

Personalized treatment strategies in glioblastoma: *MGMT* promoter methylation status

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Abstract: The identification of molecular genetic biomarkers considerably increased our current understanding of glioma genesis, prognostic evaluation, and treatment planning. In glioblastoma, the most malignant intrinsic brain tumor entity in adults, the promoter methylation status of the gene encoding for the repair enzyme *O6-methylguanine-DNA methyltransferase (MGMT)* indicates increased efficacy of current standard of care, which is concomitant and adjuvant chemoradiotherapy with the alkylating agent temozolomide. In the elderly, *MGMT* promoter methylation status has recently been introduced to be a predictive biomarker that can be used for stratification of treatment regimes. This review gives a short summary of epidemiological, clinical, diagnostic, and treatment aspects of patients who are currently diagnosed with glioblastoma. The most important molecular genetic markers and epigenetic alterations in glioblastoma are summarized. Special focus is given to the physiological function of DNA methylation – in particular, of the *MGMT* gene promoter, its clinical relevance, technical aspects of status assessment, its correlation with *MGMT* mRNA and protein expressions, and its place within the management cascade of glioblastoma patients.

Keywords: glioblastoma, *MGMT*, temozolomide, personalized treatment, outcome

Glioblastoma

Diffuse gliomas are heterogeneous neoplasms that account for half of all intrinsic brain tumors. Histological grading of these tumors according to the classification system of the World Health Organization (WHO) provides a basis for defining groups of patients for clinical assessment, but also predicts the clinical behavior of the respective neoplasm with direct impact on the applied treatment regimes. WHO grade IV gliomas are characterized by high cellularity, cellular pleomorphism, nuclear atypia, brisk mitotic activity, microvascular proliferation, and necrosis.¹ They account for 50%–60% of all astrocytic gliomas, with an increasing incidence of currently three to five new cases per 100,000 patient-years.² The cause for glioblastoma development is not clear. Some authors speculate that cytomegalovirus infection may drive the oncogenic process by modulating growth factor and receptor expressions in gliomas.³ Clinically, these tumors are rapidly progressive and ultimately fatal, with a median survival of only 15 months in trial populations.⁴ Primary glioblastomas develop with only a short clinical history and without evidence of a previous lesion of lower malignancy. They account for the vast majority of glioblastomas in older patients. Morphologically, they cannot be distinguished from about 5%–10% of glioblastomas that develop in younger patients below the age of 50 years by progression from a pre-existing lower-grade glioma.^{5,6}

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These secondary glioblastomas differ significantly in their genetic profiles from primary glioblastomas.^{7,8}

The diagnostic challenge

Routine diagnosis critically relies on magnetic resonance imaging (MRI) that frequently exhibits contrast enhancement of the lesion in T₁-weighted sequences accompanied by perifocal edema as being depicted on T₂-/fluid-attenuated inversion recovery-weighted images. Lack of focal contrast enhancement, however, does not exclude grade IV histology.^{9,10} The vast majority of glioblastomas develop in the cerebral hemispheres. However, the highly infiltrative growing pattern frequently causes tumor spread into the basal ganglia or even the contralateral hemisphere, with unfavorable effects on patients' outcome. Glioblastomas with primary location in the cerebellum, brain stem, or myelon are rare. Metabolic imaging such as positron emissions tomography (PET) utilizing amino acid tracers (eg, O-[2-{¹⁸F}fluoroethyl]-1-tyrosine) is increasingly used to assess differential diagnosis,^{11,12} biological tumor volume, and intratumoral heterogeneity,¹⁰ and to monitor therapy.^{13–15} Mainly due to its limited availability and associated costs, metabolic imaging has not yet been routinely implemented into a current standard of care algorithm for glioblastoma patients. Ultimately, tissue samples are needed for definite histological diagnosis. Neuropathological evaluation of gliomas, however, suffers from significant interobserver variability, particularly in the distinction of grade III and grade IV histology and the determination of an oligodendroglial cell component.¹⁶

