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Impact of Molecular Markers on Personalised-Treatment Concepts in Gliomas

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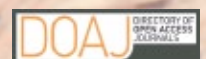
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Impact of Molecular Markers on Personalised-Treatment Concepts in Gliomas

Oliver Schnell

Abstract: Gliomas are rare brain-derived tumours classified according to mainly histopathological criteria by a 4-step grading system of the World Health Organisation (WHO grades I–IV). Differentiating between the underlying tumour cell components (eg, astrocytomas, oligodendrogliomas, ependymomas), grading intends to give clinicians a general estimation about their patients' prognoses. Increasing knowledge about the molecular genetic profile now leads to a deeper insight into the predictive or prognostic value of several molecular markers with large impact on the treatment of glioma patients.

Hypermethylation of the ⁶O-methylguanine-DNA methyltransferase (MGMT) promoter has turned out to be an important predictive marker

for patients with glioblastoma multiforme (GBM, WHO grade IV) receiving radiochemotherapy with concurrent and adjuvant temozolomide chemotherapy and may be crucial for a treatment decision between (radio-) chemotherapy versus radiation only in older patients. Moreover, mutations of the isocitrate dehydrogenase (IDH) seem to have a strong prognostic impact in malignant gliomas since GBM patients with IDH mutation showed longer median survival compared to patients with anaplastic gliomas (WHO grade III) without IDH mutation. Additionally, genetic co-deletion on chromosomes 1p and 19q (LOH 1p/19q) has been demonstrated to be a favourable prognostic factor for patients with anaplastic gliomas receiving radiation, chemotherapy with alkylating agents, or both.

Hence, histological diagnosis will not be sufficient to render the best medical treatment to glioma patients in the future. Determination especially of MGMT promoter methylation status, IDH mutation, and LOH1p/19q amongst others will have increasing influence on decision-making in order to meet the demand for more personalised therapy for glioma patients. **Eur Assoc NeuroOncol Mag 2014; 4 (3): 109–15.**

Key words: glioblastoma multiforme (GBM), low-grade glioma (LGG), predictive and prognostic molecular markers, MGMT promoter methylation, IDH mutation, LOH 1p/19q, personalised therapy

Introduction

Numerous publications have underlined the importance of molecular genetic profiling in patients with gliomas [1–13]. Having been evaluated in several clinical studies as well as demonstrating predictive or prognostic value and the possibility to analyse them on a routine basis in a constantly increasing number of neuro-oncologic centres makes molecular markers now broadly available outside clinical trials. Nevertheless, there are also uncertainties about the predictive or prognostic impact of these markers in gliomas of different WHO grades and prediction for one special therapy may be difficult to follow in patients who receive several lines of therapy during the course of their disease on a regular basis. On the other hand, their relevance for decision-making is now constantly emerging with recent publications supporting the importance of MGMT promoter status especially in the elderly [9, 10, 14] or IDH mutation in malignant gliomas [6, 11] as well as LOH 1p/19 in patients with (anaplastic) oligodendroglial tumours [15, 16]. Therefore, this review focuses on these 3 markers and their impact on decision-making in the future.

Methylation of the ⁶O-Methylguanine-DNA Methyltransferase (MGMT) Promoter

MGMT is an enzyme which repairs DNA damage on the ⁶O-guanine position of the DNA caused by alkylating chemotherapeutic drugs like temozolomide (TMZ). Therefore, it has been concluded that epigenetic silencing of MGMT by hypermethylation of its promoter region on the DNA prevents MGMT synthesis, thereby giving the alkylating agent TMZ the opportunity to induce greater damage to tumour-cell DNA. An un-

methyated MGMT promoter which does not interfere with MGMT synthesis would therefore act against chemotherapy (CT) by TMZ and combined treatment leading to worse response rates in these patients (Figure 1) [1, 17].

