it is difficult to know whether the variation was due to random variability.

The RESCUE clinical trial, published in 2010, was a study of the 50mg/m2 metronomic TMZ schedule for patients with recurrent malignant gliomas (364). 91 glioblastoma patients were divided into three categories: B1) those who had tumor progression during the first six cycles of first-line TMZ; B2) those who progressed during extended cycles of first-line TMZ, that is to say beyond the sixth cycle; and B3) those who had progression at least 2 months after the completion of first-line TMZ cycles. The median PFS, PFS-6, and OS-12 values were noticeably better in groups B1 and B3 compared to group B2, implying that patients with disease progression during the first six cycles or after successful completion of first-line TMZ therapy may benefit from metronomic TMZ, while those who have disease progression during extended cycles of first-line TMZ may not benefit from a switch to the metronomic schedule.

Optune (formerly NovoTTF) by Novocure

See *Chapter 3* for details on Optune.

Other chemotherapy agents at recurrence

While Temodar is now the drug of choice for the initial treatment of glioblastoma, the majority of patients will receive minimal benefit. Patients who have failed the standard treatment protocol often proceed to other chemotherapy drugs. These include the nitrosoureas, BCNU and CCNU (and ACNU in Europe and Japan), and also the platinum drugs, and irinotecan, a drug developed for colon cancer known also known as CPT-11.

While BCNU was the standard chemotherapy treatment for glioblastomas for decades, there never was definitive evidence of its efficacy. A recent study of patients with tumors recurring after radiation treatment is typical of the evidence (190). Of forty patients receiving BCNU at the time of tumor recurrence after radiation, the PFS-6 value was 17%, accompanied by considerable hepatic and pulmonary toxicity. Even less promising results were produced in a small Australian study in which BCNU was given to patients who had progressed when using temozolomide. Here 23 of 24 patients failed during the first six months (191).

Given that BCNU and PCV (which contains CCNU, an oral cousin of BCNU) have never been shown to be differentially effective, a somewhat surprising result has been reported using PCV for tumors recurrent after radiation (and for some patients after radiation and prior chemotherapy). In a relatively large study of 86 patients (192), PFS-6 was 38%, a value superior to that obtained for Temodar in a comparable setting, although with considerable toxicity. However, another study (193) that used PCV for patients with recurrent tumors after Temodar had failed had a PFS-6 value of only 13%. One plausible explanation for the discrepancy between the two studies is the nature of the prior treatment that had failed.

A new member of the nitrosourea family is fotemustine, now available in Europe. In a recent review of its use with a variety of different schedules for patients with recurrent tumors after the standard Stupp protocol treatment, the PFS-6 value ranged from 26 to 44% (194). The best results have been obtained when fotemustine was given every two weeks for five consecutive treatments at a dose of 80 mg/sq.-meter followed by maintenance therapy every four weeks. The PFS-6 value was 61% with a median time to progression of 6.7 months (195). In an Italian study reported at the 2014 SNO meeting, patients who had failed the initial standard protocol received either avastin or fotemustine at the time of recurrence. Survival six months after recurrence was the primary measure, which was slightly higher for patients receiving fotemustine (322).

The platinum drugs cisplatin and carboplatin have also been used as single agents. Carboplatin has increasingly become the preferred drug because it has significantly less toxicity for eyes, ears and kidneys. In a representative study of carboplatin (196), 4 of 29 patients with recurrent glioma had a partial regression and 10 achieved stable disease. However, other treatment studies using the platinum drugs have produced highly variable results, with the source of the variability not clearly identifiable.

One of the newer chemotherapy agents is CPT-11 (also known as irinotecan), which has been FDA-approved for the treatment of colon cancer. Its application to gliomas has been pioneered by Dr. Henry Friedman at Duke University and is now undergoing clinical trials at a number of other medical centers as well. The initial results from the early trial were that 9 of 60 patients with recurrent gliomas had a confirmed partial response, while an additional 33 patients had stable disease lasting more than 12 weeks (197). However, results from other reported studies have been less positive (198, 199).

 Like Temodar, CPT-11 is now being studied in various combinations with other chemotherapy regimens, notably gliadel, intravenous BCNU, and Temodar. Some results are available for the combination of CPT-11 with BCNU, which produced a PFS-6 value of 30% for patients who had failed temozolomide-based initial chemotherapy (200). One interesting sidelight about CPT-11 is that the gastro-intestinal toxicity that it produces, which can be severe, is substantially attenuated by low doses of thalidomide (see pages 36-37 for further discussion of thalidomide as a treatment agent in its own right). A recent study combining CPT-11 and thalidomide with patients who had failed both temodar and nitrosourea chemotherapy produced a PFS-6 value of 28% (201). Finally, CPT-11 has been combined with celebrex, with patients with recurrent tumors, and produced a PFS-6 value of 25% (202).

