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# Significance of complete 1p/19q co-deletion, *IDH1* mutation and *MGMT* promoter methylation in gliomas: use with caution

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**The histopathological diagnosis of diffuse gliomas often lacks the precision that is needed for tailored treatment of individual patients. Assessment of the molecular aberrations will probably allow more robust and prognostically relevant classification of these tumors. Markers that have gained a lot of interest in this respect are co-deletion of complete chromosome arms 1p and 19q, (hyper)methylation of the *MGMT* promoter and *IDH1* mutations. The aim of this study was to assess the prognostic significance of complete 1p/19q co-deletion, *MGMT* promoter methylation and *IDH1* mutations in patients suffering from diffuse gliomas. The presence of these molecular aberrations was investigated in a series of 561 diffuse astrocytic and oligodendroglial tumors (low grade $n=110$, anaplastic $n=118$ and glioblastoma $n=333$) and correlated with age at diagnosis and overall survival. Complete 1p/19q co-deletion, *MGMT* promoter methylation and/or *IDH1* mutation generally signified a better prognosis for patients with a diffuse glioma including glioblastoma. However, in all 10 patients with a histopathological diagnosis of glioblastoma included in this study complete 1p/19q co-deletion was not associated with improved survival. Furthermore, in glioblastoma patients >50 years of age the favorable prognostic significance of *IDH1* mutation and *MGMT* promoter methylation was absent. In conclusion, molecular diagnostics is a powerful tool to obtain prognostically relevant information for glioma patients. However, for individual patients the molecular information should be interpreted with caution and weighed in the context of parameters such as age and histopathological diagnosis.**
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Gliomas are the most common primary tumors of the central nervous system with an incidence of approximately five to seven new cases per 100 000/year.[1,2] Gliomas comprise a very heterogeneous group of neoplasms with regard to patient age at diagnosis, location within the central nervous system, extent of invasiveness, histological subtype, malignancy grade, tendency for progression and response to treatment.

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In adult patients, the vast majority of gliomas belong to the spectrum of diffuse gliomas, the common denominator of these tumors being extensive, diffuse infiltration of individual or small groups of tumor cells in the pre-existent brain parenchyma.[3] Diffuse gliomas can generally be subtyped as astrocytic, oligodendroglial or mixed oligo-astrocytic, and graded as WHO grade II (low grade), WHO grade III (anaplastic) or WHO grade IV (glioblastoma, gliosarcoma, glioblastoma with oligodendroglial component).

Only modest advancements in the treatment of diffuse gliomas have been made in the past decades, and curation of patients carrying these tumors is still virtually impossible. Unfortunately, glioblastoma is by far the most frequent representative of the diffuse gliomas. Even after receiving optimal therapy (including surgical resection, irradiation, and chemotherapy) the median survival for glioblastoma patients is $\sim$15 months, with $<$27% of patients surviving up to 2 years and $<$10% of patients surviving up to 5 years.[4,5]

It is well established that clinical characteristics such as patient age, Karnofsky Performance Scale, extent of surgery as well as histopathological features (glioma type and malignancy grade) provide information on the clinical course of the disease that is to be expected. More recently, molecular markers were shown to be helpful in recognizing more uniform subgroups of gliomas with regard to prognosis and response to therapy. The three markers that have gained most interest in this respect are complete deletion of both the short arm of chromosome 1 and of the long arm of chromosome 19 (complete 1p/19q co-deletion), hypermethylation of the *MGMT* promoter and mutations in the isocitrate dehydrogenase 1 or 2 gene (*IDH1*/*IDH2* mutations). All three markers are considered as indicators of favorable prognosis.[6–9] MGMT hypermethylation status is already included in the nomogram for predicting survival of patients with newly diagnosed glioblastoma, and IDH1/2 mutation was shown to be a reliable genetic marker of secondary glioblastomas and their precursor lesions.[10–12] In contrast, the presence of other markers such as loss of *PTEN* and *CDKN2A* and amplification of *EGFR* are reported to be indicative for more aggressive tumor behavior.[13]

