Glioblastoma multiforme is one of the most lethal and treatment-resistant of human tumors. Among the therapeutic triad of surgery, radiation therapy, and chemotherapy, only radiation therapy has been shown to improve survival.1 Despite 30 years of intensive efforts to find an effective chemotherapy regimen for glioblastoma multiforme, the median survival of 12 to 15 months has not changed appreciably since the introduction of radiation therapy.

This dire circumstance has finally begun to change with the recent publication of a positive phase 3 trial comparing radiation therapy (RT) alone with RT with concurrent and adjuvant temozolomide chemotherapy in patients with newly diagnosed glioblastoma multiforme (GBM).2 This study was conducted in Europe and Canada under the direction of the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC), and is the first to demonstrate a meaningful survival effect from chemotherapy in patients with GBM. A parallel translational study provided a remarkable molecular explanation for the positive effects of chemotherapy in the trial.3

This review will consider the clinical problem of GBM, discuss the details of the positive phase 3 clinical trial, and examine the results of the translational study of methylguanine methyltransferase (MGMT) gene silencing. The phase 3 trial will be used to illustrate how national practice patterns can affect the fundamental design of clinical trials. Finally, a welcome new problem in neuro-oncology—the impact of a new treatment standard on existing and future clinical trials—will be described.

THE CLINICAL PROBLEM OF GBM

A 60-year-old executive described a several-week history of worsening headache, difficulty with routine tasks (such as buttoning his shirt and finding the way home from his office), and a shuffling gait. Magnetic resonance images revealed a large ring-enhancing mass lesion in the right temporal lobe (Figure 1A). An aggressive subtotal resection was performed and the histopathological diagnosis was GBM. The postoperative magnetic resonance images revealed a small area of residual enhancement along the posterior aspect of the resection cavity (Figure 1B). The patient was enrolled in a Radiation Therapy Oncology Group (known as RTOG) clinical trial where he received involved-field RT (50 Gy in 35 fractions) and fractionated stereotactic radiotherapy (28 Gy in 4 fractions) to the area of residual enhancement. Following RT, he received oral temozolomide (200 mg/m² per day) for 5 days out of every 28 days. Magnetic resonance imaging revealed an asymptomatic recurrence 12 months following diagnosis, and despite reoperation and treatment with intravenous carmustine (1,3-bis [2-chloroethyl]-1-nitrosourea), he died 18 months from the date of diagnosis due to progressive disease. The clinical course of this patient is very typical for GBM and illustrates clearly the extreme therapeutic resistance of these tumors.
Glioblastoma multiforme is the most common primary brain tumor and accounts for about 50% of adult gliomas. It occurs with an incidence of 3 per 100,000 per year, producing about 9,000 cases annually in the United States. Cases are distributed over a broad range of ages with an average age at diagnosis of 53 years. Prognostic factors, which permit some refinement of predicted survival for individual patients, include age (with the tumors of older patients being more aggressive and more resistant to treatment) and postoperative physical performance status. Long-term survivors, defined as those who are alive 3 to 5 years following diagnosis, are rare and have young age as their only common feature.

Glioblastoma multiforme results from a cascade of genetic alterations that begin in a target brain cell and, through unregulated cell division and a panoply of other molecular abnormalities, lead to an expanding mass lesion. The normal functions of many of these altered genes are now known and include regulation of the cell cycle and apoptosis, maintenance of genomic stability, modulation of the immune microenvironment, and control of angiogenesis. Recent work has suggested that a progenitor population, rather than a differentiated glial cell, may be the cell of origin of malignant gliomas.

The causes of the initial genetic changes within the cell of origin are not known. Epidemiologic studies have not provided etiologic insights regarding potential environmental agents that would predispose individuals to gliomas. Glial tumors occur in several heritable conditions, such as the Li-Fraumeni syndrome with a constitutional p53 mutation, but these cases account for only a tiny fraction of all patients with GBM. Mutations resulting from random errors in DNA replication are a plausible explanation for the initiating events in formation of gliomas.