VAL-083 (Dianhydrogalactitol)

Phase 2 and 3 trials are underway for recurrent glioblastoma testing VAL-083, a bi-functional alkylating chemotherapy. As VAL-083 depends on a different mechanism compared to other alkylating agents used for GBM (temozolomide and nitrosoureas), and causes lesions in DNA at locations not subject to repair by the MGMT enzyme, it is hoped that VAL-083 will be effective regardless of the MGMT status of the tumors. VAL-083, also known as dianhydrogalacticol, is approved in China for chronic myelogenous leukemia (CML) and lung cancer. In addition to the two GBM clinical trials, there is also an expanded access protocol in place for patients who have exhausted standard treatment options to obtain the drug outside of trials.

14. The Role of Radiation

For many years the only treatment (other than surgery) offered to patients with glioblastomas was radiation, due to radiation being the only treatment found to improve survival time in randomized clinical trials. This continued to be the case in Europe until the last decade, but in this country chemotherapy (usually BCNU) gradually came to be accepted as a useful additional treatment component despite the absence of definitive evidence from clinical trials. Part of the reason for this acceptance of chemotherapy has been that very few patients receiving only radiation survive longer than two years (3-10%), compared to 15-25% of patients also receiving chemotherapy.

The initial approach to using radiation to treat gliomas was whole-head radiation, but this was abandoned because of the substantial neurological deficits that resulted, sometimes appearing a considerable time after treatment. Current clinical practice uses a more focused radiation field that includes only 2-3 cm beyond the periphery of the tumor site. Because of the potential for radiation necrosis, the current level of radiation that is considered safe is limited to 55-60 Gy. Even at this level, significant deficits may occur, often appearing several years after treatment. The most common causes of these deficits are damage to the myelin of the large white fibers, which are the main transmitters of information between different centers of the brain, and damage to the small blood vessels, which results in an inadequate blood supply to the brain and also increases the likelihood of strokes. An additional risk, not yet proven clinically because of the typical short survival times of glioblastoma patients, is the growth of secondary tumors due to the radiation damage to the DNA. However, experimental work with animal models has supported the reality of this risk (208). Three-year-old normal rhesus monkeys were

given whole brain radiation using a protocol similar to the common human radiation protocol and then followed for 2-9 years thereafter. A startling 82% of the monkeys developed glioblastoma tumors during that follow-up period. It is currently unclear to what degree a similar risk occurs for human patients who are long-term survivors.

The major additional use of radiation in the treatment of gliomas has been localized radiation to the tumor field, after the external-beam radiation treatment is finished (or sometimes concurrently), either by use of implanted radiation seeds (typically radioactive iodine), a procedure known as brachytherapy, the use of radiosurgery (including gamma knife), or by the insertion into the tumor cavity of an inflatable balloon containing radioactive fluid (gliasite). Previous editions of this treatment summary devoted considerable discussion to these treatments. However, these treatments now are used much less frequently. Two different randomized trials of brachytherapy failed to show a statistically significant survival benefit even though the procedure causes considerable toxicity in terms of radiation necrosis (209). A recent randomized study of radiosurgery (210) similarly failed to show a benefit. Gliasite has yet to be studied in a randomized trial.

The usual interpretation of the failure to find a benefit in the randomized trials is that the initial studies indicating a survival benefit (usually increasing survival time about a year) involved a highly selected patient population, who otherwise had a good prognosis regardless of whether they received the procedure. However, selection bias seems not to account for all of the benefits of the procedure. For example, the use of gliasite for recurrent GBM tumors produced a median survival time of 36 weeks (211), which compares favorably with a median survival time of only 28 weeks when gliadel wafers were implanted for recurrent tumors, even though eligibility criteria were similar for the two procedures. Moreover, when patients receiving gliasite as part of the initial treatment (212) were partitioned according to according to established prognostic variables, and each partition was compared to its appropriate historical control, survival time was greater for patients receiving gliasite in each of the separate partitions.

Perhaps the best results reported involving radiation boosts comes from the combination of permanent radioactive iodine seeds with gliadel (212). Median survival for patients with recurrent glioblastomas was 69 weeks, although accompanied by considerable brain necrosis. The use of gliadel alone in the same treatment center, by comparison, produced a median survival time of 28 weeks, while the use of the radiation seeds alone produced a median survival of 47 weeks.

