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CLINICAL ARTICLE

Use of telomerase promoter mutations to mark specific molecular subsets with reciprocal clinical behavior in IDH mutant and IDH wild-type diffuse gliomas

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OBJECTIVE Recent studies have established that hemispheric diffuse gliomas may be grouped into subsets on the basis of molecular markers; these subsets are loosely correlated with the histopathological diagnosis but are strong predictors of clinical tumor behavior. Based on an analysis of molecular and clinical parameters, the authors hypothesized that mutations of the telomerase promoter (TERTp-mut) mark separate oncogenic programs among isocitrate dehydrogenase 1 and/or 2 (IDH) mutant (IDH-mut) and IDH wild-type (IDH-wt) diffuse gliomas independent of histopathology or WHO grade.

METHODS Four molecular subsets of the combined statuses of IDH and TERT-promoter mutations (double mutant, IDH only, TERT only, and double negative) were defined. Differences in age, anatomical location, molecular genetics, and survival rates in a surgical cohort of 299 patients with a total of 356 hemispheric diffuse gliomas (WHO Grade II, III, or IV) were analyzed.

RESULTS TERTp-mut were present in 38.8% of IDH-mut and 70.2% of IDH-wt gliomas. The mutational status was stable in each patient at 57 recurrence events over a 2645-month cumulative follow-up period. Among patients with IDH-mut gliomas, those in the double-mutant subset had better survival and a lower incidence of malignant degeneration than those in the IDH-only subset. Of patients in the double-mutant subset, 96.3% were also positive for 1p/19q codeletions. All patients with 1p/19q codeletions had TERTp-mut. In patients with IDH-mut glioma, epidermal growth factor receptor or phosphatase and tensin homolog mutations were not observed, and copy-number variations were uncommon. Among IDH-wt gliomas, the TERT-only subset was associated with significantly higher age, higher Ki-67 labeling index, primary glioblastoma-specific oncogenic changes, and poor survival. The double-negative subset was genetically and biologically heterogeneous. Survival analyses (Kaplan-Meier, multivariate, and regression-tree analyses) confirmed that patients in the 4 molecular subsets had distinct prognoses.

CONCLUSIONS Molecular subsets result in different tumor biology and clinical behaviors in hemispheric diffuse gliomas.

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KEY WORDS glioma; isocitrate dehydrogenase; telomerase; mutation; prognosis; oncology

ABBREVIATIONS ATRX-mut = ATRX mutations; CART = classification and regression tree; EGFR = epidermal growth factor receptor; GBM = glioblastoma; H3F3A-mut = H3 histone family member 3A mutations; HDG = hemispheric diffuse glioma; IDH = isocitrate dehydrogenase 1 and/or 2; IDH-mut = IDH mutations; IHC = immunohistochemistry; MLPA = multiplex ligation-dependent probe amplification; PDGFR = platelet-derived growth factor receptor; pGBM = primary GBM; PTEN = phosphatase and tensin homolog; TERTp-mut = telomerase promoter mutations; wt = wild type.

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Recent large integrated analyses have clearly established that oncogenic molecular changes closely correlate with clinical behavior in diffuse gliomas.[3–6,19] Most of these oncogenic changes are stochastic, but some recur enough to be used as molecular markers to define clinical subsets.[7] Today, there is compelling evidence that isocitrate dehydrogenase 1 and/or 2 (IDH) mutations (IDH-mut) are indicative of a specific disease group.[3–6,19] Similarly, 1p/19q codeletions have become synonymous with oligodendrogliomas. Other authors have demonstrated that a combination of 3 molecular markers (IDH-mut, telomerase promoter mutations [TERTp-mut], and 1p/19q codeletions) can be used to categorize lower-grade gliomas and glioblastomas (GBMs) into clinically relevant molecular subsets.

With a cohort of 299 unique patients with hemispheric diffuse gliomas (HDGs), this study supports recent efforts on molecular classification of HDGs and also provides novel clinically relevant observations. Molecular tests were performed on 356 sample sets, which were acquired at initial surgeries and surgeries for recurrence in these 299 patients. This article is organized into 3 sections. The initial section focuses on activating TERTp-mut and presents our findings that support TERTp-mut as a reliable biomarker in gliomas. The second section is aimed at demonstrating that molecular markers have only a weak correlation with histopathology in HDGs. The third section focuses on molecular classification of all HDGs based on only 2 molecular markers, namely IDH-mut and TERTp-mut. When we stay blinded to the histopathological diagnosis, these 2 markers can provide strong clues on tumor biology. The 4 resultant molecular subsets differed in various aspects of tumor biology, including anatomical localization, multifocality, degree of anaplasia, Ki-67 index, and additional molecular changes harbored, and also differed in age and survival rates of affected patients.

Methods

Definition of the Molecular Subsets

The following molecular subsets were defined to enhance understandability and fluency of this work. Based on IDH-mut, TERTp-mut, and 1p/19q codeletion, Eckel-Passow et al.[7] reported 5 molecular subsets among lower-grade gliomas (WHO Grades II and III) and 4 molecular subsets among GBMs (WHO Grade IV). Although our groups were similar to and our findings parallel with those of that benchmark study, for the sake of consistency we chose to categorize HDGs of all grades into 4 subsets. Here, "double mutant" defines hemispheric diffuse gliomas with IDH-mut and TERTp-mut. This group corresponds to the combination of "triple-positive" and "TERT and IDH mutation" subsets defined by Eckel-Passow et al.[7] The "IDH-only" subset is defined as the presence of IDH-mut and absence of TERTp-mut and corresponds to the "IDH mutation only" subset of Eckel-Passow et al.[7] The "TERT-only" group is defined by the absence of IDH-mut and presence of TERTp-mut and corresponds to the "TERT mutation only" subset of Eckel-Passow et al.[7] The "double-negative" subset is defined by the absence of both IDH-mut and TERTp-mut and corresponds to the "triple-negative" subset of Eckel-Passow et al.[7]

Patients and Tumor Samples

A total of 356 HDG samples from 299 unique patients who underwent surgery at Acıbadem University School of Medicine, Department of Neurosurgery, were included. Thalamic, brainstem, cerebellar, and spinal gliomas were excluded. Only patients with HDGs were included; those with gliomatosis cerebri and specific expansile pathologies such as pilocytic astrocytomas, pleomorphic xanthoastrocytomas, glioneuronal tumors (ganglioglioma, gangliocytoma), or dysembryoplastic neuroepithelial tumors were excluded. Clinical details are presented in Table 1. Fifty-seven of the tumor samples were from recurrence events in 46 patients. In 10 WHO Grade II oligodendrogliomas (all TERTp-mut), biopsy samples were obtained from brain parenchyma 1.5 cm away from tumor T2-weighted hyperintensity (as determined by intraoperative MRI) to test for the presence of TERTp-mut in the surrounding brain parenchyma. Patients with WHO Grade II oligodendroglioma were selected for this purpose, because that is the least likely histopathological group to contain a large number of infiltrating tumor cells in the surrounding normal-appearing brain parenchyma. Clinical information and tissues were used with written informed consent from the patients. This study was approved by the Acıbadem University Ethics Committee.

Statistical Analysis

The chi-square test, t-test, and ANOVA were used for standard statistical analyses. Multivariate analysis was performed for 13 variables (age [dichotomized at 50 years], sex, Ki-67 [dichotomized at 0.15], anatomical localization, multifocality, histopathology, WHO group, WHO grade, molecular group, IDH-mut status, TERTp-mut status, 1p/19q codeletion status, and H3 histone family member 3A mutation [H3F3A-mut] status) using the Mann-Whitney U-test (2 variables) or Kruskal-Wallis test (> 2 variables). Dunn's test was used for multiple comparisons. Fisher's exact chi-square test was used for categorical data. Kaplan-Meier analyses were performed using logarithmic transformation and log-rank test and plotted using XLSTAT 2014.6.03 software (Addinsoft).

A decision-tree analysis (end point, overall survival) was used for predicting the effect of dependent and independent variables. Classification and regression-tree (CART) analysis was used to calculate the predictive power of independent variables on overall survival time (with 10-fold cross-validation). Variables were IDH-mut status, TERTp-mut status, 1p/19q codeletion status, H3F3A-mut status, phosphatase and tensin homolog (PTEN) loss on immunohistochemistry (IHC), p16 loss on IHC, nuclear TP53 accumulation on IHC, Phospho-BRAF on IDH, and epidermal growth factor receptor (EGFR) on IHC. Calculations were performed in SPSS 20 (IBM Corp.) using custom R codes.

