Imaging, Diagnosis, Prognosis

Clinical Cancer Research

IDH1 and *IDH2* Mutations Are Prognostic but not Predictive for Outcome in Anaplastic Oligodendroglial Tumors: A Report of the European Organization for Research and Treatment of Cancer Brain Tumor Group

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Abstract

Purpose: Recent studies have shown the prognostic significance of *IDH1* mutations in glioma. It is yet unclear if *IDH1* mutations are predictive for outcome to chemotherapy. We determined the effect of *IDH1* mutations on progression-free survival and overall survival (OS), and its correlation with other clinical and molecular features in the prospective randomized European Organization for Research and Treatment of Cancer study 26951 on adjuvant procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-L-nitrosourea, and vincristine (PCV) in anaplastic oligodendroglioma.

Experimental Design: *IDH1* and *IDH2* alterations of the mutational hotspot codons R132 and R172 were assessed by the bidirectional cycle sequencing of PCR-amplified fragments. *MGMT* promoter methylation was assessed using methylation-specific multiplex ligation–dependant probe amplification based on methylation-sensitive restriction analysis. Loss of chromosomes 1p, 19q, 10, and 10q and the gain of 7 and the *EGFR* gene were assessed with fluorescence *in situ* hybridization.

Results: From 159 patients, sufficient material was available for *IDH1* analysis. In 151 and 118 of these patients, respectively, the 1p/19q status and the *MGMT* promoter methylation status were known. In 73 cases (46%), an *IDH1* mutation was found and only one *IDH2* mutation was identified. The presence of *IDH1* mutations correlated with 1p/19q codeletion and *MGMT* promoter methylation, and inversely correlated with loss of chromosome 10, *EGFR* amplification, polysomy of chromosome 7, and the presence of necrosis. *IDH1* mutations were found to be prognostic in the radiotherapy- and the radiotherapy/PCV-treated patients, for both progression-free survival and OS. With Cox proportional hazard modeling for OS with stepwise selection, *IDH1* mutations and 1p/19q codeletion but not MGMT promoter methylation were independent prognostic factors.

Conclusion: In this homogeneously treated group of anaplastic oligodendroglioma patients, the presence of *IDH1* mutations was found to carry a very strong prognostic significance for OS but without evidence of a predictive significance for outcome to PCV chemotherapy. *IDH1* mutations were strongly associated with 1p/19q codeletion and *MGMT* promoter methylation. *Clin Cancer Res; 16(5); 1597–604. ©2010 AACR.*

Recent sequencing of the genome of glioblastoma identified novel mutations in the *isocitrate dehydrogenase1* gene (*IDH1*; refs. 1–5). These mutations occur in the highly conserved residue R132 in the active site of IDH1 and mostly concern Arg→His amino acid substitutions (codon CGT→CAT change). Less frequently, CGT→TGT (substitution of Arg→Cys), CGT→GGT (Arg→Gly), and CGT→AGT (Arg→Ser) mutations are

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Translational Relevance

This study within a prospective randomized study allows a better understanding of the prognostic and predictive significance of IDH1 and IDH2 mutations in relation to other frequent genomic alterations in grade III oligodendroglial tumors, in particular, the presence or absence of 1p/19q codeletions and MGMT promoter methylation. The study shows that in multivariate analysis, both the presence of IDH1 mutations and of 1p/19q codeletions have independent favorable prognostic significance for overall survival. The major prognostic significance of IDH1 mutations need to be considered in the design of future studies on grade III oligodendroglial tumors. Randomized studies in this disease and in other gliomas should consider *IDH1* mutations as a stratification factor. However, the presence or absence of *IDH1* mutations does not allow a more tailored treatment. To assess the prognosis in individual grade III glioma patients, assessment of both 1p/19q codeletion and IDH1 mutations should be considered.