Although histopathology is still the 'gold standard' for classification (ie, typing and grading) of gliomas, it is increasingly clear that the histopathological diagnosis lacks the precision that is needed for tailored treatment of individual patients. Assessment of the molecular aberrations in diffuse gliomas may well allow for a more robust and prognostically relevant classification. For instance, it was shown that complete 1p/19q co-deletion is a favorable prognostic marker in anaplastic gliomas. However, its prognostic meaning in patients with glioblastoma remains a subject of discussion. Although one study suggested that complete 1p/19q co-deletion is associated with longer survival independent of pathological diagnosis,[14] others report that deletions involving 1p and 19q are uncommon in glioblastomas but predict a shortened survival.[15] Also, it has been suggested that complete 1p/19q co-deletion and *IDH1* mutation should be considered incompatible with the diagnosis of glioblastoma.[16] Furthermore, a recent study reported that glioblastomas carrying *IDH1* mutations are associated with a better survival than anaplastic astrocytomas without these mutations.[17] Whether the recognition of an oligodendroglial component in glioblastomas has prognostic value is still under debate[18–20] and also within this glioblastoma subtype a different origin of this oligodendroglial component is hypothesized.[19] An age-dependent correlation—which was found for the prognostic effect of molecular markers like *TP53*, 1p loss and *CDKN2A*—may well contribute to the inconsistencies between studies on the prognostic relevance of specific molecular markers.[21]

The aim of this study was to further elucidate the prognostic significance of complete 1p/19q co-deletion, (hyper)methylation of the *MGMT* promoter and *IDH1* mutation, in a large set of 561 tumors covering the complete, heterogeneous spectrum of diffuse glioma patients. The patients included in this study were treated in different hospitals, often outside clinical trials, resulting in (more or less subtle) differences in therapeutic approaches that can be encountered in a routine neuro-oncological practice. The histopathological diagnosis and results of molecular analysis were correlated with relevant clinical data, ie, patient age at diagnosis, treatment and overall survival. Knowing that substantial interobserver variation exists in subtyping of diffuse gliomas (oligodendroglial *vs* oligoastrocytic *vs* astrocytic neoplasms), we chose to group the lesions based on malignancy grade (low grade/WHO grade II, anaplastic/WHO grade III and glioblastoma/WHO grade IV) rather than on histopathological subtype.

## Materials and methods

### Glioma Samples and Patient Characteristics

Samples of diffuse glioma specimens obtained by surgical biopsy or resection were retrieved from the archives at the Department of Pathology of the Radboud University Nijmegen Medical Centre, The Netherlands, the Academic Medical Center, Amsterdam, The Netherlands and from the MD Anderson Cancer Center, Houston, TX, USA. The use of brain tumor tissue for research purposes after completing histopathological diagnosis has been approved by the Regional Ethics Committees. The tumors included in this study (in total $n=561$) were typed and graded according to the WHO 2007 classification[4] as diffuse astrocytoma (AII; $n=46$), anaplastic astrocytoma (AIII; $n=34$), glioblastoma

($n=333$), including gliosarcoma ($n=4$) and glioblastoma with oligodendroglial differentiation ($n=19$), oligodendroglioma (OII; $n=47$), anaplastic oligodendroglioma (OIII; $n=67$), oligoastrocytoma (OAII; $n=17$) and anaplastic oligoastrocytoma (OAIII; $n=17$).

Because of the substantial interobserver variation that exists in subtyping of diffuse gliomas (oligodendroglial *vs* oligoastrocytic *vs* astrocytic neoplasms), we chose to group the lesions for this study by their malignancy grade: as low grade/WHO grade II, anaplastic/WHO grade III and glioblastoma/WHO grade IV.

The mean age of patients with a low-grade glioma was 40 years (range 20–71), for patients with anaplastic glioma 42 years (range 22–66) and for glioblastoma patients 54 years (range 31–78). Survival time was defined as the period from date of surgery to date of death or, when the patient was still alive or date of death was not available, the date of last follow-up.

For survival analysis with respect to MGMT, we only included patients who received chemoradiation in line with the Stupp protocol (Figure 3e) or received only irradiation (Figure 3d).[5] Patients with other treatment protocols were excluded for this part of the analysis.

