Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Long-Term Survival in Primary Glioblastoma With Versus Without Isocitrate Dehydrogenase Mutations

Christian Hartmann[1,2], Bettina Hentschel[3], Matthias Simon[4], Manfred Westphal[6], Gabriele Schackert[7], Jörg C. Tonn[8], Markus Loeffler[3], Guido Reifenberger[9], Torsten Pietsch[5], Andreas von Deimling[2], and Michael Weller[10], for the German Glioma Network

Abstract

Purpose: The determinants of long-term survival in glioblastoma have remained largely obscure. Isocitrate dehydrogenase (*IDH*) 1 or 2 mutations are common in World Health Organization (WHO) grades II and III gliomas, but rare in primary glioblastomas, and associated with longer survival.

Experimental Design: We compared clinical and molecular characteristics of 69 patients with centrally confirmed glioblastoma and survival >36 months (LTS-36), including 33 patients surviving >60 months (LTS-60), with 257 patients surviving <36 months. *MGMT* promoter methylation, 1p/19q codeletions, *EGFR* amplification, *TP53* mutations, and *IDH1/2* mutations were determined by standard techniques.

Results: The rate of *IDH1/2* mutations in LTS-36 patients was 34% (23 of 67 patients) as opposed to 4.3% in controls (11 of 257 patients). Long-term survivors with *IDH1/2*-mutant glioblastomas were younger, had almost no *EGFR* amplifications, but exhibited more often 1p/19q codeletions and *TP53* mutations than LTS patients with *IDH1/2* wild-type glioblastomas. Long-term survivors with *IDH1/2* wild-type showed no distinguishing features from other patients with *IDH1/2* wild-type glioblastomas except for a higher rate of *MGMT* promoter methylation. Similarly, among 11 patients with *IDH1/2*-mutant glioblastomas without long-term survival, the only difference to *IDH1/2*-mutant long-term survivors was less-frequent *MGMT* promoter methylation. Compared with LTS-36 patients, LTS-60 patients had less frequently *TP53* mutations and radiotherapy alone as initial treatment.

Conclusions: *IDH1/2* mutations define a subgroup of tumors of LTS patients that exhibit molecular characteristics of WHO grade II/III gliomas and secondary glioblastomas. Determinants of LTS with *IDH1/2* wild-type glioblastomas, which exhibit typical molecular features of primary glioblastomas, beyond *MGMT* promoter methylation, remain to be identified. *Clin Cancer Res; 19(18); 5146–57. ©2013 AACR.*

Introduction

According to the World Health Organization (WHO) classification of brain tumors, glioblastomas (WHO grade IV) are defined as malignant astrocytic tumors with necrosis or microvascular proliferation or both (1). They most commonly occur *de novo* and are then referred to as primary glioblastomas. Less frequently, they develop by progression from lower grade gliomas and are then referred to as secondary glioblastomas. The overall prognosis of glioblastoma has remained poor. In a prospective study of 301 primary glioblastoma patients enrolled in the German Glioma Network (GGN), we observed a median overall survival (OS) of 12.5 months (2). However, a small fraction of patients survive for more than 36 months. We have previously arbitrarily defined these patients as long-term survivors (LTS) and clinically characterized these patients as being younger at diagnosis and having a good initial performance score. Molecularly, their tumors showed an increased rate of *MGMT* promoter hypermethylation (74% vs. 30–35% in the general glioblastoma population; ref. 3).

More recently, mutations in the gene encoding cytosolic NADP+-dependent isocitrate dehydrogenase 1 (*IDH1*) were identified in a minority of glioblastoma patients. They are more common in younger patients with primary glioblastoma and in patients with secondary glioblastoma and associated with increased OS (4, 5). In contrast, *IDH1*

Authors' Affiliations: [1]Department of Neuropathology, Institute of Pathology, Hannover Medical School, Hannover; [2]Department of Neuropathology, and German Cancer Research Center (DKFZ), Clinical Cooperation Unit Neuropathology, University Hospital of Heidelberg, Institute for Pathology, Heidelberg; [3]Institute for Medical Informatics, Statistics and Epidemiology, University Leipzig; Departments of [4]Neurosurgery and [5]Neuropathology, University of Bonn, Bonn; [6]Department of Neurosurgery, University of Hamburg, Hamburg; [7]Department of Neurosurgery, University of Dresden, Dresden; [8]Department of Neurosurgery, University of Munich, Munich; [9]Department of Neuropathology, Heinrich Heine University, Düsseldorf, Germany; and [10]Department of Neurology, University Hospital Zurich, and Neuroscience Center Zurich, University of Zurich, Zurich, Switzerland

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Christian Hartmann, Department of Neuropathology, Institute of Pathology, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany. Phone: 49-511-5325237; Fax: 49-511-53218512; E-mail: hartmann.christian@mh-hannover.de

doi: 10.1158/1078-0432.CCR-13-0017

©2013 American Association for Cancer Research.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

Translational Relevance

Although the prognosis of patients with primary glioblastoma is generally poor, a minority of patients achieve long-term survival beyond 36 months. We here report that the rate of isocitrate dehydrogenase 1/2 (*IDH1/2*) mutations in such patients is 34% as opposed to 4.3% in controls and thus substantially increased. Molecular marker profiles of *IDH1/2*-mutant primary glioblastoma correspond to that of secondary glioblastomas which are typically *IDH1/2*-mutant, too. *IDH1/2* wild-type glioblastoma patients with long-term survival exhibit no characteristic marker profile distinguishing them from ordinary *IDH1/2* wild-type glioblastoma patients with poor outcome, suggesting that host-derived factors, for example, immune responsiveness, contribute to long-term survival in such patients.

mutations are detected in 60% to 90% of diffusely infiltrating gliomas of WHO grades II and III (6–12). *IDH1* mutations occur exclusively in codon 132 and around 93% are R132H alterations (7). Moreover, mutations in the gene encoding the mitochondrial NADP+-dependent *IDH2* were identified in around 3% of WHO grade II and III gliomas and secondary glioblastomas (7, 12). Thus, the differential distribution of *IDH1/2* mutations among gliomas provided strong support for the differentiation of secondary from primary glioblastoma and helped to molecularly dissect primary glioblastoma into distinct molecular subgroups (2, 10). These considerations led us to reanalyze and expand our original cohort of LTS glioblastoma patients (3) to address the question to what extent *IDH1/2* mutations account for the LTS phenotype and to identify characteristic features of the *IDH1/2* wild-type LTS phenotype.

