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MGMT Pyrosequencing Methylation Assay for Glioblastoma

Background

Glioblastoma is the most common and most aggressive malignant primary brain tumor. While occurring in only two to three cases per 100,000 people in North America, glioblastoma represents 52% of all functional tissue brain tumor cases and 20% of all intracranial tumors. Prognosis for those diagnosed with glioblastoma is poor, with a median survival time of about 14 months.[1]

Patients with glioblastoma can be treated with alkylating agents such as Temador® (temozolomide). Epigenetic silencing of the *MGMT* (O^6-methylguanine-DNA methyl-transferase) DNA-repair gene by promoter methylation compromises DNA repair and has been associated with longer survival in patients with glioblastoma who receive temozolomide.[2,3]

Temozolomide kills tumor cells by producing cross-links between DNA strands and inhibiting DNA replication. The most common alkylation site is the O^6 position of guanine. O^6-methylguanine DNA methyltransferase (*MGMT*) is a DNA repair protein that reverses such DNA alkylation and confers chemoresistance by repairing DNA damage. Temozolomide seems to work by sensitizing the tumor cells to radiation.[4]

Recent clinical studies confirm that the presence of *MGMT* promoter methylation in tumor samples corresponds to an increased likelihood that tumor cells would be responsive to temozolomide.[3,4,5,6] If the promotor was methylated, temozolomide was more effective. It is estimated that approximately 40 to 50% of glioblastoma tumors exhibit *MGMT* gene methylation, which correlates significantly with reduced DNA damage repair induced by alkylating agents and significantly enhanced chemosensitivity.[4]

According to recent clinical trials, glioblastoma patients with *MGMT* methylation respond to temozolomide two to three times better than those lacking of *MGMT* methylation. Prolonged overall and progression-free survival at 24 months was 80% for those with *MGMT* methylation vs. 20% for those lacking *MGMT* methylation.

Diagnostic *MGMT* testing requires sufficient and optimally preserved tumor tissue. Cleveland Clinic's *MGMT* methylation assay is a new, quantitative MSP test to detect the methylation status of brain tissue that has undergone thorough clinical evaluation. Our protocol calls for a minimal tissue sample size of ½-centimeter, which is much smaller than other laboratories that typically require at least a minimum 1-centimeter sample size. The best results are obtained with cryopreserved tumor specimens.

Clinical Indications

For patients diagnosed with glioblastoma to determine if a methylated *MGMT* promoter is present, which is a favorable prognostic indicator for temozolomide treatment. Individuals without a methylated *MGMT* promoter do not have such a benefit. *MGMT* "silence" is the most significant guide for the treatment of glioblastoma.This assay is to validate the methylation status of the *MGMT* gene.

Methodology

Pyrosequencing technology, which is based on the principle of sequencing by synthesis, provides quantitative data in sequence context within minutes. Real-time sequence information is highly suitable for quantification of CpG methylation. We have validated pyrosequencing-based assay in detection of *MGMT* methylation in paraffin-embeded biopsy tissue specimens. With 10% average methylation as a cutoff, *MGMT* promoter methylation was detected in glioblastoma, but not detected in non-neoplastic brain tissue. The analytical sensitivity of the assay is 5% of target cells harboring *MGMT* methylation.

Diagnostic *MGMT* testing requires sufficient and optimally preserved tumor tissue. The biopsy should be at least 0.5 cm in size and necrosis should be less than 15%. Both frozen and paraffin-embedded tissue are suitable for the pyrosequencing-based *MGMT* methylation assay.

Interpretation

Positive for *MGMT* methylation: equal or greater than 10% of methylation in any CpG island or in average of all CpG islands analyzed.

Negative for *MGMT* methylation: less than 10% of any CpG island or in average of all CpG islands analyzed.

References

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3. Hegi M, *et al*. MGMT Gene Silencing and Benefit from Temozolomide in Glioblastoma. *New England J Medicine.* 2005;352;10:997-1003.
4. Chamberlain Marc C, *et al*. "Early necrosis following concurrent Temodar and radiotherapy in patients with glioblastoma". *Journal of Neuro-Oncology*. 82(1):81-3. doi:10.1007/s11060-006-9241-y. PMID 16944309.
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6. Brandes A, *et al*. MGMT Promoter Methylation Status Can Predict the Incidence and Outcome of Pseudoprogression After Concomitant Radiochemotheraphy in Newly Diagnosed Glioblastoma Patients. *J. Clinical Oncology*. 2008; 26;13:2192-2197.

Test Overview

Test Name	*MGMT* Methylation Assay for Glioblastoma
Ordering Mnemonic	*MGMT* Methylation
Specimen Requirements	The biopsy should be at least 0.5 cm in size and necrosis should be less than 15%. The best results with methylation-specific PCR are obtained with cryopreserved tumor specimens.
Reference Range	Positive for *MGMT* methylation: ≥ of 10% of any CpG island or average of all 5 CpG islands. Negative for *MGMT* methylation: >10% of methylation or average of all CpG islands analyzed.
CPT Code	81287 (x1); G0452 (x1)

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