**TERT** promoter mutations contribute to **IDH** mutations in predicting differential responses to adjuvant therapies in WHO grade II and III diffuse gliomas

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**ABSTRACT**

**IDH** mutations frequently occur in WHO grade II and III diffuse gliomas and have favorable prognosis compared to wild-type tumors. However, whether **IDH** mutations in WHO grade II and II diffuse gliomas predict enhanced sensitivity to adjuvant radiation (RT) or chemotherapy (CHT) is still being debated. Recent studies have identified recurrent mutations in the promoter region of telomerase reverse transcriptase (**TERT**) in gliomas. We previously demonstrated that **TERT** promoter mutations may be promising biomarkers in glioma survival prognostication when combined with **IDH** mutations. This study analyzed **IDH** and **TERT** promoter mutations in 295 WHO grade II and III diffuse gliomas treated with or without adjuvant therapies to explore their impact on the sensitivity of tumors to genotoxic therapies. **IDH** mutations were found in 216 (73.2%) patients and **TERT** promoter mutations were found in 112 (38%) patients. In multivariate analysis, **IDH** mutations (p < 0.001) were independent prognostic factors for PFS and OS in patients receiving genotoxic therapies while **TERT** promoter mutations were not. In univariate analysis, **IDH** and **TERT** promoter mutations were not significant prognostic factors in patients who did not receive genotoxic therapies. Adjuvant RT and CHT were factors independently impacting PFS (RT p = 0.001, CHT p = 0.026) in **IDH** mutated WHO grade II and III diffuse gliomas but not in **IDH** wild-type group. Univariate and multivariate analyses demonstrated **TERT** promoter mutations further stratified **IDH** wild-type WHO grade II and III diffuse gliomas into two subgroups with different responses to genotoxic therapies. Adjuvant RT and CHT were significant parameters influencing PFS in the **IDH** wt/**TERT** mut subgroup (RT p = 0.015, CHT p = 0.015) but not in the **IDH** wt/**TERT** mut subgroup.
INTRODUCTION

Diffuse gliomas are the most common primary malignant brain tumors with the propensity to infiltrate adjacent brain parenchyma [1]. According to The World Health Organization (WHO), based on histological criteria, diffuse gliomas are categorized into astrocytoma, oligodendroglioma and oligoastrocytoma, and graded from grade II to IV [2]. Among astrocytic glioma, also known as glioblastoma multiforme (GBM), patients with grade IV tumors have relatively better but variable survivals than patients with grade II and III tumors. Due to their variable prognosis and difficulties in designing and evaluating clinical trials in WHO grade II and III diffuse gliomas, treatment strategies on these gliomas are still controversial [3–5].

Recently, molecular biomarkers have become important in the classification of WHO grade II and III diffuse gliomas and prediction of survival and response to treatment. Chromosome 1p/19q codeletion has been associated with favorable clinical outcome and enhanced chemoradiosensitivity in oligodendroglial tumors [6–8]. Mutations in isocitrate dehydrogenase 1 (IDH1) and IDH2 have been discovered in the majority of WHO grade II and III diffuse gliomas and secondary GBM [9–11]. Furthermore, there is strong evidence that patients with IDH mutated gliomas across all tumor grades exhibit better overall survivals compared to their wild-type counterparts [11–15]. Far less certain is whether this survival benefit can be explained by improved response to adjuvant genotoxic therapies like radiation therapy (RT) or chemotherapy (CHT) or is attributable to differences in intrinsic tumor behavior. While some prospective and retrospective studies have demonstrated greater response rates to adjuvant therapies and longer progression-free survival (PFS) in the IDH mutated subset of WHO grade II and III diffuse gliomas [14, 15], others have failed to observe the same findings [12, 13].

Frequent mutations in the promoter region of telomerase reverse transcriptase (TERT) are detected in various types of tumors, including gliomas [16–18]. The TERT gene encodes the catalytic subunit of telomerase, an enzyme that elongates telomeres in cells, and prevents chromosomal degradation from multiple rounds of mitosis [19, 20]. Somatic TERT promoter mutations, most commonly being C228T and C250T, generate a new binding site (5’-TTCC-3’) for E-twenty-six (ETS) transcription factors, which increases TERT gene transcription and indicates that TERT mutations contribute to tumorigenesis via telomerase activation [16, 17, 20, 21]. In glioma genomics, TERT promoter mutations are frequently found in over 70% of primary GBM and oligodendrogliomas, and less frequently in oligoastrocytomas and WHO grade II and III diffuse astrocytomas [18, 19, 21]. Furthermore, we and others showed that TERT promoter mutations in combination with IDH mutation, are promising prognostic indicators of survival in glioma [17, 19, 20, 22]. The role of TERT promoter mutations in predicting responses to adjuvant genotoxic therapies in gliomas remains relatively unexplored. In this study, we performed mutational analysis for TERT promoter and IDH in a large series of WHO grade II and III diffuse gliomas and summarized the patient outcome in response to adjuvant therapies.

