MGMT Promoter Methylation Conferring Sensitivity to Alkylating Agents

The UNC Hospitals Molecular Genetics Laboratory performs DNA pyrosequencing to determine promoter methylation status of the O\textsuperscript{6}-methylguanine methyltransferase (MGMT) gene. Promoter methylation is associated with low levels of MGMT protein and accompanying increased sensitivity of brain tumors, specifically glioblastomas, to alkylating agents such as BCNU (carmustine)\textsuperscript{1}. Low levels of MGMT also appear correlated with prolonged progression-free survival (PFS) in patients with gliomas treated with temozolamide\textsuperscript{2}.

**Biology of the process:** MGMT is a DNA repair enzyme that is associated with tumor resistance to alkylating agent therapy\textsuperscript{3}. MGMT rapidly reverses alkylation, including methylation, at the O\textsuperscript{6} position of guanine by transferring the alkyl group to the active site of the enzyme\textsuperscript{4}. Lack of MGMT in the cell allows accumulation of O\textsuperscript{6}-alkylguanine in the DNA which, following incorrect pairing with thymidine, triggers mismatch repair, inducing DNA damage signaling and cell death\textsuperscript{5}. Lack of MGMT expression is due to methylation of a CpG island located in the 5’ region of MGMT (bp -552 to +289) which includes 97 CpGs\textsuperscript{6}. Methyl-CpG-binding proteins will bind to aberrantly methylated sequences which leads to alterations in chromatin structure, thus preventing the binding of other transcription factors, effectively silencing MGMT\textsuperscript{6}.

**Clinical indications for MGMT promoter methylation testing:** Patients newly diagnosed with high grade gliomas (anaplastic astrocytomas and glioblastomas) or patients with gliomas who are being considered for temozolamide therapy.

**Laboratory testing for MGMT promoter methylation:** The preferred sample is a paraffin block containing at least 50% malignant cells or five unstained slides, 4-8 uM thick on plain glass, plus an H&E stained slide and a copy of the surgical pathology report. Tumor cells are enriched by macro-dissection, if needed, and the extracted DNA is subjected to a bisulfite-treatment step followed by pyrosequencing to determine methylation status of selected sites in the CpG island of the MGMT promoter. Results are interpreted by a pathologist. Turn-around time for results is expected to be two weeks.


**Questions?** Please consult a pathologist in the Molecular Genetics Lab at 919-966-4408 or e-mail Dr. Margaret L. Gulley at Margaret_gulley@med.unc.edu.