present. *IDH1* R132 mutations are present in 55% to 80% of grade II and III oligodendroglioma and astrocytoma but rarely in primary glioblastoma nor in a variety of other primary brain tumors including pilocytic astrocytoma. Although TP53 mutations and 1p/19q loss are mutually exclusive in glioma, *IDH1* mutations are present in TP53 mutated and in 1p/19q codeleted tumors. This, in combination with the findings in patients with multiple biopsies in which there were no cases that acquired *IDH1* mutations after the acquisition of either *TP53* mutations or combined 1p19q loss suggests that the occurrence of *IDH1* mutations is an early event in the tumorigenesis of diffuse glioma (6). Although the initial observations did not identify this mutation in other tumors, sequencing of the acute myeloid leukemia genome recently identified IDH1 mutations in 15 of 187 acute myelogenous leukemia patients, predominantly in patients with normal cytogenetic status (7–9). Less frequently, glial tumors show mutations in the corresponding codon 172 of the *IDH2* gene, which codes for a mitochondrial enzyme with a similar function (3). Although initial studies focused on the decreased enzymatic conversion of isocitrate to α-ketoglutarate in the presence of an *IDH1* mutation, a recent study showed that the mutated IDH1 enzyme increased the NADPH-dependent conversion of α-ketoglutatrate to R(−)-2-hydroxyglutarate (10). Of note, the presence of the wild-type IDH may actually provide the substrates required for this conversion. Inborn errors of R(−)-2-hydroxyglutarate metabolism are related to the development of brain tumors (11, 12). These data suggest that by an altered substrate affinity with a gain of function, *IDH1* mutations may indeed act as an oncogene, and presents a potentially drugable target.

Retrospective studies have found a major favorable prognostic effect of the presence of *IDH1* and *IDH2* mutations on survival of grade II and grade III glial tumors. However, because patients in these studies patients were managed heterogeneously, it is still unknown if *IDH1* and *IDH2* mutations are predictive markers for outcome to treatment or prognostic markers. Moreover, these studies are on retrospective data sets. The prospective randomized phase III European Organization for Research and Treatment of Cancer (EORTC) study 26951 investigated the benefit of six cycles of adjuvant procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-L-nitrosourea, and vincristine (PCV) chemotherapy in anaplastic oligodendroglial tumors (13). The study showed that adjuvant PCV chemotherapy improves progression-free survival (PFS) but not overall survival (OS), most likely due to the effects of crossover at the time of progression. Further details including molecular analysis of the study have been published elsewhere (14). We used this study to investigate if *IDH* mutations predict outcome to chemotherapy and to correlate *IDH* mutation status to other known markers related to outcome.

Materials and Methods

Patients were eligible for EORTC study 26951 if they had been diagnosed by the local pathologist with anaplastic oligodendroglioma or anaplastic oligoastrocytoma (AOA) with at least 25% oligodendroglial elements according to the 1993 edition of the WHO classification of brain tumors (15), had at least three of five anaplastic characteristics (high cellularity, mitoses, nuclear abnormalities, endothelial proliferation, or necrosis), were between ages 16 and 70 y, had an Eastern Cooperative Oncology Group performance status of 0 to 2, and had not undergone prior chemotherapy or radiotherapy to the skull. Patients were randomized between treatment with radiotherapy alone versus radiotherapy followed by six cycles of adjuvant PCV chemotherapy. The clinical and molecular details of this study have been published elsewhere; for eligibility, the study required written informed consent (13, 14). Patients were included on the local diagnosis, and central pathology review was part of the study. For this study, the WHO criteria 2007 were used (14). Those criteria do no longer consider necrosis consistent with the diagnosis anaplastic oligoastrocytoma; for this analysis, these tumors are considered together with glioblastoma multiforme (GBM).

All molecular studies were done using selected areas enriched for a high tumor cell percentage. DNA was extracted from formalin-fixed, paraffin-embedded tissues as previously described (16). Fluorescent *in situ* hybridization was used to assess copy number aberrations of chromosome 1p, 19q, 7, 10, and 10q, and the *EGFR* gene as described elsewhere (13, 14). *MGMT* promoter methylation was assessed with methylation-specific multiplex ligation-dependent probe amplification, as previously described (17).