RESULTS

Clinical and pathological characteristics of the cohort

Out of 295 total patients, there were 179 males and 116 females in the series with a male to female ratio of 1.54:1. The mean age at diagnosis was 42.6 ± 12.1 years. The mean duration of follow-up was 9.6 years. All patients underwent tumor resection: 153 patients had total resection, 103 patients had subtotal resection, and the extent of resection in the remaining 39 cases could not be retrieved or evaluated based on available data. 231 patients (78.3%) received postoperative RT and 180 patients (61%) received CHT. In total, 246 patients (83.4%) were receiving postoperative RT, radiation doses and fractions were available in 174 cases (75.3%). The radiation doses ranged from 52.0 Gy to 66.4 Gy with a mean dose of 59.1 Gy. In the 180 cases receiving postoperative CHT, chemotherapy strategies were available in 141 cases (78.3%). The chemotherapy protocols administered included temozolomide (TMZ, 43.3%) and alkylating agents such as semustine (MeCCNU, 39.0%), fotemustine (FCNU, 12.1%) and nimustine (ACNU, 5.7%).
IDH mutations were found in 216 (73.2%) cases while mutations in the TERT promoter were found in 112 (38%) cases. Among the 216 cases with IDH mutations, there were 206 cases harboring IDH1 mutations and 10 cases harboring IDH2 mutations. Among the 112 TERT promoter mutated tumors, C228T mutations were observed in 76 (67.9%) cases and C250T mutations were detected in 36 (32.1%) cases. Chromosome 1p/19q codeletion was detected in 73 (24.7%) WHO grade II and III diffuse gliomas. These data are shown in Table 1.

### Table 1: Clinical, pathological, and treatment characteristics of the patient cohort (n = 295)

| Factors                        | No. of cases | Percentage (%) |
|--------------------------------|--------------|----------------|
| Sex                            |              |                |
| Male                           | 179          | 60.7           |
| Female                         | 116          | 39.3           |
| Age                            |              |                |
| Mean                           | 42.6         |                |
| Standard deviation             | 12.1         |                |
| WHO grade                      |              |                |
| Grade II                       | 188          | 63.7           |
| Grade III                      | 107          | 36.3           |
| Histology                      |              |                |
| Astrocytic                     | 178          | 60.3           |
| Oligodendroglial/Oligoastrocytic | 117        | 39.7           |
| IDH mutation                   |              |                |
| Mutant                         | 216          | 73.2           |
| Wild-type                      | 79           | 30             |
| TERT promoter mutation         |              |                |
| Mutant                         | 112          | 38             |
| Wild type                      | 183          | 62             |
| 1p/19q codeletion              |              |                |
| Yes                            | 73           | 24.7           |
| No                             | 222          | 75.3           |
| Extent of resection*           |              |                |
| Complete                       | 153          | 59.8           |
| Incomplete                     | 103          | 40.2           |
| Primary RT                     |              |                |
| Yes                            | 231          | 78.3           |
| No                             | 64           | 21.7           |
| Primary CHT                    |              |                |
| Yes                            | 180          | 61             |
| No                             | 115          | 39             |

*Extent of resection in 39 cases was unavailable.
**IDH** mutations, not **TERT** promoter mutations, are independent prognostic factors in response to genotoxic therapies

We divided the entire series into two groups, based on postoperative therapies. Group A (n = 246) patients received adjuvant postoperative genotoxic therapies in the form of RT, CHT, or both and Group B (n = 49) patients had no additional treatment after surgery. Univariate analysis on Group A revealed patients with **IDH** mutated WHO grade II and III diffuse gliomas had significantly better PFS (p < 0.001) and OS (p < 0.001) than those with **IDH** wild-type WHO grade II and III diffuse gliomas (Fig. 1A-1B and Table S1). Multivariate analysis demonstrated **IDH** mutations (PFS p < 0.001, OS p < 0.001) and two other putative prognostic factors (WHO grade and extent of resection) to be independent predictors of PFS and OS in Group A (Table 2). However, the prognostic value of **IDH** mutation status was lost for PFS and OS in both univariate and multivariate analysis in Group B (Fig 1E-1F, Table 2, and Table S1). Both univariate and multivariate analyses revealed no prognostic significance for **TERT** promoter mutation status in both Group A and Group B (Fig. 1C-1D, 1G-1H, Table 2, and Table S1).