Methylation of the MGMT promoter stepped into the spotlight of neuro-oncology and glioma therapy when results of the landmark study of the European Organisation for Research and Treatment of Cancer (EORTC) and National Cancer Institute of Canada (NCIC) were published in 2005

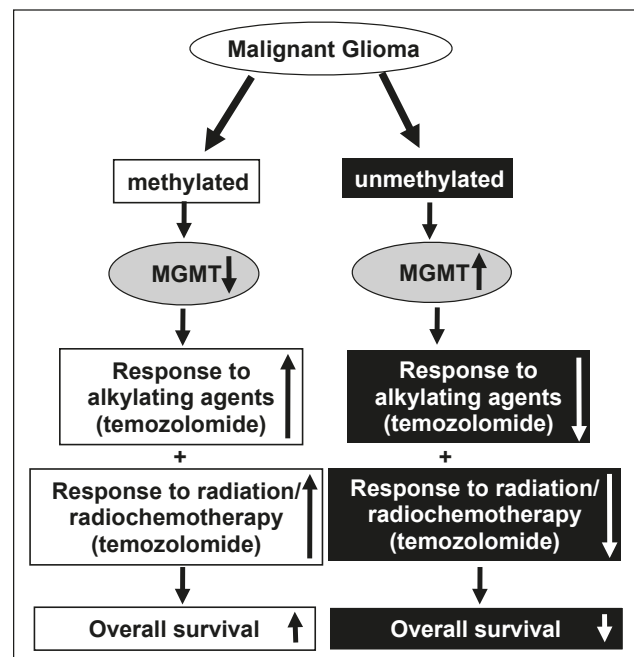


Figure 1. Effect of MGMT promoter methylation status in glioblastomas. In GBM, hypermethylation of the MGMT promoter results in reduction of MGMT protein, which in turn leads to improved chemosensitivity for TMZ chemotherapy or combined radiochemotherapy. Therefore, MGMT promoter methylation is correlated with improved overall survival in these patients. In contrast, GBM patients with unmethylated MGMT promoter status may have reduced response to radio-/chemotherapy and therefore worse prognosis (mod from [1, 17]).

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[18], demonstrating that glioblastoma patients had significantly better median survival when treated with radiotherapy plus concomitant and adjuvant TMZ versus radiotherapy alone (14.6 vs 12.1 months). Even more interesting, patients with hypermethylation of the MGMT promoter were shown to have better survival rates with this combined treatment [1]. In the following years, these findings were verified several times and led to the conclusion that MGMT promoter methylation has to be considered as a predictive molecular marker for chemotherapy with alkylating agents like TMZ in patients with glioblastomas [1, 19]. On the other hand, in patients with anaplastic gliomas WHO grade III, methylation of the MGMT promoter was not predictive for CT with alkylating agents but had favourable prognostic impact independent of the applied treatment strategy [5, 20]. This somehow confusing fact may be in part explained by the different tumorigenic pathogeneses of anaplastic gliomas and primary glioblastomas. Therefore, some authors have hypothesised that these differences might be responsible since anaplastic gliomas exhibit a higher rate of other favourable molecular markers like IDH mutation and its association with the cytosine-phosphatidyl-guanine (CpG) island methylator phenotype (CIMP) [21] or LOH 1p/19q [22].

Nevertheless, one has to keep in mind that patients > 65 or 70 years have often been excluded from many clinical trials in the past [23], which is also true for the EORTC/NCIC trial [18]. This is of importance since especially older patients often suffer from additional comorbidities and reduction of therapy-associated risks is mandatory. In elderly patients with diffuse GBM and methylated MGMT promoter, one might therefore consider giving TMZ without additional radiation therapy (RT) in order to avoid additional side effects. Different studies indeed provided evidence that elderly patients with GBM and methylated MGMT promoter have better prognoses when TMZ is applied, whereas patients with unmethylated MGMT promoter have better prognoses after RT [9, 24]. These data have now been confirmed by 2 large, randomised, controlled trials (NOA-08 and NORDIC) [10, 14]. While NOA-08 randomised patients > 65 years with malignant gliomas (anaplastic astrocytoma or glioblastoma) to either radiation therapy or dose-dense temozolomide (one week on, one week off), the NORDIC trial included only patients \geq 60 years with newly diagnosed glioblastomas and randomised them to standard radiotherapy (60 Gy over 6 weeks), hypofractionated radiation (34 Gy over 2 weeks), or cyclic temozolomide (5/28 days, 200 mg/m²). In the NOA-08 trial, there was no statistical difference between both treatment groups and temozolomide was demonstrated not to be inferior to radiation therapy in this group of patients. Yet, it turned out that patients with methylated MGMT promoter status had longer overall survival (11.9 months) and showed longer event-free survival (EFS) when being treated with temozolomide (8.4 months) compared to radiation therapy (4.6 months). In comparison, patients with unmethylated MGMT promoter showed an overall survival of 8.2 months and EFS was longer if patients underwent radiotherapy (4.6 months) than with temozolomide (3.3 months). Consistent with these findings, the NORDIC trial demonstrated better prognosis for patients with methylated vs unmethylated MGMT promoter who were treated with temozolomide (9.7 vs 6.8 months) in contrast to treatment with ra-

