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## Management of Gliomas: Relevance of Molecular Markers for Clinical Practice

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# Management of Gliomas: Relevance of Molecular Markers for Clinical Practice

Michael Weller

**Abstract:** The histological subtyping and the grading of gliomas in the World Health Organization classification of brain tumours was originally designed mainly to provide clinicians with guidance as to the natural course of disease and the necessity of further treatment beyond surgery. The profound prognostic impact of this pathological grading has been confirmed over the last decades in numerous retrospective analyses. Yet, it has also become clear that tumours virtually identical by morphology may have very different outcomes and that molecular markers may aid in deriving more detailed prognostic information. The 1p/19q co-deletion in oligodendroglial tumours became paradigmatic in this re-

gard: patients with 1p/19q-deleted tumours derive much more benefit from radiotherapy or chemotherapy than patients with tumours lacking this aberration. Thus, the 1p/19q status does not help to select among different genotoxic treatments. The first molecular marker attributed a predictive power specifically for benefit from alkylating agent chemotherapy was promoter methylation of the O<sup>6</sup>-methylguanylmethyltransferase gene. However, it now seems that this predictive effect may be limited to glioblastoma and that implementation of the according test in clinical practice is a major challenge. The identification of isocitrate dehydrogenase mutations as typical changes restricted to certain types of

gliomas and the rapid development of common mutation-specific antibodies has provided a major advance in subclassifying gliomas, but not resulted in novel treatment strategies yet. A specific type of epidermal growth factor receptor mutation, EGFRvIII, has emerged as a target for vaccination. Ongoing high-throughput analyses are likely to yield novel candidate biomarkers in due course, suggesting that molecular neuropathology will have an increasing impact in clinical neurooncology very soon. **Eur Assoc of Neuro Oncol Mag 2012; 2 (1): 6–10.**

**Key words:** MRI, DTI-FT, fMRI, neuronavigation, brain mapping, glioma

## ■ Introduction

The current World Health Organization (WHO) Classification of Tumours of the Nervous System allows for a histomorphological subtyping of brain tumours and a grading into WHO grades I–IV according to the expected degree of malignancy [1]. One major goal of the WHO classification was to provide clinicians with guidance as to the natural course of disease and the indication to withhold or offer further treatment beyond surgery, specifically radiotherapy or chemotherapy. The profound prognostic impact of the pathological grading afforded by the WHO classification has been confirmed over the last decades in numerous retrospective analyses. Yet, these studies as well as prospective interventional trials have also shown that tumours identical by morphological criteria may have highly different outcomes and that molecular markers may aid in deriving more detailed prognostic information upfront. Numerous such molecular markers in the field of glioma have been explored, many without attracting enduring attention. Some, however, continue to raise interest over years, such as p53 mutations, epidermal growth factor receptor (EGFR) mutations, and the 1p/19q co-deletion, others such as O<sup>6</sup>-methylguanylmethyltransferase (MGMT) promoter methylation and mutations of isocitrate dehydrogenase (IDH) 1 and B-Raf, are subject of ongoing studies and controversies (Table 1, Figure 1). Moreover, powerful high-throughput analyses, which also led to the identification of IDH as a mutational target [3], are likely to yield novel diagnostic and prognostic information in the very near future. The article at hand summarizes and extends previous reviews on the devel-

opment of molecular markers for the differential diagnosis and stratified management of gliomas [2, 4–7]. The potential value of molecular markers includes, but is not limited

- to aid in the differential diagnosis of brain tumours that are at times difficult to distinguish
- to allow prognostication within tumour entities
- to allow prediction of response or lack of response to a specific type of treatment.

Notably the distinction between prognostic and predictive molecular factors is not consistently made in the literature and has given rise to controversies and misconceptions, eg, the false assumption that patients with 1p/19q-co-deleted oligodendrogliomas should be treated with chemotherapy or chemotherapy plus radiotherapy rather than radiotherapy alone (see below).