RT and CHT are clinical factors independently impacting the PFS in **IDH** mutated WHO grade II and III diffuse gliomas but not in **IDH** wild-type subgroup

We further investigated the impact of genotoxic therapies on PFS in **IDH** mutated, **IDH** wild-type, **TERT** promoter mutated and **TERT** promoter wild-type WHO grade II and III diffuse gliomas. In univariate analysis, the prognostic significance of adjuvant genotoxic therapies (RT p < 0.001, CHT p < 0.001) on PFS was observed in the **IDH** mutated subgroup (n = 216), but not in the **IDH** wild-type subgroup (n = 79). Subsequent multivariate analysis demonstrated that WHO grade (p = 0.038), extent of resection (p < 0.001), RT (p = 0.001), and CHT (p = 0.026) were independent prognostic factors for PFS in **IDH** mutated WHO grade II and III diffuse gliomas (Fig. 2A-2B, Table 3, and Table S2). However, in **IDH** wild-type tumors, multivariate analysis showed that only WHO grade (p < 0.001) and extent of resection (p = 0.077) were of prognostic significance (Fig. 2C-2D, Table 3, and Table S2). As for **TERT** promoter mutated and wild-type WHO grade II and III gliomas, univariate analysis showed that RT and CHT were prognostic factors, significantly influencing PFS in the two subgroups.

![Image](image_url)

**Figure 1:** Kaplan-Meier survival curves (univariate analysis) of **IDH** and **TERT** promoter mutations for OS and PFS in WHO grade II and III diffuse gliomas with and without adjuvant therapies. **IDH** mutations were associated with significantly longer OS A. and PFS B. in WHO grade II and III diffuse gliomas treated with genotoxic therapies after surgery but **TERT** promoter mutations were not significantly associated with longer PFS C. and were significantly associated with longer OS D. In the absence of genotoxic therapies after surgery, **IDH** mutations E and F. and **TERT** promoter mutations G and H. were not associated with significantly longer OS and PFS in WHO grade II and III diffuse gliomas.
Mutations in TERT promoter categorize IDH wild-type WHO grade II and III diffuse gliomas into two subgroups with different responses to adjuvant genotoxic therapies

We further combined TERT promoter and IDH mutations to stratify WHO grade II and III diffuse gliomas into four subgroups: IDH mut/TERT mut (n = 92), IDH mut/TERT wt (n = 125), IDH wt/TERT mut (n = 20) and IDH wt/TERT wt (n = 58). In IDH mutated WHO grade II and III diffuse gliomas (IDH mut/TERT mut and IDH mut/TERT wt), Genotoxic therapies significantly influenced PFS (Fig. 3A-3D, Table 4, and Table S3), with the exception that the administration of post-operative CHT did not reach statistical significance in multivariate analysis in the IDH mut/TERT mut subgroup (p = 0.369, Table 4). Upon univariate analysis of IDH wild-type WHO grade II and III diffuse gliomas, RT and CHT were significant parameters impacting the PFS in the IDH wt/TERT mut subgroup (RT p = 0.015, CHT p = 0.015) (Fig. 3E-3F, Table S3), but not in the IDH wt/TERT wt subgroup (RT p = 0.925, CHT p = 0.403) (Fig. 3G-3H, Table S3). Subsequent multivariate analysis confirmed that adjuvant therapies were not parameters significantly influencing PFS in the IDH wt/TERT wt subgroup (RT p = 0.598, CHT p = 0.741) (Table 4). Due to the limited sample size (n = 20) of the IDH wt/TERT mut subgroup, multivariate analysis was not performed.

We further categorized IDH mutated WHO grade II and III gliomas into an IDH mutated, 1p/19q codeleted subgroup and an IDH mutated, 1p/19q intact subgroup. On multivariate analysis, only RT was an independent factor impacting the PFS of the IDH mutated, 1p/19q codeleted subgroup (p = 0.012, Table 4).
Figure 2: Kaplan-Meier survival curves (univariate analysis) of adjuvant therapies for PFS in IDH mutated, IDH wild-type, TERT promoter mutated and TERT promoter wild-type WHO grade II and III diffuse gliomas. In IDH mutated WHO grade II and III diffuse gliomas, patients who received postoperative RT A. and CHT B. had significantly better PFS than those who did not. However, in IDH wild-type WHO grade II and III diffuse gliomas, PFS of patients who received postoperative RT C. or CHT D. did not differ significantly from PFS of those who did not. As for TERT promoter mutated and TERT promoter wild-type WHO grade II and III diffuse gliomas, patients who received postoperative RT E, G. and CHT F, H. had significantly better PFS than those who did not.