Table 1. Baseline characteristics of the 159 patients with IDH1 assessment

Baseline characteristics	*IDH1*		
	Not mutated (*n* = 86) *N* (%)	Mutated (*n* = 73) *N* (%)	Total (*n* = 159) *N* (%)
Age			
<50	38 (46%)	44 (54%)	82
≥50	48 (62%)	29 (38%)	77
Previous resection for low grade			
No	81 (59%)	57 (41%)	138
Yes	4 (21%)	15 (79%)	19
Missing	1	1	2
Central diagnosis			
AOD with or without necrosis/AOA without necrosis	47 (51%)	44 (48%)	91
GBM/AOA with necrosis	32 (69%)	15 (31%)	47
LGG	5 (31%)	11 (69%)	16
Other	2	2	34
Missing	0	1	1
Necrosis			
No	19 (33%)	39 (67%)	58
Yes	67 (67%)	33 (33%)	100
Missing	0	1	1
Mitoses			
No	11 (37%)	19 (63%)	30
Yes	75 (59%)	53 (41%)	128
Missing	0	1	1
Frontal involvement?			
Yes	34 (45%)	42 (55%)	76
No	52 (62%)	31 (37%)	83
EGFR amplification			
No	46 (43%)	61 (57%)	107
Yes	35 (88%)	5 (13%)	40
Missing	5	7	12
Trisomy 7			
No	41 (44%)	53 (57%)	94
Yes	35 (76%)	11 (24%)	46
Missing	10	9	19
10 loss			
No	51 (45%)	62 (55%)	113
Yes	28 (85%)	5 (15%)	33
Missing	7	6	13
10 or 10q loss			
No	39 (41%)	55 (59%)	94
Yes	40 (77%)	12 (23%)	52
Missing	7	6	13
1p/19q loss			
No	75 (65%)	41 (35%)	116
Yes	5 (14%)	30 (86%)	35
Missing	6	2	8
MGMT			
Unmethylated	24 (89%)	3 (11%)	27
Methylated	35 (38%)	56 (62%)	91
Missing	27	14	41

Abbreviation: AOD, anaplastic oligodendroglioma.

For statistical analysis, results on *IDH1* and *IDH2* mutations were taken together and correlated to clinical characteristics [age, performance status, involved lobe (frontal versus other), molecular features (polysomy chromosome 7, *EGFR* amplification, loss of chromosome 1p/19q, loss of chromosome 10, loss of chromosome 10q, and *MGMT* promoter methylation), histologic features, diagnosis (pure versus mixed), and presence or absence of necrosis and endothelial proliferation] and to PFS and OS in treatment groups. PFS and OS were measured from the day of randomization. Patients provided written informed consent according to national and local regulations.

Assessment of IDH1 and IDH2 mutations. *IDH1* and *IDH2* alterations of the mutational hotspot codons R132 and R172, respectively, were assessed by bidirectional cycle sequencing of PCR-amplified fragments. Primers used were IDH1-forward 5′-CTCCTGATGAGAAGAGGGTTG-3′ and IDH1-reverse 5′-TGGAAATTTCTGGGCCATG-3′, and IDH2-forward 5′-TGGAACTATCCGGAACATCC-3′ and IDH2-reverse 5′-AGTCTGTGGCCTTGTACTGC-3′, respectively.

Statistical analysis. The Kaplan-Meier technique was used to estimate PFS and OS. The prognostic significance of the *IDH1* and *IDH2* mutations for PFS and OS were first univariately analyzed. For multivariate analysis, the major prognostic clinical variables used were as follows: type of surgery (resection or biopsy), WHO performance status (0, 1, 2), age (<50, ≥50), location (frontal versus nonfrontal), the central histology review diagnosis (AOD or AOA), endothelial abnormalities, necrosis, and the molecular factors combined (1p/19q loss, $EGFR^{amp}$, $CHR7^{poly}$, $CHR10^{loss}$, $CHR10q^{loss}$, and *MGMT*) promoter methylation. For the *MGMT* MLPA assay, the cutoff of 0.25 was used to distinguish between *MGMT* promoter–methylated and *MGMT* promoter–unmethylated tumors (17, 18). Association between factors except for the performance status was assessed by the Spearman correlation coefficient; the Fisher's exact test was used for inference. Correlations superior or equal to 0.2 (fair) were reported. For performance status (scored 0,1, and 2), the Wilcoxon rank-sum test was used. Survival analyses were done with the log-rank test and the Cox regression analysis with and without forward stepwise selection (5% significance). Peto's technique was used for interaction tests. Internal validation was done by bootstrap resampling technique (5% significance) to assess the generalizability of the models. Factors with a probability of inclusion in regression models of >60% based on 1,000 bootstrap samples were considered confirmed as independent prognostic factor. Factors with a probability of inclusion between 51% and 60% were retained in the final model but were not confirmed. Logistic regression with stepwise selection was used for multivariate analysis of the relationship between IDH1 and other factors (5% significance). These analyses were purely exploratory and no adjustment for multiplicity was done.

