## Title

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## Authors

Sabha, Nesrin
Knobbe, Christiane B
Maganti, Majula
et al.

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# Analysis of *IDH* mutation, 1p/19q deletion, and *PTEN* loss delineates prognosis in clinical low-grade diffuse gliomas

**Nesrin Sabha†, Christiane B. Knobbe†, Majula Maganti, Soha Al Omar, Mark Bernstein, Rob Cairns, Besmira Çako, Andreas von Deimling, David Capper, Tak W. Mak, Tim-Rasmus Kiehl, Philippe Carvalho, Evelyn Garrett, Arie Perry, Gelareh Zadeh, Abhijit Guha, and Sidney Croul**

*Arthur and Sonia Labatt Brain Tumor Centre, Hospital for Sick Children's Research Institute, Toronto, ON, Canada (N.S., G.Z., A.G.); Division of Neurosurgery, Toronto Western Hospital, Toronto, Ontario, Canada (S.A.O., M.B., P.C., G.Z., A.G.); Campbell Family Institute for Breast Cancer Research at Princess Margaret Hospital, Princess Margaret Hospital, Toronto, Ontario, Canada (C.B.K., R.C., T.W.M.); Department of Neuropathology, Institute for Pathology, University of Heidelberg, Heidelberg, Germany (A.v.D., D.C.); University Health Network Pathology, Toronto General Hospital, Toronto, Ontario, Canada (T.-R.K., S.C.); Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto Medical Sciences Building, Toronto, Ontario, Canada (B.C., T.-R.K., S.C.); UCSF Department of Pathology, San Francisco, California (E.G., A.P.); Division of Biostatistics, Princess Margaret Hospital, Toronto, Ontario, Canada (M.M.)*

**Corresponding Author:** Sidney E. Croul, MD, FRCPC, UHN Pathology, 11E426 TGH, 200 Elizabeth St, Toronto, ON M5G 2C4 Canada (sidney.croul@uhn.ca).

†These authors contributed equally to this work.

See the editorial by Jenkins, on pages 891–892.

**Background.** Grades II and III gliomas have unpredictable rates of progression, making management decisions difficult. Currently, several clinical and radiological characteristics are utilized to predict progression and survival but collectively are suboptimal.

**Methods.** In this study, we analyzed a set of 108 nonenhancing hemispheric grade II–III gliomas. Demographic variables, including patient age, tumor diameter, extent of resection, and performance status, were combined with molecular data (*IDH* mutation status [*mIDH*], 1p/19q codeletion, *PTEN* deletion, and *EGFR* amplification). A complete dataset for all variables was compiled for 70 of the 108 patients. Both univariable and multivariable analyses were performed to determine whether the molecular data singly or in combination offer advantages over tumor type and grade for prediction of overall survival (OS) and/or progression-free rate (PFR).

**Results.** Patient age, clinical variables (tumor diameter, extent of resection, performance status), and pathology (tumor type and grade) were not predictive of OS or PFR. *IDH* mutation status alone was predictive of longer OS and PFR for the entire group of tumors; 1p/19q deletion alone was predictive of OS but not PFR. In the multivariable analysis, none of the clinical or demographic factors were predictive of OS or PFR. *IDH* mutation status, 1p/19q codeletion, and *PTEN* deletion were predictive of OS ($P = .003$, $P = .005$, $P = .02$, respectively). Both *mIDH* ($P < .001$) and the interaction term of 1p/19q and *PTEN* ($P < .001$) were found to be predictive of PFR.

**Conclusions.** We conclude that the combination of *mIDH*, 1p/19q codeletion, and *PTEN* deletion may be particularly effective in discriminating good prognosis from poor prognosis hemispheric gliomas. We propose that such a scheme merits testing on larger prospective cohorts. Should our findings be confirmed, routine clinical analysis of hemispheric gliomas for *mIDH*, 1p/19q codeletion, and *PTEN* deletion would be justified.

**Keywords:** IDH, low grade glioma, mutations, overall survival, progression free rate.

Diffuse gliomas are the most common intrinsic primary brain tumors in humans. World Health Organization criteria provide a scheme to segment the diffuse gliomas from low grade (II) through intermediate or anaplastic (grade III) to malignant (grade IV; also known as glioblastoma multiforme [GBM]). Grade II gliomas have a relatively favorable but highly variable prognosis. Patients with grade III lesions have a somewhat shorter but still variable survival. Those with GBM have the shortest lifespan with the least variability. There are several histological subtypes of low- and intermediate-grade gliomas, including the more common astrocytomas (AII, AIII), oligodendrogliomas (OII, OIII), mixed oligoastrocytomas (OAII, OAIII), and ependymomas (EII, EIII).[1]

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Because prognosis is variable and response to therapy unclear, the management of patients who present with grade II–III gliomas is still controversial. The development of markers that reliably predict the behavior of these tumors would be useful both for counseling and for treatment planning of patients in this group.[2]

For some time, it has been recognized that malignant transformation of glial cells is associated with a number of genetic events, including p53 mutations, 1p/19q codeletion/translocation, loss of heterozygosity (LOH) of chromosomes 10 and 17p, as well as amplification of epidermal growth factor receptor (EGFR).[3,4] Recently, mutations in the isocitrate dehydrogenase (IDH) genes 1 and 2 (*IDH1* and *IDH2*) have been described in the majority of grade II–III astrocytomas and oligodendrogliomas, as well as in a minority of GBM.[5–8] The most common IDH mutation occurring in gliomas is CGT to CAT at codon 132 in exon 4 of IDH1, resulting in an amino acid exchange from arginine to histidine (R132H). It has also been shown that patients whose grade III and grade IV gliomas carry IDH mutations have a better overall prognosis than patients whose gliomas are IDH-mutation negative.[9–12] The impact of IDH mutation status (*mIDH*) on the prognosis of patients with grade II astrocytomas is not as clear.

The present study was designed to test whether overall survival (OS) and progression free rate (PFR) of patients with clinically low-grade gliomas can be best predicted by pathological grading alone or a combination of molecular markers.