Current treatment concepts

More recently, some progress has been achieved in the treatment of glioblastoma patients. A companion prospective multicenter study performed by the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) has shown that the addition of the alkylating agent temozolomide to radiotherapy improved 2-year survival of patients with newly diagnosed malignant glioma (mainly glioblastoma) from 11.0% to 27.3%, 3-year survival from 4.4% to 16.0%, and 5-year survival from 1.9% to 9.8%, respectively.^{17,18} Temozolomide is an orally administered chemotherapy with limited side effects and a good penetration of the blood–brain barrier. It prevents replication by alkyl group-mediated crosslinking of DNA.¹⁹ Currently, radiotherapy with concomitant and adjuvant temozolomide is the gold standard for patients with newly diagnosed glioblastoma.⁴ Most recent results

from the Phase III study AVAglio, evaluating the addition of the antiangiogenic agent bevacizumab to combined chemoradiotherapy with temozolomide, have indicated favorable effects on progression-free survival (median 10.6 months versus 6.2 months) and duration of good clinical performance (Karnofsky performance status ≥ 70 ; 9 months versus 6 months).^{20,21} However, no effect on overall survival was observed. In the elderly, the best treatment strategy is highly influenced by the patient and tumor-related factors and ranges from combined chemoradiotherapy with temozolomide for highly selected patients with good clinical performance status to either radiotherapy or temozolomide treatment alone, and supportive care only.^{22–28}

Open tumor resection also represents one of the mainstays in glioblastoma treatment and is usually considered the first step within the management algorithm.^{29,30} The highly infiltrative growing pattern of glioblastomas into surrounding brain tissues explains that curative resection generally cannot be achieved. However, there is good evidence that overall survival is improved if early postoperative MRI depicts that no residual contrast-enhancing tumor mass has been left in situ (“gross total resection”).^{31–35} In a community setting, complete resection is achieved in about 40%–45% of patients, a similar proportion receives incomplete resection, and about 10%–20% are diagnosed by biopsy only.²¹ The number of patients without residual tumors can be significantly increased if microsurgical tumor removal is performed with the aid of 5-aminolevulinic acid and intraoperative MRI.^{31,36} The prognostic impact of incomplete resection is poorly defined.³⁰ Favorable impacts on survival rates have been suggested if at least 78% of contrast-enhancing tumor volume has been successfully removed.³⁷ However, this and other studies did not compare the impact of incomplete resection with biopsy only, and there is some evidence that biopsy only with early transferral to chemoradiotherapy might be appropriate in selected patients in whom even partial resection bears a considerable risk of surgery-related sequelae.³⁸ In patients suffering from clinical deterioration due to a large space-occupying tumor burden, decompressive surgery should be attempted.³⁰

Although the use of temozolomide has improved outcome considerably, almost all patients suffer from recurrent disease. Local tumor progression (within 3 cm from the margin) is the predominant pattern (93.5%) of treatment failure.^{39–42} In general, tumor recurrence is associated with poor outcome as treatment options are limited.⁴³ Approximately one-quarter of patients with glioblastoma develop a type of recurrence that allows for local treatment including repeated

surgical resection.⁴⁴ Although reoperation might improve post-recurrence survival in selected cases, its value must be counterbalanced by the risk of surgery-related morbidity and mortality.^{45,46} Other local treatment options include stereotactic radiosurgery for small lesions, hypofractionated radiotherapy protocols, even for larger recurrent tumors, and any combination of local irradiation and chemotherapy.⁴³ Systemic therapy is generally considered essential for recurrent tumors. Various antineoplastic agents have been tested. Efficacy, however, is generally hampered by the broad chemoresistance of glioblastoma cells, in parallel with the limited bioavailability of most drugs inside the central nervous system. The most important approaches include rechallenge regimes with dose-intensified temozolomide and non-temozolomide-containing regimes.^{43,47} Since 2009 the monoclonal antibody to vascular endothelial growth factor bevacizumab has gained US Food and Drug Administration approval for recurrent glioblastoma based on response rate, with favorable effects relative to historical controls from noncomparative Phase II trials.^{48,49} The rejection in Europe, however, was based on the absence of a randomized trial with a bevacizumab-free control arm.⁴⁷ Other targeted approaches address the epidermal growth factor receptor, mammalian target of rapamycin, histone deacetylase, and many other structures in recurrent glioblastoma. However, postrecurrence survival rarely exceeds 6–9 months in most clinical study populations.