Results

A total of 368 patients had been entered into the study. From 159 patients, enough material was present to assess the mutational status of *IDH1* and *IDH2*. In 151 and 118 of these patients, respectively, the 1p/19q status and the *MGMT* promoter methylation status were known. The data from 29% of these patients have been presented previously as part of a single-center study (5). PFS and

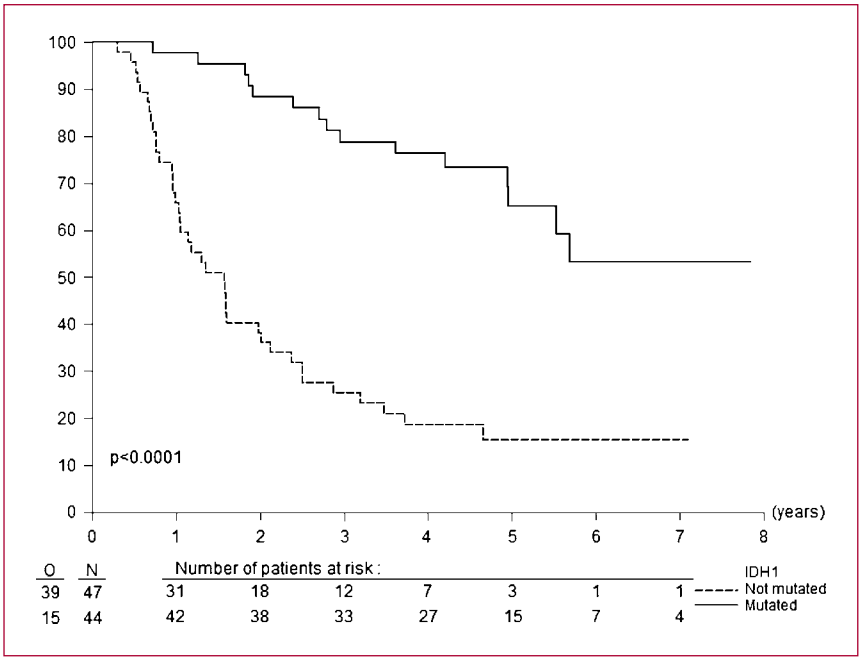

Fig. 1. OS regardless of treatment in *IDH1*-mutated and *IDH1* wild-type tumors in at central review confirmed anaplastic oligodendroglioma and anaplastic oligoastrocytoma (according to the WHO 2007 definition). N, number of patients; O, number of observed events.

OS were similar for the patients with and without *IDH1* and *IDH2* mutation assessment ($P = 0.195$ and 0.565, respectively).

IDH1 mutations were identified in 73 cases (45.9%), and only one *IDH2* mutation was identified. In all further analysis, this patient was considered among the *IDH1* mutated.