**Table 1.** Clinical data

| Variable | Total $N = 108$ Patients<br>*n* (% or mean ± STD) | $N = 70$ Patients for Mutivariable Analysis<br>*n* (% or mean ± STD) |
|---|---|---|
| Age at diagnosis, y | | |
| ≤50 | 84 (78) | 55 (79) |
| >50 | 24 (22) | 15 (21) |
| Average tumor diameter | | |
| ≤4 cm | 37 (34) | 31 (44) |
| >4 cm | 71 (66) | 39 (56) |
| KPS | | |
| ≥80 | 12 (11) | 9 (13) |
| <80 | 96 (89) | 61 (87) |
| Extent of resection | | |
| Gross total | 15 (15) | 13 (20) |
| Near total | 13 (13) | 9 (13) |
| Partial | 54 (52) | 40 (60) |
| Biopsy | 21 (20) | 5 (7) |
| Status | | |
| Alive | 51 (47) | 37 (53%) |
| Dead | 57 (53) | 33 (47) |
| Progression status | | |
| No progression | 29 (27) | 19 (27) |
| Progression | 79 (73) | 51 (73) |
| Status: Progression | | |
| Alive–No progression | 28 (26) | 18 (26) |
| Alive–Progression | 23 (21) | 19 (27) |
| Dead–No progression | 1 (1) | 1 (1) |
| Dead–Progression | 56 (52) | 32 (46) |
| Follow-up, mo | | |
| Survival time, live patients Median (range) | 70 (18–144) | 68 (18–144) |
| Progression-free time, mo, no progression cohort, median (range) | 65(18–168) | 65 (18–168) |

## Materials and Methods

### *Study Cohort*

All studies were undertaken with institutional review ethic board approval. The cohort consisted of 130 patients with gliomas cared for at the University Health Network (UHN). A clinical low grade was assigned to these tumors based on MRI lacking significant contrast enhancement or edema. The cohort had previously been used in a multi-institutional study of glioma prognostic grading.[2] The associated clinical data, including radiology, operative reports, Karnofsky performance scale, OS, and PFR were available through UHN. An independent neuropathological review (S.E.C., C.B.K., T.R.K.) was performed on all cases for tumor diagnosis. One hundred eight of the original 130 surgical specimens yielded sufficient tissue for further immunohistochemistry and DNA extraction. Sufficient data were available from 70 of the 108 specimens for multivariable analysis.

### *Tissue Microarray Construction*

A tissue microarray (TMA) was prepared from the 108 specimens to facilitate immunohistochemistry, in situ hybridization, and other histologic assays. For TMA assembly, the slides of each case were evaluated and the areas corresponding to the most cellular and diagnostic regions circled for coring. Construction of the TMA was done using a semi-automated tissue arrayer (Pathology Devices) with a 1.5-mm coring needle. The samples were distributed over 3 blocks. For all 3 blocks, a control hematoxylin and eosin stain was performed and reviewed by the neuropathologists for adequate tumor representation.

### IDH1 *Mutation Immunohistochemistry*

Prior to immunostaining, TMA sections were deparaffinized, rehydrated, and pretreated in citrate buffer, pH 6.0, for 15 min. Immunostaining was undertaken with hybridoma supernatant to *mIDH1*-R132H, developed by 2 of us (D.C. and A.v.D.), for 1 h at room temperature at a 1:3 dilution as described previously.[13] Only cases showing cytoplasmic staining for R132H-*mIDH1* were scored as positive by D.C. and A.v.D., independently of knowledge of any associated clinical data.

### IDH1 *and* IDH2 *Genotyping*

In parallel to building the TMA blocks, an adjacent core was taken for DNA extraction. Paraffin was removed by xylene incubation and centrifugation. DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen). The

**Table 2.** Pathology

| Pathology Type and Grade | Total $N = 180$<br>*n* (%) | $N = 70$ for Multivariable Analysis<br>*n* (%) |
|---|---|---|
| AII | 56 (52) | 34 (49) |
| AIII | 17 (16) | 15 (21) |
| OII/OAII | 19 (18) | 15 (21) |
| OIII/OAIII | 16 (14) | 6 (9) |

quantity of isolated DNA was assessed using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). The quality of the DNA was assessed by PCR for beta-actin. *IDH1* and *IDH2* genotypes were analyzed with the UHN Sequenom MassARRAY platform using primers directed to exon 4 of *IDH1* and *IDH2* in a blinded fashion. Each run included both positive (an oligodendroglioma known to carry an R132H mutation in *IDH1*) as well as negative controls (different samples from temporal lobe resections for epilepsy treatment). Sequencing analyses included codon 132 of *IDH1* as well as codon 172 of *IDH2*.

### *Fluorescent In situ Hybridization*

Fluorescent in situ hybridization (FISH) was used to analyze deletion of chromosomes 1p and 19q, loss of the phosphatase and tensin homolog gene (*PTEN*), and *EGFR* amplification as previously described.[14] In brief, baked slides were deparaffinized with CitriSolv (2 × 10 min; Thermo Fisher Scientific, cat. #22-143-975) and isopropanol (3 × 3 min). After air drying, slides were treated with 0.2N HCl for 20 min and rinsed for 10 min in running water, then for 3 min in distilled water. Antigen retrieval was done in citrate buffer for 20 min in a rice cooker. Slides were allowed to cool for 10 min and then rinsed. Pepsin digestion (2.5 mg/mL; Sigma-Aldrich, cat. # P7000) occurred for 25 min at 37°C, after which slides were again rinsed, then washed (1 × 5 min) in 2× saline-sodium citrate (SSC; Thermo Fisher Scientific, cat # BP1325-4). Air-dried slides were then co-denatured (13 min at 90°C) and hybridized overnight (at 37°C) with appropriate probes. Paired probes for 1p32/1q42 and 19p13/19q13, where the 1p and 19q were labeled red and the 1q and 19p were labeled green, were diluted from stock at 1:25 using denaturation solution – hybridization buffer (Insitus Biotechnologies). Other probes used were CEP7 for *EGFR* and CEP10 for *PTEN* (Vysis). The next day, slides were washed with 50:50 formamide/2× SSC (1 × 10 min, then 1 × 5 min), then 2× SSC (2 × 10 min). Tissues were then counterstained with 4′,6′-diamidino-2-phenylindole in Fluoroguard (Insitus, cat. # F203), coverslipped, and examined with fluorescence microscopy.

### *1p/19q Loss of Heterozygosity*

On selected tumors, 1p/19q analysis had previously been performed at UHN by PCR-based LOH analysis as previously described.[15] These results were included in the overall evaluation of 1p/19q status.

