OPTIMAL APPROACHES TO ASSESSING MGMT EXPRESSION

Matthias Preusser, MD
Medical University of Vienna, Vienna, Austria

Several independent clinical studies have shown a strong association between the methylation status of the 06-methylguanine-methyltransferase gene (MGMT) promoter and the response to temozolomide.1 MGMT, a DNA repair protein, is thought to protect tumor cells by removing methyl groups from the 06 position of guanine, thus acting as an antagonist against the DNA-damaging effects of alkylating agents. Methylation of MGMT promoter causes silencing of the MGMT gene, inhibiting MGMT protein expression. There is much interest in making MGMT assessment of tumor tissue specimens amenable for routine diagnostic evaluation of patients with glioblastoma. Previous studies have employed different methods and protocols for assessing MGMT. Most have used MGMT gene promoter methylation status, but others have analyzed MGMT expression at the protein or RNA levels.

The optimal method for assessing MGMT should meet the standard requirements for any diagnostic test. It should be widely available, cost effective, and reproducible in the same laboratory and in different laboratories, and correlate consistently with patient outcomes.

The processing of neurosurgical tumor samples begins with limitations. The standard formalin-fixing and paraffin-embedding techniques degrade the quality of DNA and RNA. Also, tissue fixation protocols are inconsistent. Different laboratories use different fixatives, and the duration of formalin preservation also varies. In addition, stereotactic biopsies may yield only small amounts of tumor tissue.

Many studies have used methylation-specific polymerase chain reaction (MSP) to assess MGMT promoter methylation status. However, the high dependence of MSP on tissue quality limits the usefulness of this method for routine diagnosis. Results of repeat analysis from the same formalin-fixed and paraffin-embedded tumor tissue samples are often inconsistent. Reproducibility seems to be better with frozen tissue rather than formalin-fixed tissue. However, in the routine clinical setting, frozen tumor samples are usually not available in all cases.

Promising alternatives for assessing MGMT at the DNA level are real-time quantitative polymerase chain reaction (PCR) and multiplex ligation-dependent probe amplification (MLPA).

Real-time quantitative PCR may be applied on formalin-fixed paraffin-embedded tissue; results are available within a few hours. This method provides quantitative results, in contrast to the semiquantitative results available with conventional MSP. One study involving a commercial company has shown results comparable to those obtained with MSP.2 This method has yet to be validated through interlaboratory comparisons in an academic setting and through prospective clinical trials. A disadvantage of real-time quantitative PCR is that the protocol, similar to conventional MSP, requires bisulfite modification, which is particularly problematic when using formalin-fixed material.

Multiplex ligation-dependent probe amplification analysis also works with formalin-fixed paraffin-embedded tissue, but does not require bisulfite modification. It allows copy number detection, which might be beneficial in some cases. In one study, results were shown to agree highly with those from MSP testing.3 MLPA appears to be an interesting alternative, but still requires intra- and interlaboratory comparison and validation in prospective clinical trials.

Immunohistochemistry (IHC) is another possible alternative for MGMT testing. This diagnostic method is available in most laboratories and is usually technically reliable on formalin-fixed paraffin-embedded tissue. There are MGMT antibodies commercially available. The analytical and clinical performance of
MGMT IHC as a clinical biomarker at the protein level was tested on tissue specimens of the European Organization for Research and Treatment of Cancer/National Cancer Institute of Canada trial 26981/22981. Two tissue microarrays containing 164 glioblastoma samples were immunostained using 2 anti-MGMT antibodies. Slides were evaluated by 4 observers from 3 neuropathology laboratories. Each observer evaluated the slides at baseline and 3 to 6 weeks later. Results showed a wide variability in reproducibility among observers. A possible explanation is that the many non-neoplastic cells (eg, preexisting glial cells and infiltrating hematogenous cells) within the tumor tissue were assessed differently by the different observers. Furthermore, the study found a poor correlation between IHC and PCR results, similar to what had been found in earlier studies.\(^3\),\(^5\)-\(^7\) Again it was hypothesized that the results were affected by MGMT expressed in infiltrating cells as well as in tumor cells. PCR analysis of survival showed that patients with unmethylated status had shorter survival times than those with methylated status. However, IHC for MGMT protein did not show a significant survival association in any of the assessments performed. The study conclusion is that IHC MGMT assessment does not seem to be clinically useful for patients with glioblastoma.

Several studies have suggested using Western blot, MGMT activity assays, or RNA analysis to determine MGMT expression in brain tumors.\(^8\)-\(^9\) A major disadvantage of these methods is that they require fresh frozen tissue, which is not always available in the routine clinical setting. MGMT activity assays and RNA-based methods are also sophisticated and not available to many laboratories.