## ■ p53

The tumour suppressor protein (TP) 53 acts mainly as a transcription factor that controls the transcription of several genes involved in cell cycle arrest, DNA repair, senescence, and apoptosis, eg, p21 or bax. Mutations of the p53 gene or effectors of the p53 pathway are among the most common molecular aberrations in human cancers. Among gliomas, p53 mutations are relatively common in WHO grade-II and -III tumours and, accordingly, also in secondary glioblastomas, that is, glioblastomas progressing from previously lower-grade gliomas. In contrast, p53 mutations are relatively rare in glioblastoma.

Since p53 controls DNA damage response pathways and is thought to promote either DNA repair and survival or irreversible growth arrest, traditional views held that the p53 status should correlate with responses to radiotherapy or DNA-damaging agent chemotherapy. While this can be nicely modelled in cell culture models and p53 knockout mice, such an asso-

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ciation could never be demonstrated in large prospective clinical trials, neither in gliomas nor in other tumours. In contemporary studies from the German Glioma Network, the p53 status was neither associated with progression-free survival in patients with WHO grade-II gliomas managed with surgery alone [8] and neither with progression-free or overall survival of glioblastoma patients treated with radiotherapy plus concomitant and adjuvant temozolomide [9]. Possibly further molecular changes resulting from the loss of the p53 checkpoint control make tumours too heterogeneous to allow prediction of their behaviour in response to genotoxic stress. Moreover, the p53 pathway can be disabled at multiple levels other than p53 itself, accordingly, p53-signalling alterations may be present in 87 % of all glioblastoma patients whereas the p53 mutation rate is only 15–30 % [10]. Accordingly, at present there is no need to know the p53 status for any clinical decision-making in the glioma field. There are at least 2 scenarios where this may change: (1) mutant p53 proteins exhibiting immunogenic epitopes could be explored as targets for immunotherapy and (2) p53-mimetic drugs which induce conformational changes and thereby transcriptional activation of mutant p53 variants could be assessed in subgroups of glioma patients carrying appropriate mutations.

## ■ EGFR

Amplification, constitutive activation, or increased expression of the epidermal growth factor receptor (EGFR) gene may promote EGFR-signalling and thereby proliferation, invasiveness, and resistance to cell death induction. EGFR amplification or mutational activation are rare in gliomas of lower WHO grades, but frequent in primary glioblastomas and glioblastoma in elderly patients [9, 10]. EGFR overexpression has been associated with inferior prognosis in some, but not all studies, and a major prognostic impact in glioblastoma can be ruled out. Numerous EGFR-targeted agents, including tyrosine kinase inhibitors such as gefitinib or erlotinib, as well as antibodies have been explored in patients with newly diagnosed or recurrent glioblastoma, but never produced a signal for activity justifying phase-III development. It has been argued that upfront EGFR status determination to enrich patients likely to respond to EGFR-targeted treatments might be necessary to improve the outcome of anti-EGFR trials in glioblastoma. Thus it has been proposed that patients with high EGFR expression and low levels of phosphorylated protein kinase B/Akt respond better to erlotinib than those with low levels of EGFR expression and high levels of phosphorylated protein kinase B/Akt [11]. Furthermore, it was reported that the coexpression of EGFRvIII and phosphatase-and-tensin-homolog-on-chromosome-ten (PTEN) by glioblastoma cells was associated with responsiveness to EGFR kinase inhibitors [12]. However, neither of these observations was confirmed in the prospective randomized European Organization for Research and Treatment of Cancer (EORTC) 26034 trial on erlotinib in recurrent glioblastoma [13]. Since the EGFR status is not strongly prognostic and since no EGFR-targeting agents are approved for glioma treatment, currently there is no need for the determination of EGFR mutation or expression status outside a clinical trial, and its knowledge will not influence clinical decision-

making. However, a specific type of EGFR mutation, EGFRvIII, which gives rise to a truncated receptor that is active independent of ligand, produces a neoantigen that might be amenable to immunological targeting [14, 15]. Thus the detection of EGFRvIII might assume relevance at least for inclusion into clinical trials in the next few years.

