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. https://thejns.org/doi/abs/10.3171/2016.11.JNS16973

KEY WORDS glioma; isocitrate dehydrogenase; telomerase; mutation; prognosis; oncology
Recent large integrated analyses have clearly established that oncogenic molecular changes closely correlate with clinical behavior in diffuse gliomas. Most of these oncogenic changes are stochastic, but some recur enough to be used as molecular markers to define clinical subsets. Today, there is compelling evidence that isocitrate dehydrogenase 1 and/or 2 (IDH) mutations (IDH-mut) are indicative of a specific disease group. 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 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. 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. 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. 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. 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.

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 log rank test and plotted using XSTAT 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. IHC was performed for IDH1 (Diovana, H09), Ki-67 (Dako, MIB-1), Phospho-BRAF (GeneTex), PTEN (Neomarkers, 17.A), p16INK4a (Cintec, INK4a), TP53 (Seytek, DO/7), and vascu-
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-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 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. 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. Fluorescence in situ hybridization was not used for 1p/19q testing.

**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)

**TABLE 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WHO Grade at Initial Presentation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>No. of patients</td>
<td>136</td>
<td>47</td>
</tr>
<tr>
<td>Age (median [range]) (yrs)</td>
<td>36.5 (20–77)</td>
<td>37 (18–66)</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>1.52</td>
<td>1.61</td>
</tr>
<tr>
<td>IDH-mut cases (%)</td>
<td>91.2</td>
<td>66</td>
</tr>
<tr>
<td>TERTp-mut cases (%)</td>
<td>39</td>
<td>55.3</td>
</tr>
<tr>
<td>Histology (no. [%])</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>63 (46.3)</td>
<td>16 (34)</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>49 (36)</td>
<td>17 (36.2)</td>
</tr>
<tr>
<td>Oligoastrocytoma</td>
<td>24 (17.7)</td>
<td>14 (29.8)</td>
</tr>
<tr>
<td>GBM</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Localization tumor center (no. [%])</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td>70 (51.5)</td>
<td>30 (63.8)</td>
</tr>
<tr>
<td>Insular</td>
<td>33 (24.3)</td>
<td>4 (8.5)</td>
</tr>
<tr>
<td>Temporal</td>
<td>21 (15.4)</td>
<td>9 (19.2)</td>
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<td>Parietal</td>
<td>10 (7.4)</td>
<td>4 (8.5)</td>
</tr>
<tr>
<td>Occipital</td>
<td>2 (1.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Multifocality or gliomatosis-like multilobar involvement (no. [%])</td>
<td>2 (1.5)</td>
<td>5 (10.6)</td>
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<tr>
<td>Follow-up (median [range]) (mos)</td>
<td>49.5 (1–267)</td>
<td>26 (1–189)</td>
</tr>
<tr>
<td>Deaths during follow-up (no. [%])</td>
<td>12 (8.8)</td>
<td>16 (34.0)</td>
</tr>
</tbody>
</table>
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, 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
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. 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.
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-
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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 immuno-}

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.

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-
IDH and TERTp mutation–based subsets of HDGs

Discussion

TERTp-mut Are Tumor Specific and Stable Over Time

Telomere lengthening is a fundamental hallmark of cancer, and “activating telomerase promoter mutations
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. 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. 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-
mut HDGs and 3.9% of the double-mutant tumors. In a large series reported by Eckel-Passow et al., 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. 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. 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."

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.
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 sub-
sets 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 frequency). The Double-Mutant and IDH-Only Groups Differ in Many Respects.
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 chang-
es), 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 sup-
pression 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 estab-
lished TERT-only HDGs as a subset with molecular and
clinical characteristics of pGBMs regardless of the histo-
pathology 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 clini-
cal courses similar to those of GBMs.3,16 Age is a bad prog-
nosticator 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 presen-
tation, 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 find-
ings indicate that this is not limited to GBMs; carriers of IDH-
w t 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 no-
tion 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 mo-
lecular 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-neg-
ative 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 biol-
ogy. 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 clin-
ically 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 profil-
ing of gliomas; rather, we sought only to provide a sim-
ple, 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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References


Disclosures

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

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