Materials and Methods

Patient recruitment and histology

Sixty-nine patients with primary glioblastoma and an OS of >36 months were identified by the 8 clinical centers and 2 associated clinical centers of the GGN (www.gliomnetzwerk.de). To be eligible for this analysis, it was required that the first histologic diagnosis was that of a glioblastoma according to the WHO classification of central nervous system tumors (1) confirmed by central pathology review at the Brain Tumour Reference Center of the German Society of Neuropathology and Neuroanatomy (DGNN). Clinical and part of the molecular data of 36 patients have been published previously, but were updated and complemented for *IDH1/2* mutation status for this report (3), data of 9 other patients have been included in another publication of the GGN (2). The study was approved by the local ethics committees at the GGN clinical centers and written informed consent was obtained from each patient. As a reference group, we used a cohort of 257 primary glioblastoma patients published previously, but excluded all patients who survived for more than 36 months (2).

DNA extraction and analysis for *MGMT* promoter methylation, 1p/19q codeletion, *EGFR* amplification, and *TP53* mutation

DNA was extracted by standard methods either from formalin-fixed paraffin-embedded tumor tissue or from unfixed frozen tumor tissue samples. *MGMT* promoter methylation analysis was carried out by methylation-specific PCR. *EGFR* gene dosage was determined by real-time PCR analysis. Losses on 1p and 19q were determined either by multiplex ligation-dependent probe amplification or by loss of heterozygosity analysis of 5 different microsatellite markers on each chromosomal arm. *TP53* mutations were determined by single-strand conformation polymorphism analysis followed by direct sequencing. Details of the respective molecular methods have been reported elsewhere (2, 3, 13).

IDH1 and *IDH2* mutation analysis

IDH1 codon 132 and *IDH2* codon 172 were analyzed by direct sequencing. In case of ambiguous results, the *IDH1* or *IDH2* sequences were amplified by a different set of primers (6, 7). In addition, 26 LTS patients that carried neither *IDH1* nor *IDH2* mutations in the respective codons were tested for *IDH1* codon 100 mutations (14).

Statistical analysis

Fisher's exact test or the x^2 tests were used to compare the frequencies of molecular aberrations and clinical data in the long-term survivor group versus the control group. OS was calculated from the date of first surgery for glioblastoma until the time of death or last follow-up examination. Survival curves were compared using the log-rank test. Progression-free survival (PFS) was determined from day of first surgery until tumor progression, death, or end of follow-up. Cox regression and multivariate logistic regression analyses were conducted. HR and OR with 95% confidence intervals (95% CI) were determined. All analyses were conducted using IBM SPSS Statistics Version 20.

Results

Patient characteristics

We identified 69 patients with a survival >36 months and a median follow-up of 73 months (LTS-36 cohort). Of these patients, 33 patients were ascertained to have survived for 60 months (LTS-60). These 2 cohorts were compared to a cohort of 257 glioblastoma patients who survived for less than 36 months and had a median follow-up of 23.6 months. Demographic, clinical, and molecular characteristics of these 3 cohorts are summarized in Table 1. Seventeen of 69 LTS-36 and 15 of 33 LTS-60 patients are alive. LTS-36 patients were younger than control patients ($P < 0.001$), but there was no enrichment of young patients in the LTS-60 group relative to the entire LTS-36 group of patients. The relative frequency of glioblastomas with oligodendroglial component was increased in the LTS cohorts. Initial KPS was similar among groups. There were fewer temporal tumors in LTS-36 patients ($P = 0.185$), but this difference was not significant, even if the analysis was

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

Table 1. Summary of patient characteristics

	Control group (OS < 36 months) *n* = 257	LTS > 36 months *n* = 69	LTS > 60 months *n* = 33
Age at diagnosis			
Median (years)	62	49	49
Range (years)	(19–86)	(21–74)	(22–74)
Gender			
Male	158 (61.5%)	35 (50.7%)	19 (57.6%)
Female	99 (38.5%)	34 (49.3%)	14 (42.4%)
Histologic subtype			
Glioblastoma	235 (91.4%)	60 (87.0%)	28 (84.8%)
Glioblastoma olig. comp.	5 (1.9%)	4 (5.8%)	4 (12.1%)
Gliosarcoma	7 (2.7%)	2 (2.9%)	1 (3.0%)
Giant cell glioblastoma	7 (2.7%)	3 (4.3%)	–
Glioblastoma with single giant cells	3 (1.2%)	–	–
Survival			
Median follow-up (months)	23.6	73.0	73.6
Median PFS (months, 95% CI)	6.0 (5.4–6.6)	31.7 (25.8–37.6)	45.9 (38.1–53.7)
Median OS (months, 95% CI)	10.6 (9.6–11.7)	60.2 (52.8–67.5)	83.0 (71.1–94.5)
Alive at last follow-up	4 (1.6%)	17 (24.6%)	15 (45.5%)
KPS at diagnosis			
90–100	106 (41.9%)	27 (41.5%)	13 (41.9%)
70–80	119 (47.0%)	32 (49.2%)	16 (51.6%)
<70	28 (11.1%)	6 (9.2%)	2 (6.5%)
No data	4	4	2
Tumor location			
Frontal	59 (23.1%)	17 (24.6%)	8 (24.2%)
Temporal	72 (28.2%)	14 (20.3%)	6 (18.2%)
Parietal	31 (12.2%)	11 (15.9%)	6 (18.2%)
Occipital	12 (4.7%)	1 (1.4%)	–
Not localized to one site	60 (23.5%)	20 (29.0%)	9 (27.3%)
Multifocal	3 (1.2%)	1 (1.4%)	1 (3.0%)
Others	18 (7.1%)	5 (7.2%)	3 (9.1%)
No data	2	–	–
Surgery			
Gross total resection	104 (40.9%)	32 (49.2%)	16 (50.0%)
Subtotal resection (50–99%)	95 (37.4%)	21 (32.3%)	12 (37.5%)
Partial resection (<50%)	29 (11.4%)	8 (12.3%)	3 (9.4%)
Biopsy	26 (10.2%)	4 (6.2%)	1 (3.1%)
No data	3	4	1
First-line therapy			
Radiotherapy alone	61 (24.1%)	19 (27.5%)	5 (15.2%)
Radiotherapy plus TMZ	151 (58.8%)	37 (53.6%)	21 (63.6%)
Radiotherapy plus nitrosourea	2 (0.8%)	11 (15.9%)	6 (18.2%)
Radiotherapy plus PC	1 (0.4%)	–	–
TMZ alone	10 (3.9%)	–	–
Procarbacine plus lomustine alone	–	1 (1.4%)	–
Nitrosourea alone	1 (0.4%)	–	–
No therapy	30 (11.6%)	1 (1.4%)	1 (3.0%)
Salvage therapies			
Surgery (1)	53 (20.6%)	16 (23.2%)	7 (21.2%)
Surgery (2)	11 (4.3%)	8 (11.6%)	4 (12.1%)
Surgery (>2)	1 (0.4%)	5 (7.2%)	3 (9.1%)