diation therapy (8.2 vs 7.0 months). Moreover, standard radiation was associated with worse overall survival compared to temozolomide or hypofractionated radiation therapy. Even if results from the comparison of combined radiochemotherapy to either treatment alone are still being analysed in an ongoing trial (NCT00482677), testing for MGMT promoter status has to be considered mandatory in elderly patients with malignant gliomas before deciding which therapy they should receive.

Isocitrate Dehydrogenase (IDH) Mutations

IDH is an enzyme of the Krebs cycle which leads to oxidative decarboxylation of isocitrate to α -ketoglutarate by generating NADPH from NADP⁺. While mutations of IDH1, which is present in the cytoplasm and peroxisomes, occur almost exclusively on the active binding site R132 (arginine), IDH2 is located in the mitochondria where it is involved in the Krebs cycle and shows mutations on the R172 arginine residue, which is the analogue to the R132 mutation of IDH1 [25, 26].

Genomic analyses recently demonstrated that mutations of IDH1 and IDH2 are almost exclusively present in WHO grade-II or -III astrocytomas and oligodendrogliomas or secondary GBM (ie, those that have emerged from low-grade astrocytomas), whereas ependymomas do not show IDH mutations and pilocytic astrocytomas only very rarely do [27–30]. Interestingly, the majority of low-grade astrocytomas with IDH mutation also display a mutation of the tumour suppressor gene TP53, whereas TP53 was only seldom found to be mutated unless IDH mutation was present. On the other hand, most oligodendrogliomas with IDH mutation also have a co-deletion of chromosomes 1p and 19q. Therefore, IDH mutation seems to be an early event in gliomagenesis which almost always precedes acquisition of TP53 in astrocytomas or LOH 1p/19q in oligodendrogliomas [31]. Moreover, patients with an IDH1 or IDH2 mutation have been shown to have a better prognosis and are substantially younger than those with the corresponding wild-type expression [4, 6, 32, 33].

For example, sequencing of the IDH1 gene at codon position 132 of 382 patients from the German Neuro-Oncology Group (NOA) 04 study and a prospective translational cohort of the German Glioma Network (GGN) revealed that 60 % of anaplastic astrocytomas and 7.2 % of glioblastomas carried an IDH1 mutation and that this was the most prominent single prognostic factor, even stronger than age, diagnosis, or MGMT methylation status [6]. This is remarkable since outcome analysis demonstrated that IDH mutation was significantly stronger than WHO grading and patients with anaplastic astrocytoma (AA) and IDH mutation had the most favourable prognosis, followed by GBM patients with IDH mutation who had an even more favourable prognosis than patients with AA and IDH wild type. Patients with GBM and IDH wild type experienced the worst prognosis. Furthermore, results from multivariate analysis indicated that most of the favourable prognostic effects of age may be due to the fact that IDH mutation was much more pronounced in younger patients [6]. Additionally, a recent report has demonstrated that GBM long-term survivors (> 36 months) have a significantly higher rate of IDH mutations (34 %) in contrast to patients with shorter survival (4.3 %). Again, these patients were younger and exhibited more often LOH 1p/19q or TP53 mutations

than patients who had long-term survival but no IDH mutation [12]. Since these are typical genetic alterations found in WHO grade-II or -III astrocytomas or oligodendrogliomas as well as secondary GBM, there is now increasing evidence that this might define a special glioma subgroup with a particularly favourable prognosis.