### Molecular Analysis

DNA was isolated from routinely processed, formalin-fixed, paraffin-embedded tumor samples or from snap frozen tumor tissue using the DNeasy Tissue Kit (Qiagen, Venlo, The Netherlands) as described previously[22] or the Miller salting out technique as described previously.[23] MLPA analysis was performed to detect copy number changes of multiple loci simultaneously (http://www.mlpa.com;[24]) and all assays used were prepared by MRC-Holland (Amsterdam, The Netherlands). MLPA assay P088 (lot nos. 0804, 0305, 0706 or 0608) was used to detect complete or partial losses involving chromosome arms 1p and 19q in 444 gliomas.[25] *MGMT* promoter hypermethylation was assessed with MS-MLPA assay ME-011 in 433 tumors, and *IDH1* mutation analysis was performed in 442 tumors by direct sequencing as described previously.[26–29] In addition, in 440 cases MLPA assay P105 (lot nos. 0306, 0407 or 1008) was used to detect copy number changes in the genes *CDKN2A, PTEN* and *EGFR*.[28] Owing to limited amount of DNA of some samples it was not possible to asses all molecular markers on all samples.

### Statistical Analysis

Statistical analysis was performed with the MEDCALC statistical software (http://www.medcalc.org). Kaplan–Meier curves were generated to depict the correlation between molecular markers and survival. Differences between Kaplan–Meier survival curves were calculated by the log-rank test.

## Results

The number of different glioma samples evaluated for the molecular markers is shown in Table 1. Overall, complete 1p/19q co-deletion was detected in 34% of low-grade gliomas, in 52% of anaplastic gliomas and 3% of glioblastomas. We did not detect a survival difference between patients with a glioblastoma or patients with a glioblastoma with an oligodendroglial component. Although in patients with low-grade or anaplastic gliomas complete 1p/19q co-deletion was associated with a significant survival benefit (Figures 1a and b), for patients with a histopathological diagnosis of glioblastoma this survival benefit was absent (Figure 1c). More detailed analysis of the 10 patients in this latter category revealed that the mean age was comparable with that of the rest of the glioblastoma patients (55.9 *vs* 53.9 years, respectively). Interestingly, four out of eight of these glioblastoma samples that could also be tested for *IDH1* mutation and *EGFR* status showed an *IDH1* mutation and a normal *EGFR* copy number (median survival of these four patients 135 days), whereas the other four tumors carried wild-type *IDH1* but an increased *EGFR* copy number (three low-level gain, one high copy amplification; median survival of these four patients 225 days). Histopathological review of six of these cases and more detailed investigation of the clinical history could be performed. In three cases the review diagnosis was glioblastoma with oligodendroglial component, in one of these cases an *IDH1*

**Table 1** Summary of the numbers of diffuse gliomas analyzed at the molecular level

| | *Low-grade gliomas* | | *Anaplastic gliomas* | | | *Glioblastomas* | |
|---|---|---|---|---|---|---|---|
| | *No.* | *%* | *No.* | *%* | | *No.* | *%* |
| *1p/19q co-deletion* | 66 | 35 | 53 | 51 | | 325 | 3 |
| Oligodendrogliomas | 22 | 82 | 31 | 77 | *GBM-O* | 19 | 5 |
| Oligo-astrocytomas | 16 | 12 | 15 | 13 | | | |
| Astrocytomas | 28 | 11 | 7 | 14 | | | |
| *IDH1 mutation* | 104 | 84 | 115 | 75 | | 223 | 16 |
| Oligodendrogliomas | 44 | 84 | 68 | 81 | *GBM-O* | 19 | 21 |
| Oligo-astrocytomas | 16 | 100 | 15 | 67 | | | |
| Astrocytomas | 44 | 77 | 32 | 66 | | | |
| *MGMT methylation* | 61 | 36 | 51 | 45 | | 321 | 27 |
| Oligodendrogliomas | 19 | 47 | 31 | 55 | *GBM-O* | 19 | 16 |
| Oligo-astrocytomas | 16 | 50 | 14 | 36 | | | |
| Astrocytomas | 26 | 19 | 6 | 17 | | | |

Abbreviations: IDH1, isocitrate dehydrogenase 1; MGMT, O-6-methylguanine-DNA methyltransferase; no., number analyzed; 1p/19q co-deletion, complete co-deletion of chromosome arms 1p and 19q; %, percentage with aberration.
Also the numbers and frequencies are shown for the different histopathological subtypes.