At least 2 distinct clinical and genetic pathways are known to exist in the formation of GBM. De novo or primary GBM, representing approximately 80% of cases, typically occurs in older patients who have a highly malignant tumor at the time of first clinical detection. The tumor cells of these patients demonstrate activation of the epidermal growth factor receptor gene by way of overexpression and have normal p53 status. In secondary GBM, where there is a prior known low-grade astrocytoma that has undergone anaplastic progression to GBM, younger patients have tumors with inactivated p53 but normal epidermal growth factor receptor. The striking correlations between age, genetic changes, and clinical behavior in diffuse astrocytomas suggest that distinct genetic pathways are targeted in different age groups during tumor formation.

CONVENTIONAL THERAPY FOR GBM

In the United States, the standard of care for patients with GBM during the past 2 to 3 decades has been maximal safe resection followed by involved-field irradiation and treatment with chemotherapy using standard alkylating agents such as carmustine or treatment with new chemotherapy or biological agents in the setting of clinical trials. The routine administration of chemotherapy has been based on evidence of minimal improvement in 2-year survival. The clinical trials process is very well developed (Table), but a large number of agents have been tested with disappointing results. By comparison, in Europe and Canada the routine administration of chemotherapy has been less common, a fact that played a significant role in the design of the positive phase 3 trial.

Figure 1. Glioblastoma of the right temporal lobe prior to surgery (A). A large, ring-enhancing mass lesion was present on the axial T1-weighted, gadolinium-enhanced image. Following surgery (B), a small focus of residual enhancement was present along the posterior margin of the surgical cavity (arrow).
The clinical evaluation of a new chemotherapy agent in oncology involves 3 levels, or phases, of trials (Table). Each phase has a specific goal that if met leads to the next phase. The study described here was a phase 3 trial that involved randomization of large numbers of newly diagnosed patients with GBM between 2 treatment regimens that included standard postoperative RT with or without the chemotherapy agent temozolomide. Overall survival was the primary end point. The technical terms used to describe the study are discussed in the accompanying Table.

During a 24-month period, the EORTC/NCIC trial enrolled 573 patients with biopsy-proven, newly diagnosed GBM into a 2-armed phase 3 study comparing a standard RT regimen without chemotherapy with RT with concurrent and adjuvant temozolomide chemotherapy (Table and Figure 2).3

Temozolomide is a relatively new alkylating agent that was developed specifically for the treatment of malignant glioma by the Medical Research Council, London, England. It has excellent oral bioavailability when taken on an empty stomach, it has good central nervous system penetration, and it produces only mild myelosuppression and adverse gastrointestinal effects. Temozolomide methylates DNA at O6 guanine positions, leading to cell cycle arrest and apoptosis of tumor cells. The methylation of O6 guanine is normally reversed by the cellular enzyme MGMT.