The mechanism by which IDH mutations lead to a better prognosis has so far not been completely settled. It has been assumed that IDH-mutated enzymes might bind to IDH wild type which in turn reduces IDH activity and leads to higher amounts of isocitrate and lower amounts of α -ketoglutarate. Since α -ketoglutarate is a substrate for degradation of hypoxia-inducible factor (HIF), this might lead to larger amounts of HIF, which is known to have cancerogenic potential [34]. Newer data now indicate that the IDH mutation corresponds to a gain of function since α -ketoglutarate (α KG) can be transformed into 2-hydroxyglutarate (2HG) by mutated IDH. 2HG is a competitive inhibitor of α KG-dependent dioxygenases resulting in reduction of histone demethylases and TET hydroxylases, which in turn prevents histone demethylation and increases promoter methylation of certain genes including the MGMT promoter [12]. This explains why gliomas with IDH1/2 mutation exhibit the glioma-CpG island methylator phenotype (G-CIMP) [35]. However, it remains to be elucidated if reduction of α -ketoglutarate or elevation of 2-hydroxyglutarate is of more importance for the up-regulation of HIF-1 α in glioma cells with IDH mutation [34, 36, 37].

Loss of Heterozygosity (LOH) on Chromosomes 1p and 19q

LOH 1p/19q is a chromosomal aberration which results from the co-deletion and unbalanced translocation of genetic material from chromosomes 1p and 19q and has been connected to oligodendroglial tumours [38, 39]. The tumourigenic mecha-

nism with which LOH 1p/19q leads to tumour progression has not been elucidated so far. However, two tumour suppressor genes, CIC and FUBP-1, have been linked to this location but need to be further evaluated [21, 40]. Co-deletion of 1p and 19q is especially seen in WHO grade-II and -III oligodendrogliomas or oligoastrocytomas but rarely (approximately 5 %) in GBM and a little more often (approximately 15 %) in GBM with an oligodendroglial component [22, 41, 42], which has been defined as a separate entity in the current edition of the WHO classification of tumours [43].

Regarding clinical impact, co-deletion of 1p/19q has been connected to prolonged PFS and OS by several studies which has only recently presented long-term data. The Radiation Therapy Oncology Group (RTOG) 9402 study included patients with anaplastic oligodendrogliomas (AO) or mixed anaplastic oligoastrocytomas (AOA). Patients were randomised after surgery to either CT with procarbazine/lomustine/vincristine (PCV) followed by RT or to RT only. Long-term results showed that patients with AO or AOA and LOH 1p/19q had significantly longer OS if they received PCV + RT (14.7 years) or RT (7.3 years) than patients without 1p/19q co-deletion (2.6 and 2.7 years). Since survival was twice as high if patients with LOH 1p/19q received PCV + RT versus RT alone, combined therapy was assumed to be an effective treatment strategy for patients with AO or AOA, especially if they had additional LOH 1p/19q [15]. The EORTC 26951 trial included patients with newly diagnosed AO WHO grade III and randomised them either to RT or to RT plus adjuvant PCV chemotherapy [44]. Overall survival (OS) was prolonged for patients who received PCV in addition to RT (42.3 months) versus patients who had received RT alone (30.6 months). Patients with AO and additional co-deletion of 1p and 19q had better survival rates than patients without LOH 1p/19q and there was a trend towards combined chemoradiation (OS not

Table 1. Biological impact of the most important molecular markers in gliomas of different WHO grades. Co-deletion of chromosomes 1p/19q, IDH mutation as well as MGMT promoter methylation status have diverging (un-) favourable impact as well as prognostic or predictive value in gliomas of different WHO grades as demonstrated in this table.

Adapted from [Weller M, Stupp R, Hegi ME, van den Bent M, Tonn JC, Sanson M, Wick W, Reifenberger G. Personalized care in neuro-oncology coming of age: why we need MGMT and 1p/19q testing for malignant glioma patients in clinical practice. *Neuro Oncol* 2012; 14 (Suppl 4): iv100–iv108] by permission of Oxford University Press on behalf of The Society of NeuroOncology (SNO). This table does not come under a Creative Commons license or any other open-access license and is view only.