Table 3: Multivariate analysis of clinicopathological factors for PFS in patients with IDH mutated (n = 215) and IDH wild-type (n = 77) WHO II and III diffuse gliomas

| Variables                        | PFS     | OS       |
|----------------------------------|---------|----------|
|                                  | HR(95%CI) | p-value | HR(95%CI) | p-value |
| **IDH mut**                      |         |          |
| Age                              | 1.011(0.990–1.031) | 0.302 | 1.019(0.996–1.043) | 0.105 |
| WHO grade (Grade II vs III)      | 1.720(1.030–2.873) | **0.038** | 1.884(1.059–3.354) | **0.031** |
| Complete resection (Yes vs. No)  | 2.399(1.559–3.691) | <**0.001** | 1.960(1.212–3.169) | **0.006** |
| RT (Yes vs. No)                  | 2.345(1.409–3.904) | **0.001** | 2.001(1.136–3.526) | **0.016** |
| CHT (Yes vs. No)                 | 1.646(1.062–2.552) | **0.026** | 1.295(0.781–2.149) | 0.316 |
| 1p/19q codeletion (Yes vs. No)   | 1.747(1.109–2.751) | **0.016** | 2.142(1.270–3.612) | **0.004** |
| **IDH wt**                       |         |          |
| Age                              | 1.028(1.005–1.052) | **0.017** | 1.023(0.999–1.048) | 0.056 |
| WHO grade (Grade II vs III)      | 4.030(1.978–8.208) | <**0.001** | 4.113(1.910–8.858) | <**0.001** |
| Complete resection (Yes vs. No)  | 2.398(0.908–6.330) | 0.077 | 2.825(1.072–7.445) | **0.036** |

(Continued)
### IDH, TERT promoter and 1p/19q codeletion status

| Variables | PFS | OS |
|-----------|-----|----|
|           | HR(95%CI) | p-value | HR(95%CI) | p-value |
| RT (Yes vs. No) | 1.349(0.698–2.607) | 0.373 | 1.367(0.701–2.667) | 0.36 |
| CHT (Yes vs. No) | 1.211(0.440–3.329) | 0.711 | 0.903(0.339–2.400) | 0.837 |
| Age | 1.057(1.027–1.088) | <0.001 | 1.085(1.047–1.125) | <0.001 |
| WHO grade (Grade II vs III) | 2.214(1.150–4.262) | 0.017 | 2.413(1.141–5.104) | 0.021 |
| Complete resection (Yes vs. No) | 2.387(1.307–4.358) | 0.005 | 2.206(1.027–3.998) | 0.042 |
| RT (Yes vs. No) | 2.211(1.118–4.371) | 0.022 | 2.292(1.055–4.978) | 0.036 |
| CHT (Yes vs. No) | 1.000(0.528–1.896) | 1 | 0.577(0.271–1.230) | 0.155 |
| 1p/19q codeletion (Yes vs. No) | 1.639(0.900–2.985) | 0.107 | 2.209(1.028–4.004) | 0.041 |
| Age | 1.022(1.005–1.039) | 0.011 | 1.022(1.003–1.041) | 0.02 |
| WHO grade (Grade II vs III) | 2.87(1.755–4.692) | <0.001 | 3.282(1.930–5.579) | <0.001 |
| Complete resection (Yes vs. No) | 1.967(1.241–3.118) | 0.004 | 1.856(1.128–3.054) | 0.015 |
| RT (Yes vs. No) | 1.477(0.896–2.434) | 0.126 | 1.551(0.917–2.622) | 0.101 |
| CHT (Yes vs. No) | 1.593(0.991–2.561) | 0.055 | 1.350(0.810–2.250) | 0.249 |
| 1p/19q codeletion (Yes vs. No) | 2.085(0.898–4.844) | 0.087 | 2.964(1.068–8.228) | 0.037 |
| Age | 1.045(1.003–1.089) | 0.036 | 1.066(1.010–1.125) | 0.021 |
| WHO grade (Grade II vs III) | 1.891(0.681–5.253) | 0.221 | 2.448(0.777–7.708) | 0.126 |
| Complete resection (Yes vs. No) | 2.908(1.258–6.723) | 0.013 | 3.167(1.207–8.310) | 0.019 |
| RT (Yes vs. No) | 2.404(0.953–6.066) | 0.063 | 1.471(0.460–4.702) | 0.515 |
| CHT (Yes vs. No) | 1.526(0.681–3.419) | 0.304 | 0.798(0.277–2.302) | 0.677 |
| 1p/19q codeletion (Yes vs. No) | 1.054(0.416–2.667) | 0.912 | 0.810(0.267–2.460) | 0.71 |

*p values in bold were considered statistically significant

IDH mut: IDH mutant; IDH wt: IDH wild-type; HR: hazard ratio; CI: confidence interval; RT: radiation therapy; CHT: chemotherapy.