### *Clinical Parameters*

Tumor size was determined from the preoperative radiologic assessment and categorized as >4 or <4 cm average diameter. Performance was determined by KPS preoperatively. Extent of resection was determined from the first postoperative imaging study and categorized as biopsy, partial, near total, or gross total resection. Progression-free rate was defined as the time interval between initial pathological diagnosis and date of radiological progression (unequivocal increase in fluid attenuated inversion recovery/T2 signal abnormality and/or newly detected areas of contrast enhancement on MRI) or the date of the last known MRI with no evidence of disease progression, whichever occurred first. The overwhelming majority of the patients did not receive radiation or chemotherapy

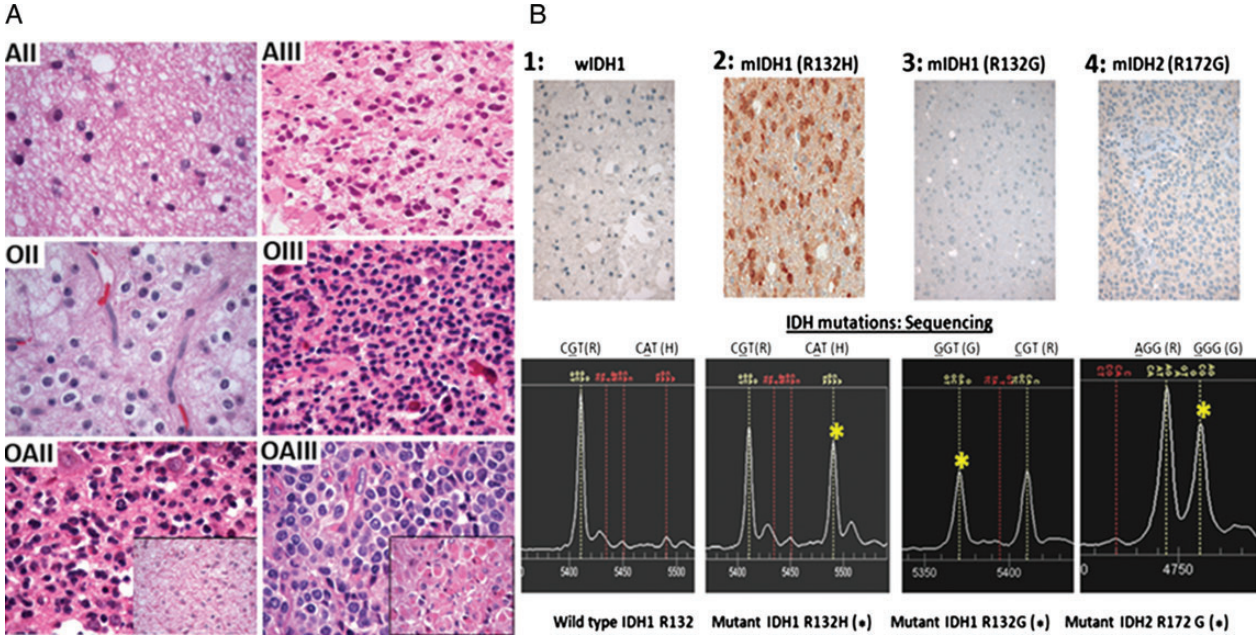


**Fig. 1.** Pathology, immunohistochemistry, and sequence analysis. (A) Montage of hematoxylin/eosin sections (40×) of low-grade gliomas: AII, OII, OAII, AIII, OAIII, and anaplastic oligodendroglioma grade III. Inserts in the OAII and OAIII tumors show regions of astrocytoma in these mixed gliomas. (B) Immunohistochemistry for mutant R132H-*mIDH1*, with monoclonal mutation specific R132H-*mIDH1*antibody (top), and corresponding sequencing histograms (bottom) on: (B1) AII astrocytoma negative for the mutant R132H-*mIDH1* by immunohistochemistry with sequence histogram demonstrating wild type CGT peak only; (B2) AII astrocytoma with cytoplasmic staining detected by the mutant specific monoclonal antibody against R132H-*mIDH1*. Corresponding sequence histogram shows 2 equal peaks comprising wild type peak CGT and mutant CAT in codon 132, indicative of CGT to CAT mutation in one allele and amino acid exchange from arginine to histidine and expression of the mutant R132H-*mIDH1*; (B3) OII oligodendroglioma with equal levels of CGT and mutant GGT in codon 132 as per sequencing data. This results in arginine to glycine exchange and expression of mutant R132G from one allele not detected by the R132H-*mIDH1*antibody; (B4) AIII anaplastic astrocytoma with mutant *IDH2* at R172. Equal amounts of wild type AGG and mutant AAG by sequencing histogram results in arginine to glycine exchange (R172G) by one allele, which is not detected by the R132H-*mIDH1* antibody.

(usually temozolomide), until there was clinical and accompanying radiological evidence of progression. Overall survival was defined as the time interval from initial pathological diagnosis to death. For patients assigned to palliative care, the last documented physician's visit was substituted.

### *Statistical Analysis*

All statistical analysis was conducted using SAS v9.1 software for Windows. Data on categorical variables were provided as percentages. Continuous variables were reported as means ± standard deviations. Median follow-up time was reported for patients who were alive, as well as for patients who had no progression. Survival and PFR estimates were obtained using the Kaplan–Meier method and compared using the log-rank test. $P \leq .05$ was considered significant.

Cox proportional hazards regression was used to test the effect of 3 biomarkers (*mIDH*, 1p/19q deletion, and *PTEN* deletion) either alone or together in the model as well as when the model was adjusted for clinical and demographic factors. These were expressed as hazard ratios with 95% confidence intervals. All possible interactions between the 3 markers were tested one by one in the Cox regression model. For ease of interpretation, the interaction term in the model was replaced by a 4-level covariate as a combination of 1p/19q status and *PTEN* status. Predicted values obtained from the Cox regression model were used to categorize the patients into low- and high-risk groups. The groups were the same regardless of whether they were based on the predicted values for survival or the predicted values for PFR.

## Results

### *Clinical Data*

From the total of 108 cases, 78% of patients were <50 years old at diagnosis, 34% had tumors <4 cm in average diameter, and 89% had a KPS of <80 preoperatively (Table 1). Resection data were available for 103 of the 108 patients. Gross total resection was achieved in 15% of patients, near total resection in 13%, partial resection in 52%, and biopsy only in 20%. Forty-seven percent of the patients were alive at the time the data for this study were compiled. Of the total, 4% were lost to follow-up. Forty-seven percent of the patients still in follow-up were alive at the time the data for this study were compiled. Of the total, 27% had shown no progression, 26% were alive without evidence of progression, 21% were alive with progression, 1% were dead without progression, and 52% were dead with progression. Median follow-up time for those still alive was 70 months (range, 18–144) and median follow-up time for patients with no progression was 65 months (range, 18–168).