Table 1 summarizes the baseline characteristics of the patients with and without *IDH1* and *IDH2* mutations. In the univariate analysis, the presence of *IDH1* mutations was positively correlated with a previous resection for a low-grade tumor ($r = 0.25$), absence of necrosis ($r = 0.33$), absence of epidermal growth factor receptor (EGFR) amplification ($r = 0.40$), absence of polysomy of chromosome 7 ($r = 0.31$), absence of loss of chromosome 10 ($r = 0.33$), presence of 1p/19q loss ($r = 0.43$), and presence of MGMT promoter methylation ($r = 0.42$; all $P < 0.01$). Weaker positive correlations ($r < 0.2$; P between 0.05 and 0.01) were found with younger age ($r = 0.16$), the absence of mitosis ($r = 0.17$), frontal involvement ($r = 0.18$), and the central review histologic diagnosis according to the WHO 2007 classification ($r = 0.18$; ref. 19). In logistic regression, the presence of *IDH1* and *IDH2* mutations was predicted by younger age ($P = 0.0021$), the absence of necrosis ($P = 0.0005$), the absence of *EGFR* amplification $P = 0.0007$), the presence of 1p/19q loss ($P = 0.001$) and the presence of *MGMT* promoter methylation ($P < 0.0001$).

Survival. Both PFS and OS were strongly correlated with *IDH1* mutational status. Median and percentage of 2-year PFS for wild-type *IDH1* patients was 7.8 months and 19% versus 50 months and 65% for patients with *IDH1*-mutated tumors [hazard ratio (HR), 0.27; 95% confidence interval (95% CI), 0.18-0.40]. In the Cox multivariate analysis with stepwise selection, the presence of IDH mutations, necrosis, frontal localization, and 1p/19q loss were selected as independent prognostic factors. With bootstrap validation, both the presence of *IDH1* mutations and of combined 1p/19q loss were confirmed.

Median and percentage of 2-year OS was 16 months and 37% for patients with *IDH1* wild-type tumors and not reached and 83% for patients with *IDH1*-mutated tumors (HR, 024; 95% CI, 0.15-0.38). In the Cox multivariate analysis with stepwise selection, the presence of *IDH1* mutations, of necrosis, and of 1p/19q loss were independent prognostic factors. Bootstrapping confirmed *IDH1* mutations and 1p/19q loss, and necrosis was of borderline significance (included in 55% of samples).

In the subgroup of 91 that were confirmed anaplastic oligodendroglial tumors according to the WHO 2007 (anaplastic oligodendroglioma with or without necrosis and AOA without necrosis) at central review, 44 had an *IDH1* mutation. This *IDH1*-mutated subgroup had a significantly better survival (HR, 0.19; 95% CI, 0.10-0.35; Fig. 1). Two-year survival for the *IDH1* intact tumors was 38.3% (95% CI, 24.6-51.8) versus 88.4% (95% CI, 74.3-95.0) for the *IDH1* mutated tumors.

For neither PFS nor OS, the presence of *IDH1* mutations was related to outcome to adjuvant PCV chemotherapy (test for interaction, respectively, 0.70 and 0.94; Fig. 2). Table 2 summarizes median and 2-year PFS in relationship to treatment (radiotherapy or radiotherapy/PCV). For PFS, the HR (95% CI) reduction in the presence of an *IDH1* mutation after radiotherapy only was 0.29 (0.17-0.50) and 0.24 (0.13-0.43) after treatment with radiotherapy/PCV.

Discussion

As expected, *IDH1* mutations were less frequent in tumors with glioblastoma features, but occurred in about half of the patients with a confirmed anaplastic oligodendroglial tumors at central review and in 86% of patients with combined 1p/19q loss. In the current data set, *IDH2* mutations were rare (1:159). *IDH1* mutations were more frequent in younger patients; patients having previously undergone a resection for a low-grade tumor; patients without the diagnosis of a glioblastoma or AOA with necrosis (synonym: glioblastoma with oligodendroglial differentiation) at central review; patients without necrosis in the tumor; patients with frontal involvement; and from the molecular perspective, patients without *EGFR* amplification, Trisomy 7, and loss of chromosome 10. These findings all fit with a low incidence of *IDH1* mutations in primary glioblastoma, but with a high incidence of these mutations in grade II and grade III tumors. In addition, a strong correlation ($P < 0.0001$) was found with *MGMT* promoter methylation. IDH1 mutations were observed in 62% of the *MGMT* promoter–methylated tumors as opposed to only 10% of the MGMT-unmethylated tumors. Others also found a strong correlation with *MGMT* promoter methylation (58% *IDH1* mutations in methylated versus 26% *IDH1* mutations in unmethylated tumors; ref. 5). This contrasts with a German study, in which both *IDH1* mutations and *MGMT* promoter methylation were independent prognostic variables, and in which analysis the presence of the 1p/19q codeletion lost its prognostic significance (20). Of note, combined loss of 1p/19q is also highly correlated with *MGMT* promoter methylation (5, 21, 22). Moreover, our current data and a previous report from our group confirm the presence of *IDH1* mutations both in patients with the 1p/19q codeletion and with *TP53* mutations, although in all the series, a substantial percentage of 1p/19q-codeleted or *TP53*-mutated tumors do not carry and *IDH1* or *IDH2* mutation (5, 6, 23).