We also explored the roles of different chemotherapy strategies in the four subgroups defined by IDH and TERT promoter status. In the IDH mut/TERT mut and IDH mut/TERT wt subgroups, the PFS of patients receiving alkylating agents (MeCCNU, FCNU and ACNU) was significantly longer than those who did not receive chemotherapy (IDH mut/TERT mut *p* = 0.007, IDH mut/TERT wt *p* < 0.001), while the PFS of patients receiving TMZ as chemotherapy did not differ significantly from that of patients receiving alkylating agents (Figure S1, Table S4). As for the IDH wt/TERT mut and IDH wt/TERT wt subgroups, there was no significant difference between the PFS of patients who received TMZ, alkylating agents, and no chemotherapy (Figure S1, Table S4).
Figure 3: Kaplan-Meier survival curves (univariate analysis) of adjuvant therapies for PFS in subgroups of WHO grade II and III diffuse gliomas defined by IDH and TERT promoter mutations. In IDH mut/TERT mut A and B, IDH mut/TERT wt C and D, and IDH wt/TERT mut tumors E and F, patients who received post-operative RT and CHT had significantly better PFS than those who did not. However, in IDH wt/TERT wt tumors, PFS of patients who received postoperative RT G, and CHT H, did not differ significantly from PFS of those who did not.

Table 4: Multivariate analysis of clinicopathological factors for PFS in subgroups of WHO II and III diffuse gliomas as defined by IDH and TERT promoter mutations

| IDH/1p/19q codeletion/TERT promoter status | PFS | OS |
|-------------------------------------------|-----|----|
| Variables                                 | HR(95%CI) | p-value | HR(95%CI) | p-value |
| All patients                              |     |     |     |     |     |
| Age                                       | 1.028(1.015–1.042) | <0.001 | 1.031(1.016–1.047) | <0.001 |
| WHO grade (Grade II vs II)                | 2.549(1.733–3.749) | <0.001 | 2.802(1.838–4.271) | <0.001 |
| Complete resection (Yes vs. No)           | 2.026(1.413–2.906) | <0.001 | 1.848(1.241–2.751) | 0.002 |
| RT (Yes vs. No)                           | 1.671(0.968–2.503) | 0.073 | 1.626(1.059–2.496) | 0.026 |
| CHT (Yes vs. No)                          | 1.337(0.917–1.951) | 0.131 | 1.126(0.741–1.710) | 0.578 |
| IDH mutation (Yes vs. No)                 | 1.456(0.961–2.205) | 0.076 | 1.803(1.176–2.763) | 0.007 |
| 1p/19q codeletion (Yes vs. No)             | 1.613(0.999–2.605) | 0.051 | 1.881(1.088–3.252) | 0.024 |
| TERT promoter mutation (Yes vs. No)       | 1.139(0.756–1.716) | 0.533 | 1.158(0.746–1.796) | 0.513 |
| IDH mut/TERT mut                          |     |     |     |     |     |
| Age                                       | 1.041(1.005–1.079) | 0.024 | 1.083(1.032–1.137) | 0.001 |
| WHO grade (Grade II vs III)               | 2.016(0.860–4.728) | 0.107 | 2.114(0.761–5.871) | 0.151 |