The prognostic network of glioblastoma patients

In glioblastoma, the patient's prognosis and response to therapy are highly influenced by clinical and molecular genetic factors that are increasingly used for prognostic profiling and individualized risk-adapted treatment considerations.⁵⁰ Classical phenotypical traits correlating with favorable survival are younger age, good performance status, and noneloquent circumscribed tumor formations.^{8,35,51–53} However, these conventional prognostic factors do not necessarily account for the highly variable clinical courses of glioblastoma patients.

Molecular biomarkers have improved our understanding of glioma development, are increasingly exploited for glioblastoma subclassification, and have gained prognostic/predictive relevance. From a clinical perspective, a biomarker should be highly sensitive and specific in providing information relevant for diagnosis, prognosis, or therapy of a disease. So far, only a few biomarkers have gained clinical relevance in glioblastoma patients.

Genetic markers relevant for glioma patients

Mutations in the gene encoding for the Krebs cycle enzyme, *isocitrate dehydrogenase (IDH)*, have been identified as one of the earliest molecular events in the pathway of glioma genesis. They are frequent in grade II/III astrocytomas, oligoastrocytomas, and oligodendrogliomas, and can be used to differentiate primary and secondary glioblastomas.^{6,54,55} These findings suggest that histologically differently appearing tumor subclasses might share common precursor cells, and that histologically indistinguishable tumors (eg, primary versus secondary glioblastomas) could be separated into biologically distinct subclasses. *IDH* point mutations are predominantly found at the arginine 132 (*IDH1*), but can also occur at arginine 172 (*IDH2*). These mutations cause alterations in the active site of the *IDH* enzyme and result in increased production of 2-hydroxyglutarate, which is associated with an increased risk of cancer and glioma progression.⁵⁶ *IDH1* mutations are associated with favorable outcome in WHO grade III and IV malignant gliomas.^{57,58} Notably, patients with *IDH1* wild-type anaplastic astrocytoma WHO grade III experience even worse outcome than those with glioblastomas WHO grade IV harboring an *IDH1* mutation.⁵⁹ The prognostic impact of the *IDH1* status in WHO grade II astrocytomas is more controversial but might be associated with unfavorable outcome until tumor progression occurs.⁶⁰ *TP53* mutations are frequent in 70% of secondary glioblastomas and seldom in primary glioblastomas.⁶¹ *TP53* modulates cell-cycle control in tumor cells and has been associated with unfavorable outcome in grade II gliomas.^{62,63} Loss of heterozygosity on chromosome arms *1p* and *19q* (*LOH 1p/19q*) is frequent in oligodendroglial tumors, and has been associated with increased chemosensitivity in grade III gliomas.^{64,65} Whether *1p*- and *19q*-codeleted tumors have a less aggressive natural course than noncodeleted tumor has not been clarified so far. In glioblastoma, *LOH 1p/19q* is rare (~5%).⁵⁰ However, an oligodendroglioma-like component (GBM-O) is seen in 15% of all glioblastomas and has been associated with improved clinical outcome.⁶⁶ Whether a favorable outcome in these patients is also influenced by *LOH 1p/19q* has not been unequivocally clarified.^{67,68} Other molecular genetic markers, such as *PTEN* mutation, epidermal growth factor receptor variant III and *CDK 4* amplifications, and *CDKN2 A* homozygous deletion, can be used to separate primary from secondary glioblastomas, indicating different biology and cells of origin.^{8,69} However, these markers have not yet gained prognostic relevance in clinical trials.⁷⁰

Besides a mutated *IDH1* status and *LOH 1p/19q*, methylation of the promoter region of the *O6-methylguanine-DNA methyltransferase (MGMT)* gene has been correlated with favorable outcome in malignant glioma patients.