Histopathology and IHC

A diagnosis was determined for each sample by a single neuropathologist according to the 2007 WHO Central Nervous System Tumor Classification Scheme.[15] IHC was performed for IDH1 (Diovana, H09), Ki-67 (Dako, MIB-1), Phospho-BRAF (GeneTex), PTEN (Neomarkers, 17.A), $p16^{Ink4a}$ (Cintec, INK4a), TP53 (Scytek, DO/7), and vascu-

TABLE 1. Patient characteristics

Characteristic	WHO Grade at Initial Presentation			Total
	II	III	IV	
No. of patients	136	47	116	299
Age (median [range]) (yrs)	36.5 (20–77)	37 (18–66)	45 (24–82)	44 (18–82)
Male/female ratio	1.52	1.61	2.05	1.72
IDH-mut cases (%)	91.2	66	8.6	55.2
TERTp-mut cases (%)	39	55.3	68.1	52.8
Histology (no. [%])				
Oligodendroglioma	63 (46.3)	16 (34)	0 (0)	95 (31.8)
Astrocytoma	49 (36)	17 (36.2)	0 (0)	78 (26.1)
Oligoastrocytoma	24 (17.7)	14 (29.8)	0 (0)	45 (15.1)
GBM	0 (0)	0 (0)	138	138
Localization tumor center (no. [%])				
Frontal	70 (51.5)	30 (63.8)	45 (38.8)	145 (48.5)
Insular	33 (24.3)	4 (8.5)	1 (0.8)	38 (12.7)
Temporal	21 (15.4)	9 (19.2)	39 (33.6)	69 (23.1)
Parietal	10 (7.4)	4 (8.5)	14 (12.1)	28 (9.4)
Occipital	2 (1.5)	0 (0)	17 (14.7)	19 (6.4)
Multifocality or gliomatosis-like multilobar involvement (no. [%])	2 (1.5)	5 (10.6)	5 (4.3)	12 (4)
Follow-up (median [range]) (mos)	49.5 (1–267)	26 (1–189)	21 (4–69)	23 (1–267)
Deaths during follow-up (no. [%])	12 (8.8)	16 (34.0)	61 (52.6)	89 (30)

lar endothelial growth factor (VEGF) (Neomarkers, VG1) (molecular subsets are in parentheses).

Sanger Sequencing and Minisequencing

IDH-mut and TERTp-mut were tested using sequencing and/or minisequencing. Minisequencing was performed for IDH1-R132G/S/C, IDH1-R132L/H/P, IDH2-R140Q/L, IDH2-R140W, IDH2-R172K/M, IDH2-R172W, hTERT-C228T, and hTERT-C250T. If no mutations were detected in any of these hotspots, IDH and TERTp were also determined by Sanger sequencing of IDH1-R132, IDH2-R140, IDH2-R172, TERTp-C228T, and TERTp-C250T. EGFR mutations were also Sanger sequenced.

Microsatellite Marker Analysis for 1p/19q Codeletions

Microsatellite marker analysis (fluorescent polymerase chain reaction [PCR] and capillary electrophoresis for D1S162, D1S199, D1S226, D1S186, D1S312, D19S112, D19S918, and D19S206) was the standard test for determining 1p/19q codeletion. Fluorescence in situ hybridization was not used for 1p/19q testing.

OncoScan Analysis

Formalin-fixed paraffin-embedded samples of 20 oligodendrogliomas (WHO Grades II and III) were tested using OncoScan 3.0 (Affymetrix) and analyzed using Nexus Express software (BioDiscovery, Inc.).

Multiplex Ligation-Dependent Probe-Amplification Analysis

A SALSA multiplex ligation-dependent probe-amplification (MLPA) probe mix P105-D1 Glioma-2 (MRC-Holland) kit was used to analyze EGFR copy-number alterations in 20 oligodendrogliomas[15] according to manufacturer recommendations.

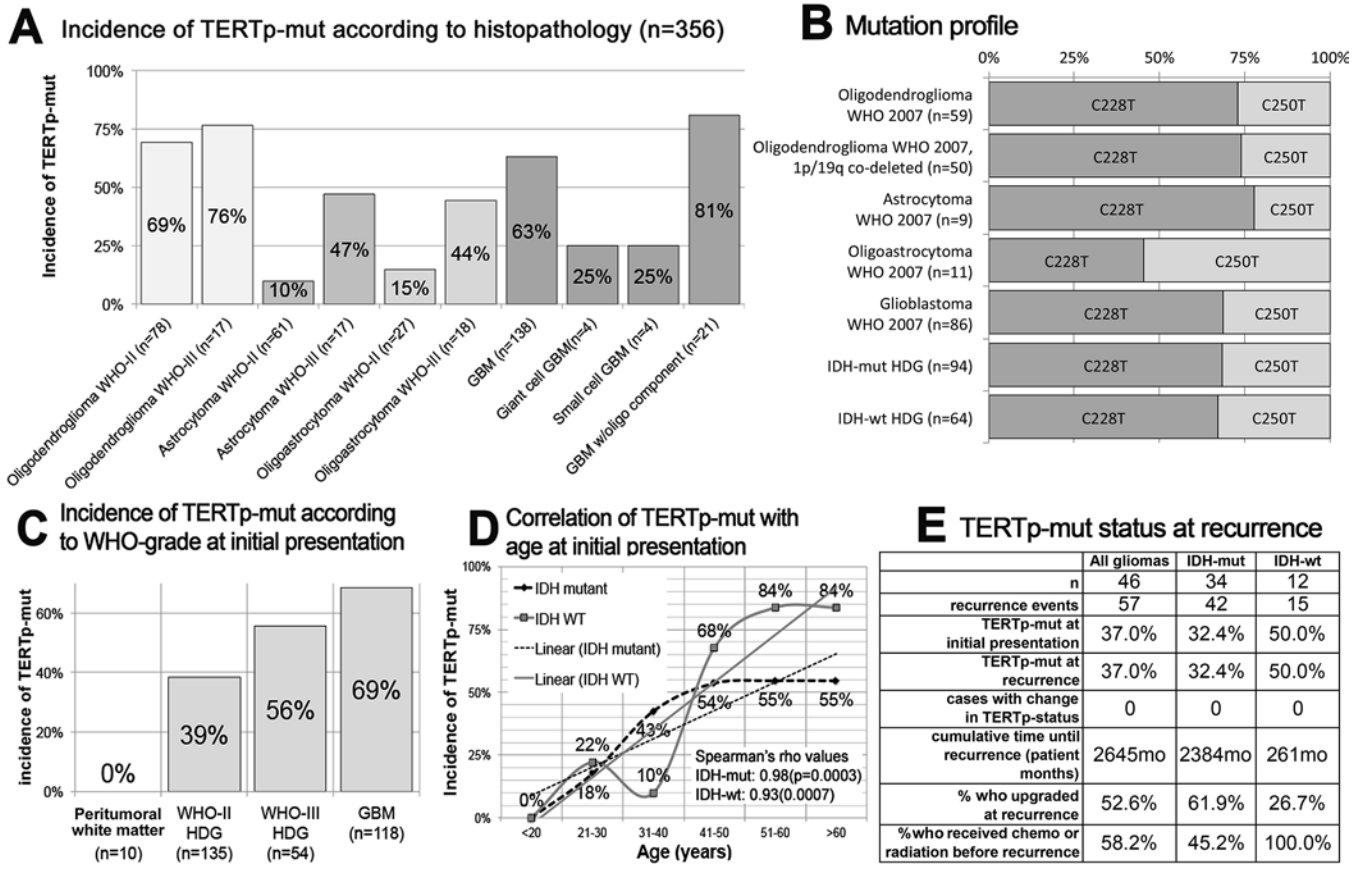
Exome Sequencing and Copy-Number Calculation

Whole-exome capture and next-generation sequencing were performed for 35 patients as described in detail previously.[8] Mutants were confirmed by Sanger sequencing. Copy-number determination was performed using the ExomeCNV tool.