(Continued)
| IDH/1p/19q codeletion/TERT promoter status | PFS | OS |
|---|---|---|
| Variables | HR(95%CI) | p-value | HR(95%CI) | p-value |
| Complete resection (Yes vs. No) | 2.628(1.273–5.423) | **0.009** | 1.843(0.775–4.380) | 0.167 |
| RT (Yes vs. No) | 2.285(1.010–5.169) | **0.047** | 1.976(0.725–5.386) | 0.183 |
| CHT (Yes vs. No) | 1.411(0.666–2.988) | 0.369 | 0.690(0.250–1.902) | 0.473 |
| 1p/19q codeletion (Yes vs. No) | 0.829(0.395–1.736) | 0.618 | 0.878(0.353–2.183) | 0.779 |
| **IDH mut/TERT wt** | | | |
| Age | 1.008(0.981–1.036) | 0.581 | 1.013(0.983–1.045) | 0.393 |
| WHO grade (Grade II vs III) | 1.838(0.923–3.659) | **0.083** | 2.051(0.973–4.320) | 0.059 |
| Complete resection (Yes vs. No) | 2.718(1.522–4.854) | **0.001** | 0.047(1.009–3.709) | **0.018** |
| RT (Yes vs. No) | 2.540(1.258–5.126) | **0.009** | 1.917(0.933–3.937) | 0.076 |
| CHT (Yes vs. No) | 1.952(1.105–3.450) | **0.021** | 1.617(0.855–3.059) | 0.14 |
| 1p/19q codeletion (Yes vs. No) | 1.984(0.823–4.782) | 0.127 | 2.849(0.986–8.229) | 0.053 |
| **IDH mut/1p/19q codeletion** | | | |
| Age | 1.045(1.003–1.089) | **0.036** | 1.061(1.010–1.115) | **0.019** |
| WHO grade (Grade II vs III) | 1.891(0.681–5.253) | 0.221 | 2.362(0.837–6.664) | 0.104 |
| Complete resection (Yes vs. No) | 2.908(1.258–6.723) | **0.013** | 3.118(1.310–7.421) | **0.01** |
| RT (Yes vs. No) | 2.404(0.953–6.066) | 0.063 | 1.069(0.387–2.951) | 0.898 |
| CHT (Yes vs. No) | 1.526(0.681–3.419) | 0.304 | 0.852(0.334–2.174) | 0.737 |
| TERT promoter mutation (Yes vs. No) | 1.054(0.416–2.667) | 0.912 | 0.890(0.349–2.267) | 0.807 |
| **IDH mut/1p/19q intact** | | | |
| Age | 1.011(0.981–1.042) | 0.476 | 1.016(0.982–1.050) | 0.373 |
| WHO grade (Grade II vs III) | 2.474(1.072–5.709) | **0.034** | 2.402(0.998–5.783) | 0.051 |
| Complete resection (Yes vs. No) | 3.050(1.513–6.145) | **0.002** | 2.576(1.158–5.727) | **0.02** |
| RT (Yes vs. No) | 3.207(1.293–7.951) | **0.012** | 3.187(1.250–8.125) | **0.015** |
| CHT (Yes vs. No) | 1.807(0.895–3.652) | **0.099** | 1.830(0.832–4.025) | 0.133 |
| TERT promoter mutation (Yes vs. No) | 10.141(1.376–74.740) | **0.023** | 2.782(0.753–9.166) | 0.265 |
| **IDH wt/TERT wt** | | | |
| Age | 1.014(0.986–1.042) | 0.325 | 1.010(0.981–1.040) | 0.509 |
| WHO grade (Grade II vs III) | 5.114(2.075–12.602) | **<0.001** | 5.460(2.083–14.317) | **0.001** |

(Continued)
DISCUSSION

Previously, we evaluated the frequency, distribution, and prognostic significance of TERT promoter mutations when combined with IDH mutations in WHO grade II to IV gliomas [19, 22]. The present study focused on the roles of TERT promoter mutations and IDH mutations in predicting responses to adjuvant genotoxic therapies. The results presented here confirmed that WHO grade II and III diffuse gliomas with IDH mutation are more sensitive to DNA-damaging therapies. Furthermore, tumors with TERT promoter mutations could further stratify IDH wild-type WHO grade II and III diffuse gliomas into two subsets with different responses to genotoxic therapies.

The discovery of IDH mutations is one of the most important findings in glioma genomics in recent years. The fact that IDH mutations confer a favorable prognosis for both PFS and OS in gliomas has been well established by numerous studies [3, 13–15, 23–26]. However, far fewer studies have addressed whether the superior PFS and OS of IDH mutated gliomas are due to less aggressive intrinsic tumor biology or due to improved sensitivity to genotoxic therapies. Dubbink et al observed no relationship between improved response to temozolomide chemotherapy and IDH mutations in progressive low-grade gliomas [13]. Likewise, a report from EORTC gave no indication that the presence of IDH mutations predicted improved response to procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-L-nitrosourea, and vincristine (PCV) chemotherapy in WHO III anaplastic oligodendrogliomas and hypothesized that the favorable survival in IDH mutated gliomas was primarily due to a less aggressive biological behavior, rather than enhanced chemotherapeutic sensitivity [12]. Other studies, however, have drawn discrepant conclusions. Survival analysis of patients who never received post-operative adjuvant RT or CHT may be the closest approximation of the natural course of glioma and has been proposed to be the ideal model for studying the impact of IDH mutations on clinical outcomes [27].

Houillier et al. studied 171 patients without adjuvant therapies until first progression and demonstrated that spontaneous PFS did not differ significantly in patients with IDH mutated wild-type low-grade gliomas [14]. Hartmann et al. demonstrated similar results in an analysis of PFS in 89 patients with low-grade gliomas who received no additional genotoxic therapy after surgery [27]. More recently, in a study based on long-term follow-up data of RTOG trial 9402, IDH mutations were identified as a predictive biomarker that conferred survival benefit to patients with WHO III anaplastic oligodendrogliomas receiving PCV chemotherapy, but not in those without PCV chemotherapy [15]. In our cohort comprising patients who received (Group A) and did not receive (Group B) genotoxic therapies after surgery, we showed in univariate and multivariate analyses that IDH mutations were independent biomarkers significantly influencing PFS and OS in Group A but not in Group B (Table 2). Further analysis revealed that genotoxic therapies were independent clinical parameters impacting PFS in IDH mutated WHO grade II and III diffuse gliomas but not in IDH wild-type subgroups (Table 3). Moreover, multivariate analysis of the entire cohort and multivariate analyses of IDH mutation status revealed that both genotoxic and IDH mutation status lost significance in the Cox regression model, demonstrating these variables are not independent prognostic factors and may in fact interact with one another (Table 4). These results raise the possibility that the favorable effects observed based on IDH mutation status and administration of adjuvant genotoxic therapy may be co-dependent. Thus, our study reinforces previous data, substantiating the hypothesis that IDH mutations confer improved survival due to enhanced chemotherapeutic sensitivity rather than from a more benign, intrinsic tumor biology.