REFERENCES


PITFALLS IN RESPONSE ASSESSMENT* AND PSEUDO-PROGRESSION AFTER RADIOTHERAPY AND TEMOZOLOMIDE: IS IT OF CLINICAL SIGNIFICANCE?†

Martin J. van den Bent, MD, PhD,* and Walter Tio, MD†
Daniel den Hoed Cancer Center, Erasmus University Medical Center, Rotterdam, The Netherlands

INTRODUCTION

In all areas of medicine, therapeutic efficacy is evaluated by assessing the response to therapy. With high-grade glioma, the process is more complicated. Most recurrences that develop after radiation therapy (RT) are diagnosed because of increased enhancement visible on magnetic resonance imaging (MRI) scanning. Macdonald’s criteria, which mainly use the area of enhancement on MRI, are widely accepted as a tool to diagnose progression, but enhancement does not equal tumor size.\(^1\) Enhancement also occurs when the blood-brain barrier is disrupted by abnormal vessel permeability, for example in inflammation, postsurgical scarring, seizures, early radiation-induced changes, or radiation necrosis. Recently we observed the purported occurrence of progressive MRI lesions immediately after the
end of RT and temozolomide treatment, followed by spontaneous improvement without further treatment other than adjuvant temozolomide. This phenomenon is termed pseudo-progression. Clinicians must be aware of these and other pitfalls in assessing brain scans, of the incidence of pseudo-progression, of the mechanisms by which pseudo-progression occurs, and how to manage patients when MRI suggests the possibility of early progressive lesions shortly after the end of RT.

**Pitfalls in Response Assessment**

When monitoring a patient with glioma, brain scans are used to monitor the efficacy of therapy and to detect disease progression. Problems occur when scans are misinterpreted. A false diagnosis of progression may lead to discontinuing a treatment that is actually effective or to concluding that the tumor resolved because of the treatment, when actually there was no progression to be treated.

To avoid misinterpretation, it is very important for the clinician to examine the scans personally. All imaging should follow a strict protocol, while using the same sequence every time. Because low-grade gliomas do not enhance, assessing therapeutic response to these tumors with MRI can be extremely difficult. The most recent scans should be compared with all informative scans made in the past. Metabolic imaging with amino acid positron emission tomography (PET) scanning may be helpful, but should then be done routinely to allow comparison with a baseline scan. Several pitfalls can interfere with proper response assessment.

Currently, in high-grade tumors, changes in enhancement are used to assess the response to treatment.¹ However, enhancement does not equal tumor size, but reflects leaking of the contrast medium from the vessel, blood-brain barrier disruption, or changes in regional cerebral blood volume. Several factors can cause this disruption.

Corticosteroid drugs decrease enhancement by downregulating the effects of hypoxia-inducible factor/vascular endothelial growth factor (VEGF) signaling.² Accurate monitoring of the steroid dosage is important for reliable comparisons of MR data. Other VEGF- and platelet-derived growth factor inhibitors, such as imatinib, bevacizumab, or AZD2171, also decrease enhancement.³⁻⁵ For this reason, T1-weighted contrast-enhanced MR scans are of limited value when these agents are used, but other MR techniques, such as perfusion imaging or diffusion imaging, may be useful. However, further research is needed to identify the most reliable techniques.

Postsurgical effects that may increase enhancement include ischemic lesions, hemorrhages, inflammation, seizures, and scarring. Ischemic infarction can be identified by using diffusion scan imaging instead of the usual T2- and T1-weighted images.⁶ Enhancement due to postsurgical scarring is usually described as a thin rim of enhancement, but the rim may actually be quite thick and have a nodular aspect because of ischemic lesions around the surgical cavity.

Hemorrhages visible as increased signal intensity can be misdiagnosed as enhancement and thus as tumor progression, particularly if MR scans have not been organized properly. It is important to have T1-weighted images always available before and after contrast administration.

Postsurgical enhancement usually occurs as soon as 2 or 3 days after surgery and can persist for months.⁷ Therefore, to identify any residual tumor it is preferable to perform scans within 24 hours after surgery, but no later than 48 hours. Diffusion-weighted imaging may aid in identifying areas of ischemic infarction. If chemotherapy is started immediately after surgery without an adequate baseline scan, the disappearance of hemorrhages may be mistaken for response.

Seizures can cause edema and areas of enhancement; occasionally these areas can be quite large.⁸ If treating the seizures stabilizes the clinical condition, it may be prudent to wait until the suspected seizure enhancement has decreased, repeating the scans in 1 or 2 months. The result may be complete resolution of the edema and the enhancement.