### Table. Definition of Terms Used in Brain Tumor Clinical Trials*

| Phase 1 trial | The first step in evaluating a new agent in human subjects. The goal is to determine maximum tolerated dose (MTD) of the new agent. Patients in the salvage setting are given the new agent in cohorts of 3 or 4 per group, with each cohort receiving a higher dose. When a certain percentage of patients develop a predefined level of toxic reaction, that dose is considered to be the MTD. Difficulties with phase 1 studies include the unknown risk of the new agent, the considerable logistical burden imposed on patients with advancing tumors, and the fact that heavy pretreatment with other drugs could result in underestimating the MTD. About 15 patients are enrolled in typical phase 1 trials. |
| Phase 2 trial | The goal is to detect drug activity. Patients in the salvage setting are enrolled in a single-arm study of the new agent at the MTD. Response is measured using magnetic resonance imaging examinations and clinical status. A typical outcome measure is 6-month progression-free survival, which is used as a surrogate for overall survival. This outcome is then compared with a historical control group, with the latter being as similar as possible to the study group. Prior therapy, choice of control group, and use of a surrogate for survival are all problems with phase 2 studies. Approximately 50 patients are treated in a phase 2 trial. |
| Phase 3 trial | With evidence of activity in the phase 2 study, the agent then is taken to the phase 3 trial. The goal is to demonstrate improved overall survival with the new agent. Patients in the adjuvant setting are randomized between 2 different treatment arms that differ only in the administration of the new agent vs a current best-available agent. Stratification (for example, by age or extent of surgery) occurs prior to randomization and is designed to ensure balanced patient populations in the 2 arms. A positive phase 3 trial leads to approval of the new agent by the US Food and Drug Administration’s Oncology Drugs Advisory Committee. Five hundred or more patients may be enrolled. |
| Maximum tolerated dose | Maximum tolerated dose is the dose of a new drug at which a predetermined level of toxicity occurs in a certain percentage of patients in the dose-escalating phase 1 trial. Definition of these toxicity criteria are standardized (available at http://ctep.cancer.gov/reporting/ctc.html). |
| Adjuvant setting | Any therapy that targets residual tumor cells following primary treatment. Surgical excision usually represents primary therapy and in this case, radiation therapy (RT) and chemotherapy given immediately following surgery would both be considered adjuvant. In patients with brain tumors for which significant resection is not possible, RT is the primary therapy and chemotherapy is adjuvant therapy. |
| Neoadjuvant setting | Chemotherapy agents can be tested prior to administration of RT when there is substantial residual disease after surgery. The rationale is to give the new agent the best possible chance to demonstrate an effect. Radiographic response rate is used as the outcome measure. |
| Salvage setting | This refers to administration of chemotherapy when the tumor has shown progression after surgery, RT, and adjuvant chemotherapy. Most phase 1 and 2 trials are performed in this setting. |
| Stratification and randomization | In a phase 3 trial, patients are randomized to 1 of 2 treatment arms. Because randomization can lead to unbalanced study arms through chance alone, patients are often first stratified into subgroups. Stratification factors are chosen for their influence on outcome, for example, patients of younger and older age. |
| Overall survival and median survival | This is the end point of phase 3 trials and is measured in terms of median survival (the time at which 50% of patients enrolled in the trial remain alive) or survival at some fixed time point (eg, 2-year survival). This is the standard gold for measuring activity of a new agent prior to US Food and Drug Administration approval. |
| Progression-free survival | Criterion for drug activity in many phase 2 trials in neuro-oncology. Complete response and partial response are often not used for glioblastoma multiforme because these types of responses are uncommon. Instead, the duration of stable disease, as measured by magnetic resonance imaging is used, typically at a set interval such as 6-month progression-free survival. |

*Terms in boldface are those defined elsewhere in the table for cross-reference.

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**THE POSITIVE PHASE 3 EORTC/NCIC TRIAL**

The treatment schema for the positive phase 3 trial. RT indicates radiation therapy; TMZ, temozolomide. (The rad equivalent for 60 Gy is 6000.)

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and 14.6 months for patients receiving combined therapy (Table) of 12.1 months for the patients with RT alone. Survival. Analysis of the data revealed a median survival of 12.1 months for patients receiving combined therapy compared to 9.7 months for patients receiving RT alone. This difference was statistically significant (P < .001).

In addition, the survival curves for the two groups diverge with time. The benefits of the combined therapy are more apparent in the later stages of the disease, indicating a possible delay in the progression of the disease.

This crossover at the time of progression would tend to reduce the likelihood that the combined therapy arm would show a relative benefit. This is because patients who receive RT first and then temozolomide might have a higher chance of receiving RT alone if they progress early, while patients in the temozolomide plus RT group might have a higher chance of receiving both therapies if they progress early.

The improved survival likely represents the presence of a subgroup of patients in whom combined treatment was most effective. A partial explanation for this phenomenon was provided by an analysis of the MGMT gene in the tumor tissue of study patients. Methylation of the MGMT gene in tumor cells was determined from tumor tissue. Methylguanine methyltransferase repairs the O6 methylation induced by temozolomide and other alkylating agents. In the parallel MGMT study, the methylation status of the MGMT gene promoter in tumor cells was determined from tumor tissue taken at the time of initial surgery. The analysis was carried out by a polymerase chain reaction assay in which methylated promoter DNA fails to amplify. Methylation at cytosine-guanine dinucleotides in the promoter DNA is known to result in loss, or silencing, of cellular expression of MGMT.

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Correlation of the MGMT status with survival revealed a remarkable result. In temozolomide-treated patients whose tumor showed MGMT silencing, 2-year survival was a phenomenal 46% compared with 23% in temozolomide-treated patients without MGMT silencing (Figure 3B). Indeed, MGMT silencing accounted for the majority of the effect of temozolomide therapy.