Impressive results have also been obtained with the addition of fractionated radiosurgery to the standard Stupp protocol for newly diagnosed patients (213). For 36 GBM patients median survival (from diagnosis) was 28 months and two-year survival was 57%. Median progression-free survival (from study entry) for the GBM patients was 10 months.

The foregoing results suggest that supplementary radiation procedures do provide some benefit, but it is important to appreciate that all only a portion of patients will be eligible for such treatment. Radiation necrosis caused by the treatment must be considered as well.

Hyberbaric oxygen and other radiosensitizers

A potentially important modification of the standard radiation protocols involves the use of hyperbaric oxygen prior to each radiation session. In a study conducted in Japan (214), 57 high-grade glioma patients received the standard radiation protocol with the addition of hyperbaric oxygen 15 minutes prior to each radiation session. Four rounds of chemotherapy were also administered, the first during the radiation period of treatment. For the 39 glioblastoma patients, the median survival time was 17 months, with a very high rate of tumor regression. For the 18 patients with anaplastic astrocytoma, median survival was 113 months. Two-year survival was reported separately for recursive partioning categories I-IV and V-VI, the latter including only glioblastoma patients. For categories I- IV, two-year survival was 50%; for categories V and VI, two-year survival was 38%.

A long-standing goal of radiation oncology has been to find a radiation sensitizer that does not increase toxicity to normal tissue. One of the most promising advances toward this goal was reported at the 2011 ASCO meeting (215). A new drug derived from the taxane family, with the name OPAXIO, was combined with the standard Temodar + radiation protocol during the radiation phase of the treatment. The response rate for 25 patients (17 GBM) was 45% with 27% having a complete response. With a median follow-up of 22 months, median progression-free survival was 14.9 month (13.5 months for GBM patients). Median overall survival had not been reached at the time of the report. Note that the median PFS for the standard treatment without OPAXIO is 6.9 months.

Proton radiation therapy

An alternative to the standard X-ray radiation is the use of proton beams, although only a few treatment centers have the required equipment. To date, there has been no meaningful comparison of the efficacy of proton-beam radiation and the normal procedure. However, one recent study in Japan did report unusually positive results when the two forms of radiation were combined, the standard procedure in the morning, and the proton-beam radiation in the afternoon (216). Also used was ACNU, a chemical cousin of BCNU and CCNU. Median survival for 20 patients was 21.6 month, with -1-year and 2-year progression-free rates of 45% and 16%. However, there were six cases of

radiation necrosis that required surgery, indicating a considerably higher toxicity than normally occurs with the standard radiation procedure.

Radiation via Monoclonal Antibodies

An alternative for providing a radiation boost beyond the standard external field radiation involves attaching radioactive iodine-131 to a monoclonal antibody that targets a specific antigen, tenascin, which occurs on almost all high-grade glioma tumors and not on normal brain cells. The monoclonal antibodies are infused directly into the tumor cavity over a period of several days, and reportedly produces much less radiation necrosis than either brachytherapy or radiosurgery. The median survival time from a phase 2 clinical trial of this treatment for recurrent GBM tumors was 56 weeks (217). In the first study that reported using this approach as initial treatment (218) patients received the monoclonal antibodies, followed by the standard external-beam radiation and then a year of chemotherapy. Of 33 patients, only one required re-operation for necrotic tissue caused by the radiation. Median survival time was 79 weeks for the patients with glioblastoma (27 of 33 of total patients) and 87 weeks for all patients. Estimated two-year survival rate for GBM patients was 35%. A subsequent report of the results for an expanded number of patients indicated a mean progression-free survival of 17.2 months, compared to 4-10 months for other treatment procedures (219). Median overall survival measured from the time of diagnosis was 24.9 months. At the present time, however, only one treatment center (Duke University) has used this procedure. A multi-center clinical trial was planned, but the company sponsoring the trial apparently has shelved those plans for the indefinite future.

A second type of monoclonal antibody treatment, developed at Hahneman University Medical School in Philadelphia, targets the epidermal growth factor receptor, which is overexpressed in the majority of GBM tumors (220) For patients who received the MAB treatment in combination with standard radiation, median survival time was 14.5 months; For patients who received the same protocol but with the addition of temodar, median survival was 20.4 months.