(Continued on the following page)

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

Table 1. Summary of patient characteristics (Cont'd)

	Control group (OS < 36 months) *n* = 257	LTS > 36 months *n* = 69	LTS > 60 months *n* = 33
Radiotherapy (first)	4 (1.6%)	1 (1.4%)	1 (3.0%)
Radiotherapy (re-irradiation)	19 (7.4%)	10 (14.5%)	5 (15.2%)
Chemotherapy (1)	53 (20.6%)	20 (29.0%)	13 (39.4%)
Chemotherapy (2)	25 (9.7%)	11 (15.9%)	4 (12.1%)
Chemotherapy (3)	6 (2.3%)	5 (7.2%)	–
Chemotherapy (>3)	1 (0.4%)	4 (5.8%)	2 (6.1%)
Other medical therapies (1)	20 (7.8%)	7 (10.1%)	2 (6.1%)
Other medical therapies (>1)	4 (1.6%)	1 (1.4%)	–
Including every drug not classified as chemotherapy, e.g., bevacizumab and tyrosine kinase inhibitors			
None	145 (56.4%)	21 (30.4%)	8 (22.2%)
MGMT promoter methylation status (MSP)			
Methylated	85 (33.6%)	41 (60.3%)	22 (66.7%)
Weakly methylated	15 (5.9%)	16 (23.5%)	7 (21.2%)
Unmethylated	153 (60.5%)	11 (16.2%)	4 (12.1%)
No data	4	1	–
1p/19q codeletion			
Yes	20 (8.4%)	6 (9.7%)	2 (6.5%)
No	217 (91.6%)	56 (90.3%)	29 (93.5%)
No data	20	7	2
EGFR amplification			
Yes	109 (43.4%)	20 (29.4%)	7 (21.2%)
No	142 (56.6%)	48 (70.6%)	26 (78.8%)
No data	6	1	–
TP53 mutation			
Yes	37 (15.0%)	14 (21.5%)	4 (12.5%)
No	210 (85.0%)	51 (78.5%)	28 (87.5%)
No data	10	4	1
IDH1 mutation			
Yes	9 (3.6%)	22 (32.8%)	12 (36.4%)
No	239 (96.4%)	45 (67.2%)	21 (63.6%)
No data	9	2	–
IDH2 mutation			
Yes	2 (0.8%)	1 (1.5%)	1 (3.1%)
No	239 (99.2%)	64 (98.5%)	31 (96.9%)
No data	16	4	1

restricted to the control cohort versus the LTS-60 cohort. LTS-36 patients had only slightly more often a gross total resection and slightly less often a biopsy than control patients ($P = 0.379$). Compared with control patients, LTS-36 patients had more often 2 or more further surgical interventions ($P < 0.001$), were more often re-irradiated ($P = 0.066$), and received 1 or 2 lines of salvage chemotherapy more often ($P < 0.001$). First-line treatment after surgery was overall similar except that LTS-60 patients received more often radiotherapy plus chemotherapy than radiotherapy alone relative to the other groups (n.s.). PFS with the first therapeutic intervention was 5-fold as long in LTS-36 patients compared with control patients. Seven patients in the LTS-60 cohort have not suffered progression: 5 are alive, 1 died of renal cell carcinoma, and cause of death is unknown in the other patient.

Molecular markers

The results of molecular analyses are summarized in Table 1 and Fig. 1. LTS-36 patients had more often *MGMT* promoter-methylated tumors than the control group ($P < 0.001$). 1p/19q codeletions were rare and not more common

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

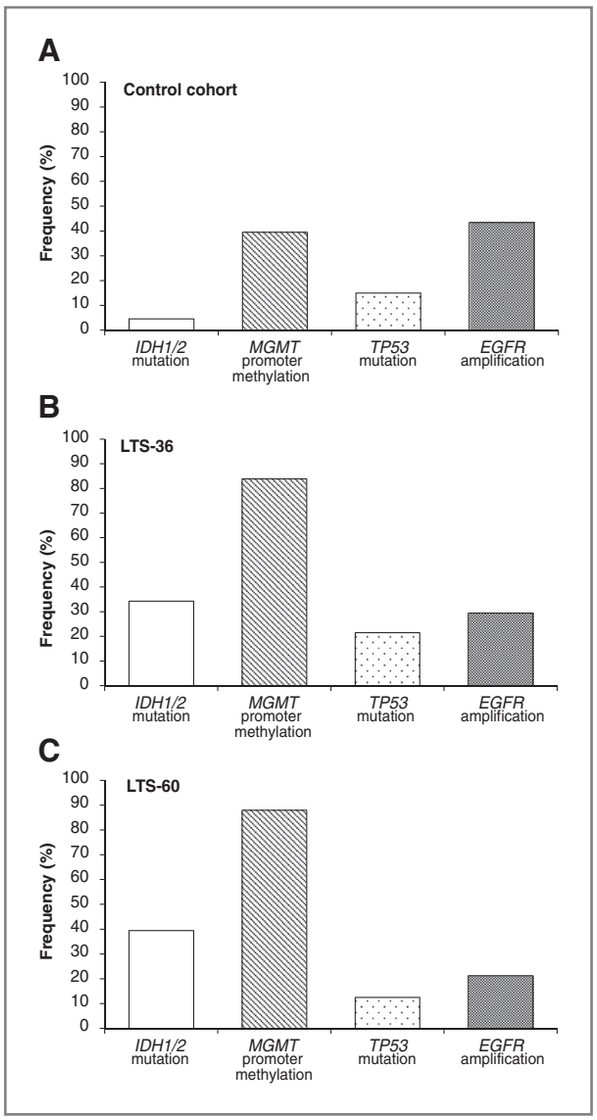

Figure 1. Relative frequency of molecular marker aberrations by cohort: *IDH1/2* mutation status, *MGMT* promoter methylation status, *TP53* mutation status, or *EGFR* amplification status in control (A), LTS-36 (B), and LTS-60 (C) patients.

in LTS patients. *EGFR* amplification was less common in LTS-36 patients ($P = 0.037$). The relative frequencies of *TP53* mutations were similar in LTS-36 and control patients ($P = 0.203$). *IDH1* mutations were much more common in the LTS-36 cohort (32.8%; 22 of 67) than in the control cohort (9 of 248; $P < 0.001$). Among LTS patients, an *IDH1* R132H mutation was identified in 20 patients, an *IDH1* R132S mutation in 1 patient and an R132C mutation in 1 patient. In the control group, 9 *IDH1* mutations were found, all of the R132H type. One *IDH2* mutation was identified in 65 LTS-36 patients and 2 *IDH2* mutations in the control cohort. Among the 33 LTS-60 patients, fewer patients had *TP53* mutations than in the entire LTS-36 cohort. We observed 13 patients in the LTS-60 cohort that had no salvage therapy, 6 with *IDH1/2*-mutant tumors and 7 with *IDH1/2* wild-type tumors. The tumors of 6 patients with *IDH1/2* mutation (median age 39 years) were *MGMT* promoter methylated and had no *EGFR* mutation. Six of 7 patients with *IDH1/2* wild-type tumors (median age 60 years) had *MGMT* promoter methylation and 4 of 6 patients had tumors without *EGFR* mutation.