**IMPLICATIONS OF THE PHASE 3 STUDY**

This well-designed and well-powered study provides strong support for the use of temozolomide in conjunction with RT in the treatment of newly diagnosed GBM. The US Food and Drug Administration approved temozolomide for the treatment of newly diagnosed GBM only 2 weeks after publication of the phase 3 study, reversing its rejection decision of several years ago regarding GBM. While impres-

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Figure 3. Overall survival curves from the phase 3 trial (A). The survival curves for temozolomide-treated patients whose tumors had or did not have methylguanine methyltransferase (MGMT) gene silencing (B).

Figure 4. Silencing of the methylguanine methyltransferase (MGMT) gene by promoter methylation. TMZ indicates temozolomide.

Prerandomization stratification (Table) was based on the following 3 factors: World Health Organization performance status, debulking surgery vs biopsy, and treatment center. After randomization, the 2 arms were found to be well balanced. All patients received a standard radiation regimen of 60 Gy (ie, 6000 rads) in 30 fractions. Half of the patients also received temozolomide 75 mg/m² daily throughout radiation treatment, followed by temozolomide (150-200 mg/m² daily) for 5 days every 28 days for 6 cycles (Figure 2). The other half of the study group did not receive temozolomide. More than 90% of patients in both arms completed RT, and 88% of those in the RT plus temozolomide arm completed intended chemotherapy. Temozolomide was discontinued in 12% of patients because of toxicity (5%), tumor progression (4%), or other reasons (3%). More than 50% of patients randomized to RT alone received temozolomide or some other chemotherapy at the time of tumor progression. This crossover at the time of progression would tend to reduce the likelihood that the combined therapy arm would show a relative benefit.

The primary end point of the study was overall survival. Analysis of the data revealed a median survival (Table) of 12.1 months for the patients with RT alone and 14.6 months for patients receiving combined therapy (P < .001) (Figure 3A). Two-year survival was 10.4% for patients with RT alone vs a remarkable 26.5% for patients receiving combined therapy (P < .001). Chemotherapy was associated with improved survival in all groups except those with biopsy only, those with poorer World Health Organization performance scores, and significantly, in those without silencing of the MGMT gene. These results represent the first time a clinically meaningful improvement in survival has been seen with chemotherapy in patients with GBM.

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sive, 26% survival for 2 years remains a sobering statistic and there is clearly a need for further improvement. We finally have the evidence that improvements can be achieved.

Many unresolved issues remain. The relative value of the concurrent vs post-RT portions of the chemotherapy is unclear. This is not a trivial issue because each portion of the chemotherapy costs about $10,000. Could a higher dose of temozolomide be employed and give even better results? Methylguanine methyltransferase testing is not yet available for routine clinical use and the proper role for testing outside of clinical trials is not understood.

It is interesting to consider whether this study could have been conducted in the United States. Half of the patients were randomized to receive no chemotherapy and chemotherapy has been considered a part of standard treatment for GBM here for more than 2 decades, despite only minor hints of improved 2-year survival in large trials. It is likely, therefore, that most neuro-oncologists in the United States and their patients would have been uncomfortable with the arm that did not include chemotherapy. Thus, this trial could be seen as a cautionary tale about firmly embedded practice patterns based on minimal evidence, a phenomenon which is widespread in every field of medicine.

On the other hand, chemotherapy for newly diagnosed GBM is now the standard around the world, not just in the United States. With the newly published data, however, administration of chemotherapy is based on statistically clear data using a new treatment approach (ie, temozolomide given concurrently with RT followed by adjuvant temozolomide) that sheds light on an important mechanism of drug activity in GBM.

The data also provide a new standard that has had a major impact on ongoing and new clinical trials. A substantial number of clinical trials for newly diagnosed GBM that were under way when this data was first made public were rewritten to include temozolomide. Obviously, alteration of a treatment regimen during the conduct of a study would have serious implications for statistical analysis. New studies for GBM will include temozolomide as part of standard treatment. This phenomenon has been seen with the emergence of active agents in many diseases, including multiple sclerosis, and is a price of progress.

A number of new trials involving temozolomide are now in the planning stage or are under way. The EORTC and RTOG are planning a large joint study to examine such issues as inclusion of MGMT status in stratification, temozolomide dose intensification, and genomic comparisons between tumors that respond or do not respond as predicted based on MGMT status. Hopefully this activity will lead to further rapid advances in the treatment of malignant gliomas.

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