## ■ 1p/19q

Combined losses of genetic material from chromosomal arms 1p and 19q, now commonly referred to as 1p/19q co-deletions, result from an unbalanced translocation which leads to the loss of one hybrid chromosome and thereby loss of heterozygosity (LOH) [16]. These observations early on indicated the presence of tumour suppressor genes on 1p or 19q. 1p/19q co-deletions are almost never found in non-glioma tumours and are strongly associated with oligodendroglial morphology. In WHO grade-II gliomas, the absence or presence of this biomarker does not correlate with progression-free survival in patients treated by surgery alone, but neither with radiotherapy nor chemotherapy [8, 17]. However, they identify anaplastic glioma patients with a superior outcome independent of whether these patients are treated with radiotherapy or chemotherapy or both [18–20]. In glioblastoma, 1p/19q co-deletions are rare and of unknown biological significance. The higher effectivity of radiotherapy and chemotherapy in oligodendroglioma patients with 1p/19q co-deletions has given rise to the hypothesis that these chromosomal regions harbour not only tumour suppressor genes but also important genes which may determine cellular sensitivity to genotoxic stress or cell death stimuli in general. However, only very recently the first convincing candidate genes have been identified. By means of coding exon sequencing, mutations in 2 genes hitherto not related to gliomas, the CIC gene, a homologue of the drosophila gene *capicua*) on chromosome 19q, and the FUBP1 gene, which encodes the “far upstream element (FUSE) binding protein” on chromosome 1p, were found in a relevant proportion of oligodendroglial tumours [21]. To what extent mutations in CIC or FUBP1 contribute to oligodendrogliomagenesis or the radiochemosensitivity of 1p/19q-co-deleted oligodendrogliomas is currently under intense investigation.

## ■ MGMT

MGMT is a DNA repair enzyme that reverses the alkylation of DNA induced by alkylating agent chemotherapy, including nitrosoureas and temozolomide. The MGMT protein is consumed during this process by its targeting to the proteasome and is therefore often classified as a suicide enzyme. An association of MGMT expression and the efficacy of alkylating agent chemotherapy in glioblastoma has been proposed for more than 20 years [5]. However, most studies reporting a prognostic impact of MGMT in glioblastoma examined promoter methylation of the MGMT promoter rather than protein levels or activity in the tumour cells. In fact, there was generally poor correlation between promoter methylation and loss of protein, and promoter methylation correlated better with outcome than MGMT protein determination by immunohistochemistry [5, 22]. The reasons for these discrepancies may have included poor-quality antibodies or difficulties in distin-

guishing MGMT-expressing tumour from non-tumour host infiltrating cells. Alternatively, MGMT promoter methylation may not always result in loss of protein expression and may signify a biological feature with significance beyond MGMT, that is, there may be a pattern of gene silencing by methylation that predicts a better outcome [23]. The MGMT promoter methylation status shows little intratumoural heterogeneity [24] and is preserved at recurrence in most glioblastomas [25, 26].

In the context of the pivotal trial that showed the superiority of concomitant and adjuvant temozolomide plus radiotherapy over radiotherapy alone [27], MGMT promoter methylation was strongly associated with the extent of benefit from the addition of chemotherapy in the experimental arm [28], but had only minor prognostic impact on progression-free survival in patients treated with radiotherapy alone. This set of data is currently the only to demonstrate that MGMT promoter methylation is not only an overall prognostic factor, but predictive for benefit of chemotherapy, if only in glioblastoma. The strong prognostic role of MGMT promoter methylation has recently been confirmed in the RTOG 0525/EORTC/NCCTG Intergroup Study that investigated 3 weeks on one week off adjuvant temozolomide dose intensification in comparison with the standard EORTC/NCIC treatment schedule. While there was no difference in progression-free or overall survival, the primary endpoint, between both treatment arms, overall survival was 23.2 months in patients with MGMT promoter-methylated tumours as opposed to 16 months in patients with unmethylated tumours [29]. The PCR-based assay used in that trial is being prepared for commercial use by MDX Health (Ghent, Belgium).