The patients in the database who were lost to follow-up were included in the analyses as being censored at last follow-up. All of these were included in the univariable analysis.

One of the patients had missing data on 1p/19q, and that patient was excluded from multivariable analysis. The other patients who were lost to follow-up were included in the multivariable analysis.

From the total 108 cases, clinical, pathological, and molecular data were complete on 70, allowing multivariable analysis on that cohort. The data for the cases in the multivariable analysis showed a similar percentage distribution to the total (Table 1).

### *Pathology*

Of the 108 cases analyzed in this study, 70% were grade II and 30% were grade III gliomas (Table 2). The grade II gliomas included 52% AIIs and 18% OIIs or OAIIs. The grade III gliomas included

**Table 3.** Summary of pathological diagnoses and molecular data

| Pathological Diagnosis | Number of Cases | *mIDH1* IHC | *mIDH* Sequenom | *mIDH* Total | 1p/19q Deleted | *PTEN* Deleted | *EGFR* Amplified |
|---|---|---|---|---|---|---|---|
| AII | 56 | 39 | 38<br>*IDH1: 32 R132H*<br>*3 R132C*<br>*3 R132G* | 46 | 10 | 7 | 0 |
| AIII | 17 | 13 | 14<br>*IDH1: 11 R132H*<br>*1 R132S*<br>*IDH2: 1R172 G*<br>*1R1732K* | 15 | 3 | 3 | 0 |
| OII | 10 | 6 | 8<br>*IDH1: 6 R132H*<br>*1 R132C*<br>*1 R132G* | 9 | 8 | 2 | 1 |
| OAII | 9 | 8 | 9<br>*IDH1:8 R132H*<br>*IDH2: 1 R172K* | 9 | 4 | 5 | 0 |
| OIII | 10 | 9 | 8<br>*IDH1: 8 R132H* | 9 | 7 | 1 | 0 |
| OAIII | 6 | 5 | 6<br>*IDH1:5 R132H*<br>*IDH2: 1 R172G* | 5 | 2 | 1 | 0 |
| Total | 108 | 80 | 83 | 93 | 34 | 19 | 1 |

16% AIIIs and 14% OIIIs or OAIIIs. The proportions of the 70 cases that qualified for multivariable analysis were similar (70% grade II, 30% grade III, 49% AII, 21% AIII, 21% OII and OAII, 9% OIII and OAIII). Representative examples are illustrated in Fig. 1A.

### *Molecular Data*

#### IDH *mutational analysis*

Immunohistochemistry of the TMAs yielded interpretable analyses in 107/108 (99%) samples (Table 3). The only failure was due to inadequate amounts of tissue in the TMA. None were due to difficulty interpreting the immunohistochemical staining pattern. Immunohistochemistry detected R132H mutations in 80/107 (75%) interpretable samples (AII: 39/55 [71%], AIII: 13/17 [76%], OII/OAII: 14/19 [74%], OIII/OAIII: 14/16 [88%]). Satisfactory DNA for *IDH1* and *IDH2* genotyping was obtained in 106/108 (98%) samples. Representative examples are shown in Fig. 1B. By sequencing, *mIDH* was detected in 83/108 (76%) gliomas, with *mIDH1*-R132H accounting for 70 (84%) of these mutations. In addition, there were 4 (5%) *mIDH1*-R132C, 4 (5%) *mIDH1*-R132G, 1 (1%) *mIDH1*-R132S, 2 (2%) *mIDH2*-R172G, and 2 (2%) *mIDH2*-R172K (AII: 32/57 [56%] R132H, 3/57 [5%] R172C, 3/57 [5%] R172G; AIII: 11/17 [65%] R132H, 1/17 [6%] R132S, 1/17 [6%] R172G, 1/17 [6%] R172K; OII/OAII: 14/19 [74%] R312H, 1/19 [5%] R132C, 1/19 [5%] R132G, 1/19 [5%] R172G/K; OIII/OAIII: 13/16 [81%] R132H, 1/16 [6%] R172G). Examples of Sequenom data are in Fig. 1C. Combining the 2 methods, a total of 93 cases with *IDH* mutations was detected (86% of total cohort), including 80 R132H, 4 R132C, 4 R132G, 1 R132S, 2 R172G, and 2 R172K. There was an 11% rate of disagreement between assays: 9 R132H mutations detected by immunohistochemistry were not detected by genotyping, 2 R132H mutations were not detected by immunohistochemistry but were detected by genotyping, and 1 R132H mutation detected by immunohistochemistry was registered as R132S by genotyping.

**Table 4.** Cases for univariable and multivariable analysis

| | Univariable Analysis Cases, *n* (%) | Multivariable Analysis Cases, *n* (%) |
|---|---|---|
| *IDH* | (108) | (70) |
| Mutant | 93 (86) | 64 (91) |
| Wild type | 15 (14) | 6 (9) |
| 1p/19q | (83) | (70) |
| Deleted | 34 (40) | 27 (39) |
| Not deleted | 50 (60) | 43 (61) |
| *PTEN* | (79) | (70) |
| Deleted | 19 (24) | 16 (23) |
| Not deleted | 59 (76) | 54 (77) |

### *Analysis of 1p/19q deletion*

Analysis of 1p/19q deletion was completed in 83 of the cases, 43 of which were analyzed by FISH alone, 18 by LOH, and 22 by both methods. Thirty-four of the 83 (41%) showed 1p/19q codeletion: 10/40 (25%) AII, 8/9 (89%) OII, 4/8 (50%) OAII, 3/15 (20%) AIII, 7/7 (100%) OIII, and 2/4 (50%) OAIII.