Prognostic factors for LTS-36

Next we asked which factors influenced the likelihood to become an LTS-36 patient and conducted a multivariate logistic regression analysis. We included age ($\leq$50 vs. >50), KPS ($\geq$80 vs. <80), extent of resection (total vs. no total) and 4 genetic alterations (*MGMT* promoter methylation, *IDH1/2* mutation, *EGFR* amplification, *TP53* mutation) as main factors. The most important prognostic factors for surviving for at least 36 months were *MGMT* status, *IDH1/2* status, and age (Table 2). Table 3 shows a comparison of control patients as well as LTS-36 and LTS-60 patients with versus without *IDH1/2*-mutant tumors.

IDH1/2-mutant glioblastoma versus *IDH1/2*-wild-type glioblastoma with LTS

LTS patients with *IDH1/2*-mutant tumors were younger ($P = 0.001$), had more often frontal as opposed to temporal or parietal tumors ($P = 0.013$), had more often an oligodendroglial component ($P = 0.099$), and were treated with radiotherapy alone initially more often ($P = 0.169$). The molecular profile of *IDH1/2*-mutant tumors of LTS-36 patients was characterized by a higher rate of *MGMT* promoter methylation ($P = 0.307$), 1p/19q codeletions ($P = 0.046$), and *TP53* mutations ($P = 0.345$), and the absence of *EGFR* amplification ($P = 0.001$). Individual profiles are provided in Supplementary Tables S1 and S2. Among patients with wild-type *IDH1/2* status, *MGMT* promoter methylation provided a HR for death of 0.47 (95% CI, 0.22–1.0; $P = 0.053$). Only 2 patients had *IDH1/2*-mutant tumors lacking *MGMT* promoter methylation. Conversely, wild-type *TP53* status conferred a risk reduction both in patients with *IDH1/2*-mutant (0.28; 95% CI, 0.09–0.82; $P = 0.020$) and *IDH1/2* wild-type (0.59; 95% CI, 0.24–1.45; $P = 0.250$) tumors. We also assessed survival probability by molecular marker status in glioblastoma patients surviving for at least 36 months (LTS-36 cohort). Figure 2 shows that, among the LTS-36 patients, *IDH1/2* status was prognostically irrelevant ($P = 0.414$) whereas *MGMT* promoter methylation ($P = 0.078$), wild-type *TP53* status ($P = 0.015$) and absence of *EGFR* amplification ($P = 0.106$), with descending magnitude of effect, were associated with increased survival. Figure 3 shows the relative distribution of patients with combinations of *IDH1/2* mutation and *MGMT* promoter methylation among the 3 cohorts, control, LTS-36, and LTS-60 patients.

IDH1/2-mutant LTS versus IDH1/2-mutant non-LTS

Columns 2 and 4 of Table 3 allow to compare 23 LTS-36 patients with *IDH1/2* mutations versus 11 patients with *IDH1/2* mutations who survived for less than 36 months.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

Table 2. Multivariate logistic regression model for LTS-36 versus control group

	OR	95% CI	*P* value
Age			
≤50 vs. >50 (ref.)	5.0	2.3 to 10.5	<0.001
KPS			
≥80 vs. <80 (ref.)	1.5	0.6 to 3.6	0.359
Extent of resection			
Total vs. no total (ref.)	1.1	0.5 to 2.2	0.806
IDH1/2			
Mutant vs. wild type (ref.)	4.0	1.5 to 11.0	0.007
MGMT			
Methylated vs. unmethylated (ref.)	6.5	2.9 to 14.4	<0.001
EGFR			
Not amplified vs. amplified (ref.)	1.4	0.6 to 3.0	0.409
TP53			
Mutant vs. wild type (ref.)	1.0	0.4 to 2.6	0.954

Their median survival was 12 months and thus similar to the median survival of the general non-LTS cohort (Table 1). Death from tumor progression was confirmed in all 11 patients. None of these tumors had an oligodendroglioma component. The only other remarkable difference between these 2 cohorts of patients with *IDH1/2*-mutant glioblastoma were the rates of *MGMT* promoter methylation: there were 17 methylated, 4 weakly methylated, and only 2 unmethylated tumors in *IDH1/2*-mutant LTS, but 4 methylated, 1 weakly methylated, and 6 unmethylated tumors in the *IDH1/2*-mutant control patients ($P = 0.016$).

IDH1/2 wild-type LTS versus IDH1/2 wild-type non-LTS

Columns 3 and 5 of Table 3 allow to compare 44 LTS-36 patients without *IDH1/2* mutations versus 229 patients without *IDH1/2* mutations who survived for less than 36 months. The major differences between these groups were (expectedly) a higher number of patients who were not treated at all after surgery among control patients and a strong enrichment of patients with *MGMT* promoter methylation in the LTS group.

Discussion

The clinical and molecular determinants of extended survival in glioblastoma remain uncertain, but are currently being elucidated using various approaches. Using one of the largest patient populations ever reported, this study defines *MGMT* status, *IDH1/2* status, and age as the most important prognostic factors for surviving the diagnosis of primary glioblastoma for at least 36 months (Table 2). Most LTS patients showed a classical glioblastoma morphology, although there was an enrichment for glioblastoma with oligodendroglial component in the LTS patients, in particular in the LTS-60 group. This histologic subtype was more common in *IDH1/2* mutant tumors (Table 3). In contrast, extent of resection was not different between control group and LTS-36 patients ($p = 0.541$). Moreover, although fewer patients in the LTS-60 group received radiotherapy alone as initial treatment, no such difference was seen between LTS-36 and control patients, and the differences in salvage treatment are unlikely to account for LTS because one might well argue that patients with less aggressive tumors are more likely to be eligible for multiple salvage therapies.