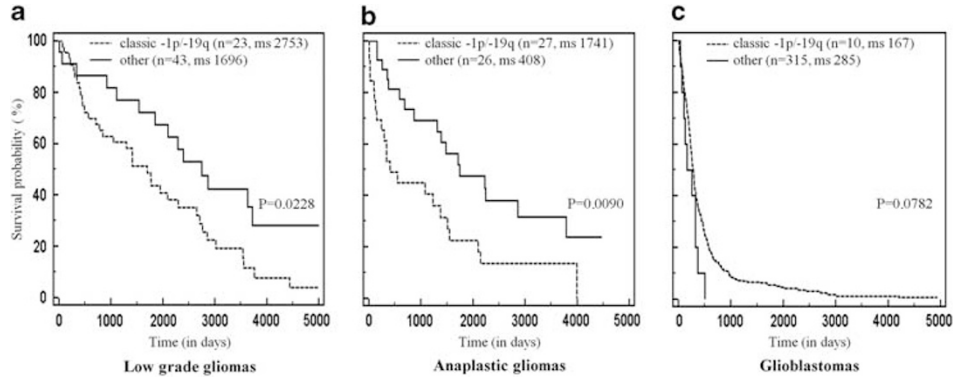


**Figure 1** Kaplan–Meier survival curves depicting survival of patients with diffuse gliomas with or without complete 1p/19q co-deletion. In (**a**) low-grade gliomas, (**b**) anaplastic gliomas and (**c**) glioblastomas. The blue lines indicate patients of which the gliomas showed complete 1p/19 co-deletion, the red lines patients with tumors containing other (combinations of) 1p/19q losses or a normal 1p/19q copy number. Note that survival advantage is seen for the presence of complete co-deletion of 1p and 19q in low-grade and anaplastic glioma patients but not for patients with a histopathological diagnosis of glioblastoma/gliosarcoma. ms, median survival; *n*, number of patients.

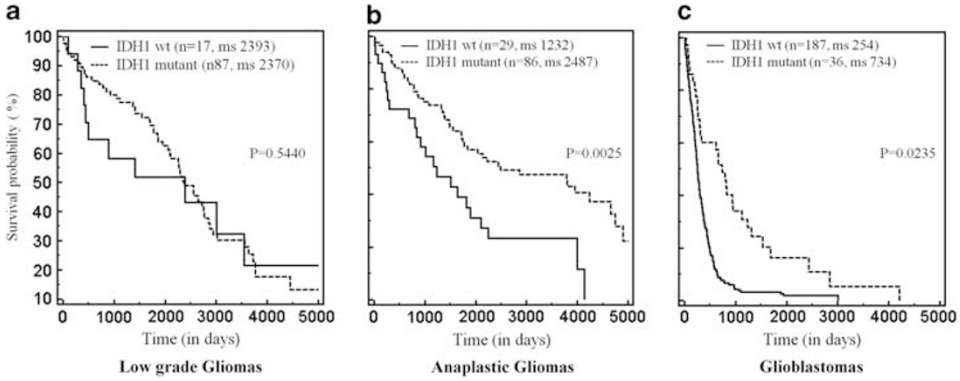


**Figure 2** Kaplan–Meier survival curves depicting survival with respect to *IDH1* mutation in (**a**) low-grade gliomas, (**b**) anaplastic gliomas and (**c**) glioblastomas. The blue lines indicate survival of patients with *IDH1* mutant glioma, in red the patients with a wild-type *IDH1* glioma. Note that in this series the favorable prognostic significance of *IDH1* mutations is only present for the patients with a histopathological diagnosis of anaplastic glioma or glioblastoma. ms, median survival; *n*, number of patients.

mutation was present; in two patients the review diagnosis was gliosarcoma (no clear oligodendroglial phenotype) but one of these patients was operated upon 7 years earlier and at that time a diagnosis of low-grade oligodendroglioma was rendered. In the remaining patient the review diagnosis remained glioblastoma. Interestingly, for patients with a diagnosis of glioblastoma carrying an *IDH1* mutation with a normal 1p or a partial loss of 1p, the survival was significantly better than for the patients with a complete 1p/19q co-deletion (see also Table 2).