Recently there has been increased interest in RT-induced changes. On classical MRI using T1- and T2-weighted images, it is impossible to distinguish radiation necrosis from tumor progression.⁹ There is evidence that necrosis may occur earlier and more frequently when RT is combined with temozolomide chemotherapy.¹⁰ The risk of necrosis also increases with the radiation dosage (particularly if the cumulative dosage exceeds 65 Gy), with stereotactic radiosurgery, and with inhomogeneous RT fields. The Figure shows imaging for a patient with a glioblastoma who was treated with 75 Gy RT. The follow-up scan showed increasing enhancement and edema, but the patient remained asymptomatic and was not treated. On further follow-up, the enhancement had disappeared. This provides a good example of non-tumoral increase
of enhancement and shows that radiation necrosis can self-resolve.

**Pseudo-Progression After Radiotherapy and Temozolomide: Is It of Clinical Significance?**

A persistent problem in treating patients with glioma is to determine whether enhancement on MR scans indicates disease progression or pseudo-progression. Others have addressed the subject of transient, non-tumoral progression with or without clinical deterioration. To improve our understanding about the incidence of pseudo-progression, its possible mechanisms, and how to manage patients with early progression, we performed a retrospective analysis of all patients who received RT and temozolomide therapy at the Daniel den Hoed Cancer Center between 2000 and July 2006.

Our study included 85 patients who received RT/temozolomide for high-grade glioma (Table). Glioblastoma was diagnosed in 80%, and anaplastic glioma in 20%; 69% had resections and 31% had biopsies. Scans were obtained before RT, 4 weeks after, and then every 3 months. MRI changes were analyzed using Macdonald's criteria. Early progression was defined as progression 4 weeks after RT. Actual progression was defined as further progression within the following 6 months. Pseudo-progression was defined as at least a partial response after 3 months or a stable condition for at least 6 months after RT. Results showed that 36 of the 85 patients (42%) had early progression directly after RT; 17 of the 36 had at least stable disease for the following 6 months, or pseudo-progression according to our criteria. In 7 patients disease was stable for 6 months; in another 7 the enhancing lesion disappeared partially, and in 3 patients the lesion disappeared completely. One of the 36 patients had a resection 4 months after RT; histologic examination showed mainly necrosis. From these data we concluded that 50% of the patients with suspected early progression actually had pseudo-progression.

A similar study involved 51 patients with glioblastoma who received RT and temozolomide. Results showed that 26 of the 51 (50%) had progression within 6 months, and 15 underwent subsequent surgery. Histopathology showed that 7 of the 15 had radionecrosis.

We know that RT can injure the brain during the acute treatment phase, in the subacute phase up to 12 weeks post-radiation, and in late radiation injury, months to years post-radiation. Symptoms of subacute injury are presumably caused by vasodilatation and edema. During the acute phase, there are signs of increased intracranial pressure, such as headache; the subacute phase is associated with hypersomnolence syndrome. These symptoms are mostly transient and.

---

**Table. Retrospective Analysis of Patients with High-Grade Glioma**

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Median age, yr (range)</th>
<th>50 (18–68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathology, N (%)</td>
<td>Glioblastoma multiforme</td>
<td>68 (80)</td>
</tr>
<tr>
<td></td>
<td>Anaplastic astrocytoma</td>
<td>11 (13)</td>
</tr>
<tr>
<td></td>
<td>Anaplastic oligodendroglioma</td>
<td>3 (3.5)</td>
</tr>
<tr>
<td></td>
<td>Anaplastic oligoastrocytoma</td>
<td>3 (3.5)</td>
</tr>
<tr>
<td>Complete/partial resection vs biopsy (%)</td>
<td>69 vs 31</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Results</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Early progression, N (%)</td>
<td>36 of 85 (42)</td>
<td></td>
</tr>
<tr>
<td>Pseudo-progression, N (%)</td>
<td>18 of 36 (50)</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>7 of 18</td>
<td></td>
</tr>
<tr>
<td>Partial disappearance of lesion</td>
<td>7 of 18</td>
<td></td>
</tr>
<tr>
<td>Complete disappearance of lesion</td>
<td>3 of 18</td>
<td></td>
</tr>
<tr>
<td>Histology of necrosis at second operation</td>
<td>1 of 18</td>
<td></td>
</tr>
</tbody>
</table>
reversible, and are often relieved by corticosteroids. Late radiation effects, attributed to a severe local tissue reaction, are often progressive and irreversible. These include leukoencephalopathy syndrome with gait disturbances, cognitive decline, urine incontinence, a variety of vascular lesions, and true radiation necrosis. Radiation necrosis is evident within 3 to 12 months as contrast-enhancing masses, indistinguishable from true progression. The clinical course of radiation necrosis varies. Some patients remain asymptomatic, whereas others have progression until death. Sometimes recovery is spontaneous. Dexamethasone is often effective, but surgery may be required. Pseudo-progression occurs before the typical time period in which classical radiation necrosis is usually diagnosed. From the data of Chamberlain et al, it seems there is a continuum between pseudo-progression and “classic” radiation necrosis. Possibly, the underlying mechanism is the same and the radiation necrosis in pseudo-progression occurs earlier because of more intense treatment.