We delineate 2 main cohorts of primary glioblastoma patients with extended survival beyond 36 months, patients with *IDH1/2* mutations and patients without *IDH1/2* mutations. Furthermore, *IDH1/2* wild-type tumors with and without *MGMT* promoter methylation can be distinguished (Fig. 3). *IDH1/2* mutations have been associated with overall better prognosis across all diffusely infiltrating malignant astrocytic entities (9, 10, 12, 15, 16) and their absence in elderly glioblastoma patients may explain in part why age is a negative prognostic factor in this disease (17). However, it has never been explored whether tumors with *IDH1/2* mutations are enriched in long-term survivors of glioblastomas. Importantly, this study only included patients with primary glioblastoma, that is, there was no evidence of a prior lower-grade lesion in any of these patients. We report a rate of *IDH1/2* mutations of 34.3% (23 of 67) in LTS-36 patients which was much higher than in the general population of glioblastoma patients (5–10%). The molecular profile of these LTS-36 patients with *IDH1/2*-mutant primary glioblastoma was characterized by an increased prevalence of *TP53* mutations and the absence of *EGFR* amplification (Table 3). Although a less malignant precursor lesion escaped clinical detection in this group of patients, the pattern of molecular alterations in their tumors was typical of secondary glioblastoma (9). Accordingly, the concept of primary and secondary glioblastoma should probably be replaced by a molecular concept, for example, of *IDH1/2*-mutant glioblastoma rather than by the absence or presence of a clinically recognized precursor lesion, because future therapeutic decision making is likely to be more commonly based on molecular profiles.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

Table 3. Comparison of control group, LTS-36, and LTS-60 patients: *IDH1/2* mutant versus *IDH1/2* wild type

	Control group (OS < 36 months) *IDH1/2* mutant $n = 11$	Control group (OS < 36 months) *IDH1/2* wild type $n = 229$	LTS-36 *IDH1/2* mutant $n = 23$	LTS-36 *IDH1/2* wild type $n = 44$	LTS-60 *IDH1/2* mutant $n = 13$	LTS-60 *IDH1/2* wild type $n = 20$
Age at diagnosis						
Median (years)	44	63	41	55	39	60
Range (years)	27–79	19–86	(22–71)	(21–74)	(22–54)	(32–74)
Gender						
Male	5 (45.5%)	141 (61.6%)	12 (52.2%)	23 (52.3%)	7 (53.8%)	12 (60.0%)
Female	6 (54.5%)	88 (38.4%)	11 (47.8%)	21 (47.7%)	6 (46.2%)	8 (40.0%)
Histologic subtype						
Glioblastoma	9 (81.8%)	209 (91.3%)	20 (87.0%)	38 (86.4%)	10 (76.9%)	18 (90.0%)
Glioblastoma olig. comp.	–	5 (2.2%)	3 (13.0%)	1 (2.3%)	3 (23.1%)	1 (5.0%)
Gliosarcoma	–	7 (3.1%)	–	2 (4.5%)	–	1 (5.0%)
Giant cell glioblastoma	–	7 (3.1%)	–	3 (6.8%)	–	–
Glioblastoma with single giant cells	2 (18.2%)	1 (0.4%)				
Survival						
Median follow-up (months)	–	21.1	73.9	73.0	73.9	73.6
Median PFS (months, 95% CI)	6.8 (3.0–10.5)	6.0 (5.4–6.6)	31.7 (13.9–49.5)	30.8 (25.3–36.4)	47.5 (0–97.2)	44.8 (33.0–56.5)
Median OS (months, 95% CI)	11.8 (5.1–8.5)	10.5 (9.5–11.6)	64.8 (47.4–82.2)	59.1 (51.0–67.2)	103.1 (-)	75.4 (69.3–81.4)
Alive at last follow-up	0	3 (1.3%)	8 (34.8%)	9 (20.5%)	8 (61.5%)	7 (35.0%)
KPS at diagnosis						
90–100	6 (54.5%)	91 (40.4%)	7 (30.4%)	18 (46.3%)	5 (38.5%)	8 (44.4%)
70–80	3 (27.3%)	110 (48.9%)	14 (60.9%)	19 (43.9%)	7 (53.8%)	9 (50.0%)
<70	2 (18.2%)	24 (10.7%)	2 (8.7%)	4 (9.8%)	1 (7.7%)	1 (5.6%)
No data	–	4	–	3	–	2
Tumor location						
Frontal	4 (36.4%)	52 (22.8%)	9 (39.1%)	8 (18.2%)	5 (38.5%)	3 (15.0%)
Temporal	1 (9.1%)	64 (28.1%)	1 (4.3%)	13 (29.5%)	–	6 (30.0%)
Parietal	3 (27.3%)	27 (11.8%)	1 (4.3%)	8 (18.2%)	1 (7.7%)	5 (25.0%)
Occipital	–	11 (4.8%)	1 (4.3%)	–	–	–
Not localized to one site	3 (27.3%)	55 (24.1%)	9 (39.1%)	11 (25.0%)	5 (38.5%)	4 (20.0%)
Multifocal	–	3 (1.3%)	–	1 (2.3%)	–	1 (5.0%)
Others	–	16 (7.0%)	2 (8.7%)	3 (6.8%)	2 (15.4%)	1 (5.0%)
No data	–	1	–	–	–	–
Surgery						
Gross total resection	6 (54.5%)	95 (42.0%)	10 (45.5%)	22 (53.7%)	7 (58.8%)	9 (47.4%)

(Continued on the following page)

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

Table 3. Comparison of control group, LTS-36, and LTS-60 patients: *IDH1/2* mutant versus *IDH1/2* wild type (Cont'd)