TERT promoter mutations frequently occur across all types of gliomas, suggesting regulation of telomere elongation by telomerase may play an important role in the pathogenesis of gliomas [17, 18, 21]. Interest in the

| Variables                  | PFS HR(95%CI)   | p-value | OS HR(95%CI)   | p-value |
|---------------------------|----------------|---------|----------------|---------|
| Complete resection (Yes vs. No) | 2.753(0.870–8.711) | 0.085   | 3.115(0.966–10.041) | 0.057   |
| RT (Yes vs. No)           | 1.243(0.553–2.791) | 0.598   | 1.442(0.629–3.310) | 0.387   |
| CHT (Yes vs. No)          | 1.230(0.361–4.191) | 0.741   | 0.894(0.272–2.937) | 0.854   |

*p values in bold were considered statistically significant
IDH mut: IDH mutant; IDH wt: IDH wild-type; TERT mut: TERT promoter mutant; TERT wt: TERT promoter wild-type; HR: hazard ratio; CI: confidence interval; RT: radiation therapy; CHT: chemotherapy.
The clinicopathological value of TERT promoter mutations has grown considerably in recent years. We are the first group to explore the potentially predictive role of TERT promoter mutations on response to genotoxic therapies in gliomas (Table 3). Notably, we found that TERT promoter mutations, in combination with IDH mutations, contribute to a survival benefit. We previously identified TERT promoter mutations as a favorable prognostication in tumors with IDH mutation, 1p/19q intact and an aggressive subset of tumors with wild-type IDH [22]. At that time, we also reported in a separate study utilizing a different cohort of WHO grade II to IV gliomas that IDH and TERT promoter mutations categorized four distinct subgroups in grade III and grade IV gliomas [19]. In this study, we sought to investigate the sensitivities to genotoxic therapies in subgroups with different IDH and TERT promoter mutations. We demonstrated that adjuvant therapies (RT and CHT) are significant clinical factors influencing PFS in three subgroups (IDH mut/TERT mut, IDH mut/TERT wt, IDH wt/TERT mut), but not in the IDH wt/TERT wt subgroup. These findings suggest that TERT promoter mutations may further stratify IDH wild-type gliomas, a subset previously considered to be less sensitive to adjuvant therapies than IDH mutated gliomas, into two subgroups with differential responses to genotoxic therapies. IDH wt/TERT mut gliomas were previously shown to exhibit a dismal prognosis and were most prevalent in primary GBM [19, 20]. Nonetheless, in our study, the IDH wt/TERT mut WHO grade II and III diffuse gliomas were more sensitive than IDH wt/TERT wt tumors to genotoxic therapies, raising the possibility that the intrinsic biological behaviors of this subtype might be more aggressive than others and that intensified treatment may be justified. Interestingly, we found that IDH wt/TERT wt WHO grade II and III diffuse gliomas did not respond to genotoxic therapies as well as other gliomas with either IDH mutations or TERT promoter mutations. While further investigations are needed, this finding supported previous work postulating that IDH wt/TERT wt WHO grade II and III diffuse gliomas represent a biologically and clinically distinct group [19]. Our data also suggested that the therapeutic efficacy of current genotoxic therapies in this subgroup was limited.

Recently, Suzuki et al. categorized grade II and grade III gliomas into three distinct subtypes characterized by IDH mutations and 1p/19q codeletion [29]. Type I tumors were defined by the presence of both IDH mutations and 1p/19q codeletion. Type II tumors comprised of those tumors with IDH mutations and without 1p/19q codeletion, and type III tumors were IDH wild-type grade II and grade III gliomas. The three subsets were demonstrated to have distinctly genetic alterations and clinical behaviors. When we stratified our patient cohort into these three subsets, we found that only RT was an independent factor significantly influencing the PFS in type II (IDH mutated, 1p/19q intact) tumors (Table 4). Taking this into consideration with our findings that genotoxic therapies significantly prolonged PFS in IDH mut/TERT mut and IDH mut/TERT wt subgroups, we hypothesized that the IDH mutation may be a more important predictive marker than 1p/19q codeletion and TERT promoter mutations. Genotoxic therapies were independent prognostic factors in IDH mutated grade II and grade III gliomas but lost significance in IDH mutated subgroups divided by the status of 1p/19q codeletion and TERT promoter mutations. Furthermore, multivariate analysis on grade II and grade III gliomas with 1p/19q codeletion demonstrated that neither RT nor CHT was a significant, independent prognostic factor (Table 3). As such, IDH mutations may be predictive markers for genotoxic therapies in grade II and grade III gliomas as a whole, while 1p/19q codeletion status may only be predictive in certain histology types such as oligodendroglial and oligoastrocytic gliomas. Lastly, our study identified a potential role for TERT promoter mutations in classifying IDH wild-type tumors into two subsets with differential sensitivities to adjuvant genotoxic therapies as previously discussed.