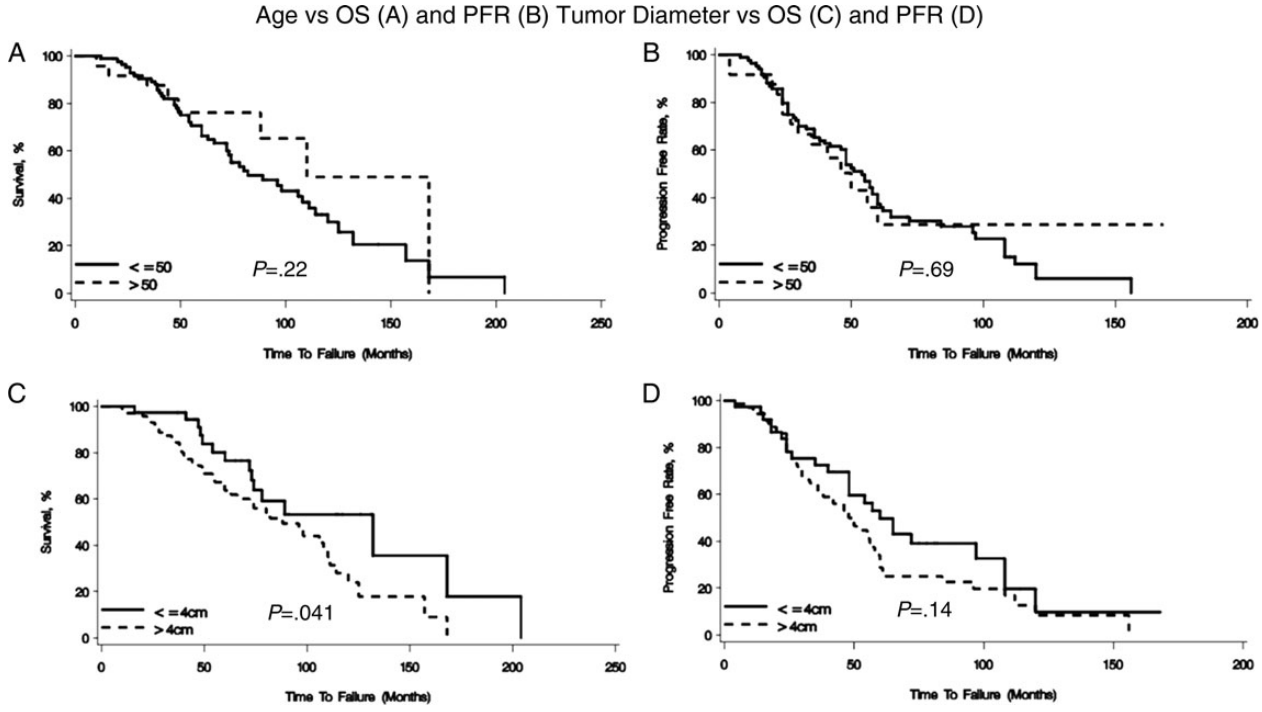


**Fig. 2.** Survival as a function of age and tumor diameter. Patient age at time of diagnosis is not associated with either OS (A) or PFR (B). Average tumor diameter $\leq$4 cm is significantly associated with longer OS (C) but not associated with PFR (D).

### PTEN *deletion*

*PTEN* FISH was interpretable in 79 of the cases—19 (24%) showed *PTEN* deletion (AII: 7/40 [18%], OII/OAII: 7/16 [44%], AIII: 3/15 [20%], OIII/OAIII: 2/8 [25%]).

### EGFR *amplification*

*EGFR* FISH was interpretable in 83 cases. Only 1 case (OII) demonstrated amplification.

## *Comparison of All Cases and Cases for Multivariable Analysis*

There were similar percentages of *IDH* mutant (87 vs 91), 1p/19q deleted (40 vs 39), and *PTEN* deleted (24 vs 23) tumors in the total set of 108 cases and the 70 cases amenable to multivariable analysis (Table 4).

## *Outcome Analysis*

### *Overall survival and progression-free rate*

#### ***Univariate Analysis***

*Clinical data* There was no significant difference in either OS or PFR for age >50 or <50 years ($P = .2$ and $P = .69$, respectively; Fig. 2A and B, Table 5). Tumor diameter <4 cm was associated with significantly longer OS ($P = .041$) but was not associated with longer PFR ($P = .14$; Fig. 2C and D, Table 5). There was no significant association between KPS >80 or <80 and OS or PFR (*P*s = .68 and .55; Fig. 3A and B, Table 5). Extent of resection was also not associated with OS or PFR ($P = .23$ and .72; Fig. 3C and D, Table 5).

*Pathological diagnosis* For the entire cohort, there was no significant difference in OS or PFR between the pathological diagnostic groups ($P = .29$ and $P = .70$; Fig. 4A and B, Table 5).

IDH *mutation* Combining the results of immunohistochemistry and genotyping, the *mIDH* group showed significantly longer OS ($P < .001$; Fig. 4C, Table 5) and a longer PFR ($P < .001$; Fig. 4D, Table 5).

*Deletion of 1p/19q* For the entire cohort, there was a significant difference in OS and a near significant difference in PFR between cases based on 1p/19q deletion alone ($P = .01$, $P = .063$; Fig. 5A and B, Table 5).

PTEN *deletion* There was no significant difference in survival between cases based on *PTEN* deletion alone (OS $P = .31$, PFR $P = .69$; Fig. 5C and D, Table 5).

**Table 5.** Summary of survival analysis

| OS | | | PFR | |
|---|---|---|---|---|
| Variable | 3-y KM Estimate ± SEE (%) | Log Rank, *P* | 3-y KM Estimate ± SEE (%) | Log Rank, *P* |
| All patients ($n = 108$) | 88.8 ± 3 | NA | 67.3 ± 4.5 | NA |
| Age | | | | |
| <=50 | 89.2 ± 3.4 | .22 | 68.8 ± 5.1 | .69 |
| >50 | 87.5 ± 6.7 | | 62.2 ± 9.9 | |
| Tumor diameter | | | | |
| ≤4 cm | 97.3 ± 2.7 | .041 | 72.5 ± 7.4 | .14 |
| >4 cm | 84.5 ± 4.3 | | 64.8 ± 5.7 | |
| KPS | | | | |
| ≥80 | 87.4 ± 3.4 | .68 | 65.3 ± 4.9 | .55 |
| <80 | 100 | | 66.7 ± 13.6 | |
| Extent of resection ($n = 105$) | | | | |
| Gross total | 100 | .23 | 80 ± 10.3 | .72 |
| Near total | 100 | | 84. ± 10.01 | |
| Partial | 88.7 ± 4.3 | | 66.2 ± 6.5 | |
| Biopsy | 71.4 ± 9.9 | | 47.6 ± 10.9 | |
| Pathology | | | | |
| AII | 87.3 ± 4.5 | .29 | 67.5 ± 6.3 | .70 |
| AIII | 94.1 ± 5.7 | | 52.9 ± 12 | |
| OII | 94.7 ± 5.1 | | 78.9 ± 9.3 | |
| OIII | 81.3 ± 9.8 | | 62.5 ± 12.1 | |
| *IDH* | | | | |
| Mutant | 92.4 ± 3 | <.001 | 73.9 ± 4.5 | <.001 |
| Wild type | 66.7 ± 12 | | 20 ± 10 | |
| 1p/19q ($n = 84$) | | | | |
| Deleted | 97.0 ± 2.9 | .01 | 82.3 ± 6.6 | .063 |
| Not deleted | 89.9 ± 4.3 | | 61.4 ± 6.9 | |
| *PTEN* ($n = 79$) | | | | |
| Deleted | 84.2 ± 8.3 | .30 | 68.4 ± 10.6 | .68 |
| Not deleted | 94.9 ± 2.9 | | 67.8 ± 6.1 | |

Abbreviation: KM, Kaplan–Meier.