Although it is clear that there is a high incidence of pseudo-progression, there is still no good way to differentiate it from true progression. Repeat biopsy may yield answers, but is an invasive procedure with a mortality rate of 5%, which is difficult to justify when the patient has no overt signs or symptoms of progression. Prospective studies of sufficient size are needed to provide more information about the usefulness of newer imaging techniques, such as PET, spectroscopy, or diffusion-weighted imaging. For now, we advise continuing with adjuvant temozolomide in case the findings indicate true early progression. Corticosteroids can be effective in controlling edema and the high intracranial pressure. Anti-VEGF therapy may be effective, but more studies are needed. For symptomatic patients, surgery may be indicated.

REFERENCES


TARGETING THE PI3 KINASE PATHWAY

W. K. Alfred Yung, MD
MD Anderson Cancer Center, Houston, Texas

Molecular targeted therapy is a promising development for the treatment of glioblastoma multiforme (GBM), because we know much about its molecular fingerprints. Primary GBM, which accounts for approximately 60% to 70% of all GBM cases, has phosphatase and tensin homolog (PTEN) gene mutation in approximately 30% of cases, epidermal growth factor receptor (EGFR) amplification in approximately 40%, and EGFR overexpression in approximately 60%. In secondary GBM, PTEN alterations are rare, approximately 5%, and p53 mutation is greater than 65%. These molecular features are very important. A GBM may appear clinically to be a primary tumor, but molecular genotyping may indicate that it is actually a secondary tumor.
Glioma progression is governed by changes in major and often overlapping signaling pathways, such as EGFR, platelet-derived growth factor receptor (PDGFR), mitogen-activated protein kinase, and phosphoinositide 3 kinase (PI3K; Figure). These overlapping key junction points are targets of drug development. For example, inhibiting tyrosine kinase phosphorylation of the PI3K pathway prevents receptor activation signals from traveling downstream to the nucleus. Similarly, the Ras gene pathway can be blocked; Ras activation and neurofibromatosis type 1 gene mutations have been identified in GBM.

How do we choose which pathway or receptor to block? The success of imatinib increased interest in developing other molecular drugs. Imatinib inhibits bcr-abl, c-kit, and PDGFR. The prototypical mechanism of action for most tyrosine kinase inhibitors (TKIs) is to block binding to the adenosine triphosphate (ATP) pocket. The small-molecule inhibitor competes with this pocket so that ATP cannot bind and phosphorylate the downstream effector substrates.

As PDGFR expression is relatively high in GBM, a good response to imatinib would be expected. But early testing was very disappointing, reporting only 1 partial response in 29 patients and only 3% of patients alive by 6 months. It became apparent that imatinib did not cross the blood-brain barrier (BBB) to reach the target.

The most critical factor to consider in targeted therapy is what factor really drives proliferation of the tumor cell. PDGFR is only a minor driver for GBM; the major driver is EGFR. Thus, targeting imatinib to PDGFR has little impact on the tumor cell.

Epidermal growth factor receptor is attractive as a molecular target in GBM, because approximately 60% of cases have EGFR amplification and 30% express a mutant EGFR. The expression of EGFRviii is associated with poor survival. One study showed that EGFR expression is associated with resistance to RT and chemotherapy; Several drugs have been developed to target EGFR, including EGFR antibodies and small-molecule inhibitors. However, in GBM, EGFR antibody is too large and does not cross the BBB.

An alternative to EGFR antibody is small-molecule agents, such as TKIs that target the receptor signaling ATP binding site. Gefitinib and erlotinib are more specific against EGFR, whereas lapatinib has activity against EGFR and human EGFR-2. Gefitinib data in GBM are inconsistent. One study showed a poor response rate and another showed a good response rate. In the latter study of 38 GBMs, partial response occurred in 5 GBMs, 1 anaplastic oligodendroma, and 1 anaplastic oligoastrocytoma. The response rate was favorable, at more than 10%, but the 6-month progression-free survival (PFS) was only 9%. The standard PFS is 15%, which is achieved when using temozolomide as a single agent for recurring GBMs.