Physiological function of DNA methylation and its role in glioma formation

One of the first and most important epigenetic modifications studied in humans is DNA methylation, which describes the covalent addition of a methyl group preferentially at the 5'-position of a cytosine or guanine nucleotide. These CpG dinucleotides tend to cluster to so-called CpG islands, being located in the promoter regions of more than half of all human genes, or to CpG island shores, which are regions of lower CpG density that lie in close proximity to CpG islands.^{71,72} DNA methylation is mediated by the DNA methyltransferase family of enzymes. These enzymes catalyze the transfer of a methyl group from S-adenosyl methionine to DNA and are responsible for maintaining the methylation pattern (eg, during DNA replication).^{73,74} Generally, CpG methylation is closely associated with transcriptional inactivation. Less frequently, when occurring at gene bodies, it can cause transcriptional activation. Genome-wide methylation patterns have been associated with tumor initiation and progression in several cancers, including colon, breast, and lung.^{75,76}

DNA methylation in glioblastoma

The genome of glioblastoma cells shows broad hypomethylation with specific areas of hypermethylation.^{77–80} This characteristic pattern has been associated with increased genetic instability, silencing of tumor suppressors such as *TP53* and *PTEN*, and activation of oncogenes. Hypermethylation mostly occurs at the promoter CpG island of genes that are associated with tumor suppression,^{81,82} DNA repair,⁸³ cell-cycle regulation,⁸⁴ apoptosis,^{85,86} invasion,^{87,88} and migration.⁸⁹ Interestingly, the methylation patterns differ between gliomas of WHO grade II–IV.⁹⁰

The Cancer Genome Atlas project has identified a glioma CpG island methylation phenotype that correlated with younger age, a proneural gene expression profile,⁹¹ and longer overall survival in glioblastoma patients.⁹² Moreover, a high frequency of *IDH1* mutations indicates a link between metabolic alterations and epigenetic modification in these tumors.^{93,94} Increased production of the metabolite 2-hydroxyglutarate interferes with α -ketoglutarate-dependent enzyme, including histone demethylases and the TET

family of 5-methylcytosine hydroxylases,^{56,95} which causes genome-wide histone modifications,⁹⁶ and alterations of DNA methylation status⁹⁷ in malignant glioma cells.^{98,99}

MGMT promoter methylation in glioblastoma

In glioblastoma, promoter methylation of the gene encoding for *MGMT* is undoubtedly the genetic fingerprint with highest impact on clinical practice. The *MGMT* gene is located at chromosome 10q26 and codes for a ubiquitously expressed suicide DNA repair enzyme that removes alkyl adducts from the O⁶-position of guanine.¹⁰⁰ As O⁶-alkylated guanine leads to double-strand breaks and base mispairing, thereby inducing apoptosis and cell death, *MGMT* protects normal cells from carcinogens. Unfortunately, it also protects tumor cells from normally lethal effects of chemotherapy with alkylating agents such as temozolomide.¹⁰⁰ *MGMT* is consumed when counteracting TMZ-induced DNA damage, and it has been predicted that the intracellular level of *MGMT* correlates with chemoresistance.¹⁰¹ However, analysis of *MGMT* protein expression in glioblastoma tissue by immunohistochemistry failed to correlate with survival under chemoradiotherapy.¹⁰²

Clinical relevance

Methylation of the *MGMT* promoter is found in 35%–45% of malignant gliomas (WHO grades III and IV) and in about 80% of WHO grade II gliomas.^{60,103} *MGMT* methylated and unmethylated glioblastomas seem to differ in primary location,¹⁰⁴ pattern of contrast enhancement¹⁰⁵ and the apparent diffusion coefficient in MRI analysis,¹⁰⁶ the incidence of pseudoprogression,^{107,108} and pattern of recurrence as determined by ¹⁸FET-PET imaging.¹⁰⁹ However, none of these methods sufficiently allows for noninvasive determination of *MGMT* promoter methylation status for the individual patient.^{104,105,110}