## *Multivariable Analysis*

### *Overall survival*

The only significant variables for OS were *mIDH*, *PTEN*, and 1p/19q ($P = .003$, $P = .02$, $P = .005$). None of the interactions were significant. The hazard ratios and 95% confidence intervals associated with significant predictors are reported in Table 6A. Based on this model, the patients were categorized into low- and high-risk groups. Figure 6A shows the survival probability of these groups.

### *Progression-free rate*

In the multivariable model for PFR, *mIDH* ($P < .001$) and the interaction between 1p/19q and *PTEN* ($P < .01$) were significant. For an easier interpretation, we combined 1p/19q and *PTEN* into a 4-level covariate (Table 6B). Based on the predicted values, risk scores were calculated for each patient, and the cohort was divided into those at low and high risk. Figure 6B shows the PFR for the 2 groups.

Interestingly, the low- and high-risk groups derived from the OS data were the same as those derived from the PFR data (Table 6C). If we define as risk factors *mIDH* wild type, *PTEN* deleted, and 1p/19q not deleted, then the high-risk group was defined as the patients with 2 or 3 risk factors and the low-risk as the patients with 1 or no risk factor.

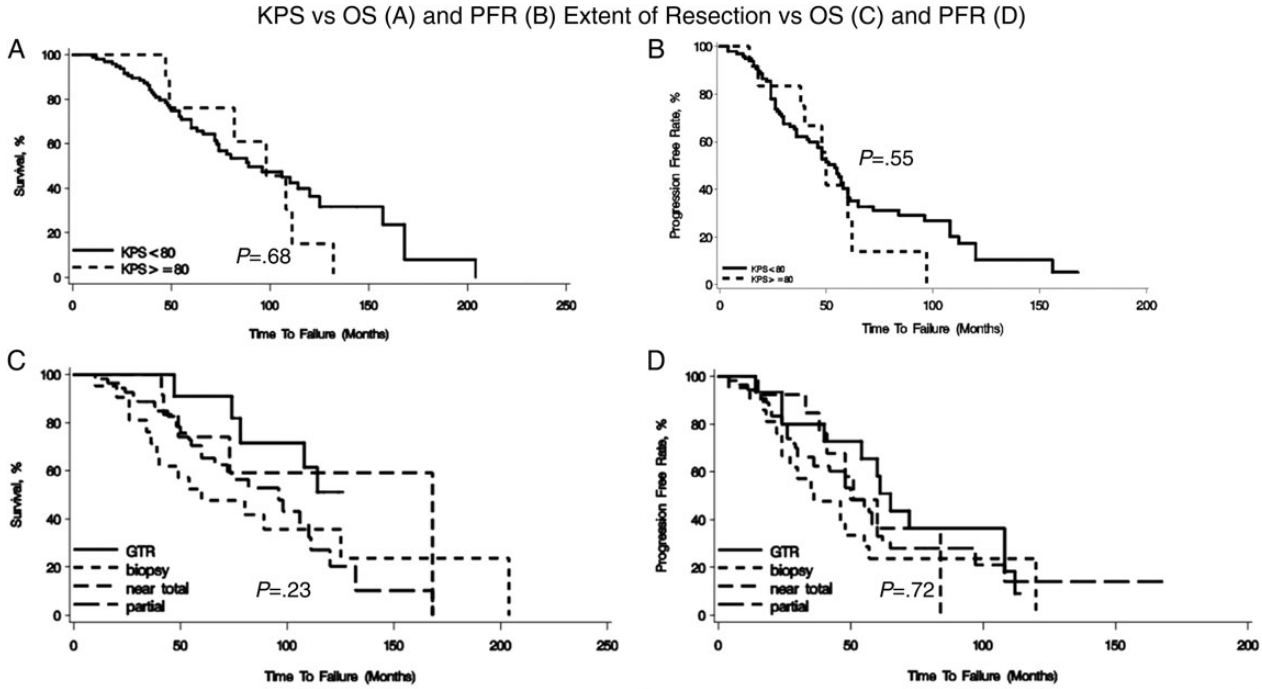


**Fig. 3.** Survival as a function of performance status and extent of resection. Neither preoperative KPS (A and B) nor extent of resection (C and D) were predictive of OS or PFR.