Erlotinib crosses the BBB better than imatinib, but again clinical trials produced mixed results. In one trial the response rate was 18% with erlotinib alone or combined with temozolomide. In another trial that involved 4 institutions, the response rate was only 5%, back to the same level as temozolomide alone. The 6-month PFS was in the range of 15%, as good as temozolomide but not better. The question is: why is erlotinib not very effective as a single agent?
A recent study showed evidence that glioma cells may be driven by more than 1 growth factor receptor. Most of the cells in this study had 2 or 3 receptors activated at the same time. Thus, if EGFR is blocked, PDGFR might be activated. These results suggest that targeting more than 1 receptor is needed to achieve better control.

Another reason for the failure of single-agent receptor blockers, such as erlotinib, is that the receptor actually sends signals to 2 parallel pathways. One pathway is through Ras, MEK, and ERK. The other pathway is through PI3K, protein kinase B (PKB)/Akt, mammalian target of rapamycin (mTOR), glycogen synthase kinase 3, nuclear factor κB, and vascular endothelial growth factor (VEGF) to promote angiogenesis, survival, invasion, migration, and growth. Due to loss of PTEN, PI3K and AKT are constitutively activated. Thus, despite inhibition of EGFR signal, activated AKT will continue to promote growth and survival.

When the University of California, San Francisco examined tumor samples from the 49 patients in the erlotinib trial for EGFR and PKB/Akt activities, the 8 responders had very low PKB/Akt activity, whereas the nonresponders had high PKB/Akt activity. This suggests that high PKB/Akt activity may be driving the malignant phenotype, even though EGFR is blocked. This also suggests that the presence of PTEN or low PKB/Akt activity is necessary for the tumor to respond to single-agent imatinib, erlotinib, or gefitinib.

Thus far, PKB/Akt appears to be a promising target for drug development. However, as it regulates many normal physiologic functions, including insulin and glucose metabolism, the toxicity that occurs when PKB/Akt is blocked is quite substantial and not acceptable.

Another interesting downstream target is mTOR. The toxicity produced by blocking mTOR is quite tolerable. mTOR inhibitors now being used in clinical trials include sirolimus, temsirolimus, AP23573, and everolimus (RAD001). However, it has been shown that mTOR has 2 signaling functions. One goes through the p-70S6K and S6, blocking the protein translation process. The other is an inhibitory signal to the insulin-like glucose factor 1 receptor (IGF1R). When mTOR is shut down for a prolonged period, this inhibitory signal is lifted and IGF1R is now activated with downstream activation of AKT. This may be why we see little clinical activity with CCI-779, a sirolimus analog, when it is used alone. In fact, some patients have rapid tumor progression. mTOR blocking also is associated with adverse effects, including mucositis and increased risk of infection.

The newer PI3K inhibitors under development are directed at blocking the signal pathway upstream, before it reaches mTOR. These PI3K inhibitors include PX-866, PI-103, XL765, XL147, SF1126, and BEZ235. BEZ235, which blocks both PI3K and mTOR, has been shown to be active in vitro against a series of cell lines. Blocking mTOR alone allows reactivation of PKB/Akt upstream, but blocking PI3 from upstream prevents the reactivation of PKB/Akt. BEZ235 does not induce apoptosis, but causes cell death by autophagy. It also blocks VEGF, inhibiting angiogenesis.

Studies that combined gefitinib or erlotinib with sirolimus have shown that mTOR inhibition enhances response to EGFR inhibitors in PTEN-deficient and PTEN-intact glioma cells. In one study of patients with GBM, the partial response was 19% and the 6-month PFS was 25%. These results are encouraging, but this combination also increases the risk of rash and mucositis, which can be very troubling for the patient.

Results of ongoing and future trials will provide more information on the best ways to design molecular targeted therapy and to provide the best combinations with enhanced efficacy and safety.

REFERENCES

SUMMARIES


IMMUNOTHERAPY: ALIVE AND KICKING?
Stefaan Van Gool, MD, PhD
University Hospital Gasthuisberg, Laboratory of Experimental Immunology, Leuven, Belgium

Currently, the prognosis for a patient with a high-grade glioma (HGG) is dismal. The possible therapeutic role of vaccination with mature dendritic cells (DCs) loaded with tumor lysates derived from the resected tumor is under investigation. Vaccination has been studied in a translational research program that included in vitro experiments with human cells, in vivo trials with orthotopic mouse models, phase I/II trials for patients with relapsed HGG, and a phase I/II trial that integrated immunotherapy into the primary treatment strategy for patients with newly diagnosed HGG. Clinical studies have shown that even with the best available neurosurgery, radiation therapy (RT), and chemotherapy, the median survival for patients with grade IV HGG is only 15 months. At time of relapse, all patients die within 18 months despite temozolomide treatment. Because immunotherapy has been successful in treating prostate cancer, lymphoma, melanoma, and renal cell carcinoma, trials were conducted to determine whether it might be effective for brain tumors.