Results

Incidence of TERTp-mut in HDGs

TERTp-mut (at C228T or C250T) were detected in 157 (52.5%) of 299 cases. The incidences of TERTp-mut in different histopathologies, tumor grades, and patient ages are presented in Fig. 1A–D. The TERTp-mut incidence was highest in oligodendrogliomas (71% [67 of 95 WHO Grade II and III oligodendrogliomas]) and in GBMs (63% [78 of 117]). The mutation was not detected in the normal-appearing white matter surrounding the tumor (1.5 cm away from T2-weighted hyperintensity) in any of the 10 patients with oligodendrogliomas, according to Sanger sequencing (Fig. 1C). The mutational status did not change in 57 recurrence events (no-gain events and no-loss events) in 46 patients over a cumulative period of 2645 patient-months (220.4 patient-years) between subsequent surgeries (Fig. 1E). Seventeen (37%) of the recurrent tumors had TERTp-mut. Twenty-eight, 7, and 11 patients had WHO Grade II, III, or IV tumors, respectively, at initial diagnosis; 58.2% had received radiation and/or chemotherapy between subsequent tumor resections, and in 52.6% of them,



	All gliomas	IDH-mut	IDH-wt
n	46	34	12
recurrence events	57	42	15
TERTp-mut at initial presentation	37.0%	32.4%	50.0%
TERTp-mut at recurrence	37.0%	32.4%	50.0%
cases with change in TERTp-status	0	0	0
cumulative time until recurrence (patient months)	2645mo	2384mo	261mo
% who upgraded at recurrence	52.6%	61.9%	26.7%
%who received chemo or radiation before recurrence	58.2%	45.2%	100.0%

FIG. 1. A: In 356 tumors, no exclusive association between the histopathological diagnosis and TERTp-mut status was noted. **B:** The C228T hotspot was approximately twice as common as C250T. **C:** No TERTp-mut were detected in the peritumoral brain parenchyma. **C and D:** TERTp-mut tumors tended to be more anaplastic, and the patients tended to be older. **E:** These findings do not indicate an accumulation of TERT mutations over time, because the initial mutational status remained stable at all recurrences over long follow-up periods despite malignant degeneration and administration of mutagenic adjuvant therapies such as temozolomide and radiation. chemo = chemotherapy.

the tumor recurred at a WHO grade higher than that of the original tumor.

The C228T mutation was more common than the C250T mutation, and this pattern was consistent in all histopathologies and also in IDH-mut and IDH wild-type (IDH-wt) tumors, except for oligoastrocytomas, in which the C250T mutation was more common (55%) (Fig. 1B). The incidences of the C228T mutation were 41 (67.2%) of 61 in double-mutant HDGs and 63 (68.5%) of 92 among TERT-only tumors.

Correlation of Oncogenic Changes With Histopathology in HDGs

There was only a weak correlation between oncogenic changes and histopathological diagnosis, and there was much overlap in the sets of oncogenic changes observed in different histopathologies (Fig. 2A). Of all oligodendrogliomas,[15] 8.9% were IDH-wt and 25% were not 1p/19q codeleted. Similarly, 5.6% of all nonoligodendroglial WHO Grade II and III gliomas harbored the 1p/19q codeletion. TERTp-mut consistently appeared in 2 separate populations, each with different oncogenic profiles (Fig. 2B). A similar dichotomization persisted when tumors of the same histological group but of different grades were considered (Fig. 2C). The oncogenic profile pattern in IDH-wt gliomas resembled that in primary GBMs (GBMs), even when they were WHO Grade II or III (Fig. 2D).

Four Molecular Subsets Based on 2 Recurrent Mutations (IDH-mut and TERTp-mut)

In contrast to the weak correlation with histopathology, TERTp-mut were much better correlated to the oncogenic profiles that were the most common in oligodendrogliomas (IDH-mut, 1p/19q codel, ATRX intact) and hemispheric diffuse astrocytomas (IDH-mut, 1p/19q-nondel, nuclear ATRX expression loss).[16] In 1p/19q-codeleted cases (n = 51 [48 oligodendrogliomas and 3 oligoastrocytomas]), the incidence of TERTp-mut was 100%. All of these tumors (n = 51) were also IDH-mut. Based on the very strong concordance between 1p/19q codeletions and TERTp-mut in IDH-mut HDGs and the significantly older patient age, higher Ki-67 labeling index, and poorer patient survival, regardless of WHO grade or group, in IDH-wt HDGs, we hypothesized that all HDGs (Grades II, III, and IV) could be categorized on the basis of IDH-mut and TERTp-mut

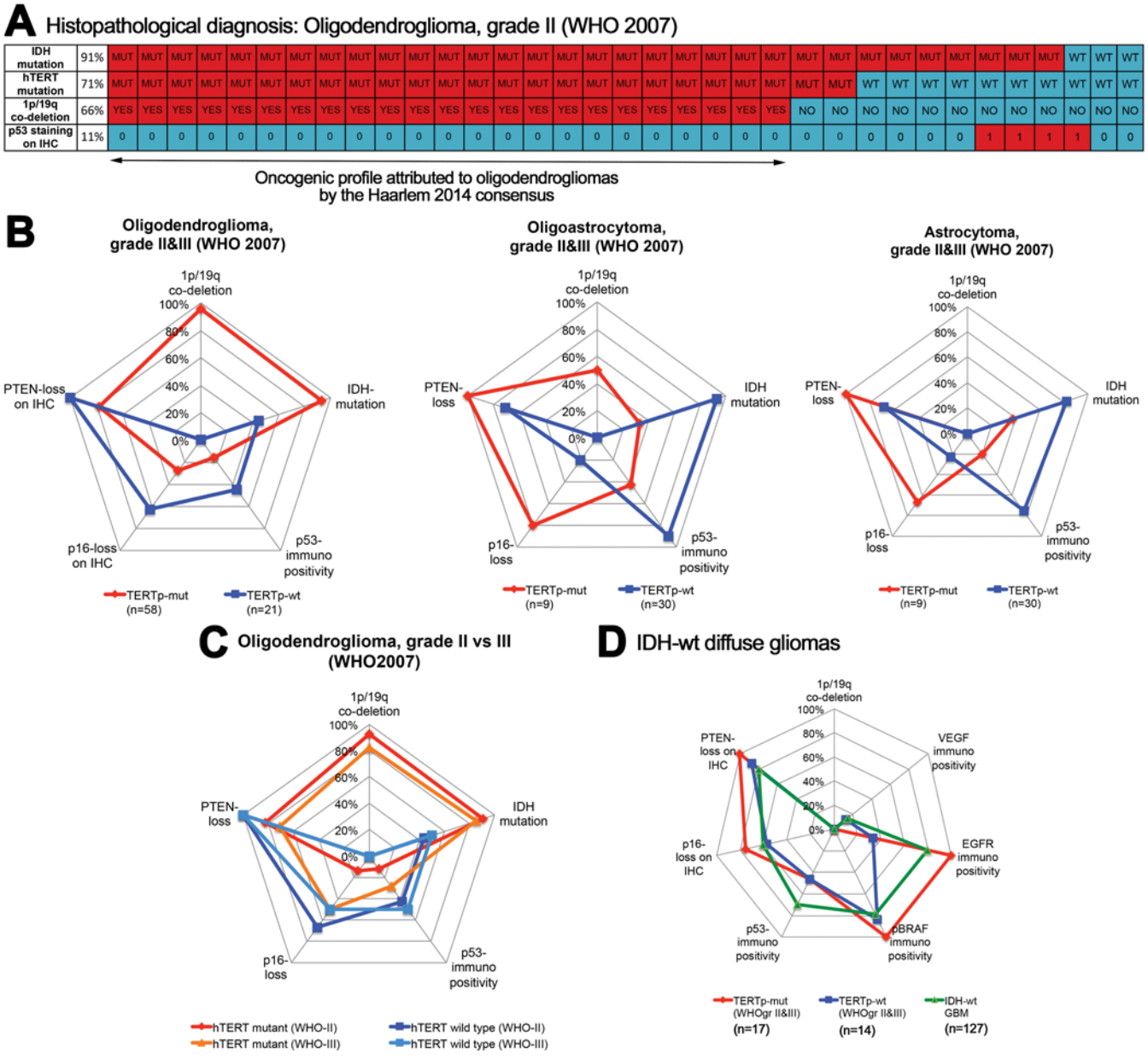


FIG. 2. A: A discrepancy between the histopathological diagnosis and oncogenic signatures was found. Even in oligodendroglioma, WHO Grade II, only two-thirds of the patients carried the "prototypic" IDH mutations and 1p/19q codeletions. **B and C:** TERTp-mut separated at least 2 oncogenic signatures in oligodendrogliomas, astrocytomas, and mixed tumors of all grades. **D:** IDH-wt WHO Grade II and III gliomas also exhibited oncogenic signatures similar to those of primary GBMs.

statuses into 4 molecular subsets that reflect their tumor biology. The double-mutant subset made up 21.4% of the cases, the IDH-only subset 33.8%, the double-negative subset 13.4%, and the TERT-only subset 31.4% (Fig. 3A–C). Forty-nine (76.6%) of 64 double-mutant tumors were WHO Grade II, and the histopathologies were oligodendroglioma in 84.4%, GBM in 6.3%, astrocytoma in 4.7%, and oligoastrocytoma in 4.7%. Eleven patients with double-mutant tumor had tumor recurrence, and 4 (36.4%) of these patients had a higher-grade tumor at recurrence. All recurrences of the double-mutant subset (with or without malignant degeneration) were local. At initial presentation, 75 (74.3%) of 101 IDH-only tumors were WHO Grade II, and the histopathological diagnoses were astrocytoma in 42.6%, oligoastrocytoma in 27.7%, oligodendroglioma in 17.8%, and GBM in 11.8%. Of 20 IDH-only tumors that recurred during the follow-up period, 13 (65%) recurred at a higher grade. Most IDH-wt gliomas had an anaplastic histology at presentation. Thirty-two (80%) of 40 double-negative tumors were either WHO Grade III or IV at presentation, and the histopathological diagnoses were GBM in 75%, astrocytoma in 12.5%, oligodendroglioma in 7.5%, and oligoastrocytoma in 5%. Ninety (95.7%) of the 94 TERT-only tumors were WHO Grade III or IV at pre-