	Control group (OS < 36 months) *IDH1/2* mutant	Control group (OS < 36 months) *IDH1/2* wild type	LTS-36 *IDH1/2* mutant	LTS-36 *IDH1/2* wild type	LTS-60 *IDH1/2* mutant	LTS-60 *IDH1/2* wild type
	n = 11	*n* = 229	*n* = 23	*n* = 44	*n* = 13	*n* = 20
Subtotal resection (50–99%)	2 (18.2%)	85 (37.6%)	7 (31.8%)	13 (31.7%)	4 (30.8%)	8 (42.1%)
Partial resection (<50%)	3 (27.3%)	22 (9.7%)	4 (18.2%)	4 (9.8%)	2 (15.4%)	1 (5.3%)
Biopsy	–	24 (10.6%)	1 (4.5%)	2 (4.9%)	–	1 (5.3%)
No data	–	3	1	3	–	1
Review diagnosis						
Glioblastoma	11 (100.0%)	215 (93.9%)	23 (100.0%)	39 (88.6%)	13 (100.0%)	19 (95.0%)
Gliosarcoma	–	7 (3.1%)	–	2 (4.5%)	–	1 (5.0%)
Giant cell glioblastoma	–	7 (3.1%)	–	3 (6.8%)	–	–
First-line therapy						
Radiotherapy alone	2 (18.2%)	58 (25.3%)	9 (39.1%)	10 (22.7%)	4 (30.8%)	1 (5.0%)
Radiotherapy plus TMZ	8 (72.7%)	132 (57.6%)	10 (43.5%)	25 (56.8%)	7 (53.8%)	14 (70.0%)
Radiotherapy plus nitrosourea	–	2 (0.9%)	2 (8.7%)	9 (20.5%)	1 (7.7%)	5 (25.0%)
Procarbacine plus lomustine alone	–	–	1 (4.3%)	–	–	–
Radiotherapy plus PC	–	1 (0.4%)				
TMZ alone	–	9 (3.9%)				
Nitrosourea alone	1 (9.1%)	–				
No therapy	–	27 (11.8%)	1 (4.3%)	–	1 (7.7%)	–
Salvage therapies						
Surgery (1)	2 (18.2%)	50 (21.8%)	4 (17.4%)	12 (27.3%)	–	7 (35.0%)
Surgery (2)	1 (9.1%)	9 (3.9%)	5 (21.7%)	2 (4.5%)	3 (23.1%)	1 (5.0%)
Surgery (>2)	–	1 (0.4%)	–	5 (13.9%)	–	3 (15.1%)
Radiotherapy (first)	1 (9.1%)	3 (1.3%)	2 (8.7%)	–	1 (7.7%)	–
Radiotherapy (re-irradiation)	1 (9.1%)	17 (7.4%)	3 (13.0%)	8 (18.2%)	1 (7.7%)	4 (20.0%)
Chemotherapy (1)	3 (27.3%)	47 (20.5%)	8 (34.8%)	13 (29.5%)	5 (38.5%)	9 (45.0%)
Chemotherapy (2)	2 (18.2%)	23 (10.0%)	3 (13.0%)	8 (18.2%)	1 (7.7%)	3 (15.0%)
Chemotherapy (3)	–	5 (2.2%)	–	5 (11.4%)	–	–
Chemotherapy (>3)	–	1 (0.4%)	1 (4.3%)	3 (6.8%)	1 (7.7%)	1 (5.0%)
Other med. therapies (1)	1 (9.1%)	18 (7.9%)	1 (4.3%)	6 (13.6%)	1 (7.7%)	1 (5.0%)
Other med. therapies (>1)	–	3 (1.3%)	–	1 (2.3%)	–	–
None	5 (45.5%)	127 (55.5%)	8 (34.8%)	13 (29.5%)	6 (46.2%)	7 (35.0%)
MGMT promoter methylation status						
Methylated	4 (36.4%)	76 (33.8%)	17 (73.9%)	24 (54.5%)	10 (76.9%)	12 (59.1%)
Weakly methylated	1 (9.1%)	13 (5.8%)	4 (17.4%)	11 (25.0%)	2 (15.4%)	5 (22.7%)
Unmethylated	6 (54.5%)	136 (60.4%)	2 (8.7%)	9 (20.5%)	1 (7.7%)	3 (18.2%)

(Continued on the following page)

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

Table 3. Comparison of control group, LTS-36, and LTS-60 patients: *IDH1/2* mutant versus *IDH1/2* wild type (Cont'd)

	Control group (OS < 36 months) *IDH1/2* mutant	Control group (OS < 36 months) *IDH1/2* wild type	LTS-36 *IDH1/2* mutant	LTS-36 *IDH1/2* wild type	LTS-60 *IDH1/2* mutant	LTS-60 *IDH1/2* wild type
	n = 11	*n* = 229	*n* = 23	*n* = 44	*n* = 13	*n* = 20
1p/19q codeletion						
Yes	2 (20.0%)	18 (8.4%)	4 (19.0%)	1 (2.6%)	2 (16.7%)	–
No	8 (80.0%)	196 (91.6%)	17 (81.0%)	38 (97.4%)	10 (83.3%)	19 (100.0%)
No data	1	15	2	5	1	1
EGFR amplification						
Yes	1 (9.1%)	102 (45.5%)	1 (4.3%)	19 (43.2%)	–	7 (35.0%)
No	10 (90.9%)	122 (54.5%)	22 (95.7%)	25 (56.8%)	13 (100.0%)	13 (65.0%)
TP53 mutation						
Yes	4 (40.0%)	32 (14.5%)	7 (30.4%)	7 (17.5%)	2 (15.4%)	2 (10.5%)
No	6 (60.0%)	189 (85.5%)	16 (69.6%)	33 (82.5%)	11 (84.6%)	17 (89.5%)
No data	1	8		4		1

The biological consequences of *IDH1/2* mutations are currently being elucidated. Mutant IDH1 proteins generate excessive amounts of 2-hydroxyglutarate (2HG) from α-ketoglutarate (18). 2HG is a competitive inhibitor of various α-ketoglutarate–dependent dioxygenases (19). Accordingly, increased 2HG levels are predicted to inhibit histone demethylases and TET hydroxylases, thereby preventing histone demethylation and increasing promoter methylation of multiple genes (19, 20), including *MGMT* promoter methylation, and explaining why *IDH1/2*-mutant gliomas commonly exhibit the glioma-CpG island methylator phenotype (G-CIMP; 21, 22).

Both LTS cohorts, patients with and without *IDH1/2* mutations, share an increased prevalence of *MGMT* promoter methylation. All patients received alkylating agent chemotherapy at least once in the course of disease. Accordingly, it is impossible to dissect a prognostic versus predictive impact of *MGMT* promoter methylation in this cohort. Because most *IDH1/2*-mutant tumors exhibit *MGMT* promoter methylation, an independent association of *MGMT* promoter methylation with outcome cannot be assessed in this cohort. The frequent *MGMT* promoter methylation in *IDH1/2* mutant glioblastomas suggests that *MGMT* belongs to the G-CIMP gene set (23). This association may explain why *MGMT* promoter methylation in patients with anaplastic gliomas, tumors with a high frequency of *IDH1/2* mutations, confers a prognostic benefit independent of the therapy regime (24, 25). In contrast, *MGMT* promoter methylation retained an (almost significant) prognostic significance in patients with *IDH1/2* wild-type glioblastoma. The molecular mechanisms inducing *MGMT* promoter methylation in glioblastomas of such patients remain to be identified. The speculation that these tumors carry alterations in other genes involved in the same pathway as *IDH1/2* has not been substantiated so far and is unlikely, given their differential genomic and epigenomic profiles. We confirm a lower rate of *EGFR* amplification in the LTS-36 cohort (26), but this can now be linked to its virtual absence in patients with *IDH1/2*-mutant tumors. Interestingly, the *EGFR* amplification rate in *IDH1/2* wild-type tumors was similar in LTS-36 patients (43.2%) and in the control cohort (45.5%). Somewhat surprisingly, among the LTS-36 patients, patients with *TP53* mutant tumors had an inferior outcome. This observation corresponds to a similar observation at long-term follow-up in low-grade astrocytoma patients (27) and was more prominent in the *IDH1/2*-mutant LTS-36 patients. Altogether, the determinants of long-term survival in patients with *IDH1/2* wild-type glioblastomas, which exhibit the typical molecular phenotype of glioblastomas, beyond *MGMT* promoter methylation remain to be identified, and even the absence of both *IDH1/2* mutations and *MGMT* promoter methylation does not preclude long-term survival. Finally, except of the report of a small, 9 patient series in 2006 (28), this study represents the only characterization of a primary glioblastoma cohort surviving for 5 years or more, and molecular data on such patients have never been reported before.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