In our series, *IDH1* mutations were found in 84% of low-grade gliomas, 75% of anaplastic gliomas and 16% of glioblastomas. In the group of low-grade gliomas *IDH1* mutations were not associated with significant survival advantage, but in patients with anaplastic gliomas and glioblastomas the survival benefit was significant (Figure 2). As age is an important prognostic factor in patients with glioblastoma, we explored the correlation between patient age and prognostic value of *IDH1* mutations by performing survival analysis for different age categories (ie, patients under 40, 45, 50 and 55 *vs* >40, 45, 50 and 55 years, respectively). In this analysis, 50 years of age was identified as the turning point above which *IDH1* mutations no longer had a positive prognostic value for patients with glioblastoma (Figure 3a).

**Table 2** Association of *IDH1* mutations and different types of chromosome arm 1p losses in glioblastomas and correlation with survival

| *Glioblastomas* | *IDH1 mutation* | *Median survival (in days)* | |
|---|---|---|---|
| | *Number (%)* | *IDH1 mutated* | *IDH1 wild type* |
| All | 36/226 (16) | 734 | 254 |
| Normal 1p | 25/148 (17) | 818 | 255 |
| 1p/19q co-deleted | 4/8 (50) | 135 | 225 |
| Partial loss 1p | 1/30 (3) | 828 | 233 |

Abbreviation: IDH1, isocitrate dehydrogenase 1.
Gliosarcomas and glioblastomas with oligodendroglial features are included in the group of glioblastomas.

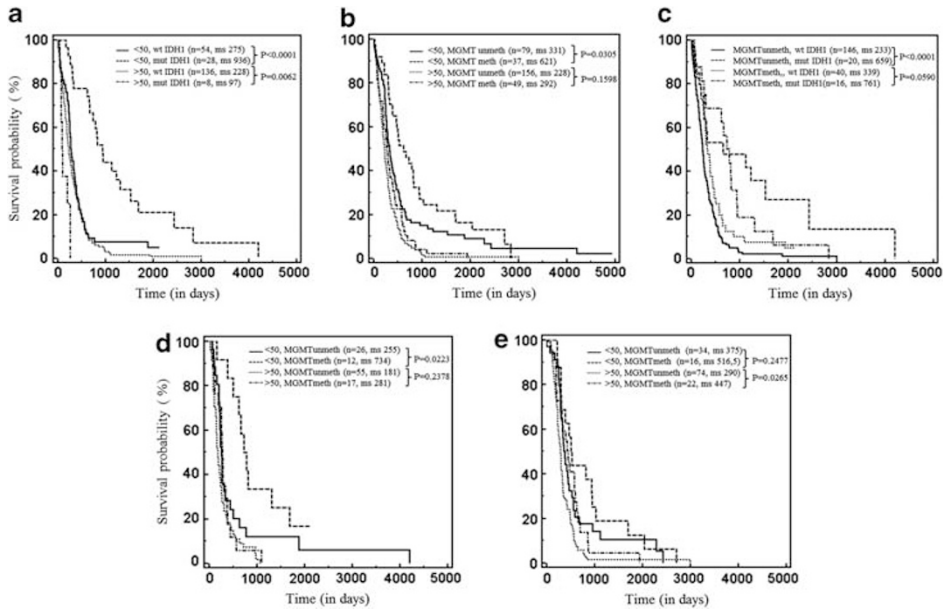


**Figure 3** Kaplan–Meier survival curves showing survival in glioblastoma patients with respect to (**a**) *IDH1* mutation by age at diagnosis $<50$ and $>50$; (**b**) *MGMT* promoter hypermethylation by age at diagnosis $<50$ and $>50$; (**c**) Combination of *IDH1* mutation and *MGMT* promoter hypermethylation; (**d**) *MGMT* promoter methylation in patients $<50$ and $>50$ only receiving radiotherapy; and (**e**) *MGMT* promoter methylation in patients $<50$ and $>50$ receiving chemoradiation. M*GMT*meth/*MGMT*unmeth is methylated *vs* unmethylated promoter of the *MGMT* gene. ms, median survival; *n*, number of patients.