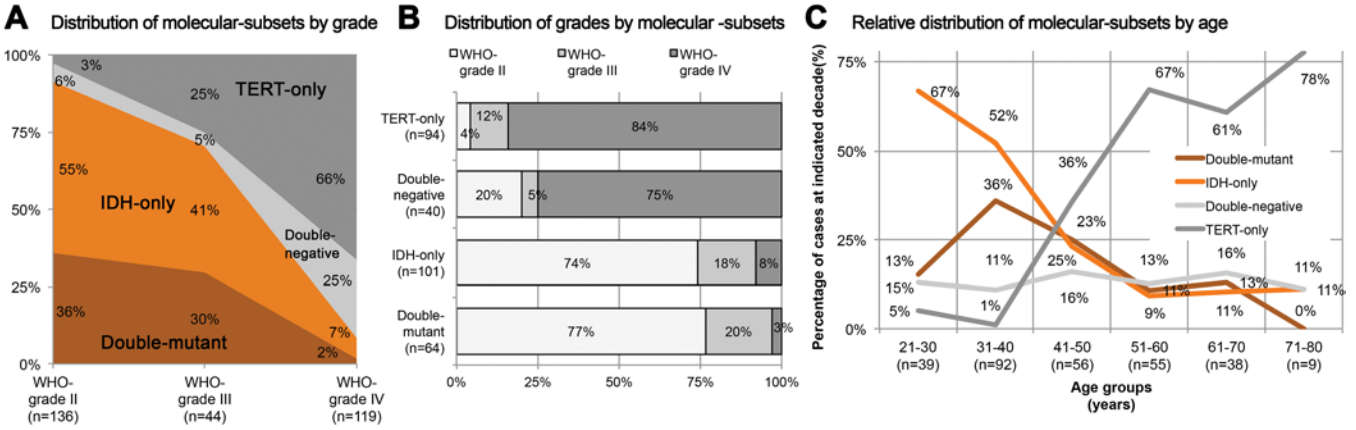


FIG. 3. When we remain blinded to histopathology results, the combination of IDH-mut and TERTp-mut status defines 4 molecular subsets. **A:** Relative incidences of these molecular subsets vary according to tumor grade. **B:** IDH-wt gliomas display mostly anaplastic features, whereas IDH-mut gliomas are predominantly lower grade at presentation. The incidence of IDH-mut peaks at younger patient ages. **C:** The incidence of TERT-only cases increases with age, whereas that of double-negative cases remains relatively constant over time.

sentation, and the histopathological diagnoses were GBM in 84.0%, oligoastrocytoma in 6.4%, astrocytoma in 5.3%, and 4 oligodendroglioma in 4.3%.

Demographic, Clinical, and Molecular Characteristics of the 4 Molecular Subsets

Patient Age at Presentation

The incidence of TERTp-mut increased significantly with patient age regardless of the IDH-mut status (Fig. 1D). Each of the 4 molecular subsets had a unique age profile (Fig. 3C). The IDH-only and double-mutant subsets were most common in the 3rd and 4th decades, respectively. The median age for the IDH-only subset was 34 years (range 17–77 years). For the double-mutant subset, it was 38 years (range 24–68 years). The relative incidence of TERT-only tumors increased persistently from 5% in the 3rd decade to 87% in the 8th decade of life. The incidence of double-negative tumors remained fairly constant at 13% (SD ± 2) from the 3rd through the 8th decade of life. The median age for patients in the TERT-only subset was 57 years (range 24–85 years) and for those in the double-negative subset was 46.5 years (range 21–71 years). We observed a significant correlation between the degree of anaplasia (WHO grade) and patient age; in the double-mutant, IDH-only, and double-negative subsets, older patients had higher WHO grades. This finding reached significance in the IDH-only and double-negative subsets ($p = 0.03665$ and 0.07113, respectively, ANOVA) but not in the double-mutant subset ($p = 0.050354$, ANOVA). In contrast, in the TERT-only subset, we found neither a significant difference nor a trend in age between WHO grades ($p = 0.261827$, ANOVA).

Anatomical Localization

The anatomical distributions of the 4 subsets followed different patterns (Fig. 4A and B). The frontal lobe was the most common site for all the molecular subsets. The double-mutant subset had a very strong predilection for the frontal lobe; 74% of the tumors were localized. IDH-only tumors made up 70% of those located at the insula. Parietal and occipital localizations were far less common for IDH-mut tumors (Fig. 4A and B). Only 2% of the double-mutant and 1% of the IDH-only tumors were located in the occipital lobe. Multifocality was not observed in the double-mutant or double-negative subsets. It was observed most commonly in the TERT-only (4%) and IDH-only (1%) subsets.

Survival

Overall survival rates were significantly different for each molecular subset when all 299 patients (WHO Grade II, II, and IV) were analyzed as a single cohort (Fig. 5A–F). The IDH-mut was uniformly associated with better prognosis. In contrast, TERTp-mut were associated with reciprocal clinical behavior in IDH-mut and IDH-wt gliomas. In the IDH-mut subset, TERTp-mut were associated with significantly better survival (patients of all grades analyzed together, $p = 0.043$, log rank) despite the short median follow-up period (42 months [range 1–267 months]). Only in the double-mutant subset was there no correlation between WHO grade and overall survival ($p = 1$, log rank). Also, the proliferative (Ki-67) indexes were comparable despite increasing WHO grades ($p = 0.77194$, ANOVA). In the IDH-only subset, however, overall survival was significantly worse in patients with tumors with a higher WHO grade ($p = 0.002$, log rank), and we noted a trend toward higher proliferative indexes in IDH-only gliomas of increasing WHO grades ($p = 0.067257$, ANOVA).

In patients with IDH-wt tumors, TERTp-mut were associated with poorer survival ($n = 134$, $p = 0.004$, log rank). When individual WHO grades were analyzed, TERTp-mut were associated with significantly poorer survival in patients with WHO Grade II ($n = 12$, $p = 0.046$, log rank) or WHO Grade IV ($n = 104$, $p = 0.03$, log rank) tumors. We found no survival difference for patients with WHO Grade III tumors based on TERTp-mut status. For patients in the TERT-only subset with tumors at different WHO grades, survival rates were comparable ($p = 0.56$, log rank), and the Ki-67 proliferative indexes at different WHO grades were also comparable ($p = 0.075570$, ANOVA). A multi-

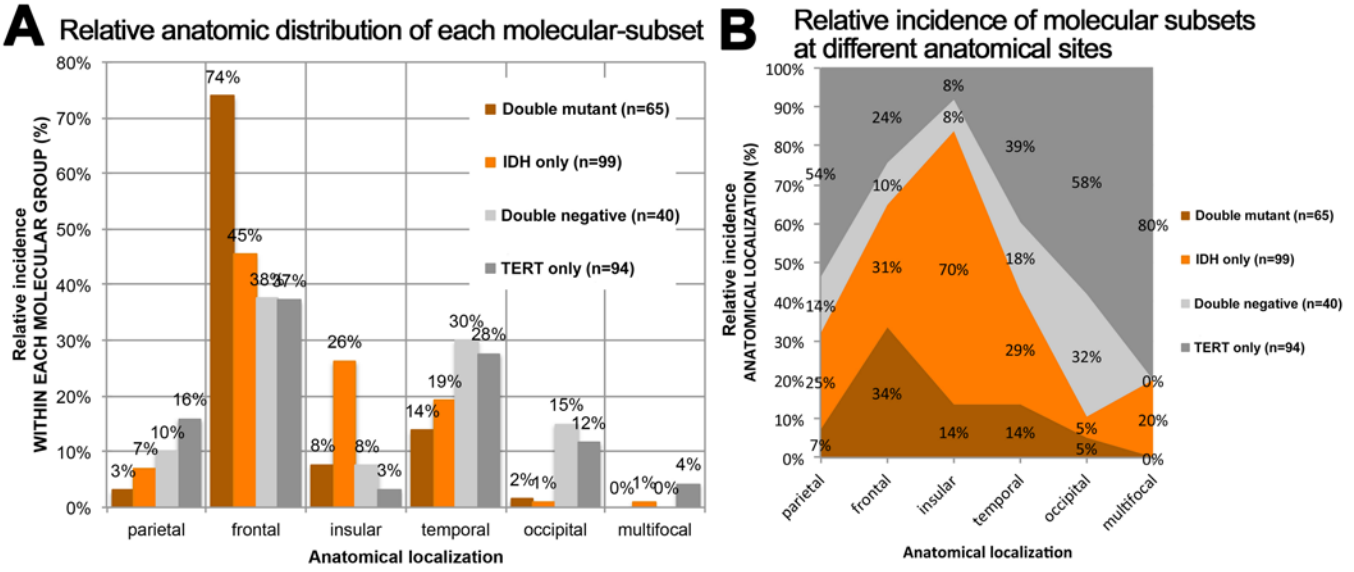


FIG. 4. The anatomical distribution of each molecular subset followed a unique pattern. The frontal lobe was the most common site for all molecular groups. The double-mutant subset had a very strong predilection for the frontal lobe. At the insula, a strong predilection for IDH-only tumors was found. IDH-mut tumors were encountered infrequently in the parietal and occipital lobes. Multifocality was observed only in the TERT-only and IDH-only subsets.