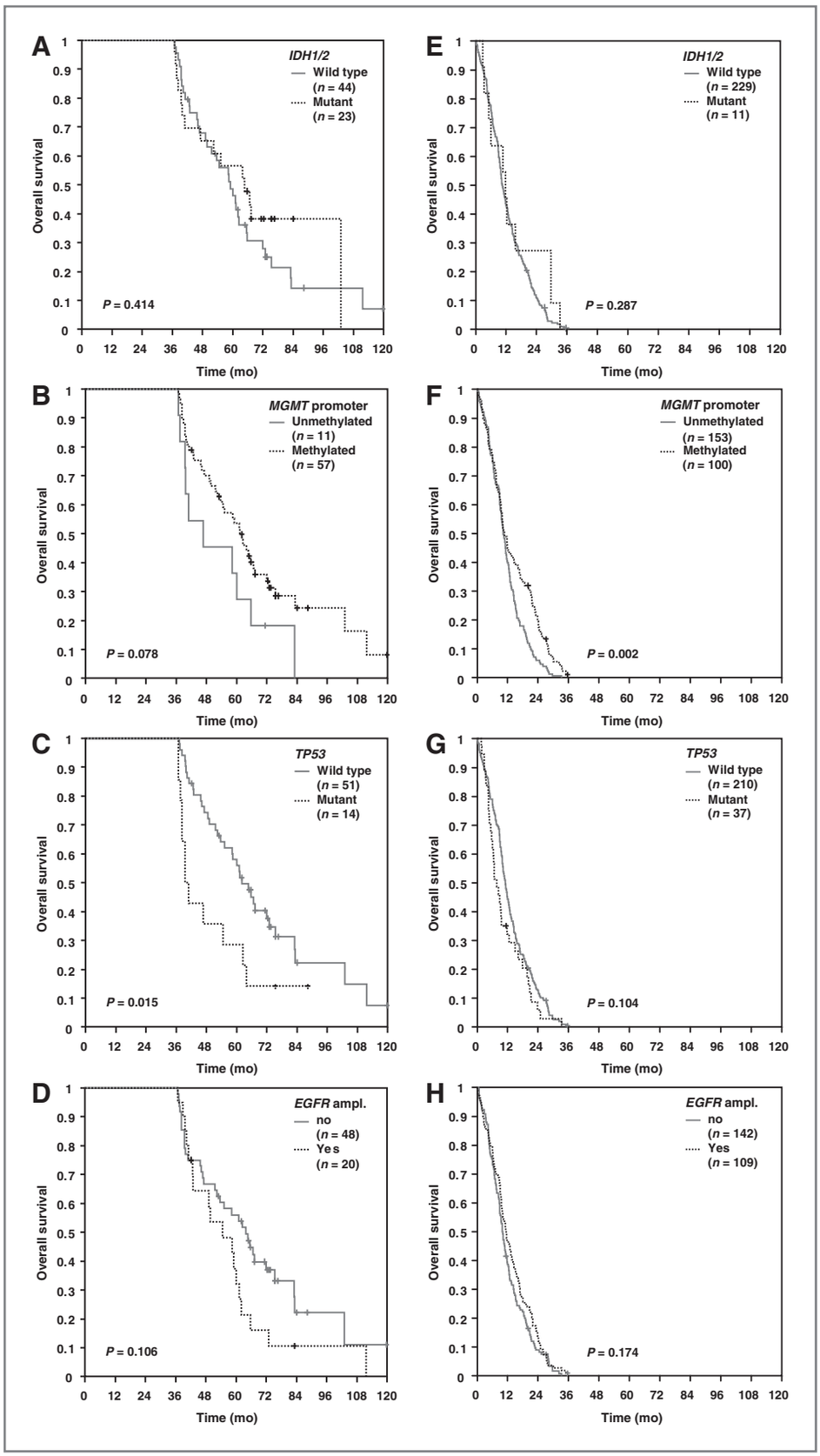

Figure 2. Survival by molecular marker status in the LTS-36 cohort. Overall survival is shown for the LTS-36 patients (left) and compared with the control cohort (right) stratified by *IDH1/2* mutation status (A, E), *MGMT* promoter methylation status (B, F), *TP53* mutation status (C, G), or *EGFR* amplification status (D, H).

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

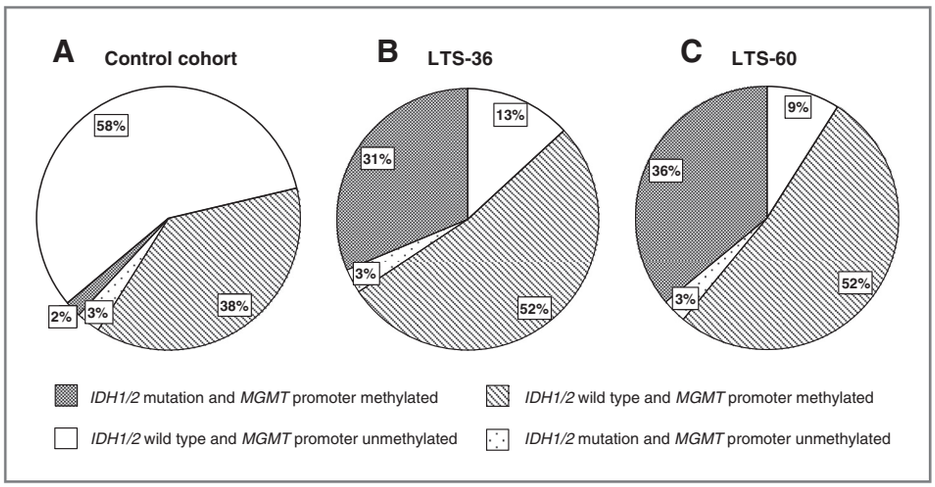

Figure 3. Distribution of glioblastomas by *IDH1/2* mutation and *MGMT* promoter methylation status among the 3 patient groups: control (A), LTS-36 (B), and LTS-60 (C) patients.