A third type of monoclonal antibody, named Cotara, is designed to bind with proteins that are exposed only when cells are dying, with the result that adjacent living tumor cells are radiated by the radiation load carried by the monoclonal antibody. This rationale is based on the fact that that centers of GBM tumors have a large amount of necrosis. This approach has been under development by Peregrine Pharmaceuticals, a small biotech with limited funding. Recently they reported the long-term results from 28 recurrent GBM patients studied over a nine-year period (221). Seven of the 28 patients survived more than one year, while 3 of the 28 survived longer than five years (2 more than 9 years). Median survival was 38 weeks.

Concluding Remarks

With each passing year the information about treatment options has expanded, making it increasingly difficult for the newly diagnosed patient, or their families, to discern which is the best treatment plan to follow. Keep in mind that I am not a physician with direct contact with patients and the valuable information that provides. On the other hand, my opinions are not constrained by the conventions of the medical system, which often hamstring oncologists in considering the possible options.

My first piece of advice is to seek treatment at a major brain tumor center. Their surgical techniques are more likely to be state-of-the-art, which in turn means the patient will be more likely to receive a complete resection, now known to be a strong contributor to longer survival. Also important is that major centers will be better equipped to retain tumor samples that will allow various tests of genetic markers that have important implications for which treatments are most likely to be successful for the individual patient. Patients should request prior to surgery that their tumor tissue be frozen and preserved for later use.

Unlike even ten years ago, there now are meaningful choices for effective treatment protocols, although several of the most promising are still in clinical trials and not generally available. Vaccine and viral therapies such as dendritic cell vaccine targeted to CMV pp65 (preferably combined with tetanus-diptheria toxoid preconditioning and/or basiliximab) for newly diagnosed GBM, and therapies for recurrent GBM such as polio-rhinovirus and DNX-2401 adenovirus have all seen particularly strong results in early stage trials and are currently recruiting patients. Recently initiated trials combining vaccines with PD-1 antibodies likely represent the next step in the advancement of immunotherapy for high grade glioma (NCT02529072, NCT02798406). Optune (Tumor Treating Fields) has been approved by the FDA and as of July 2016 has been added to the NCCN guidelines as a standard treatment for newly diagnosed glioblastoma.
Patients ineligible for promising trials may still make use of other options described throughout this document, such as repurposed drugs, non-prescription supplements, dietary and lifestyle interventions. While such an experimental approach is best done in co-operation with a knowledgeable physician, the absence of a truly effective standard of care for this disease means that patient experimentation is inevitable, with or without the guidance of a licensed medical practitioner. This document aims to provide information to make such an approach as informed and educated as possible, with the ultimate aim of improving outcomes beyond what is expected with standard treatments alone.

See Appendix B for further resources.

Appendix A: Summary of major revisions

2017

Chapter 2, small randomized trial compared 6 versus 12 months of adjuvant temozolomide for newly diagnosed glioblastoma in the *How many cycles of TMZ?* Section

Chapter 3, updated data from the EF-14 trial of Optune for newly diagnosed GBM

Chapter 6, *Rapamycin (sirolimus) plus hydroxychloroquine*

Chapter 6, *Methadone*

Chapter 7, *Metabolic therapy with sodium R lipoate plus hydroxycitrate*

Chapter 7, Efficacy results of small randomized trial testing Sativex (THC:CBD) for recurrent glioblastoma. *Cannabis* section

Chapter 8, ICT-107 trial suspended

Chapter 8, Nivolumab monotherapy not superior to Avastin monotherapy in Checkmate-143 trial

Chapter 8, *Hyperprogression following anti PD-1/PD-L1 therapy*

Chapter 8, *EGFRvIII-directed CAR T-cells*

Chapter 8, *IL13Rα2-targeted Chimeric Antigen Receptor T-cells (CAR T-cells)*

Chapter 9, *ABT-414*

Chapter 9, *MDNA55*

Chapter 13, *Baseline blood neutrophil counts predict efficacy of bevacizumab in recurrent glioblastoma*

Chapter 13, *Angiotensin system inhibitors plus Avastin*

Chapter 13, section on the RESCUE study rewritten in the *Rechallenging with Temodar* section

Chapter 13, new section on *VAL-083 (Dianhydrogalactitol)*

2016

Chapter 1, Anaplastic astrocytoma section

Chapter 1, Dexamethasone section

Chapter 2, How many cycles of TMZ? section: discussion of prolonging temozolomide cycles to 12, or more than 12 cycles.

Chapter 3, Optune plus chemoradiation section: discussion of FDA approval of Optune for newly diagnosed GBM, and the continuation of Optune at first recurrence.

Chapter 5, Hormones and Cancer Therapy chapter created

Chapter 5, *Angiotensin-II Receptor Blockers* section

Chapter 5, Beta-blockers section

Chapter 5, Thyroid Hormone T4 section

Chapter 6, Disulfiram section: results of the phase 1 dose and pharmacodynamics trial for glioblastoma.