To obtain material for immunotherapy, patient-derived immature dendritic tumor cells are loaded with tumor lysate and stimulated to become mature, expressing patient-derived tumor surface antigens. The cells are injected into the patient and migrate to the lymph nodes, where they should activate T cells that recognize the antigen and then travel to the tumor site to control the tumor.

Several in vitro studies with human cells demonstrated clearly that immature DCs were able to internalize HGG tumor protein and then stimulate T cells to generate tumor antigen-specific activity. Antitumoral T-cell activity was generated only in the presence of persistent interleukin-10 (IL-10) production during stimulation and the effector phase, indicating a complex cytokine regulatory mechanism in which the generation of cell-mediated cytotoxicity is strengthened by the cytokine IL-10.

For in vivo studies, transplanting splenocytes from vaccinated mice induced tumor-specific protection in the recipients. The transplanted mice died if they were inoculated with untreated tumor cells or with DCs that contained the wrong tumor antigens. The mice responded and were likely to survive if treated with DCs loaded with the RNA or lysate of the glioblastoma cell line. The mouse studies also showed that it was important to balance the immune suppressor and effector mechanisms.

Human clinical trials involved 131 children and adults with relapsed HGG in 4 cohorts. Patients received a median of 6 million mature DCs by intradermal injection; some also received imiquimod locally at the place of DC injection. For the 97 patients evaluable, median progression-free survival (PFS) was 3.6 months and overall survival (OS) was 11.25 months (2-year OS = 21.8%). Results were better for younger patients than for older patients. For the 72 patients aged older than 20, median PFS and OS were 3.7 months and 9.9 months, respectively (2-year OS =
12.9%). For the 25 patients aged younger than 20, median PFS and OS were 3 months and 16.9 months, respectively (2-year OS = 41.9%). On univariate analysis, the extent of resection influenced PFS but not OS. However, at least subtotal resection was a requirement for 3 of the cohorts. During the trials, the only adverse event was repetitive vaccine-related peritumoral edema in 1 patient with gross tumoral disease. The Karnofsky performance scores among the vaccinated patients remained stable. Patients also reported a quite good quality of life with almost no symptoms and with some improvement of cognitive function.

In the same clinical trials, separate data were collected on a subgroup of 71 patients with relapsed glioblastoma multiforme (GBM). The median age in this group was 44 years (range 7–77) and the median PFS and OS were 3.5 months and 9.9 months, respectively (2-year OS = 16.1%). In the 57 patients aged older than 20, the median PFS and OS were 3.7 months and 9.6 months, respectively (2-year OS = 7%). In the 14 patients aged younger than 20, the median PFS and OS were 2.4 months and 16.9 months, respectively (2-year OS = 42.3%). Extent of resection did not influence PFS or OS. Shortening the intervals between vaccinations produced significant improvement of PFS in the total group of adults with relapsed HGG and the subgroup with relapsed GBM. The addition of imiquimod produced a further significant improvement in PFS. The conclusion of these trials was that immunotherapy for patients with relapsed HGG is feasible without major adverse events.

Research on the efficacy of immunotherapy is being continued in a study on patients with newly diagnosed GBM that integrates immunotherapy into the primary multimodal treatment. The study plan is to enroll 60 patients. After subtotal or total resection the patient undergoes leukapheresis, then RT and chemotherapy followed by 4 weekly vaccinations with autologous GBM lysate-loaded DCs. A lysate boost is given during temozolomide maintenance therapy. Preliminary results include data on 42 patients with a median age of 57 years (range 17–70). At 6 months, 67.7% of patients had no disease progression. Median PFS and OS were 9.5 months and 20.6 months, respectively (2-year OS = 44.3%). Progression occurred in 30% of patients during RT or chemotherapy, but was still treated. After 4 weekly DC injections, there was an increase in the CD25+ fraction in CD8+ T cells. In 4 of 8 patients who were monitored for tumor antigen-reacting interferon-γ-producing T cells, there were increased numbers of these T cells at the time of vaccines 4 and 7 when compared to pre-vaccination samples. One patient had the sudden onset of hemiplegia and aphasia at the 8-month follow-up; this was diagnosed as ischemic and possibly inflammatory. No patient had any clinical autoimmune reaction. Data on functional disability and quality of life are pending.

Experience from these early trials is promising, and several centers in Europe are now collaborating on a phase III trial with overall survival as an end point. The plan is to organize randomized parallel trials for adults and children.

Areas of possible future investigation include whether varying chemotherapy regimens may yield different results. It is possible that immunotherapy does not actually kill the tumor, but may reorganize the body’s natural defenses against the tumor. More basic science research, including immune monitoring and tumor biology, will help to increase knowledge about tumors and their interactions with host immune systems. Ultimately, more effective vaccines will be created. To accomplish this there is a great need for data from well-designed international multicenter randomized trials involving large numbers of patients.