variate analysis for overall survival identified age (older than 50 years), Ki-67 labeling index (greater than 0.15), pathological diagnosis, WHO group, WHO grade, IDH-mut status, TERTp-mut status, and 1p/19q codeletion status as independent significant variables (Table 2). Sex, laterality, multifocality, and H3F3A-mut status were not significant variables. In addition to Kaplan-Meier and multivariate analyses, a third method was also used. An unsupervised CART analysis of molecular markers with an end point of overall survival in 299 patients confirmed IDH-mut status, 1p/19q codeletion status, TERTp-mut status, nuclear TP53 expression on IHC, and numeric Ki-67 index as significant independent variables. When the same analysis was performed with WHO grade and age as covariates, the same independent variables were identified. These decision trees are presented in Figs. 6 and 7.

Oncogenic Molecular Changes Across HDGs

We observed different patterns of molecular oncogenic changes in the 4 molecular subsets defined according to the IDH-mut and TERTp-mut statuses. Among IDH-mut cases, the double-mutant and IDH-only subsets had slightly different oncogenic molecular changes. Non-R132H IDH-mut were significantly more common in the IDH-only subset than in the double-mutant subset (12.8% vs 3.2%, respectively; $p = 0.041$, chi-square). Nuclear TP53 expression was significantly more common in the IDH-only subset (79.2%) than in the double-mutant subset (21.9%) ($p < 0.001$, chi-square test).

EGFR and PTEN alterations are considered a hallmark of primary GBMs (pGBMs). We did not observe any EGFR mutations in IDH-mut tumors (n = 30, Sanger sequencing). In the double-mutant subset, no EGFR copy-number alterations were detected (n = 19). In IDH-only cases, EGFR amplifications were observed in 3 (27%) of 11 cases. It is interesting to note that a semiquantitative immunohistochemical comparison for EGFR protein expression in 18 double-mutant versus 5 IDH-only cases was insignificant ($p = 0.3081$, t-test). PTEN mutations, which are also characteristic of pGBMs, were not observed in any of the IDH-mut cases (tested by Sanger sequencing in 19 double-mutant and 11 IDH-only cases). Copy-number alterations were also rare, and heterozygous losses were observed in 1 of 19 double-mutant cases (as tested by OncoScan) and 1 of 11 IDH-only cases (as tested by MLPA). In contrast, the loss of PTEN protein expression, as demonstrated by IHC, was very common in all 4 molecular subtypes (72.7% in double-mutant, 77.1% in IDH-only, 82.6% in TERT-only, and 78.4% in double-negative subsets).

Glioma-associated oncogenic changes (PTEN, EGFR, PDGFRA, CDKN2A, CDK4, RB1, p53, and H3.3) were analyzed according to the molecular subsets in 68 HDGs using a combination of techniques (Fig. 8). This study was performed as a proof-of-principle analysis to support the idea that the 4 molecular subsets had different oncogenic molecular changes suggestive of each of them having a different tumor biology, but a comprehensive characterization of all molecular changes in HDGs was not intended. However, the results of this analysis provide support for the notion that the double-negative subset was the most heterogeneous group. In addition to oncogenic changes, this group was also heterogeneous in terms of age, Ki-67 index, and overall survival. When double-negative tumors of different WHO grades were analyzed, they also each exhibited different tumor biology. WHO Grade II tumors made up 20% of the double-negative subset, were from young patients, had very low Ki-67 indexes, and resulted in very good survival. In contrast, the majority (80%) of the double-negative tumors were WHO Grade III or IV and resulted in a dismal prognosis. These cases included those with histone H3.3 (*H3F3A*) mutations (a mutation originally identified in diffuse intrinsic pons gliomas of the pediatric popula-

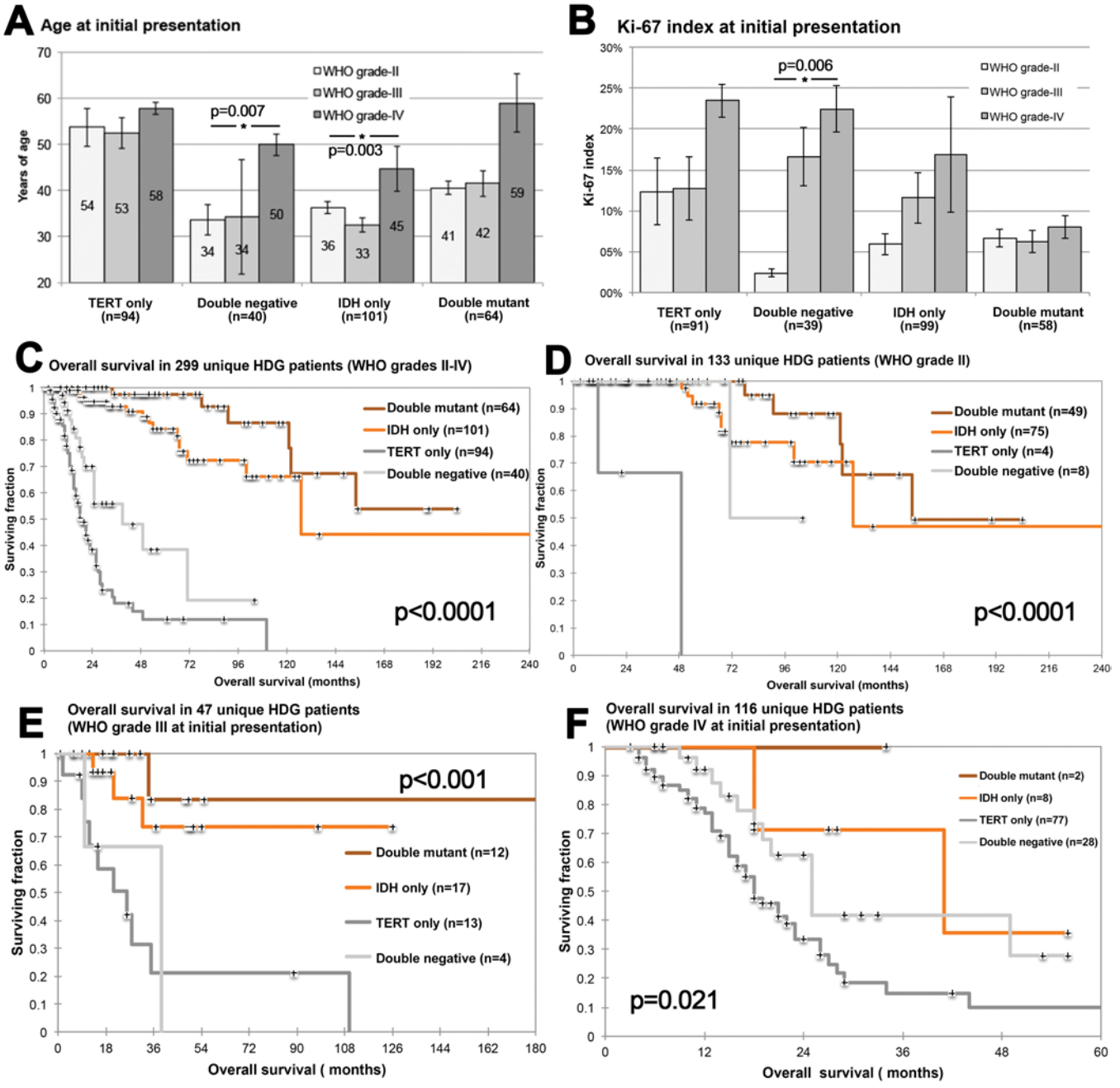


FIG. 5. Each of the 4 molecular subsets has distinct clinical characteristics. **A:** Regardless of the molecular subset, patients with a WHO Grade IV tumor at initial presentation were significantly older than those with a lower-grade tumor. Patients of the TERT-only subset were also significantly older at lower tumor grades than patients with tumors of the same grade of different subsets. **B:** These patients with a WHO Grade II or III tumor also exhibited higher proliferative indexes compared to those of other subsets. **C–F:** The TERT-only subset was associated with poorer prognosis than all subgroups at all WHO grades. The double-negative subset contained young patients with a low proliferative index and good survival at WHO Grade II and older patients with a high proliferative index and poor survival at higher tumor grades (A, B, D, E, and F). These results hint at further subgroups in this molecular subset. IDH-mut gliomas were associated with a much better prognosis than IDH-wt HDGs (C, D, E, and F). Mean values are presented at the center of the columns. *Error bars* indicate the standard error. Significant results are marked with *stars*, and the p values are indicated.

tion) and had a very poor prognosis (median survival 11 months, maximum survival 16 months). For WHO Grade IV tumors, however, the double-negative subset was associated with a significantly better prognosis than the TERT-only subset ($p = 0.044$, log rank).