The methylation status of the *MGMT* promoter has been identified as a strong and independent predictive factor of favorable survival in glioblastoma patients undergoing chemotherapy with alkylating agents.^{103,111–113} The median survival for patients with a methylated *MGMT* promoter was 21.7 months compared with 12.7 months for patients without. Accordingly, a high frequency of *MGMT* promoter methylation was noted in long-term survivors of glioblastoma who received repetitive alkylating chemotherapy during the course of the disease.^{23,114} Generally, carriers of the methylated form of the *MGMT* promoter respond substantially better to therapy with temozolomide as compared

with those with an unmethylated *MGMT* promoter.^{17,58} This predominant influence of *MGMT* promoter methylation is independent of surgical treatment. Moreover, a methylated *MGMT* promoter status seems to stratify outcome even in the recurrent disease.¹¹⁵ Postrecurrence survival was better in those *MGMT* methylated patients who receive alkylating therapy for recurrent disease.¹¹⁵ However, a methylated *MGMT* promoter status does not influence outcome in solely irradiated glioblastoma patients (without alkylating therapy), whereas in grade III gliomas favorable outcome in methylated patients seems to be irrespective of the applied treatment regime.^{58,116} It is currently unclear, however, whether this observation indicates a true prognostic value for *MGMT* methylation in all grade III gliomas.¹¹⁷ In grade II gliomas, *MGMT* promoter methylation shows a Janus head-like correlation with shortened progression-free survival but prolonged overall survival under radio and/or chemotherapy.⁶¹

Determination of the *MGMT* promoter methylation status

Currently, there is no consensus about the most suitable technique for determination of the *MGMT* promoter methylation status. This concerns both the tissue sampling technique as well as the molecular assays currently used in the clinical setting.³⁰ *MGMT* promoter methylation status can be efficiently determined even from small (1 mm³) formalin-fixed, paraffin-embedded tissue samples obtained from stereotactic biopsy procedures.²³ Analysis of *MGMT* promoter methylation status of multiple tissue samples harvested from serial biopsy procedures throughout entire tumor volumes has proven that this molecular fingerprint is homogeneous in glioblastoma formations.²³ Notably, only vital (non-necrotic) tumor specimens must be used for subsequent analysis in order to avoid false-negative results.^{23,118} Analyses from paired tumor samples harvested from primary and recurrent glioblastomas revealed that *MGMT* promoter methylation status is unchanged during the course of the disease.¹¹⁵

In respect of the currently available methods for *MGMT* testing, nonquantitative methylation-specific polymerase chain reaction (MSP)¹¹⁹ seems to be the most appropriate one in a clinical setting.^{120,121} Commonly, two pairs of primers – each specific to either the methylated or the unmethylated *MGMT* promoter region – are used for MSP as originally described by Esteller et al.¹²² However, discrepancies exist between sequencing analyses¹²³ and results from MSP. Tumors classified as “unmethylated” by MSP could turn out to be “methylated” or at least “partially methylated” using sequencing analysis.¹²⁴ A recent study systematically

analyzed the impact of specific *CpG* sites within the *MGMT* promoter on the transcriptional regulation of *MGMT* using a luciferase reporter assay.¹²⁵ This study revealed that MSP is located within the optimal region for *MGMT* testing. However, the study also showed that substitution of a single *CpG* outside the MSP region almost completely inhibited the promoter activity. Also, there was a high variability regarding the methylated positions. Hence, promoter methylation-mediated gene silencing seems to be strongly dependent on the location of the methylated *CpGs* and the extent of the overall *CPG* island methylation, which considerably complicates classification.^{117,126} Given these facts, determination of all individual *CpG* sites of the recently identified optimal region might predict the transcriptional activity and sensitivity to alkylating substances more confidently.¹¹⁷ More clinical data are needed to support this assumption.

MGMT determination by immunohistochemistry has also been suggested. However, this method lacks standardization, reproducibility, and correlation with outcome.

MGMT promoter methylation and RNA expression

Differential regulation of *MGMT* mRNA expression might also explain why *MGMT* promoter methylation is not unequivocally linked to a favorable treatment response. It has been shown that not all patients with a methylated promoter reveal similar response to temozolomide treatment and that a considerable number of unmethylated tumors experienced a surprisingly favorable course of the disease.¹²⁷ These observations could be explained by discordant correlations between *MGMT* promoter methylation (as being determined by both MSP and sequencing analysis) and *MGMT* mRNA expression pattern. High (low) mRNA *MGMT* expression was detected in approximately 25% of glioblastomas despite a methylated (unmethylated) *MGMT* promoter. Notably, those patients with low transcriptional activity exhibited a better treatment response, which was independent of *MGMT* promoter methylation. These observations were confirmed by another study verifying low *MGMT* immunostaining in 23% of unmethylated patients and high *MGMT* expression in 8% of methylated patients.¹²⁸ The underlying mechanisms of discordance still remain unclear. It has been hypothesized that low *MGMT* expression levels combined with an unmethylated promoter might result from transcript destabilization and/or transcription-repressing factors, such as miRNA regulation or histone modifications.^{127,129} Additionally, variable outcomes in the unmethylated glioblastoma population could be mediated by