REFERENCES


RE-IRRADIATION IN RECURRENT GliOBlastoma
Brigitta G. Baumert, MD, PhD
GROW (Research Institute Growth and Development) and Maastricht University Medical Center, Maastricht, The Netherlands

INTRODUCTION
Until relatively recently, glioblastoma multiforme (GBM) was rapidly fatal. Now, data from the European Organization for Research and Treatment of Cancer study 26982-22892 show that treatment with temozolomide and radiation therapy (RT) can extend the life span of a patient with GBM for 2 years or longer.1 In this study, the 2-year survival rate with combined therapy was 26.5%. Based on these results, it is probable that the number of patients with recurrent tumors will increase. Treatment options for recurrence include surgery, re-irradiation, chemotherapy, or a combination of these treatments. Chemotherapy can be a problem for a patient who already has reduced bone marrow reserves from previous chemotherapy. A promising option for such patients is high-precision RT.

RADIOTHERAPY TECHNIQUES
The goal of high-precision RT is to increase the dose to the tumor while sparing the surrounding normal tissues. New techniques, such as stereotactic radiotherapy or intensity-modulated radiotherapy (IMRT), provide a high level of accuracy when delivering radiation to tumor tissue. The result is a substantial improvement in treatment results.

Conventional RT involved irradiating the tumor and a large area of surrounding normal brain tissue, often by using 2 opposing beams (2-dimensionally planned RT). High-precision conformal RT uses computerized tomography, magnetic resonance imaging, and 3-dimensional (3D) RT dose calculation to direct 3D beams. Radiotherapy beams are tailored so that the prescribed RT dose conforms closely to the shape of the target, while sparing surrounding normal tissue.

IMRT allows modulation of the beams to provide higher doses in specific subareas of the tumor by dividing each individual beam into several subunits (beamlets) with different dose levels (Figure). IMRT can also be used to avoid irradiation of normal brain tissue, especially crucial areas, such as the hypothalamic region or the brain stem. Compared to conventional RT, 3D high-precision RT can reduce 30% of normal brain tissue treated to a higher dose level and 50% of the overall volume of normal brain tissue irradiated.

High-precision RT for re-irradiation of GBM may be administered by a variety of techniques, including conformal treatment techniques, such as radiosurgery with a linear accelerator or a gamma knife, fractionated stereotactic RT, or intraoperative brachytherapy given with catheters. Stereotactic RT uses stereotactically guided 3D definition of an intracranial lesion as a 3D target calculation in the brain, using a neuronavigation system (external immovable rigid reference system). Stereotactic irradiation can be given as a single high-dose fraction, known as stereotactic radiosurgery. Stereotactic irradiation also can be given in a fractionated schedule, known as stereotactic RT.

Figure. Schematic Drawing of Different Radiotherapy Techniques

1. Conventional Radiotherapy
2. 3D-Conformal Radiotherapy
3. Intensity-Modulated Radiotherapy

For conventional radiotherapy opposing beams are used. For 3D-conformal radiotherapy, 3-dimensionally directed beams based on 3D planning are used. Irregular volumes can best be irradiated with intensity-modulated radiotherapy. This is reached by a variation or modulation of the beam intensity. (Gray areas with bolts represent RT beams; the gray checked volume is the irradiated area containing the tumor; and light gray shaded volume represents normal brain tissue.) 3D = 3 dimension; RT = radiation therapy.
OVERALL SURVIVAL AND TREATMENT RESPONSE

A literature search for the results of re-irradiation for patients with GBM yields several retrospective, single-center studies, but no randomized trial data. Most data concern high-grade glioma (HGG), although some include other primary brain tumors, in addition to low-grade astrocytoma. The data summarized in this article involve approximately 1700 patients with primary brain tumors who received re-irradiation ranging from conventional RT to high-tech RT, such as radiosurgery or stereotactic fractionated RT. There are fewer data available about re-irradiation of HGG, and it is difficult to compare studies because they use different treatment techniques, end points, response definitions, total dose applied, and fractionation schemes.

Data from studies with conventional fractionated RT most often report total dose level ranges within 45 to 50 Gy, in fractions of 1.5 to 2 Gy.3-8 The results are quite encouraging, with a median survival of approximately 10 months. Only a few patients had necrosis of normal brain tissue. The largest study with conventionally fractionated stereotactic re-irradiation was associated with a median overall survival of 8 months post-treatment, with only 1 clinically relevant brain necrosis reported.5

Studies on re-irradiation with brachytherapy reported a high toxicity, notably bacterial meningitis, and most required reoperation.9-18 The median survival was approximately 10 months.