Discussion

TERTp-mut Are Tumor Specific and Stable Over Time

Telomere lengthening is a fundamental hallmark of cancer, and "activating telomerase promoter mutations

TABLE 2. Multivariate analysis of 13 variables for overall survival

Variable	p Value
Age (yrs)	<0.001*
>50	
<50	
Sex	0.597
Female	
Male	
Ki-67 labeling index	0.042*
>0.15	
<0.15	
Laterality	0.750
Left	
Right	
Bilateral	
Anatomical localization	0.006*
Frontal	
Temporal	
Parietal	
Occipital	
Insular	
Multifocality	0.250
Unifocal	
Multifocal	
Pathological diagnosis (WHO grade)	<0.001*
Oligodendroglioma (II)	
Oligodendroglioma (III)	
Astrocytoma (II)	
Astrocytoma (III)	
Oligoastrocytoma (II)	
Oligoastrocytoma (III)	
GBM	
WHO group	<0.001*
Oligodendroglial	
Astrocytic	
Oligoastrocytic	
GBM	
WHO grade	<0.001*
II	
III	
IV	
IDH-mut status	<0.001*
Mutated	
Wild type	
TERTp-mut status	0.026*
Mutated	
Wild type	
1p/19q codeletion status	0.005*
Codeleted	
Non-codeleted	
H3F3A-mut status	0.283
Mutated	
Wild type	

CONTINUED IN NEXT COLUMN »

» CONTINUED FROM PREVIOUS COLUMN

TABLE 2. Multivariate analysis of 13 variables for overall survival

* Significant result.

(TERTp-mut)" rank first among regulatory mutations in cancer.[20] The telomere-lengthening process in most gliomas depends on either TERTp-mut or ATRX mutations (ATRX-mut), and these oncogenic changes are, in most cases, mutually exclusive.[11] Analyzing our surgical cohort, we identified several clinical correlations of TERTp-mut. The mutations were present in more than half (52.7%) of the patients. When TERTp-mut were present, they were present homogenously within the tumor but not present in the surrounding normal-appearing brain parenchyma. The TERTp-mut status of a tumor did not change over time, or in recurrences, despite adjuvant therapies. These observations led us to the hypothesis that TERTp-mut are an early clonal event and hence part of an oncogenic program that is indispensable for the formation and maintenance of the glioma.

In analyzing our cohort, we also noted that the clinical behavior that could be attributed to the presence of a TERTp-mut was context dependent and relied on the IDH-mut status. In IDH-mut gliomas, TERTp-mut correlated very well with the 1p/19q codeletion, which is considered synonymous with oligodendrogliomas. In IDH-wt gliomas, however, TERTp-mut tumors were associated universally with demographic, molecular, and clinical characteristics long associated with pGBMs. As the TERTp-mut–associated clinical behavior was reciprocal, in IDH-mut and IDH-wt gliomas we classified all HDGs (WHO Grades II–IV) into 4 molecular subsets based on IDH-mut and TERTp-mut statuses (Fig. 3). These molecular subsets differed in various aspects, including age of onset, anatomical localization, degree of anaplasia, oncogenic molecular changes, and clinical behavior. Although these molecular groups differed in the degree of observed anaplasia (WHO grade), there was a significant discrepancy between the histopathological group and the molecular subsets. Therefore, we decided to stay blinded to the histopathologic group (WHO groups, astrocytic and oligodendroglial tumors, GBMs).

Most of the Double-Mutant HDGs Are Oligodendrogliomas With 1p/19q Codeletions

The association between TERTp-mut and oligodendrogliomas was almost exclusive when oligodendrogliomas were defined with the presence of IDH-mut and 1p/19q codeletions.[16] All of the 52 IDH-mut 1p/19q-codeleted HDGs and 0 of the 57 IDH-mut, ATRX-mut, and TERTp-wt HDGs carried TERTp-mut at initial presentation. However, there was a minority in the double-mutant subset that were not 1p/19q codeleted and made up 1.8% of all IDH-

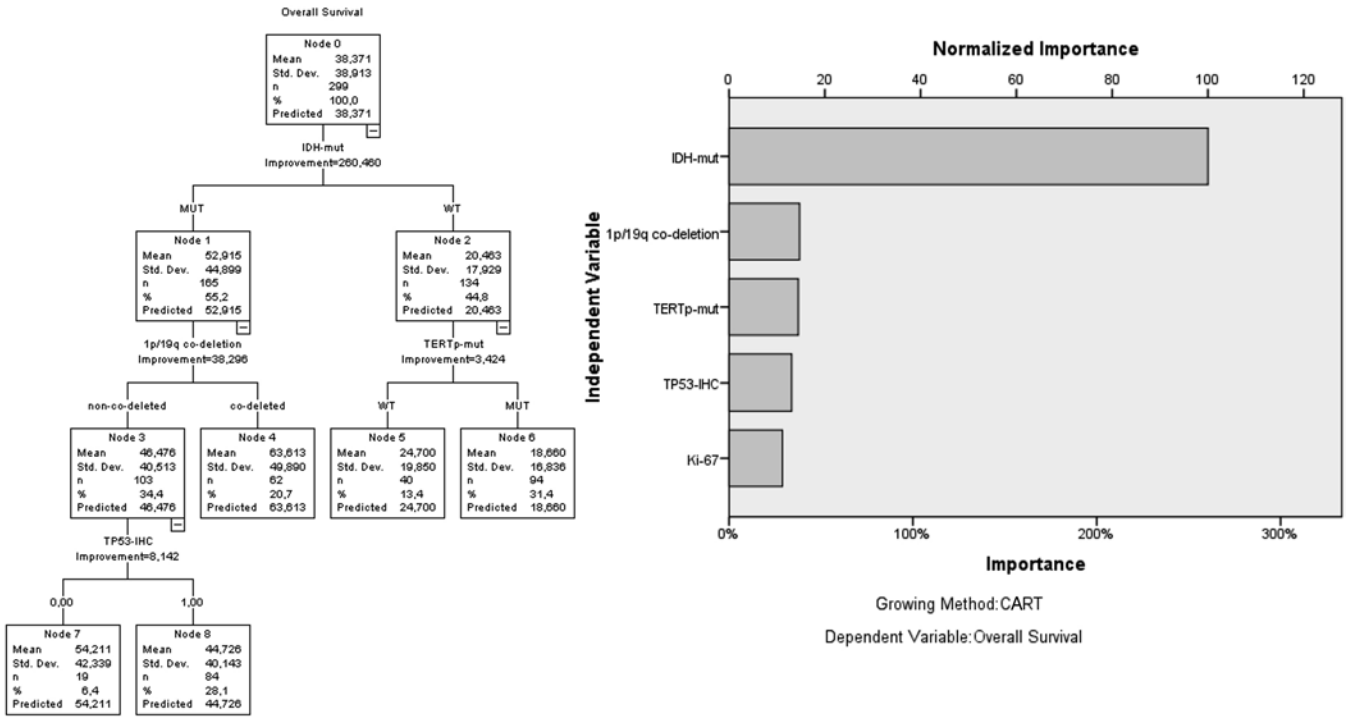


FIG. 6. Unsupervised CART analysis based on overall survival was performed with data from 299 unique patients. 1p/19q codeletion and p53 statuses were identified as significant indicators in IDH-mut tumors. One should consider that in this cohort, all 1p/19q-codeleted tumors also included TERTp-mut and that 96.3% of patients in the double-mutant subset carried the 1p/19q codeletion. Compared with IDH-mut status, the predictive power of each of the significant variables is small but significant, as indicated by the "normalized importance."

mut HDGs and 3.9% of the double-mutant tumors. In a large series reported by Eckel-Passow et al.,[7] such double-mutant but not 1p/19q-noncodeleted tumors made up 7.9% of all IDH-mut HDGs and 18.8% of the double-mutant tumors. The same authors reported that the TERT-mut and IDH-mut tumors were not prognostically different from the triple-positive tumors.[9] This group of tumors needs further clinical analysis and evaluation to conclude whether double-mutant tumors are different from IDH-mutant and TERTp-mut tumors.

