

[A **technical** review]

MGMT Gene Methylation Assay

A Biomarker for Therapy Consideration in Glioblastoma

Brain Cancer Background

Each year more than 20,500 people in the US are diagnosed with brain or central nervous system cancers. Approximately 13,000 people die each year of primary malignant brain tumors.¹ The relative risks of brain cancers are higher for men, the elderly, Caucasians, and those living in metropolitan areas.¹

The diagnosis of brain cancer in an adult, and even more so in a child, conveys a very poor prognosis for the patient. While malignant brain tumors represent only a small fraction of all cancers (1.4%), the outcome is typically fatal, with a 5-year survival rate for all brain cancers of just 30%.¹

The most common primary brain tumors are called gliomas as they begin in the glial tissues. Gliomas account for approximately 36% of all brain tumors and 81% of malignant tumors.¹ There are several types of gliomas, with glioblastomas and astrocytomas accounting for 75% of all gliomas.¹ Astrocytomas arise from star-shaped glial cells called astrocytes. A grade III astrocytoma is sometimes called an anaplastic astrocytoma.² A grade IV astrocytoma is usually called a glioblastoma multiforme (GBM). In the US there are 8400 to 9800 individuals diagnosed with GBM each year.² GBM is the most aggressive of all brain cancers with high morbidity and mortality. Despite recent advances in therapy, the median survival for patients with GBM is only 12 to 15 months.² In an effort to improve this outlook, important advances have led to a better understanding of the molecular pathogenesis of malignant gliomas and response to treatment, particularly with DNA-damaging drugs such as alkylating agents.²

Treatment Options for GBM

Treatment options for GBM and other high-grade gliomas are evolving rapidly. Standard therapeutic choices include radiation therapy in addition to surgery or surgery combined with chemotherapy. In the last several years, a major change in chemotherapy for high-grade gliomas has occurred with the introduction of temozolomide (Temodar[®]), a next-generation alkylating agent.³

Temozolomide is an oral chemotherapeutic agent that has been noticeably effective (compared to other chemotherapeutic agents) in the treatment of glioma. A recent phase III clinical trial⁴ comparing the regimen of temozolomide plus radiation versus radiation alone in patients with newly diagnosed glioblastoma showed that patients exhibited significant improvement in time-to-progression and overall survival when treated with radiotherapy and temozolomide, versus radiotherapy alone.⁴ Median survival for patients being treated with the combination of radiation and temozolomide was 14.6 months, compared to 12.1 months for patients receiving radiation only, a difference that was statistically significant ($P < 0.001$).⁴ More impressive was the difference in the 2-year survival rate, which was 26.5% for the patients receiving radiation therapy and temozolomide, but only 10.4% for those receiving radiation alone.⁴ Following the report of this study, temozolomide was approved for use in patients with newly diagnosed glioblastoma in conjunction with radiotherapy.

The main cytotoxic action of temozolomide is affected by its role as an alkylating agent, with the antitumor activity of temozolomide due to the creation of cross-links between the strands of the tumor DNA, which when they are not adequately repaired, result in cell death.⁵

MGMT Gene Methylation and Response to Alkylating Agents

Gene methylation is a DNA-based control mechanism that regulates gene expression. In cancer, gene promoter regions can have abnormal or increased levels of methylation (hypermethylated). This epigenetic modification to DNA blocks gene function, resulting in the protein encoded for by the gene not being produced or being produced at a diminished level.

In a healthy individual, O6-methylguanine-DNA methyltransferase (MGMT) is an essential DNA repair enzyme. The activity or function of this gene is frequently lost during cancer development. Loss of the MGMT gene or MGMT function makes an individual more susceptible to DNA damage and prone to tumor development. Once the cancer has developed, however, the presence or absence of MGMT activity plays a critical role in response to therapy. The loss of or silencing of MGMT expression (which made the cell more likely to become a cancer cell in the first place) makes the tumor cells more sensitive to radiation therapy and alkylating drugs such as BiCNU® (BCNU, carmustine), CeeNu® (CCNU, lomustine), Temodar® (temozolomide), and Cytosan® (cyclophosphamide).⁵ Several studies have shown that approximately 40% to 45% of GBM tumors exhibit MGMT gene methylation.^{5,6} If the MGMT gene is methylated, then the tumor is more likely to respond favorably (more tumor death) when alkylating agents are administered.