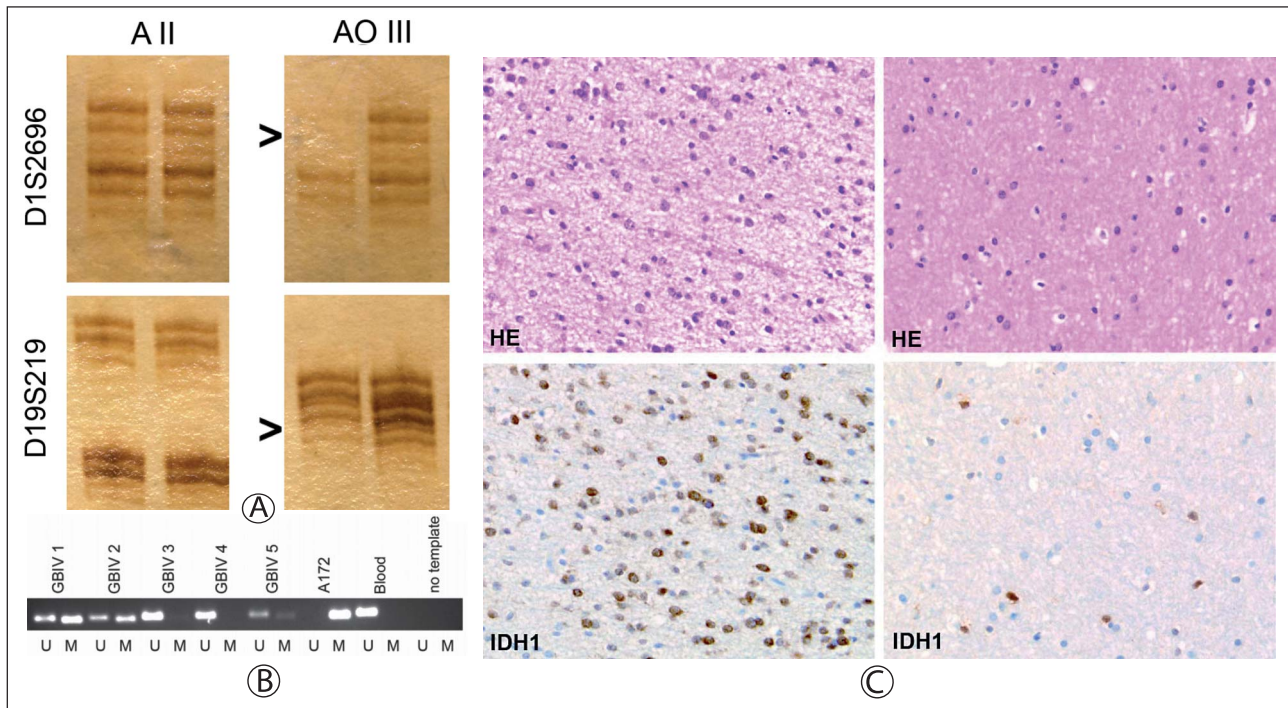
## ■ IDH

Somatic mutations of isocitrate dehydrogenase (IDH) genes 1 and 2 have only recently been described, but have already had a major impact in diagnostic neurooncology [3, 7, 30, 31]. The IDH1 gene encodes cytosolic NADP<sup>+</sup>-dependent IDH whereas the IDH2 gene encodes mitochondrial NADP<sup>+</sup>-dependent IDH. IDH mutations cluster at codon 132 of the IDH1 respectively codon 172 of the IDH2 gene, suggesting that these mutations afford a gain of function to tumour cells. If merely loss of IDH function was the main mechanism, any type of mutation resulting in loss of functional enzyme would be tumorigenic. Present concepts include that the mutant IDH variants exhibit an altered substrate specificity which results in the production of a putative oncometabolite, D-2-hydroxyglutarate. While this oncometabolite has been considered a possible biomarker to monitor disease activity in acute myeloid leukaemia, the only other cancer with a relatively high rate of IDH mutations, D-2-hydroxyglutarate levels may be too low in the serum of glioma patients to be of diagnostic value [32].