Given limitations of tissue availability, we were unable to explore whether the LTS phenotype in our cohort is linked to recently characterized gene expression or DNA methylation signatures (29–31). The first high-throughput approaches have indeed suggested links between LTS and decreased retinoic acid signaling (32), enhanced immune-related gene expression (33), or distinct DNA methylation profiles (34), but more studies with larger patient populations seem to be required to decide whether tumor rather than host factors are chiefly responsible for LTS.

Disclosure of Potential Conflicts of Interest

J.C. Tonn has honoraria from Speakers Bureau of Roche and MerckSerone. He is also a consultant/advisory board member in Roche and MerckSerone. G. Reifenberger has honoraria from Speakers Bureau of Honoraria for Advisory Boards by Merck Serono. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C. Hartmann, B. Hentschel, M. Loeffler, G. Reifenberger, M. Weller
Development of methodology: C. Hartmann, M. Loeffler, A. von Deimling, M. Weller
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Hartmann, M. Simon, M. Westphal, G. Schackert, J.C. Tonn, G. Reifenberger, T. Pietsch, A. von Deimling, M. Weller
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Hartmann, B. Hentschel, M. Simon, M. Loeffler, T. Pietsch, A. von Deimling, M. Weller
Writing, review, and/or revision of the manuscript: C. Hartmann, B. Hentschel, M. Simon, M. Westphal, G. Schackert, J.C. Tonn, M. Loeffler, G. Reifenberger, T. Pietsch, M. Weller
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Hartmann, M. Weller
Study supervision: M. Weller

Grant Support

This work and the German Glioma Network were supported by the German Cancer Aid (Deutsche Krebshilfe 70-3163-Wi 3).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 11, 2013; revised July 2, 2013; accepted July 17, 2013; published OnlineFirst August 5, 2013.

References

1. Louis DNInternational Agency for Research on Cancer. WHO classification of tumours of the central nervous system. 4th ed. Lyon: International Agency for Research on Cancer; 2007.
2. Weller M, Felsberg J, Hartmann C, Berger H, Steinbach JP, Schramm J, et al. Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German Glioma Network. J Clin Oncol 2009;27:5743–50.
3. Krex D, Klink B, Hartmann C, von Deimling A, Pietsch T, Simon M, et al. Long-term survival with glioblastoma multiforme. Brain 2007;130:2596–606.
4. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. Science (New York, NY) 2008;321:1807–12.
5. Weller M, Wick W, von Deimling A. Isocitrate dehydrogenase mutations: a challenge to traditional views on the genesis and malignant progression of gliomas. Glia 2011;59:1200–4.
6. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A. Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 2008;116:597–602.
7. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol 2009;118:469–74.
8. Ichimura K, Pearson DM, Kocialkowski S, Backlund LM, Chan R, Jones DT, et al. IDH1 mutations are present in the majority of common adult gliomas but are rare in primary glioblastomas. Neuro Oncol 2009;11:341–7.
9. Nobusawa S, Watanabe T, Kleihues P, Ohgaki H. IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin Cancer Res 2009;15:6002–7.
10. Sanson M, Marie Y, Paris S, Idbaih A, Laffaire J, Ducray F, et al. Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J Clin Oncol 2009;27:4150–4.
11. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H. IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol 2009;174:653–6.
12. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765–73.
13. Felsberg J, Rapp M, Loeser S, Fimmers R, Stummer W, Goeppert M, et al. Prognostic significance of molecular markers and extent of resection in primary glioblastoma patients. Clin Cancer Res 2009;15:6683–93.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025

14. Pusch S, Sahm F, Meyer J, Mittelbronn M, Hartmann C, von Deimling A. Glioma IDH1 mutation patterns off the beaten track. Neuropathol Appl Neurobiol 2011;37:428–30.
15. Combs SE, Rieken S, Wick W, Abdollahi A, von Deimling A, Debus J, et al. Prognostic significance of IDH-1 and MGMT in patients with glioblastoma: one step forward, and one step back? Radiat Oncol 2011;6:115.
16. SongTao Q, Lei Y, Si G, YanQing D, HuiXia H, XueLin Z, et al. IDH mutations predict longer survival and response to temozolomide in secondary glioblastoma. Cancer Sci 2012;103:269–73.
17. Hartmann C, Hentschel B, Wick W, Capper D, Felsberg J, Simon M, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. Acta Neuropathol 2010;120:707–18.
18. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 2009;462:739–44.
19. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell 2011;19:17–30.
20. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature 2012;483:474–8.
21. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 2010;17:510–22.
22. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 2012;483:479–83.
23. Bady P, Sciuscio D, Diserens AC, Bloch J, van den Bent MJ, Marosi C, et al. MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status. Acta Neuropathol 2012;124:547–60.
24. van den Bent MJ, Dubbink HJ, Sanson M, van der Lee-Haarloo CR, Hegi M, Jeuken JW, et al. MGMT promoter methylation is prognostic but not predictive for outcome to adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors: a report from EORTC Brain Tumor Group Study 26951. J Clin Oncol 2009;27:5881–6.
25. Wick W, Hartmann C, Engel C, Stoffels M, Felsberg J, Stockhammer F, et al. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. J Clin Oncol 2009;27:5874–80.
26. Pelloski CE, Ballman KV, Furth AF, Zhang L, Lin E, Sulman EP, et al. Epidermal growth factor receptor variant III status defines clinically distinct subtypes of glioblastoma. J Clin Oncol 2007;25:2288–94.
27. Stander M, Peraud A, Leroch B, Kreth FW. Prognostic impact of TP53 mutation status for adult patients with supratentorial World Health Organization Grade II astrocytoma or oligoastrocytoma: a long-term analysis. Cancer 2004;101:1028–35.
28. Steinbach JP, Blaicher HP, Herrlinger U, Wick W, Nagele T, Meyermann R, et al. Surviving glioblastoma for more than 5 years: the patient's perspective. Neurology 2006;66:239–42.
29. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006;9:157–73.
30. Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell 2012;22:425–37.
31. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010;17:98–110.
32. Barbus S, Tews B, Karra D, Hahn M, Radlwimmer B, Delhomme N, et al. Differential retinoic acid signaling in tumors of long- and short-term glioblastoma survivors. J Natl Cancer Inst 2011;103:598–606.
33. Donson AM, Birks DK, Schittone SA, Kleinschmidt-DeMasters BK, Sun DY, Hemenway MF, et al. Increased immune gene expression and immune cell infiltration in high-grade astrocytoma distinguish long-term from short-term survivors. J Immunol 2012;189:1920–7.
34. Shinawi T, Hill VK, Krex D, Schackert G, Gentle D, Morris MR, et al. DNA methylation profiles of long- and short-term glioblastoma survivors. Epigenetics 2013;8:149–56.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/19/18/5146/2012386/5146.pdf by guest on 13 January 2025