Glioma subtype		IDH mutation	LOH 1p/19q	MGMT promoter methylation
Biological impact		Linked to DNA and histone methylation, energy metabolism, and pro-angiogenic pathways	Mode of action not elucidated yet; linked to oligodendroglial morphology	DNA repair enzyme
WHO grade II	Oligodendroglioma Diffuse astrocytoma	Favourable	Significance unclear, probably favourable Seldom present	Unknown
WHO grade III	Anaplastic oligodendroglioma Anaplastic astrocytoma	Favourable in patients treated with either radiation and/or chemotherapy	Favourable in patients treated with radiation and/or chemotherapy; predictive for benefit from addition of PCV to radiation Seldom present	Favourable in patients treated with radiation and/or chemotherapy
WHO grade IV	Primary glioblastoma Secondary glioblastoma	Rarely present Favourable; suggestive of sGBM	Rarely present, significance unclear	Predictive for benefit from alkylating chemotherapy

sGBM: secondary glioblastoma multiforme; PCV: procarbazine/lomustine/vincristine

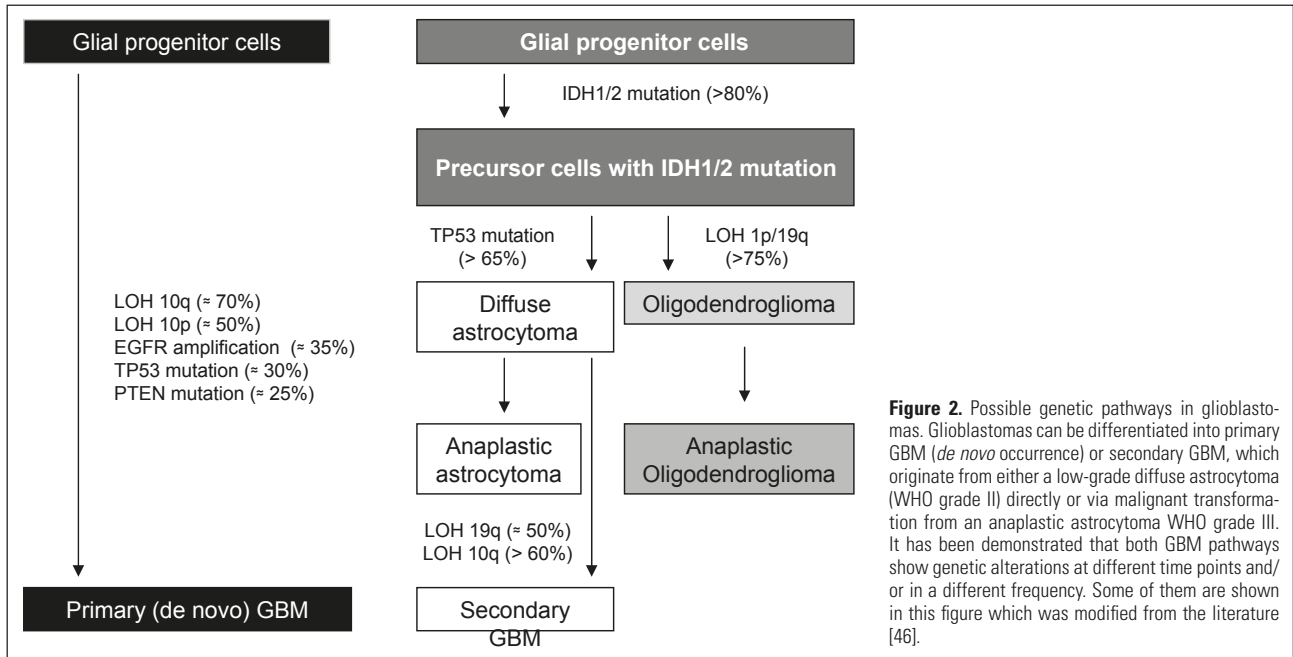


Figure 2. Possible genetic pathways in glioblastomas. Glioblastomas can be differentiated into primary GBM (*de novo* occurrence) or secondary GBM, which originate from either a low-grade diffuse astrocytoma (WHO grade II) directly or via malignant transformation from an anaplastic astrocytoma WHO grade III. It has been demonstrated that both GBM pathways show genetic alterations at different time points and/or in a different frequency. Some of them are shown in this figure which was modified from the literature [46].

yet reached after 140 months median follow-up) compared to RT alone (112 months) in these patients [16, 45]. Additionally, the NOA-04 study also included patients with anaplastic gliomas (WHO grade III) and randomised them into 3 different treatment groups: (1) RT, (2) PCV chemotherapy, or (3) TMZ chemotherapy. In case of toxicity or progression, patients were transferred from RT to CT with either PCV or TMZ and from both CT regimens to RT in a cross-over study design. The present analysis once again resulted in better prognosis for patients with LOH 1p/19q in contrast to patients without this co-deletion but so far there is no difference between treatment groups regarding PFS and OS [5]. Yet, in light of both aforementioned studies, the current follow-up period is considered possibly too short for final evaluation of prediction to different treatment options (Table 1) [21].