Alkylating agents inhibit cellular proliferation by cross-linking DNA, resulting in cell death.⁵ The damaging action of alkylating drugs can be reversed by DNA repair enzymes, such as MGMT, that remove the cross-linked structures, facilitating DNA repair. Hypermethylation of MGMT results in diminished levels or loss of MGMT expression. Tumor cells that do not express MGMT will not survive when treated with alkylating agents. In this manner, a patient's MGMT gene methylation status can help predict a patient's clinical response to treatment. Studies indicate that patients whose tumors exhibit methylated MGMT have shown a more favorable response to alkylating agent-based therapy than those with normally functioning (unmethylated) MGMT.⁵

Studies such as those by Hegi et al⁶ have demonstrated a correlation between MGMT gene methylation status and

response to alkylating agents. In these analyses, the majority of the survival benefit for patients being treated with radiotherapy and temozolomide was realized by those patients whose tumors were methylated for MGMT.⁶ The difference in survival between patients with methylated versus unmethylated MGMT was statistically significant ($P < 0.001$), favoring patients with tumors that were MGMT methylated.⁶

Table—1. Effect on Survival of Methylated MGMT Gene vs Unmethylated MGMT Gene⁶

Treatment with RT and TMZ		Methylated MGMT	Unmethylated MGMT
Progression-free Survival	Median Duration (months)	10.3	5.3
	Rate at 6 months (%)	68.9%	40.0%
Overall Survival	Median Duration (months)	21.7	12.7
	Rate at 2 years (%)	46.0%	13.8%

Adapted from *N Engl J Med.* 2005;352:999.

Numerous studies have supported the conclusions of Hegi et al⁶, which demonstrated that a patient's MGMT gene methylation status could help identify those patients who are most likely to benefit from alkylating agent-based therapy. Equally important could be identification of those patients who are least likely to benefit and would therefore warrant consideration for alternative approaches to temozolomide therapy based on different dosing regimens, or in combination with other therapies or MGMT inhibitors.^{7,8}

MGMT Methylation by Methylation-specific PCR (MSP)

The MGMT methylation assay utilizes methylation-specific PCR (MSP) technology to measure MGMT gene promoter methylation levels in paraffin-embedded tumor tissue samples. Unmethylated cytosine residues are the targets for bisulfite treatment and are converted to uracil, whereas methylated cytosines are not affected by this pretreatment step.^{6,9} MSP assays then use unique and separate primers to distinguish methylated from unmethylated DNA sequences. The real-time PCR format of the MSP assay correlates well with the gel-based assays that were used to assess response to treatment.^{6,8-9}


As methylation of MGMT results in decreased expression, studies have evaluated the correlation between

MGMT gene methylation status and MGMT expression using immunohistochemical techniques (IHC). In most of these studies, MGMT expression has shown poor to moderate correlation with MGMT gene methylation status and no significant association with patient outcome. Immunohistochemical assays such as these are often impacted by several operational limitations, such as inter-observer variability, expression from nonneoplastic cells, and antibody/methodologic variability, which underscore the difficulties and limitations in applying IHC techniques for determination of MGMT function in tumor tissue.^{10,11}

MGMT Gene Methylation Assay Clinical Utility

Results from retrospective studies have demonstrated that GBM patients with methylated MGMT have a longer progression-free survival and better overall survival than patients with unmethylated MGMT when treated with alkylating agent-based therapies such as temozolomide. While the results of the various retrospective studies support the potential prognostic and predictive importance of methylation status of the MGMT gene promoter, confirmation of the retrospective study data requires additional prospective validation studies.

Currently, several prospective studies are underway to evaluate MGMT gene methylation status relative to alternative approaches for GBM therapy, such as dose-intensified temozolomide schedules, combination therapies, and MGMT resistance-modulating strategies.^{3,8} The MGMT gene methylation assay can provide standardized guidance for consideration in such trials.^{12,13}

The determination of the methylation status of key genes, such as MGMT, is becoming an important diagnostic method in oncology, and along with technologies that characterize gene mutation status and gene expression levels are providing the tools to diagnose, manage, and treat a variety of cancer types. 

MGMT (O6-Methylguanine-DNA Methyltransferase) Gene Methylation Assay 489280

CPT 83891; 83896(x2); 83898(x2); 83907; 83912

Synonym MGMT Methylation Assay, O6-Methylguanine-DNA Methyltransferase

Special Instructions Please provide a copy of the pathology report. Direct any questions regarding this test to customer service at 800-533-0567.