The advantage of studying prognostic and predictive factors within a prospective randomized study is that the treatment heterogeneity that is usually disturbing retrospective studies is limited. This allows the assessment of the effect of the molecular marker on the outcome to the randomized treatment, thus making a distinction between markers of prognostic and of predictive significance. Clearly, the presence of *IDH1* mutations are of major prognostic significance for outcome in this group of anaplastic oligodendroglial tumors. However, the present study gives no indication that the presence of *IDH1* mutations predicts the outcome to adjuvant PCV chemotherapy. In a previous report on temozolomide chemotherapy

in progressive low-grade astrocytoma, we observed no relationship between outcome and IDH mutations. This suggests that at present, the improved survival in *IDH1*-mutated tumors is primarily due to a less aggressive biological behavior, and not because of an improved outcome to chemotherapy treatment (24).

Previous analysis of EORTC study 26951 has shown that adjuvant PCV after radiotherapy increased PFS but not OS, presumably because of crossover at the time of recurrence. Combined loss of 1p/19q was found to be of prognostic but not of predictive significance for outcome to adjuvant PCV chemotherapy. Subsequent molecular analysis confirmed that because of the unclear histologic boundaries between oligodendroglial tumors and astrocytic tumors, a considerable percentage of the tumors included in this EORTC study carried a genotype that was resembles more the genetic alterations usually observed in primary glioblastoma (EGFR amplification, loss of 10, polysomy of 7; ref. 14). This also explains the relatively low frequency of IDH mutations in this series (46%) that contrast to most series on grade III gliomes (with IDH mutations in the range of 60-80%). Surprisingly, in the EORTC 26951 study, *MGMT* promoter methylation was found to be of prognostic significance, for both PFS and