Subsequently, we investigated how the prognostic significance of (absence of) *IDH1* mutation in glioblastomas was associated with the presence of molecular markers that are described to have a negative prognostic connotation (Table 3). As survival was comparable for glioblastoma patients with wild-type *IDH1* and age older or younger than 50 years, patients with *IDH1* wild-type glioblastomas were included in a single group. Interestingly, in the group of *IDH1*-mutated glioblastomas, no significant difference was found in frequency of *EGFR* amplification, *CDKN2A* loss or *PTEN* loss between patients younger *vs* older than 50 years of age. Survival in this latter group of patients (ie, *IDH1*-mutated glioblastoma, $>50$ years of age) was similar to that of patients with *IDH1* wild-type glioblastomas, but the *IDH1*-mutated lesions less frequently also harbored molecular aberrations involving *EGFR*, *CDKN2A* and/or *PTEN* (Figure 3a, Table 3).

*MGMT* promoter methylation was assessed with MS-MLPA, and methylation was defined as MS-MLPA ratio $>0.5$. Overall, 38% (23/61) of the low-grade gliomas, 45% (23/51) of the anaplastic gliomas and 27% (87/321) of the glioblastomas showed methylation of the *MGMT* promoter.[26] Survival analysis revealed that *MGMT* promoter methylation correlated with increased overall survival in low-grade and anaplastic diffuse gliomas as well as in glioblastomas (data not shown). When evaluating the interrelationship of *MGMT* status and age of glioblastoma patients again (similar to the situation for *IDH1* mutations), *MGMT* promoter methylation no longer signified survival benefit in patients older than 50 years of age (Figure 3b).

In total, 12% (20/166) of glioblastomas without *MGMT* promoter methylation had an *IDH1* mutation and 29% (16/56) of tumors with *MGMT* promoter methylation had an *IDH1* mutation. Survival analysis indicates that the presence of an *IDH1* mutation in glioblastomas has a more favorable prognostic impact for patients without *MGMT* promoter methylation than for those with such methylation (Figure 3c).

As *MGMT* promoter methylation was originally described as a marker predicting favorable response to therapy using alkylating agents, we investigated the association between therapeutic modality (irradiation alone *vs* chemoradiation in line with Stupp protocol), *MGMT* status and survival. Patients younger than 50 years of age with methylated *MGMT* promoter, and only irradiation showed better survival than those without *MGMT* promoter methylation; in patients older than 50 years this survival advantage was not evident (Figure 3d). In contrast, in our series in patients receiving chemoradiation a significantly longer median survival was found for patients older than 50 years of age and with a methylated *MGMT* promoter, whereas for patients under 50 years this advantage was not evident (Figure 3e).

## Discussion

During the last decade, the clinical potential of molecular characterization of gliomas has become increasingly clear. Of the available molecular markers the diagnostic, prognostic and/or predictive importance of especially complete co-deletion of chromosome arms 1p and 19q, of *IDH1* mutations