Specimen Formalin-fixed, paraffin-embedded (FFPE) tissue. Please send four precut, unstained slides from paraffin block in 5-µM sections.

Volume Four unstained slides at 5 µM or FFPE tissue block

Minimum Volume Two unstained slides at 5 µM

Collection Please provide four unstained slides at 5 µM or tissue block.

Storage Instructions Maintain blocks/slides at room temperature.

Causes for Rejection Tumor block containing insufficient tumor tissue; tumor fixed in a heavy metal fixative; broken or stained slides

Use Determination of methylation status of the MGMT gene promoter region

Limitations Preparation of DNA from tissue samples is dependent on the quality of the provided specimen. Inadequate DNA extraction may occur in a significant number of paraffin-embedded samples. This procedure may be considered by Medicare and other carriers as investigational and, therefore, may not be payable as a covered benefit for patients.

Methodology Quantitative methylation-specific polymerase chain reaction (PCR)

Additional Information MGMT (O6-methylguanine-DNA methyltransferase) is a DNA repair enzyme that is involved in the repair of damage caused by a variety DNA cross-linking compounds, including alkylating agents. Increased methylation of the MGMT gene promoter region causes diminished or silenced expression of the gene, making cells more sensitive to DNA damage. This relationship has been shown for glioblastomas and alkylating agents such as temozolomide (Temodar®). Approximately 40% to 45% of glioblastoma multiforme (GBM) tumors exhibit MGMT gene methylation. Retrospective studies have shown that detection of MGMT promoter methylation in tumor samples is associated with an increased likelihood of a favorable response to temozolomide.

References

- Vlassenbroeck I, Califice S, Diserens A-C, et al. Validation of real-time methylation-specific PCR to determine O6-methylguanine-DNA methyltransferase gene promoter methylation in glioma. *J Mol Diagn*. 2008 Jul; 10(4):332-337.
- Hegi ME, Diserens A, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005 Mar 10; 352(10):997-1003.
- Brandes AA, Franceschi E, Tosoni A, et al. MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. *J Clin Oncol*. 2008 May 1; 26(13):2192-2197.
- Hau P, Stupp R, Hegi ME. MGMT methylation status: The advent of stratified therapy in glioblastoma? *Dis Markers*. 2007; 23(1-2):97-104.

References

1. Central Brain Tumor Registry of the United States. CBTRUS 2007-2008 Primary Brain Tumors in the United States Statistical Report 2000-2004. 2008.
2. Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med*. 2008;359:492-507.
3. Gilbert MR, Armstrong TS. Management of patients with newly diagnosed malignant primary brain tumors with a focus on the evolving role of temozolomide. *Ther Clin Risk Manag*. 2007;3:1027-1033.
4. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987-996.
5. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med*. 2000;343:1350-1354.
6. Hegi ME, Diserens A, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005;352:997-1003.
7. Mrugala MM, Chamberlain MC. Mechanisms of disease: Temozolomide and glioblastoma – look to the future. *Nat Clin Pract Oncol*. 2008;58:476-486.
8. Hegi ME, Liu L, Herman JG, et al. Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcome in glioblastoma and clinical strategies to modulate MGMT activity. *J Clin Oncol*. 2008;26:4189-4199.
9. Vlassenbroeck I, Califice S, Diserens A-C, et al. Validation of real-time methylation-specific PCR to determine O6-methylguanine-DNA methyltransferase gene promoter methylation in glioma. *J Mol Diagn*. 2008;10:332-337.
10. Rodriguez FJ, Thibodeau SN, Jenkins RB, et al. MGMT immunohistochemical expression and promoter methylation in human glioblastoma. *Appl Immunohistochem Mol Morphol*. 2008;16:59-65.
11. Preusser M, Janzer RC, Felsberg J, et al. Anti-O6-methylguanine-methyltransferase (MGMT) immunohistochemistry in glioblastoma multiforme: Observer variability and lack of association with patient survival impede its use as a clinical biomarker. *Brain Pathol*. 2008;18:520-532. [Epub 2008 Apr 8].
12. Brandes AA, Tosoni A, Franceschi E. Adjuvant therapy in glioblastomas: false steps and real advances. *Expert Rev Anticancer Ther*. 2007;7:607-608.
13. Iafrate AJ, Louis DN. “MGMT for pt mgmt”: Is methylguanine-DNA methyltransferase testing ready for patient management. *J Mol Diagn*. 2008;10:308-310.



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