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Can MGMT promoter methylation status be used as a prognostic and predictive marker for glioblastoma multiforme at the present time?

A word of caution

Editorial

Several studies have addressed the prognostic and predictive value of the methylation status of the O6-methylguanine-DNA methyltransferase (MGMT) gene in patients with glioblastoma multiforme (GBM). In a translational study analyzing the randomized EORTC-26981-22981/NCIC-CE3 trial by Stupp et al. [9], Hegi and coworkers [4] first demonstrated that the benefit of combined radiotherapy and temozolomide chemotherapy over radiotherapy alone was significant in GBM patients with a methylated MGMT promoter (median survival: 21.7 versus 15.3 months; p<0.001), but only slight and borderline significant in those with an unmethylated status (median survival: 12.7 versus 11.8 months; p<0.06). MGMT promoter methylation was found to be an independent prognostic factor for survival (hazard ratio: 0.41; p=0.001). However, these initial results were partly offset by the results of a five-year analysis published by the same group at a later date [8]. In the latter study, the addition of temozolomide to radiotherapy also had a significant beneficial effect in patients with an unmethylated MGMT promoter (median survival: 12.6 vs. 11.8 months; p=0.035).

The predictive value of MGMT promoter methylation status was also demonstrated in two recent studies on the treatment of elderly GBM patients over 65 [11] and 60 years of age [5]: in the groups treated with temozolomide alone, patients with a methylated MGMT promoter had a better prognosis than those without methylation. Within these groups, the NOA-08 trial [11] showed an advantage in terms of event-free survival (median: 8.4 versus 3.3 months; p=0.01), while the Nordic study by Malmstrom et al. [5] demonstrated an additional benefit in terms of overall survival (median: 9.7 vs. 6.8 months; p=0.02). Conversely, MGMT promoter methylation status had no impact among patients treated with radiotherapy alone.

Consequently, there are now frequent demands for the routine use of MGMT promoter methylation as a predictive and prognostic factor in clinical practice. For example, Platten and colleagues [6] stated that the decision to forego radio- or chemotherapy should depend on the methylation status of the MGMT gene. For individuals over 65 years of age, this means that temozolomide chemotherapy alone should be administered in patients with a methylated MGMT promoter and radiotherapy alone in those with an unmethylated MGMT promoter. Nowadays, more and more studies are being designed for the exclusive inclusion of patients with a specific MGMT status (compare the CENTRIC, CeTeG and Glarius studies). This assumes a widespread availability of standardized, reliable and validated methods for the determination of MGMT promoter methylation status.

However, we would like to point out that there are still many uncertainties associated with the practical implementation of MGMT promoter methylation analysis. In our view, it would be premature to use the MGMT status as a biomarker for treatment selection in routine clinical practice at this stage. Our opinion is supported by a recent review published by Berghoff and colleagues in Austria [1].

What are the current arguments against the routine use of MGMT promoter methylation status in clinical practice?

- Various methods for the determination of MGMT promoter methylation status have been developed and are currently available. However, sufficient evidence demonstrating the intra- and interlaboratory reproducibility of any of these tests is still lacking. Therefore, in their clinical neuropathology practice guide, Berghoff et al. demand a scientific analysis of the reproducibility of these tests, stating"This lack of evidence impedes recommendation of MGMT testing for routine clinical use" [1].
- The MGMT gene promoter contains a total of 97 potential CpG methylation sites. However, it is frequently the case that only a small sample (5 to 9) of these sites are analyzed, depending on the analytical method used. The methylation patterns show some heterogeneity between different patients, but there is evidence that the prognostic significance of the individual methylation sites differ [7]. Consequently, the prognostic and predictive value of MGMT status must always be

Tab. 1 Current studies on O6-methylguanine-DNA methyltransferase (MGMT) gene promoter methylation status in patients with glioblastoma multiforme (GBM)