**Table 3** Overview of co-occurence of molecular aberrations in glioblastomas in relation to *IDH1* status

| | *IDH1 wild type* | | *IDH1 mutated <50 years* | | *IDH1 mutated >50 years* | |
|---|---|---|---|---|---|---|
| | *Absolute* | *%* | *Absolute* | *%* | *Absolute* | *%* |
| *1p/19q* | | | | | | |
| Normal 1p/19q | 81 | 57 | 21 | 91 | 4 | 50 |
| −1p/−19q | 4 | 3 | 1 | 4 | 3 | 38 |
| Other 1p/19q | 56 | 40 | 1 | 4 | 1 | 12 |
| *MGMT* | | | | | | |
| Unmethylated | 146 | 78 | 15 | 54 | 5 | 62 |
| Methylated | 40 | 22 | 13 | 46 | 3 | 38 |
| *EGFR* | | | | | | |
| Normal | 41 | 22 | 19 | 76 | 7 | 88 |
| Gain | 65 | 34 | 6 | 24 | 1 | 12 |
| Amplification | 23 | 12 | 0 | 0 | 0 | 0 |
| HCA | 60 | 32 | 0 | 0 | 0 | 0 |
| *CDKN2A* | | | | | | |
| Normal | 50 | 26 | 12 | 48 | 4 | 50 |
| Loss | 45 | 24 | 7 | 28 | 2 | 25 |
| HD | 82 | 43 | 4 | 16 | 2 | 25 |
| Gain | 12 | 6 | 2 | 8 | 0 | 0 |
| *PTEN* | | | | | | |
| Normal | 57 | 30 | 13 | 52 | 5 | 62 |
| Loss | 118 | 62 | 12 | 48 | 3 | 38 |
| HD | 10 | 5 | 0 | 0 | 0 | 0 |
| Gain | 4 | 2 | 0 | 0 | 0 | 0 |

Abbreviations: CDKN2A, cyclin-dependent kinase inhibitor 2A; EGFR, epidermal growth factor receptor; HCA, high copy amplification; HD, homozygous deletion; IDH1, isocitrate dehydrogenase 1; MGMT, O-6-methylguanine-DNA methyltransferase; PTEN, phosphatase and tensin homolog.

and of *MGMT* promoter methylation are relatively well studied. Our study on a large set of tumors covering the complete heterogeneous spectrum of glioma patients corroborates the prognostic significance of all three markers. Of course, translation of such information for individual patients remains difficult. This study shows that also for certain subgroups of patients with a diffuse glioma prognostication based on these markers should be performed with caution. More precisely, in our study in the small group of patients with a histopathological diagnosis of glioblastoma and complete 1p/19q co-deletion, the favorable significance of this (with or without *IDH1* mutation) was lost. Also, the prognostic significance of *IDH1* mutation and of *MGMT* promoter methylation was lost in glioblastoma patients >50 years of age. Finally, in our study population the assessment of the prognostic meaning of *MGMT* promoter methylation is complex. These findings will now be discussed in somewhat more detail.

In our large series we identified 10 glioblastomas with complete 1p/19 co-deletion. Recently, it was suggested that the presence of 1p/19q co-deletion and *IDH1* mutations may have to be considered as incompatible with the diagnosis of glioblastoma.[16] Our observation that complete 1p/19q co-deletion lacks prognostic meaning for glioblastoma patients argues against this point of view. Another study recently reported a similar finding and suggested that true genomic instability was the reason for shortened survival of patients with glioblastoma and complete 1p/19q co-deletion.[15] Furthermore, in the study of Gravendeel *et al*,[30] 10 out of 175 glioblastoma cases showed complete 1p/19q co-deletion but no explicit correlations with overall survival for these 10 cases were made. However, most studies do not report this lack of favorable prognostic impact of 1p/19q co-deletion in glioblastoma patients. This discrepancy may partly be caused by different techniques to identify 1p/19q losses. LOH and FISH, investigating only a limited numbers of loci on these chromosomes, are often used in such studies and may not allow for a clear identification of tumors with a complete 1p/19q co-deletion as they cannot be accurately distinguished from other types of 1p/19q aberrations.[31] The inclusion of different types of 1p/19q aberrations in one group may prohibit identification of the true clinical value for the individual subgroups.[32] Moreover, differences in criteria used to diagnose glioblastoma (*vs*, eg, anaplastic (oligo)astrocytoma) may have contributed to masking this lack of favorable prognosic impact of 1p/19q co-deletion. Still, histopathological review of the glioblastomas with complete 1p/19q co-deletion in our series revealed that, although some of them had some oligodendroglial features (allowing for a diagnosis of glioblastoma with oligodendroglial component according to the WHO 2007 classification) or evolved from an oligodendroglioma, the diagnosis remained that of a WHO grade IV glial tumor (glioblastoma/gliosarcoma) in all cases. In our opinion, it is thus too early to discard a diagnosis of glioblastoma for lesions showing complete 1p/19q co-deletion (with or without *IDH1* mutation) as the patients carrying these tumors may well have a grim prognosis.