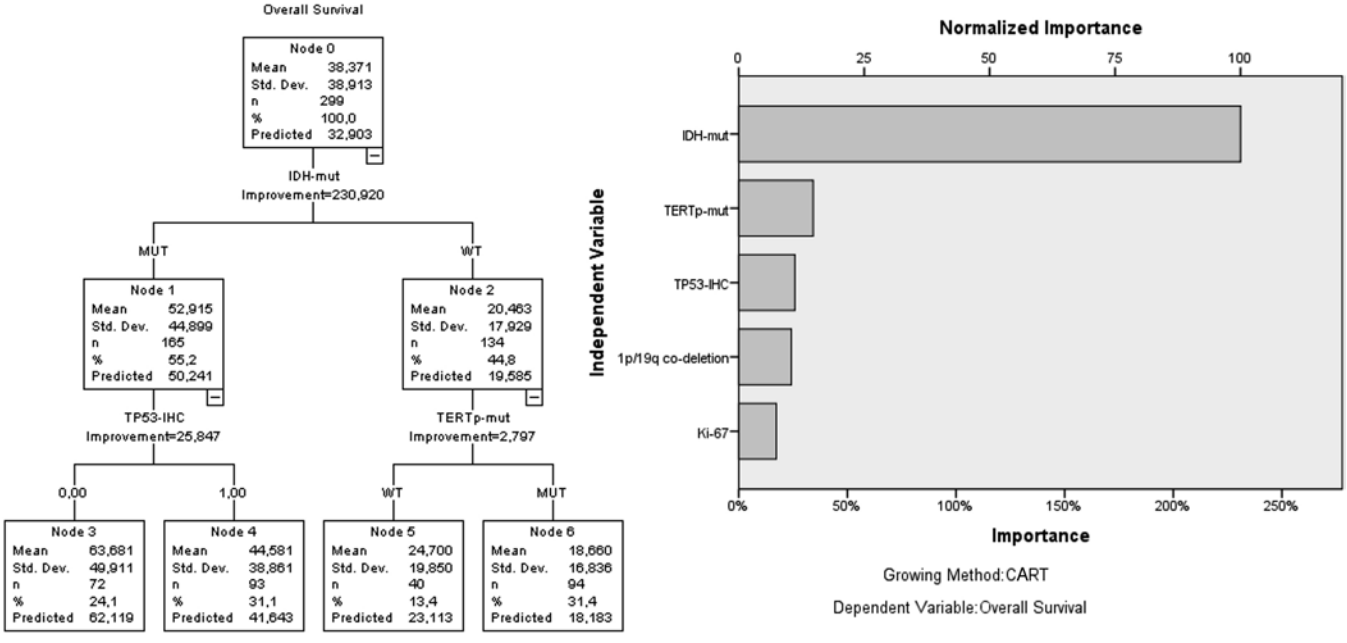


FIG. 7. The same unsupervised CART analysis based on overall survival analysis, as shown in Fig. 6, was performed with patient age and WHO grade as covariates and yielded very similar findings.

molecular subset	double-mutant																			IDH-only										
Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Age & gender	38F	32M	40M	49M	45M	42M	44M	38M	48M	36F	47F	37M	56M	37M	37F	28F	37M	45M	28F	68M	31M	30F	33M	34M	38F	31F	31M	37M	31M	37M
WHO group	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	A	OD	OD	OD	OA	OD	A	OD
WHO grade	II	II	II	II	II	II	II	II	II	II	II	II	II	II	III	III	III	III	III	II	II	II	II	II	II	II	III	III	III	III
1p/19q co-deletion	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
IDH 1/2-mut	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	S	S	G
H3F3A-mut	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TERT-mut	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
ATRX-mut	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
PTEN	0	0	0	0	0	0	0	HE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	HE
EGFR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	xN	XN	0	0	0	xN
PDGFRA	0	0	0	0	0	0	0	0	0	0	HE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	xN	0	0	0
CDKN2A	0	0	0	0	0	0	0	HE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	HE
CDK4	0	0	0	0	0	0	0	0	xN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RB1	0	0	0	0	0	0	0	0	0	HE	HE	HE	HE	HE	0	0	0	0	0	0	0	0	N/A	HO	0	0	N/A	N/A	N/A	HE
Nuclear TP53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1
Ki-67-index	0	0.1	0	0	0	0.1	0	0.3	0.1	0.3	0	0.1	0	0.1	0	0.1	0	0.1	0	0	0	0	0	0.1	0	0	0.3	0	0	0.6
technique of analysis	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	ONC + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG

molecular subset	Double-negative									TERT-only									
Patient	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Age & gender	40F	54F	21M	19M	44M	50M	60M	68F	52F	64M	56F	47M	52F	65M	49M	41M	70F	51M	62F
WHO group	OD	A	A	OD	GBM	GBM	GBM	GBM	GBM	OA	A	A	OD	OA	GBM	GBM	GBM	GBM	GBM
WHO grade	II	II	II	III	IV	IV	IV	IV	IV	III	III	III	III	III	IV	IV	IV	IV	IV
1p/19q co-deletion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH 1/2-mut	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H3F3A-mut	0	0	0	G34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TERT-mut	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
ATRX-mut	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PTEN	0	0	0	0	HE	xN	HO	HO	0	HO	HE	HE	HO	HE	HE	HE	HO	HO	HO
EGFR	0	0	0	HE	1	XN	XN	0	xN	XN	XN	XN	xN	XN	XN	XN	XN	XN	XN
PDGFRA	0	0	0	0	1	0	0	HO	0	0	0	0	HE	0	xN	XN	0	0	0
CDKN2A	0	0	0	0	1	HO	0	0	XN	HO	HO	HO	0	0	HO	HO	HO	HO	HO
CDK4	0	0	0	HE	0	0	XN	0	0	0	0	0	0	0	XN	XN	0	HE	0
RB1	N/A	N/A	N/A	0	1	0	HO	HO	HE	0	0	0	0	0	0	HO	0	HO	0
Nuclear TP53	0	0	1	1	1	1	0	1	1	1	0	1	0	1	1	1	1	1	1
Ki-67-index	0	0	0	0.3	N/A	0.5	0.2	0.5	0.4	0.1	0	0.2	0.1	0.1	0.2	0.4	0.3	0.4	0.3
technique of analysis	MLPA + MSM + SNG	MLPA + MSM + SNG	MLPA + MSM + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG

molecular subset	TERT-only (cont.)																		
Patient	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
Age & gender	68F	58M	66M	85F	82M	67F	60M	59F	61M	49M	58F	43M	62M	51M	30M	51M	48M	62M	58M
WHO group	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM	GBM
WHO grade	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV
1p/19q co-deletion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IDH 1/2-mut	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H3F3A-mut	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TERT-mut	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ATRX-mut	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PTEN	HO	HO	HE	HO	HO	HO	HE	HE	HE	HE	HE	HE	0	0	0	0	0	0	0
EGFR	XN	xN	XN	XN	0	0	XN	XN	XN	xN	xN	xN	xN	xN	xN	XN 1	XN 1	0	0
PDGFRA	0	HO	0	0	0	0	XN	0	0	0	0	0	0	XN	0	xN	0	0	0
CDKN2A	HO	HO	HO	0	0	0	xN	0	0	0	0	HE	HE	0	0	0	HO	xN	0
CDK4	0	HE	0	0	0	0	0	0	XN	XN	0	0	0	XN	0	0	0	0	0
RB1	HO	HO	0	HO	HO	HE	HE	0	0	0	0	0	0	0	0	0	HE	HE	0
Nuclear TP53	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	0	0	1
Ki-67-index	0.1	0.1	0.4	0.4	0.2	N/A	0.3	N/A	N/A	0.1	0.1	N/A	0.5	N/A	0.2	0.5	0.5	N/A	0.2
technique of analysis	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG	NGS + SNG