Study	Histopathology	Analyzed CpG positions	Method	Institution	Tumor cell content	Successfully determined MGMT methylation status
NOA-08 (Wick et al. [11])	GBM: 88.7% AA: 10.7% NA: 0.5%	1) CpG 75–79 and CpG 87–89 2) CpG 73–76 and CpG 86–88	Two methylation-specific PCR assays 1) Quantitative real-time PCR (Vlassenbroeck et al. [10]); 182 samples 2) Conventional methylation-specific PCR (Felsberg et al.[2]); same 182 samples and 70 additional samples from stereotactic biopsies) In case of conflicting results (4 samples), results from real-time PCR were used	1) MDxHealth, Liège, Belgium (quantitative real-time PCR) 2) Brain Tumor Reference Centre, Germany (conventional methylation-specific PCR)	≥ 80%	56% (209/373 patients)
Nordic Trial (Malmström et al. [5])	Only patients with histologically confirmed GBM eligible	CpG 75–79 and CpG 87–89	Quantitative real-time PCR (Vlassenbroeck et al. [10])	MDxHealth, Liège, Belgium	Not specified	59% (203/342 patients)
EORTC 26981 (Hegi et al. [4]; Stupp et al. [8])	Only patients with histologically confirmed GBM eligible	CpG 75–79 and CpG 83–86	Nested Methylation-Specific PCR	Laboratory of Tumor Biology and Genetics, Department of Neurosurgery, Lausanne, Switzerland	Not specified (adequate tissue not available in 266 patients)	36% (206/573 patients)
Felsberg et al. [3]	GBM	1) CpG 73–76 and CpG 86–88 2) CpG 73–77	1) Conventional methylation-specific PCR (80/80 patients) 2) Pyrosequencing (48/80 patients; Qiagen PyroMark Q24 MGMT kit)	Brain Tumor Reference Center, Düsseldorf, Germany	≥ 80% (all except for 3 of 80 patients)	100% (80/80 patients); 70/80 frozen tissue sections; 10/80 formalin-fixed and paraffin-embedded tissue
Shah et al. [7]	GBM	1) All 97 CpG sites 2) CpG 8, CpG 22 and CpG 80	1) Quantitative bisulfite sequencing 2) Methylation-specific multiplex ligation-dependent probe amplification	Swedish Neuroscience Insti-tute, Seattle, USA	Not specified	100% (70/70); frozen tissue sections]

CpG sites as defined by Shah et al. [7]. *GBM* glioblastoma, *AA* anaplastic astrocytoma, *NA* not available.

interpreted in terms of the particular method used and the specific methylation sites assessed. In our opinion, it is therefore problematic to transfer the significance of the MGMT status as defined in the aforementioned studies to alternative methods or different methylation sites in an uncritical manner. The different methods and CpG sites studied in the cited publications are listed in **Tab. 1**.

- The previously published studies of MGMT promoter methylation status set high standards for the sample materials used for analysis. In the NOA-08 study, for example, only those tissue samples with a tumor cell content of at least 80% were accepted for analysis [11]. In our experience, this restricts the possibility of determining the MGMT status in practice to just 40 to 60% of all GBM patients. The tumor cell content of the remaining samples will be too low to ensure the reliable determination of MGMT promoter methylation status under the present conditions. Currently available studies show similarly low rates of analysis (**Tab. 1**). Furthermore, the two studies with a 100% success rate used frozen tissue sections, which are not always available in routine practice. This means that, in clinical practice, the MGMT promoter methylation status cannot be determined in a significant proportion of patients due to a lack of suitable sample material.
- In the EORTC-26981-22981/NCIC-CE3 study [9], the addition of temozolomide to radiotherapy resulted in a small, yet statistically significant survival benefit for GBM patients with an unmethylated MGMT status. This implies that MGMT-negative GBM patients might also benefit from temozolomide. It would thus seem that MGMT status does not play a relevant role in treatment decisions for patients <70 years of age (Stupp et al. included patients <70 years in their

2005 study). On the other hand, the studies by Wick et al. [11] and Malmström et al. [5] provided evidence demonstrating the predictive value of MGMT status in elderly patients treated with temozolomide alone, whereas the effect of radiotherapy was independent of the MGMT status. Combined treatment with temozolomide and radiotherapy has not yet been tested in this age group. In our opinion, it is therefore not possible to draw reliable conclusions regarding the treatment of elderly GBM patients using this approach.

Taken together, these arguments suggest that:

- MGMT promoter methylation status cannot be reliably determined at all routine laboratories at the present time.
- It is unclear which method and which tumor material should or could be used.
- It is unclear which methylation sites allow a reliable predictive and/or prognostic assessment.
- There are still too few independent studies on the prognostic and predictive value of MGMT promoter gene methylation to permit its use in treatment decisions relating to the use of combined radiotherapy and chemotherapy in elderly GBM patients.

Therefore, it is imperative that well-defined and reproducible conditions for the determination of MGMT promoter methylation status are established prior to the introduction of MGMT status in routine diagnostics and daily clinical practice (e.g., when making treatment decisions for older GBM patients).

From a radio-oncological perspective, it is necessary to clearly identify the treatment regimens (e.g., combined radiotherapy and chemotherapy) in which MGMT status is really significant.

We are confident that MGMT status can play an important role in individualized treatment planning for GBM in the future. However, its precise role remains to be clarified in further studies. At present, routine use of MGMT status should be exercised with extreme caution as it is subject to the aforementioned limitations and difficulties associated with the interpretation of the results.

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Compliance with ethical guidelines

Conflict of interest. R. Fietkau, F. Putz, G. Lahmer, S. Semrau and R. Buslei state that there are no conflicts of interest.

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