■ Interdependence of Molecular Markers

MGMT promoter methylation, IDH mutation, and 1p/19q co-deletion have been demonstrated to have their own prognostic or predictive value in different glioma subtypes. Yet, there is growing evidence of an interrelation of these markers, even if the mechanisms responsible for it have not been completely elucidated so far.

IDH mutation is an early event in gliomagenesis and seems to occur before co-deletion of chromosomes 1p and 19q in oligodendrogliomas or TP53 mutation in astrocytomas (Figure 2) [46]. While LOH 1p/19q has been linked to better treatment response to chemotherapy in oligodendrogliomas, its prognostic relevance in WHO grade-II or -III oligodendrogliomas or glioblastomas with oligodendroglial components has not been clarified so far. On the other hand, IDH1 mutation has been associated with better prognosis in malignant gliomas WHO grades III and IV but not in diffuse low-grade gliomas WHO grade II; at least not until tumour progression and intervention with radiation or chemotherapy [11]. Moreover, after screen-

ing 98 human glioblastoma samples in a recent study for genetic and epigenetic alterations in 10 genes and chromosomal loci, a 2-gene survival predictor was developed for patients with glioblastoma, consisting of the combination of MGMT hypermethylation and presence or absence of IDH1 mutation [47]. Patients with IDH1 mutation and MGMT promoter methylation encountered the longest overall survival, followed by patients with IDH mutation but unmethylated MGMT promoter or patients with wild-type IDH but MGMT promoter methylation. The worst prognosis was therefore observed in patients with absence of IDH mutation and unmethylated MGMT promoter (Figure 1).

■ Impact of Molecular Markers on Decision-Making for Glioma Therapy

Neuro-oncology is an oncological field which is not reserved for one single medical specialty but requires close collaboration between different subspecialties. Therefore, the quickly expanding knowledge about molecular markers and their predictive or prognostic value for glioma patients influences all specialists involved in patient management and glioma treatment.

For example, neurosurgeons aim at total resection if it can be achieved without increased surgical risk. On the other hand, since molecular markers are homogeneously distributed within the active tumour parts and only small amounts of tissue are necessary for proper histological and genetic diagnoses, this also allows to perform stereotactical biopsy only in cases where surgery may be considered too risky. However, additional information from metabolic imaging methods may be required for proper diagnosis [48–51]. Moreover, pure insular gliomas have been shown to have a higher rate of the favourable prognostic IDH mutation compared to gliomas which extend into the frontal or temporal paralimbic area [52]. Additionally, tumours with methylated MGMT promoter might re-

spond to neoadjuvant chemotherapy, allowing for a higher extent of surgical resection afterwards [53].

Since test validity is of great importance in order to provide the clinician with reliable information [54], this will also have large impact on the diagnostic routine of neuropathologists. Some additional markers have already been linked to prognostic relevance and may be helpful to discover which patients qualify for certain therapies. Unfavourable prognostic impact for PFS and OS has been demonstrated for low-grade astrocytomas and oligoastrocytomas grade II if TP53 mutation status is present [55]. Since mutations of TP53 and its pathway appear very early in the tumourigenesis of glial tumours [56] they are also present in approximately 65 % of secondary GBM but only in 30 % of primary GBM [57, 58]. Furthermore, almost 40 % of patients with primary GBM show amplification of the endothelial growth factor receptor (EGFR) but patients with secondary GBM only rarely do [57]. Apart from wild-type EGFR, there is a high expression of mutant EGFRvIII, which has been demonstrated to show high tumourigenic activity [59]. Signal transduction of EGFR and its mutant EGFRvIII include RAS, NF1, PTEN, PI3K as well as AKT and the mammalian target of rapamycin (mTOR) [58], some of which have already been identified as possible targets for new therapies [60, 61]. Apart from genetic alterations, there has also been an intention to deliver targeted therapies, eg, against vascular endothelial growth factor (VEGF), $\alpha_v\beta_3$ -integrin, or the extracellular matrix protein tenascin, for selective antiangiogenic or radioimmunotherapy to GBM patients [62–65]. With emerging techniques for extensive genetic or protein profiling via microarray analysis, TP53, EGFR/EGFRvIII, and other signal transduction molecules may only be a small number of possible targets for novel treatment. Therefore, expression profiling for additional possible targets may become relevant in the future.