further factors, such as a heterogeneous expression pattern of the DNA repair enzyme *APNG*, which confers resistance to temozolomide treatment.¹³⁰

MGMT for decision making in glioblastoma patients

MGMT promoter methylation status has been established as an important clinical biomarker in neuro-oncology. Accordingly, determination of *MGMT* promoter methylation status is of utmost interest for prognostication of adult patients suffering from newly diagnosed glioblastoma. This also includes those patients with nonresectable tumors that undergo biopsy only. However, with a lack of established alternative treatment options and in the absence of any clinical consequence of routine determination of the *MGMT* promoter, methylation status does not yet add to the management of glioblastoma patients outside clinical trials. In the absence of potent alternative drugs, temozolomide chemotherapy should not be withheld from unmethylated glioblastoma patients younger than 70 years of age in general practice. Moreover, discordant responses even within the subgroups of methylated and unmethylated patients indicate that treatment decision in respect of chemotherapy cannot be based on this biomarker alone.

The effects of *MGMT* promoter methylation in malignant gliomas seem to also be dependent on WHO grading. In anaplastic glioma, favorable progression-free survival in *MGMT* methylated patients was also seen under radiation therapy alone.⁵⁸ This difference might be explained by the high incidence of other favorable molecular markers in WHO grade III gliomas, such as *IDH1* mutation, 1p/19q deletion, or yet to be identified novel aberrations.

MGMT in the elderly

In the older glioblastoma patient, *MGMT* promoter methylation status is on the verge of entering clinical decision making. Combined chemoradiotherapy comprising temozolomide might be too toxic for the elderly, with increased side effects.^{22,131,132} For the elderly with malignant glioma, two recently published Phase III trials have evaluated the place of dose-dense/conventional temozolomide regimes alone as compared with conventional/hypofractionated radiotherapy.^{25,133} Overall survival in methylated patients was better if temozolomide treatment was applied, whereas in unmethylated patients radiotherapy alone was more effective. Thus, *MGMT* promoter methylation is an important biomarker for personalized treatment strategies in the elderly subpopulation.

MGMT and clinical trials

MGMT promoter methylation testing has recently been introduced as a marker for patient selection within clinical trials.^{113,134–137} Results from a Phase I/IIA trial for cilengitide have shown that treatment effects were better in *MGMT* promoter methylated patients.¹³⁸ Accordingly, the ongoing Cilengitide, Temozolomide, and Radiation Therapy in Treating Patients With Newly Diagnosed Glioblastoma and Methylated Gene Promoter Status (CENTRIC) study (NCT00689221) was one of the first prospective multicenter studies that evaluated alternative treatment (cilengitide, temozolomide, and radiation therapy) in *MGMT* methylated glioblastoma patients only. The Radiation Therapy Oncology Group (RTOG) 0825 trial also incorporated *MGMT* promoter methylation testing for primary glioblastoma patients who underwent bevacizumab administered with radiotherapy compared with conventional concurrent chemoradiotherapy (temozolomide).¹³⁹

Conclusion

There is an urgent need to identify biomarkers in malignant glioma patients in order to indicate patients at risk of tumor relapse, treatment failure, or adverse events, and to allow for prognostication and clinical decision making.¹²² Currently, the methylation status of the *MGMT* gene promoter seems to separate different subtypes of malignant glioma patients and will certainly influence future studies in respect of stratification of patient groups who are more likely to respond to a certain therapy. The usefulness of *MGMT* testing in a routine clinical setting will be of fundamental relevance when distinct treatment strategies for methylated and unmethylated glioblastoma patients will be available.

Disclosure

The authors report no conflicts of interest in this work.

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