The IDH mutation rate in astrocytic and oligodendroglial gliomas of grades II and III is in the range of 60–80 % whereas the rate does not exceed 10 % in glioblastomas, indicating a differential cellular origin of these tumours. Other brain tumours such as ependymomas lack IDH mutations. This differential distribution and the development of an antibody-recognizing mutant IDH1<sup>R132H</sup> protein [33], which accounts for > 90 % of all mutants in gliomas, explains why IDH assessment was rapidly introduced into the diagnostic repertoire of neuropathology in many centres.

**Table 1.** Molecular markers in glioma: an overview

|               | Biological significance                                                                                   | Method of assessment                                | Clinical relevance                                                      |                                                                                         |                                                                                             |
|---------------|-----------------------------------------------------------------------------------------------------------|-----------------------------------------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
|               |                                                                                                           |                                                     | Grade-II gliomas                                                        | Grade-III anaplastic gliomas                                                            | Grade-IV glioblastoma                                                                       |
| <b>p53</b>    | Cell cycle checkpoint, DNA repair                                                                         | PCR, sequencing, and immunohistochemistry           | None                                                                    | None                                                                                    | None                                                                                        |
| <b>EGFR</b>   | Proliferation, invasion                                                                                   | PCR or immunohistochemistry                         | None                                                                    | Suggests possible undergrading → consider reclassification as glioblastoma              | Often overexpressed (40 %), EGFRvIII mutation (25 %) as a possible target for immunotherapy |
| <b>1p/19q</b> | Biological role unclear, co-deletion of chromosomal arms 1p and 19q linked to oligodendroglial morphology | PCR, FISH                                           | Controversial, probably prognostically favourable                       | Prognostically favourable in patients treated with radiotherapy or chemotherapy or both | Rare, significance unclear                                                                  |
| <b>MGMT</b>   | DNA repair                                                                                                | Methylation-specific PCR                            | Controversial                                                           | Prognostically favourable in patients treated with radiotherapy or chemotherapy or both | Predictive for benefit from alkylating agent chemotherapy                                   |
| <b>IDH</b>    | Biological role unclear, possible link to energy metabolism and pro-angiogenic pathways                   | PCR or immunohistochemistry (IDH <sup>R132H</sup> ) | Prognostically favourable                                               | Prognostically favourable in patients treated with radiotherapy or chemotherapy or both | Rare, prognostically favourable, suggestive of secondary glioblastoma                       |
| <b>B-Raf</b>  | Cell growth and division                                                                                  | PCR                                                 | Possibly identifying patients responsive to B-Raf kinase inhibitors (?) | Rare, no significance                                                                   | Rare, no significance                                                                       |



**Figure 1.** Molecular markers in glioma. **A**, 1p/19q co-deletion. Microsatellite-PCR-based analysis of allelic losses of 1p and 19q in an astrocytoma (WHO grade II) (A II) and an anaplastic oligodendroglioma (WHO grade III) (AO III). Losses of both markers are indicated by arrowheads in AO III, but not A II. **B**, MGMT promoter methylation. Methylation-specific PCR for unmethylated (U) and methylated (M) promoter sequences in 5 glioblastoma samples, including the glioblastoma cell line A172 as a positive control for methylated and peripheral blood cells as a control for unmethylated promoter sequences, as well as water as a negative control (empty). **C**, IDH mutation. Grade-II oligoastrocytoma, upper panels: HE staining, lower panels: IDH immunostaining, left panels: tumour centre, right panels: infiltration zone (Courtesy: Jörg Felsberg, Düsseldorf, Germany). Reprinted from [2].

Within glioma entities II–IV, patients with IDH-mutant tumours show a longer survival than patients with IDH-wild-type tumours [20, 34, 35]. Yet, the IDH status, like the 1p/19q status, does not predict benefit from a specific type of treatment, eg, radiotherapy versus chemotherapy [20, 36, 37]. Interestingly, the survival of glioblastoma patients with IDH mutation is superior to that of anaplastic astrocytoma without IDH mutation [38], illustrating that molecular features can profoundly improve the prognostic power of the current, largely morphological neuropathological assessment.