Definition of radiation volumes will have to be adjusted in light of all available information since recurrence pattern after radiochemotherapy in glioblastoma patients may be influenced by MGMT promoter status [66]. Therefore, requirements for neuroradiologists will increase in the future. The question of pseudo-progression in patients with methylated MGMT promoter [67] or imaging alteration after anti-angiogenic therapy [68] resulted in new criteria which have been recommended by the Response Assessment in Neurooncology Working Group (RANO) in order to deal with these problems [69–71].

Discrimination between treatment response and real tumour recurrence/progression may be achieved by positron emission tomography (PET) additionally to standard MRI [72]. New tracers in nuclear medicine might help to identify molecular marker profiles non-invasively in order to select patients most suitable for certain therapies (eg, integrins) [73] or analysis of proliferation or cellular turnover [72, 74].

At present, identification of MGMT promoter status in the elderly is important for all neuro-oncologists in order to include this information into decision-making since recent data from prospective and randomised trials clearly favour TMZ chemotherapy alone or in combination with radiotherapy in case of

methylated MGMT promoter and radiotherapy only in patients with unmethylated MGMT promoter [10, 14]. In the future, LOH 1p/19q analysis might be crucial in order to select patients with anaplastic oligodendrogliomas or oligoastrocytomas who might profit from (PCV) chemotherapy [10, 15, 16, 75]. An increasing number of clinical trials already randomise patients depending on their MGMT promoter methylation status [76–78]. Therefore, at least stratification for molecular markers will be of increasing importance also in upcoming trials to get unbiased results. On the other hand, the issue will be which biomarkers have to be considered without reducing statistical power by subdividing treatment arms into too-small groups.

■ Conclusion

Histopathological diagnosis of gliomas based to the WHO criteria will not be sufficient in the future to allocate patients into the best available treatment group. Patient prognosis is also influenced by age, neurological performance status, imaging features, extent of resection, and the molecular genetic signature of these tumours.

MGMT promoter methylation has turned out to be an important predictive marker for chemotherapy in patients with glioblastomas and especially elderly patients profit from treatment adjustment according to the MGMT promoter status favouring chemotherapy in methylated and radiotherapy in unmethylated MGMT promoter cases. IDH mutation is an important prognostic factor, which groups prognostically favourable patients with malignant gliomas. Even if it is not predictive for a certain therapeutic scheme, its knowledge may be of importance in cases of doubt, where more aggressive treatment may be considered for patients with IDH wild type. Co-deletion of 1p/19q should be determined at least in patients with WHO grades II and III, where LOH 1p/19q will be necessary to identify which patients qualify for (additional) chemotherapy with PCV or other agents like TMZ.

Besides the determination of the MGMT gene promoter methylation status, IDH mutation, and LOH 1p/19q, there might be other factors to analyse in the future and the question will be, which markers are most likely to achieve clinical relevance next. For example, several trials have already tried to determine the impact of EGFRvIII inhibition in glioblastoma patients. Despite controversial results, a large international trial is currently ongoing, using vaccination against EGFRvIII as a novel strategy in these patients (ACT IV). Furthermore, as already mentioned, the interdependence of already known as well as possible upcoming markers will have to be elucidated in more detail in the future. Modern personalised treatment concepts in neuro-oncology will have to take these into account in order to provide the best possible care for patients. The primary target in the treatment of gliomas will therefore not be to define one overall standard for each WHO grade which has to be rendered to every patient, but to expand our toolbox of possible therapies to choose the particular therapy that suits the individual patient best. Hence, our current and future knowledge of molecular markers will help to develop more personalised treatment concepts for our patients.

■ Conflict of Interest

None.

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