Studies of a single high-dose treatment with stereotactic radiosurgery produced similar median survival times of 8 to 10 months.5,11,19-23 However, the use of a single high-dose treatment produced an increased range of brain necrosis, 6% to 22%. This is seen less often with fractionated re-irradiation, which reduces the dose administered to normal tissue.

A summary of data on hypofractionated stereotactic RT includes results up to 2007.24-30 Different schedules were used, mainly based on a fraction size of 5 Gy (eg, 6 times 5 Gy, for a total dose of 30 Gy). In dose-escalation studies, the total dose aimed for was 50 Gy. However, total doses greater then 40 Gy resulted in a higher toxicity. Again, the median survival in these studies was approximately 9 to 10 months.

Clinical response in the studies cited was usually defined as improvement of pretherapeutic neurologic symptoms and radiologic findings.3,4,6-7,24,31-39 Again, there were no uniform response criteria, and the response range reported was quite broad, ranging from 22% to 78%.

TOXICITY

There is some concern about the risk of late toxicity after re-irradiation; for example, radionecrosis of normal brain tissue can occur months to years after retreatment. The problem of brain necrosis after re-irradiation was analyzed in a dose-escalation study using fractionated stereotactic RT.24 In general, the risk of necrosis increased with the total dose applied and the volume of tissue irradiated, in particular a dose greater than 40 Gy and an irradiated volume greater than 35 cm³, were associated with an increased risk of necrosis.

A study published in 2008 describes a systematic review of 21 studies on late toxicity following re-irradiation.40 The authors found that the major factor in the risk of necrosis was the total dose received. The risk of necrosis did not increase significantly unless the total cumulative dose from the first and second irradiation was greater than 100 Gy equivalent in 2-Gy fractions. High-precision RT using a higher fraction dose did not increase the risk of brain necrosis. Time between irradiations had no effect on necrosis; the minimum interval between treatments was 3 months. The influence of treatment volume is less clear because most studies selected patients with relatively small volume targets. The conclusion was that high-precision RT, because it limits the volume of normal brain tissue exposed to RT, allows re-irradiation of recurrent HGG with an acceptable probability of radionecrosis.

PROGNOSTIC FACTORS

Several studies have examined the effects of proper patient selection on prognosis after re-irradiation.5,6,27,30,34 A summary of multivariate analysis data from these studies shows a more favorable prognosis with an initial diagnosis of a low-grade tumor, a greater extent of resection (radical resection), frontal-lobe tumors, age younger than 50 at diagnosis, good performance status, complete response rate after the first irradiation, and a relatively long interval between irradiations (>24–36 months). One study reported that the combination of RT and temozolomide as treatment for recurrence improved prognosis.27
PATIENT SELECTION

An overview from the New Approaches to Brain Tumor Therapy Central Nervous System consortium pooled data from 10 prospective phase I and II trials that involved a total of 333 patients with recurrent HGG. Patients were treated with chemotherapy or brachytherapy; a very small group received re-irradiation. The median overall survival for the population as a whole was 7 months. A recursive partitioning analysis was applied in order to define prognostic factors. Data showed the longest median survival was 26 months for patients with tumors localized in the frontal lobe, no initial GBM histology, and a good performance status (Karnofsky performance status ≥80). Multivariate analysis showed that the prognosis improved with younger age, better performance status, if the initial histology was not GBM, or if the patient did not receive corticosteroids. The defined prognostic recursive partition analysis groups need to be validated in prospective studies.

CONCLUSIONS

A review of relevant literature confirms the value of re-irradiation for recurrent GBM. Survival time after re-irradiation is approximately 9 months, whether or not chemotherapy is added. Radiologic response is approximately 30%. The interval between first treatment and re-irradiation should be at least 3 months; 10 to 12 months would be ideal. Re-irradiation produces no significant acute toxicity if applied in a fractionated manner. Brain necrosis depends on the RT technique used, ranging from 4% to 9% for fractionated RT to 13% to 22% for single-fraction RT and 50% for brachytherapy. The risk of brain necrosis increases as tumor volume increases. Currently, most centers re-irradiate using hyperfractionated stereotactic or high-precision RT. The minimum dose for hyperfractionated stereotactic RT should be 30 Gy in fractions of 5 Gy, and the maximum would be 40 Gy (which would be calculated as 55 Gy for a conventional fractionation scheme). The maximum dose for conventional fractionated RT is 54 Gy; 46 Gy is often recommended. The prognosis may be improved by adding chemotherapy or temozolomide. Other factors that affect prognosis include the interval between irradiations, age, performance status, and tumor grade. As these data are from retrospective studies, there is a great need for well-designed prospective studies.

REFERENCES

SUMMARIES