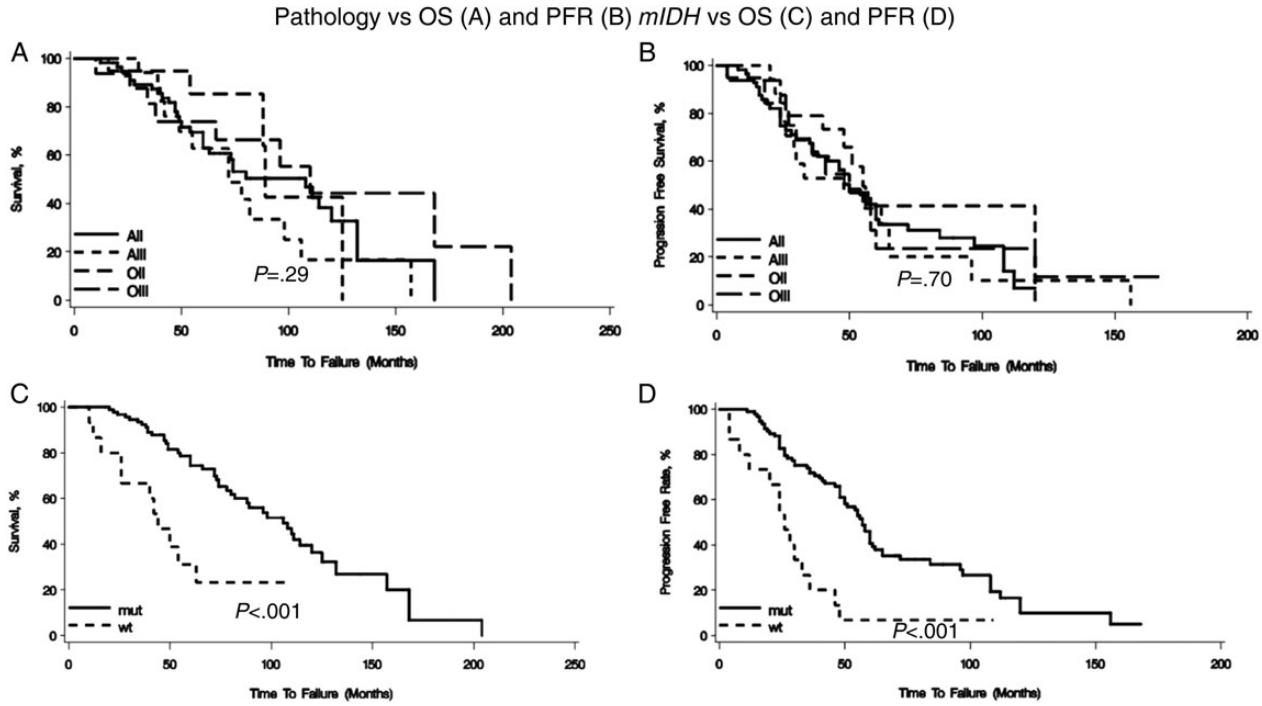


**Fig. 4.** Survival as a function of pathology and *mIDH*. Tumor pathology (both type and grade) failed to predict either OS (A) or PFR (B). When *mIDH* is defined as the combination of tumors that are R132H-*mIDH1* by immunohistochemistry in addition to those that are *mIDH* by sequencing, the group of *mIDH* tumors shows significantly longer OS (C) and PFR (D) than the group of wild-type *IDH* tumors.

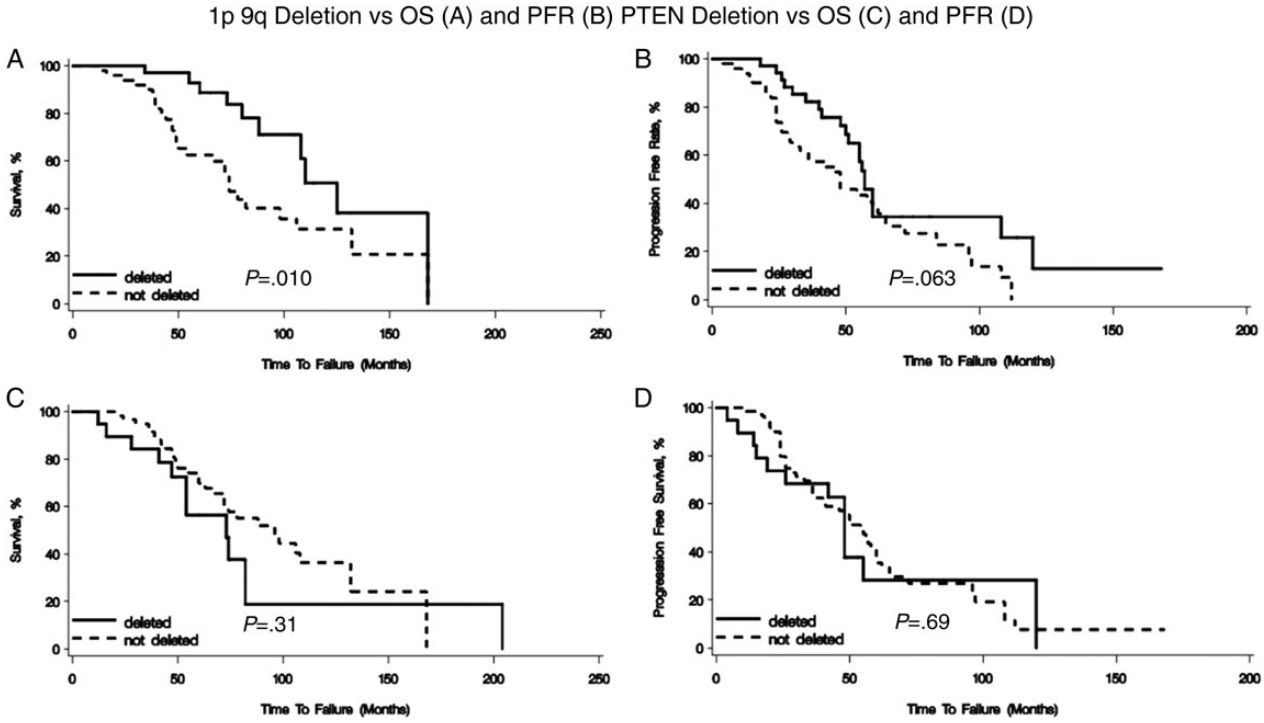


**Fig. 5.** Survival as a function of 1p/19q deletion and *PTEN* deletion. There was a significant difference in OS (A) and a near significant difference in PFR (B) between cases based on 1p/19q deletion. *PTEN* deletion alone is not predictive of OS or PFR.

**Table 6.** Multivariable analysis

| Variable | Hazard Ratio | 95% Confidence Interval | *P* | |
|---|---|---|---|---|
| A: Model for OS | | | | |
| *mIDH* | | | | |
| Wild type vs mutant | 4.9 | 1.7–14.5 | .003 | |
| *PTEN* FISH | | | | |
| Deleted vs not deleted | 2.9 | 1.2–6.8 | .02 | |
| 1p/19q | | | | |
| not deleted vs deleted | 4.1 | 1.6–11.1 | .005 | |
| B: Model for PFR | | | | |
| *mIDH* | 6.9 | 2.6–18.6 | .0001 | |
| Combination variable of *PTEN* and 1p/19q | | | .01[a] | |
| *PTEN* deleted & 1p/19q not deleted | | | | |
| vs *PTEN* not deleted & 1p/19q not deleted | 4.7 | 1.9–11.6 | .0008 | |
| *PTEN* not deleted & 1p/19q deleted | | | | |
| vs *PTEN* not deleted & 1p/19q not deleted | 0.89 | 0.45–1.8 | .74 | |
| *PTEN* deleted &1p/19q deleted | | | | |
| vs *PTEN* not deleted & 1p/19q not deleted | 0.56 | 0.17–1.9 | .34 | |
| Risk group | *mIDH* | *PTEN* FISH | 1p/19q | Number of patients |
| C: Risk grouping for OS/PFR | | | | |
| Low risk ($n = 56/59$[b]) | Mutant | Not deleted | Deleted | 17[b] |
| | Mutant | Not deleted | Not deleted | 30[b] |
| | Mutant | Deleted | Deleted | 9 |
| High risk ($n = 11$) | Wild type | Deleted | Not deleted | 2 |
| | Wild type | Not deleted | Not deleted | 4 |
| | Mutant | Deleted | Not deleted | 5 |

[a]This is the overall *P* value for the 4-level covariate (*PTEN* and 1p/19q).
[b]$n = 59$ for the low-risk group in the PFR model with number of patients being 18 and 32, respectively, in the first 2 rows of the table.