FIG. 8. This heatmap plots the IDH, TERT, H3.3, and ATRX mutational statuses against most common tyrosine-kinase receptor pathway changes (PTEN, EGFR, and PDGFRA) and key cell-cycle regulators (CDKN2A, CDK4, and RB1) in 68 patients with HDGs. WHO group, WHO grade, TP53 and Ki-67 index statuses, age, and survival data were also included to create a more comprehensive picture. IDH-mut tumors had few tyrosine-kinase or cell-cycle regulator changes. No EGFR, PDGFR, or PTEN mutations were observed in IDH-mut tumors. Only EGFR amplification events were observed in IDH-mut tumors, and they were in the IDH-only subset. The TERT-only subset had the highest incidences of EGFR, PTEN, and cell-cycle regulator changes. At least 4 oncogenic profiles were noted in the double-negative group, which consisted of an ATRX-mut profile that resulted in good overall survival, an ATRX-mut and H3.3 mutant profile that resulted in extremely poor survival, an ATRX-mut profile with receptor tyrosine kinase and PTEN changes that resulted in poor survival, and an ATRX wild-type profile that again resulted in poor survival. This heatmap is meant to be seen as a proof of principle for the fact that the 4 molecular subsets carry different oncogenic profiles, but it does not show a comprehensive analysis. A = astrocytoma; G = IDH1-R132G mutation present; G34 = H3.3-G34V mutation present; H = IDH1-R132H mutation present; HE = heterozygous deletion; HO = homozygous deletion; N/A = not available; OA = oligoastrocytoma; OD = oligodendroglioma; ONC = Affymetrix-OncoScan analysis; MSM = microsatellite marker analysis; NGS = next-generation sequencing; S = IDH1-R132S mutation present; SNG = Sanger sequencing; xN = low-level amplification; XN = high-level amplification.

The Double-Mutant and IDH-Only Groups Differ in Many Respects

In our cohort, we found demographic, anatomical, clinical, and molecular differences between the double-mutant and IDH-only subsets. Both IDH-mut glioma subsets presented most commonly for clinical attention in the 4th decade of life, but the double-mutant tumors were diagnosed approximately 5 years later than those in the IDH-only subset. Double-mutant tumors had a very strong predilection for the frontal lobe, whereas IDH-only tumors were located most commonly around the sylvian fissure in the frontal, insular, or temporal lobes (in decreasing

order of frequency) (Fig. 4). More than two-thirds of the insular gliomas were in the IDH-only subset. This finding is supported by the previously reported rarity of 1p/19q codeletions in insular gliomas.[9] Among all 4 subsets, the double-mutant tumors resulted in the best prognoses, and only within this group was there no correlation between WHO grade and overall survival or between WHO grade and the proliferative index. Overall survival was longer in the double-mutant subset than in the IDH-only subset, despite the short median follow-up time of 42 months. In this cohort, non-R132H IDH-mut were observed more commonly in the IDH-only subset. The higher percentage of nuclear TP53 expression we observed in the IDH-only subset compared with that of the double-mutant subset is in parallel with results in the published literature.[2,3,19] EGFR, platelet-derived growth factor receptor (PDGFR), and PTEN changes (mutations and/or copy-number changes), which are characteristic of pGBMs, were uncommon in IDH-mut gliomas in our cohort, which is consistent with results in previous literature.[1,14,15] We found no EGFR mutations in IDH-mut tumors, but EGFR amplifications were seen in approximately one-fourth of the IDH-only subset tumors. No PTEN mutations were noted in any of the 2 IDH-mut subsets, and copy-number losses were also uncommon. These results might be explained by previous reports of epigenetic deregulation of the PTEN tumor suppressor gene.[21]

The TERT-Only Subset Was Correlated With the Worst Prognosis Among HDGs Independent of WHO Grade

This and other studies have indicated a high incidence of TERTp-mut in pGBMs.[4,10–12] Our findings also established TERT-only HDGs as a subset with molecular and clinical characteristics of pGBMs regardless of the histopathology or WHO grade. Only a minority (14%) of the TERT-only HDGs were WHO Grade II or III, and these tumors were similar to their WHO Grade IV counterparts in age at presentation, Ki-67 index, overall survival, and oncogenic changes. This finding is consistent with the well-established fact that a significant proportion of WHO Grade II and III gliomas have genetic alterations and clinical courses similar to those of GBMs.[3,16] Age is a bad prognosticator for gliomas of all histopathologies and WHO grades.[17] Patients in the TERT-only subset were a median of 2 decades older than those with IDH-mut gliomas and 1 decade older than those in the double-negative subset (Figs. 3 and 4). In the TERT-only subset, the age at presentation, the Ki-67 proliferative index, and overall survival were not significantly different at different WHO grades. Among patients with a WHO Grade IV tumor, those in the TERT-only molecular subset fared worst, with a median overall survival of 21 months; a shorter survival rate was found only in patients with H3.3 mutant adult gliomas, which made up a small fraction of the double-negative tumor subset.[18] Previous studies reported poor prognosis for those with GBMs that have TERTp-mut.[10,13] Our findings indicate that this is not limited to GBMs; carriers of IDH-wt and TERTp-mut are present among diffuse gliomas of all WHO grades, and these tumors persistently result in the worst survival among patients with gliomas of the same WHO grade (Fig. 5). The molecular findings in this TERT-only subset are consistent with the well-established molecular characteristics of pGBMs (with EGFR changes in 86.2%, PTEN changes in 75.9%, and at least 1 change in 1 of the key cell-cycle regulators [CDKN2A, CDK4, or RB1] in 75.9% of tumors). These findings support the notion that regardless of the histopathology and WHO grade, the IDH-wt TERTp-mut signature is correlated with high risk.

The remainder of the IDH-wt gliomas were wild type for both IDH and TERTp and, hence, double negative. These tumors were heterogeneous in their clinical and molecular biological characteristics, which is a hint that this group was composed of a mixture of tumors with various oncogenic programs (Fig. 8). Although most double-negative tumors resulted in a poor prognosis, there were also subsets that resulted in a very favorable prognosis among patients with double-negative HDGs; therefore, we believe that all IDH-wt gliomas should not be lumped together.

Clinical Significance of the Molecular Subsets

The combination of IDH-mut and TERTp-mut statuses turns out to be a good predictor of individual tumor biology. The resulting 4 molecular subsets differed from each other in many respects, as described above. Most important is that these 4 molecular subsets were statistically different in their prognoses, which makes these clinical subsets clinically significant (Fig. 5). Our findings are in parallel with those of other reports previously published by independent groups.[7,10,14] Similarly, multivariate analysis confirmed TERTp-mut as an independent and significant variable (Table 2), despite the fact that TERTp-mut are associated with reciprocal clinical behavior in relation to IDH-mut status (i.e., better survival for patients with IDH-mut and worse survival in those with IDH-wt tumors). Furthermore, unsupervised CART analyses (with overall survival the end point) revealed that TERTp-mut is a significant variable among IDH-wt gliomas (Fig. 6), which held true when age and the degree of anaplasia (WHO grade) were calculated as confounding variables (Fig. 7). It was also reassuring to find that most of the nodes confirmed as significant in these unsupervised decision-tree analyses are well-known biomarkers of gliomas that are associated with specific clinical pictures (Fig. 7).

It is not the intent of this molecular grouping to test or replace the current histopathological classification scheme or to devalue the need for sophisticated molecular profiling of gliomas; rather, we sought only to provide a simple, fast, inexpensive, and reliable means to guide daily practice. Because both TERTp-mut and IDH-mut are gain-of-function mutations (at a few hotspots), they can be demonstrated more reliably and easily than copy-number variations such as the 1p/19q codeletions. Both IDH-mut and TERTp-mut are stable mutations (and hence not lost or gained over the course of the disease), and because both mutations are found diffusely within tumors, they are easy to test using molecular biological means in daily practice and yield reliable and reproducible results. As an example, TERTp-mut reconfirm the presence of 1p/19q codeletions, because all 1p/19q-codeleted tumors also have TERTp-mut.

Conclusions

Four molecular subsets can be defined among HDGs by testing for IDH-mut and TERTp-mut. These subsets have distinct demographic, anatomical, clinical, and prognostic correlations, which makes such classification clinically useful.

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Disclosures

Dr. von Deimling is a patent holder in Ventana/Roche and DIANOVA.

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Conception and design: Özduman. Acquisition of data: Özduman, Akyerli, Yüksel, Can, Erson-Omay, Oktay, Ülgen, Erdemgil, Sav, Günel, Yakıcıer, Pamir. Analysis and interpretation of data: Özduman, Akyerli, Yüksel, Can, Oktay, Coşgun, Ülgen, von Deimling, Günel, Yakıcıer. Drafting the article: Özduman, Akyerli. Critically revising the article: Özduman, Akyerli, Yüksel, Can, Oktay, Coşgun, von Deimling, Günel. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Özduman. Statistical analysis: Özduman, Coşgun. Administrative/technical/material support: Özduman, Erson-Omay, Erdemgil, Sav, Yakıcıer, Pamir. Study supervision: Özduman, Akyerli, Oktay, Yakıcıer, Pamir.

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