## ■ B-Raf

B-Raf is a member of the Raf kinase family which belongs to the serin threonine kinases and regulates cell growth and division *via* mitogen-activated protein kinase (MAPK) and extracellular signal-related kinases (ERK). Duplications of B-Raf are frequent in pilocytic astrocytomas [39, 40]. Since B-Raf mutations are rare in diffuse astrocytic gliomas of WHO grades II–IV, their absence or presence may aid in the differential diagnosis of pilocytic and higher-grade astrocytomas. An activating point mutation, B-Raf<sup>V600E</sup>, is found in approximately 60–70 % of pleomorphic xanthoastrocytomas and 20 % of gangliogliomas, but again very rarely in other types of glioma [41, 42]. The detection of B-Raf pathway activation might assume therapeutic relevance since specific inhibitors of B-Raf, such as PLX-4032, are already explored for efficacy in malignant melanoma. Moreover, less selective multikinase inhibitors such as sorafenib inhibit B-Raf, too. Finally, inhibitors of heat shock protein (HSP) 90, which destabilize mutant B-Raf, may be of interest in this regard.

## ■ Is Molecular Neuropathology Ready for Clinical Use?

Major progress has been made in recent years to supplement the morphological framework of the WHO classification [1] with molecular markers of diagnostic and prognostic significance (Table 1, Figure 1). MGMT promoter methylation stands out as a marker where choice of assay and technical standardization have turned out to be most challenging [5]. EGFR amplification, 1p/19q co-deletion, IDH mutation, and B-Raf alterations are characteristic of specific tumours whereas MGMT promoter methylation is not. 1p/19q co-deletion, IDH mutation, and MGMT promoter methylation are strongly prognostic in anaplastic gliomas. Importantly, these 3 favourable markers may not be independent, eg, the 1p/19q co-deletion lost significance upon the multivariate analysis of the NOA-04 trial when MGMT and IDH status were included in the analysis [20]. Only IDH mutations are confirmed to be prognostic in grade-II gliomas. In glioblastoma, IDH mutations indicate the origin from a prior lower-grade lesion, and MGMT promoter methylation is probably both prognostic and predictive for benefit from alkylating-agent chemotherapy. Accordingly, molecular neuropathology provides a lot of diagnostic and prognostic information, but is still largely dispensable for individual clinical decision-making.

## ■ Molecular Markers and Clinical Trial Design

Molecular markers have been introduced into the design of clinical trials to enrich patient populations, that is, to define



more homogeneous patient populations which are more likely to respond to treatment in a uniform manner. This strategy has been employed for MGMT promoter methylation in the CEN-TRIC trial for newly diagnosed glioblastoma, based on the analysis of a small phase-II trial [43] and, based on robust historical data [18, 19], for the 1p/19q co-deletion in the multinational CATNON and CODEL trials which are also open in many European countries. As one of the next steps, it seems reasonable to exclude glioblastoma patients with IDH mutations from future glioblastoma trials. The most convincing use of molecular marker analysis would be the analysis of the EGFRvIII mutation in glioblastoma as the requirement for inclusion into a vaccination trial targeting specifically this common type of mutation [15].

## ■ Outlook

Further areas of biomarker research that are far less advanced in terms of implementation in the clinic include the diagnostic and prognostic use of glioma stem cell markers as well as the identification of predictive markers for benefit from specific types of antiangiogenic treatment. These could include not only tumour tissue markers but also plasma biomarkers such as vascular endothelial growth factor (VEGF) family members or their soluble receptors [44, 45] or vessel structures amenable to advanced imaging techniques such as integrins expressed on the luminal vessel wall [46].

## ■ Conflict of Interest

The author has received research grants from MSD, Merck Serono, and Roche and honoraria for lectures or advisory boards from Magforce, MSD, Merck Serono, and Roche.

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