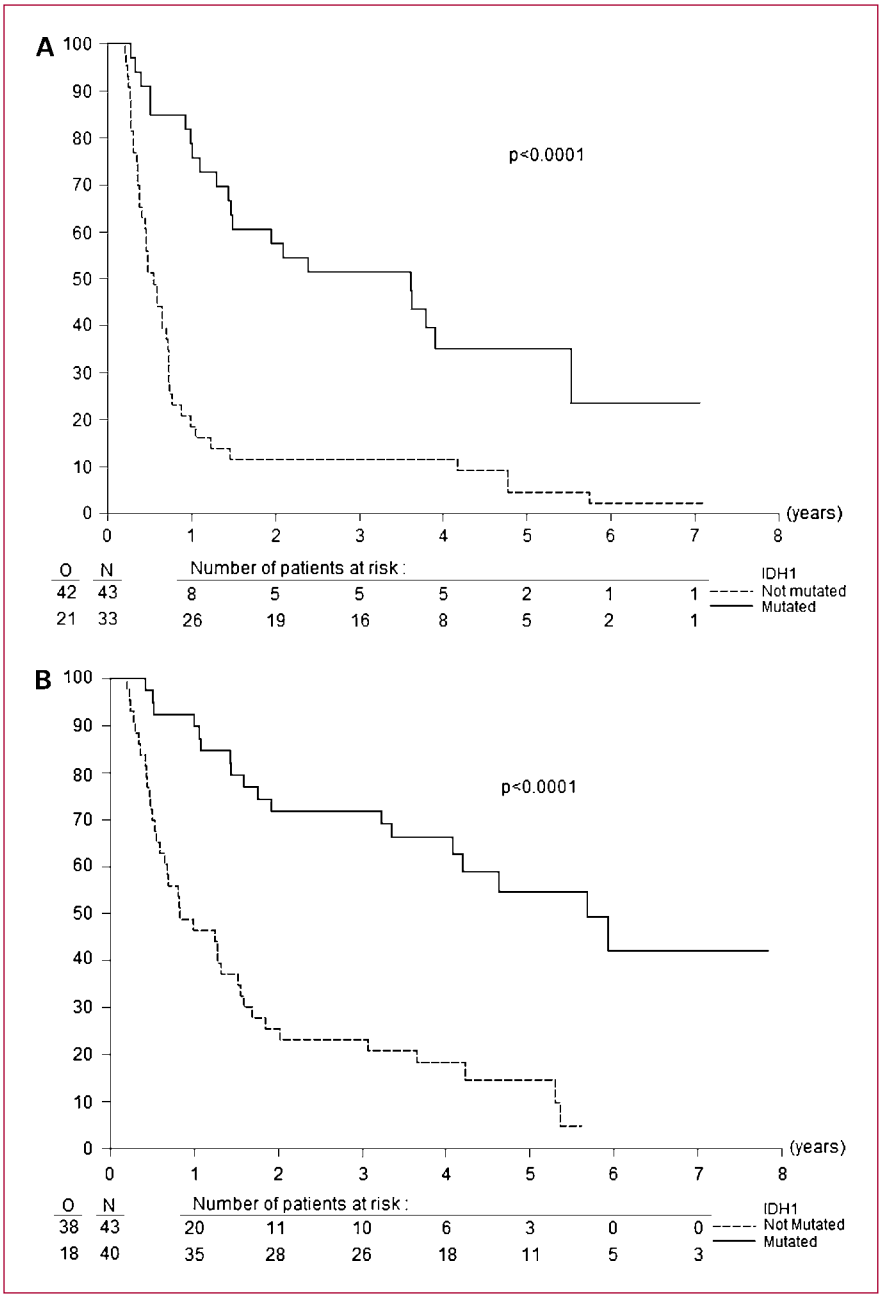

Fig. 2. A and B, PFS in *IDH1*-mutated and wild-type tumors in the radiotherapy arm (A) and the radiotherapy plus PCV arm (B): in both treatment arms, the outcome of patients with *IDH*-mutated tumors is superior compared with patients with *IDHwt* tumors. N, number of patients; O, number of observed events.

Table 2. Median (in mo) and 2-y survival (%) in the patients randomized to radiotherapy and to radiotherapy/PCV in relation to the IDH mutational status

	Radiotherapy arm				Radiotherapy plus PCV arm			
IDH1	*n*	Median (95% CI%) in mo	% at 2 y (s%; 95% CI%)	HR (95% CI%)	*n*	Median (95% CI%) in mo	% at 2 y (s%; 95% CI%)	HR (95% CI%)
Not mutated	43	14.2 (12.4-19.1)	25.6 (13.8-39.1%)	1.00	43	19.0 (15.5-34.5%)	48.8 (33.3-62.7%)	1.00
mutated	33	59.4 (43.3-NR)	84.6 (66.9-93.3%)	0.23 (0.12-0.44)	40	Not reached	82.1 (66.0-91.0%)	0.25 (0.13-0.48)

Abbreviation: NR, not reached.

OS, and not predictive for outcome to PCV chemotherapy (17). To these data, the *IDH1* and *IDH2* data are now added. In the current analysis, which includes *IDH* status, the independent prognostic significance of *MGMT* promoter methylation is lost. This might be due to a lack of power in these exploratory analyses.

To conclude, the presence of *IDH1* mutations do not predict outcome to adjuvant PCV chemotherapy. For grade III oligodendroglial tumors, the assessment of both the 1p/19q codeletion and *IDH1* mutations offer additional prognostic information. The recent report of increased conversion of α-ketoglutaraat to R(−)-2-hydroxyglutarate by the mutated IDH1 enzyme suggests this mutation indeed acts as an oncogene and actually may present drugable targets (10). This opens an entire new horizon of potential therapeutic strategies for grade II and grade III glioma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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