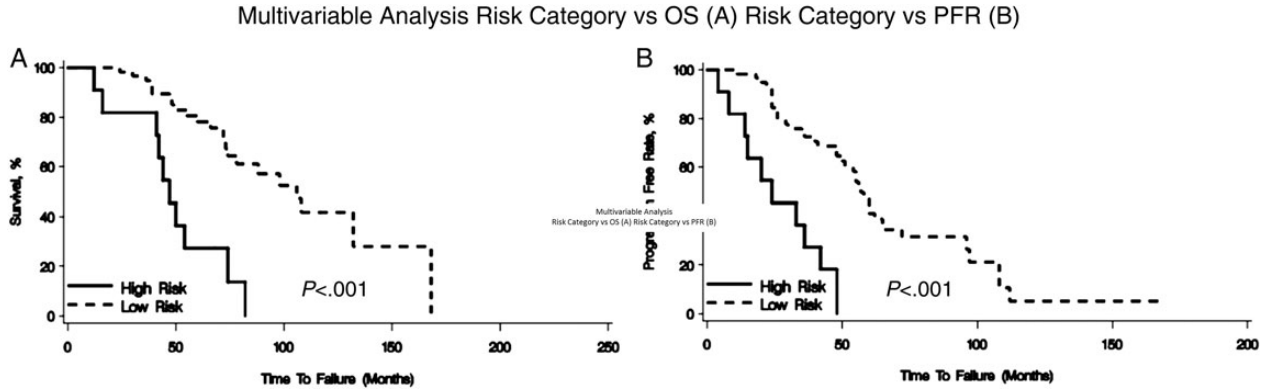


**Fig. 6.** Survival as a function of multivariate analysis. Cox regression analysis was applied to both OS and PFR statistics. For OS, high- and low-risk groups were constructed from *IDH*, *PTEN*, and 1p/19q data. The Kaplan–Meier curve for those groups is shown in (A). The Kaplan–Meier curve of PFR for high- and low-risk groups is shown in (B).

## Discussion

Currently, clinical and radiological parameters are used in many centers to guide clinicians in their management choices for patients with nonenhancing, presumptive grade II or III hemispheric gliomas. These choices include observation alone, surgery for biopsy or resection, radiation, and chemotherapy. Certainly, an initial surgical approach to these tumors adds, among other things, a pathological diagnosis. However, it remains unclear whether pathological diagnosis alone or in conjunction with mutational analysis of the tumor provides additional prognostic or therapeutic information for this subset of patients. The current study was designed to address that issue through a retrospective analysis of a cohort of nonenhancing hemispheric gliomas managed at a single institution and previously used as part of a multi-institutional clinical-radiological prognostic study.[2]

Stratification of the cases by multiple clinical variables showed that only tumor size had a significant effect on OS in univariate analysis. None of these variables affected PFR. Tumor pathology (both type and grade) did not predict OS or PFR. Of the molecular studies performed, *IDH* mutational analysis was the only study that by itself produced both OS and PFR groups that were significantly different. Analysis of 1p/19q deletion was a significant predictor of OS ($P = .01$) and a near significant predictor of PFR ($P = .06$). Multivariable analysis allowed the construction of low-risk/high-risk groups that had greater predictive value than one factor alone. The groups were based on the predictive value from the Cox regression and were the same for OS and PFR. Interestingly, pathological diagnosis was not significant in the multivariable analysis.

The failure of pathology to predict outcome in this study in all probability reflects selection bias. Rather than representing a true cross section of grades II and III hemispheric gliomas from our institution, this cohort was selected solely from cases that lacked MR evidence of tumor enhancement. As such, this excludes a large number of tumors, and in particular those with grade III histology.

Our finding that immunohistochemistry and genetic analysis together identified mutations of either *IDH1* or *IDH2* in 85% of clinical low-grade gliomas is similar to previously reported findings based on direct sequencing[8,9,16,17] but somewhat higher than other reports of 70% mutations using sequencing,[11,12] 72% using fluorescence melting curve analysis,[18] and 56%–70% using immunohistochemistry.[19,20] Some of these differences are probably methodological. Notably, our immunohistochemistry detected *IDH1*-R132H mutations in 73% of the total tumors. Using genetic analysis, *mIDH1*-R132H was detected in 57%, R132C in 4%, R132G in 5%, R132S in 1%, R172G in 2%, and R172K in 2%. The 85% total was reached by combining datasets. In addition, it is possible that our cohort, selected for nonenhancing hemispheric tumors, contributed to the percentage and types of IDH mutations.

That *mIDH* confers a better prognosis for both PFR and OS mirrors previous reports.[9,10,11,12,16,21,22,23,24] As well, the finding that the combination of *mIDH*-1p/19q deletion confers good prognosis is consistent with previous reports that *mIDH* is associated with 1p/19q deletion[16,25,26] and that the combination prognosticates for good outcome in grade III oligodendroglial tumors.[11,12,23]

The novel finding of this study is that analysis for *PTEN* deletion in combination with *mIDH* and 1p/19q analyses helps to better define prognostic groups of patients. Despite small numbers, this finding is significant. This is consistent with a previous report[12] that loss of chromosome 10 is among the factors negatively associated with *mIDH* and 1p/19q deletion in anaplastic oligodendrogliomas. Similarly, Gorovets et al[21] have identified wild-type *IDH* and *PTEN* loss as markers of poor prognosis in low-grade gliomas. Although they found that this poor prognosis group also demonstrated *EGFR* amplification, that feature was not replicated in our data.

These data and those published by others suggest that together with World Health Organization grading of gliomas, the presence or absence of *IDH* mutations, 1p/19q loss, and *PTEN* deletion in combination may better define prognosis. Given that the number of patients in this study is small and that this represents a reanalysis of retrospective data, larger prospective cohorts will need to be studied. Nonetheless, this opens up the possibility that testing adult grades II and III hemispheric gliomas for *mIDH*, 1p/19q deletion, and *PTEN* loss may lead to better prognostication and potentially more specific therapies for these patients.

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*Conflict of interest statement*. None declared.

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