Chapter 6, Valproic acid section: continuing debate based on new large retrospective study by Happold, Weller et al.

Chapter 7, Gamma-Linolenic Acid section re-written

Chapter 8, Agenus Prophage vaccine section: discussion of greatly superior outcomes in patients with low expression of PD-L1

Chapter 8, Dendritic cell vaccine targeting cytomegalovirus section: discussion of impressive preliminary outcomes in trials combining CMV-pp65 vaccine with tetanus-diptheria toxoid preconditioning or basiliximab. Currently recruiting trials of CMV pp65 vaccine.

Chapter 8, Rindopepimut section: the failure of the phase 3 trial (ACT IV) of rindopepimut for EGFRvIII positive newly diagnosed glioblastoma. The success of the randomized phase 2 ReACT trial for recurrent glioblastoma.

Chapter 8, Wilms Tumor 1 peptide vaccine

Chapter 8, Immune checkpoint inhibitors section: new data on nivolumab with or without ipilimumab for small cohorts of recurrent glioblastoma.

Chapter 9, Parvovirus (with bevacizumab)

Chapter 10, Toca 511 / TocaFC section: published results for phase 1 trial of Toca 511 injected into the resection cavity for recurrent high-grade glioma.

Chapter 11, Photodyamic Therapy: updated results of photodynamic therapy for high-grade glioma in Japan.

Chapter 12, Avastin section: evidence based on the AVAglio trial that upfront Avastin prolongs survival in patients who receive no further therapy upon disease recurrence. A new subsection on *Lower dose Avastin* summarizes favorable evidence for doses lower than the standard dose.

Chapter 14, new *Concluding Remarks*

2015

Chapter 2, Combatting chemoresistance section: discussion of clinical trial of hydroxychloroquine added to standard of care for newly diagnosed glioblastoma. Evidence for sensitivity of EGFR-overexpressing GBM cells to chloroquine.

Chapter 2, Optimizing the Schedule of Chemotherapy section: discussion of retrospective study showing benefit of metronomic temozolomide schedule for EGFR-overexpressing or EGFR-amplified glioblastoma.

Chapter 3, Patient Registry Dataset section: discussion of Optune (Novocure tumor treating fields) including the PRiDe dataset and results from the recent phase 3 trial for newly diagnosed glioblastoma.

Chapter 5, Accutane section: evidence for low dose Accutane plus interleukin-2 in other cancers

Chapter 5, Keppra section: Keppra (levetiracetam) extends survival when added to standard of care chemotherapy for newly diagnosed glioblastoma

Chapter 5, Thalidomide section: thalidomide for advanced secondary GBM

Chapter 5, Valproic acid section: valproic acid combined with chemoradiation for newly diagnosed glioblastoma

Chapter 5, A trial of 3 repurposed drugs section: updated results for a trial of 3 repurposed drugs (Accutane, Celebrex, thalidomide) plus Temodar

Chapter 7, DCVax section: new results for "informational arm" of DCVax-L trial (ie. outcomes for patients who were disqualified from the phase 3 trial due to early disease progression, but who received the vaccine on compassionate use basis)

Chapter 7, ICT-107 section: updated results of randomized phase 2 trial of ICT-107 vaccine in newly diagnosed glioblastoma

Chapter 7, Dendritic cell vaccine targeting Cytomegalovirus section: results of trial testing CMV-targeted vaccine with or without preconditioning with tetanus/diptheria toxoid

Chapter 7, Rindopepimut section: updated results of the phase 2 ACTIII trial of rindopepimut (anti-EGFRvIII vaccine) for newly diagnosed glioblastoma

Chapter 7, Immune checkpoint inhibitors section: preliminary results of nivolumab combined with ipilimumab for recurrent glioblastoma

Chapter 8, Genetically modified poliovirus (PVS-RIPO) for recurrent glioblastoma

Chapter 8, DNX-2401 adenovirus

Chapter 9, Toca 511 / TocaFC section: preliminary results of Toca 511/TocaFC therapy for recurrent high-grade glioma

Chapter 11, Avastin section: discussion of optimal timing of Avastin treatment (upfront versus delayed)

Chapter 11, Avastin combined with CCNU

Appendix B: Additional Resources

Virtualtrials.com

Surviving Terminal Cancer (film)

Anti Cancer Alliance (includes information on the proposed CUSP-ND trial)

Astrocytomaoptions.com

Our brain tumor cocktails and stories (online forum)

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