In our study population, the expected correlation between improved survival and *IDH1* mutation was very clear for glioblastoma and anaplastic glioma patients but less obvious for low-grade glioma patients. This latter finding is in concordance with the study of Kim *et al*[33] who showed in a large series of low-grade gliomas ($n=360$) that the presence of IDH1 mutations was not prognostic for the survival. In the group of glioblastoma patients we identified two exceptions to the rule that an *IDH1* mutation is a favorable prognostic marker: (1) patients with a glioblastoma harboring an *IDH1* mutation that co-occurred with a complete 1p/19q co-deletion (see above); (2) patients with a glioblastoma harboring an *IDH1* mutation that are older than 50 years of age. Interestingly, the tumors in this latter group relatively infrequently showed copy number changes in *EGFR*, CDKN2A or *PTEN*, ie, markers reported to be indicative of aggressive biological

behavior. Although *IDH1* mutations are infrequently detected in glioblastomas from older patients (eg, in 8/144 older than 50 years (current study) or in 2 out of 126 glioblastoma patients older than 60 years,[17,34] these patients do not show a survival benefit, underlining once again that patient age in our study ($>50$) should be considered when using molecular markers for assessment of prognosis. Knowing that IDH1 mutations are especially common in low-grade and anaplastic diffuse gliomas, in secondary glioblastomas and in younger patients, the lack of favorable prognostic meaning of IDH1 in the two groups just mentioned might be explained by assuming that molecular analysis was performed in these patients relatively late in their disease process.

The relatively short survival of glioblastoma patients showing both a 1p/19q co-deletion and an *IDH1* mutation was unexpected. Evaluating the co-occurrence of *IDH1* mutations and *MGMT* promoter methylation shows that an *IDH1* mutation in combination with *MGMT* promoter methylation is more favorable, than in combination with an unmethylated *MGMT* promoter, which is in concordance with a previous report of Hartmann *et al.*[17] Both observations clearly show that when using molecular markers for predicting prognosis, the status of multiple markers should be considered in the context of histopathological tumor classification as well as patient age.

Over the past years, a lot of studies have been dedicated to assessing the prognostic and/or predictive value of *MGMT* promoter methylation in gliomas. Again, the fact that different approaches were used (MS-PCR or MS-MLPA) and different CpG islands were evaluated may account for the discrepancies reported in this respect. Unfortunately, studies that critically compare all assays and systematically analyze which CpG sites best reflect treatment outcome and patient survival are still lacking.[35] In line with previous reports, our results show that patients with a glioma harboring a methylated *MGMT* promoter generally had a longer overall survival. However, in our series in glioblastoma patients aged $>50$, *MGMT* promoter methylation no longer signified survival benefit. This observation is in concordance with the apparent discrepancy between the high rate of *MGMT* promoter hypermethylation as detected in elderly glioblastoma patients and their generally poor(er) outcome.[36]

Our study underscores that molecular diagnostics is a powerful tool to obtain prognostic relevant information for patients with a diffuse glioma. However, our results also show that it is too early to use the molecular information to overrule the histopathological diagnosis. Rather, a 'smart synthesis' of morphological and molecular diagnosis is needed for optimal prediction of prognosis for patients with a diffuse glioma.[37] A more complex model is needed, in which clinical data such as age, histopathological diagnosis and co-occurrence of molecular markers such as complete 1p/19q co-deletion, *IDH1* mutation and *MGMT* promoter methylation should be integrated. It is well known that age is one of the strongest prognostic factors and therefore it is included together with therapy administered, extent of surgery, Mini Mental Score Examination, administration of corticosteroids and WHO Performance Status in the nomograms for predicting survival of GBM patients,[11] which are available on the website of the EORTC http://www.eortc.be/tools/gbmcalculator. Thusfar, only *MGMT* promoter methylation status is included as a molecular marker in the nomograms, *IDH1* mutation and 1p/19q co-deletion are not.

Much more detailed information on the molecular background of tumors will rapidly become available. The challenge will be to implement this information in daily clinical practice in such a way that it will substantially improve tailored treatment of individual patients suffering from diffuse glioma.

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## Disclosure/conflict of interest

The authors declare no conflict of interest.

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