There are several limitations and weaknesses in the present study. Although the total number of this patient cohort was relatively large, distribution among each subgroup was uneven and thus multivariate analysis could not be performed in one subgroup. Secondly, since the study was retrospective, protocols of adjuvant genotoxic therapies were not consistent. Therefore, the results of the present study should be used as a guide for future confirmation with standardized treatment protocols or clinical trials.

In conclusion, our study demonstrated that IDH mutated WHO grade II and III diffuse gliomas exhibit better PFS and OS than IDH wild-type subgroups when patients received genotoxic therapies post-operatively and that this survival benefit was lost when genotoxic therapies after surgery were absent. Our data also revealed that genotoxic therapies were independent favorable factors significantly influencing the outcome in IDH mutated WHO grade II and III diffuse gliomas but not in IDH wild-type tumors. Importantly, TERT promoter mutations stratified IDH wild-type WHO grade II and III diffuse gliomas into two subgroups with differential responses to adjuvant therapies. Overall, our study supports the role for TERT promoter mutations to complement IDH mutations in prognosticating WHO grade II and III diffuse gliomas in clinical practice.

**MATERIALS AND METHODS**

**Patients and tissue samples**

This study was approved by the Ethics Committee of Huashan Hospital, Fudan University and the New Territories East Cluster-Chinese University of Hong Kong Ethics Committee. A total of 295 patients pathologically diagnosed...
with WHO grade II and III diffuse gliomas in Huashan Hospital (Shanghai, China) and Prince of Wales Hospital (Hong Kong, China) between January 1990 and December 2013 were included in this study. The cohort of the study was partly overlapped with previous study [22]. Formalin-fixed paraffin embedded (FFPE) tissues, clinical data and follow-up data were analyzed. All cases were stained with hematoxylin & eosin (H&E) and centrally reviewed according to the 2007 World Health Organization (WHO) criteria by two senior neuropathologists (H.K.N. and Y.W.) [2]. In the series, there were 96 diffuse astrocytomas (WHO grade II; AII), 82 anaplastic astrocytomas (WHO grade III; AIII), 29 oligodendrogliomas (WHO grade II; OII), 10 anaplastic oligodendrogliomas (WHO grade III; AOIII), 63 oligoastrocytomas (WHO grade II; OAI) and 15 anaplastic oligoastrocytomas (WHO grade III; AOAI). Clinical and follow-up data were collected from medical charts, central radiological systems of the hospitals, out-patient clinics and telephone interviews. Progression-free survival (PFS) was measured from the date of pathological diagnosis to the date of initial tumor recurrence or progression (radiologically or pathologically). Radiological recurrence or progression was confirmed by magnetic resonance imaging (MRI) or computed tomography (CT). Pathological progression was confirmed by pathologists after second operation. Overall survival (OS) was measured from the date of pathological diagnosis to the date of death or last follow-up. The date of death was determined by cancellation of social ID.

Analysis of molecular markers

Tumor DNA was extracted from FFPE tissue samples in all 295 cases in this cohort. Mutational hotspots of IDH1 at codon 132 and IDH2 at codon 172 were evaluated by direct sequencing as previously reported [23]. Mutational hotspots [chr5, 1, 295, 228 (C228T) and 1, 295, 250 (C250T)] in the TERT promoter region were evaluated by direct sequencing as previously reported [22]. Chromosome 1p/19q status was examined by fluorescence in situ hybridization as previously reported [22, 28].

Statistical analysis

Survival curves were constructed using Kaplan-Meier methods. Differences in PFS and OS between subgroups of patients were analyzed by log-rank tests (univariate analysis). Suitable prognostic factors influencing the survival of WHO grade II and III diffuse gliomas were selected and subsequently put into Cox proportional hazards regression models to identify independent prognostic factors (multivariate analysis). Statistical significance was defined as a p-value of less than 0.05. Statistical analyses were performed using IBM SPSS Statistics 19 software (IBM Corp., Armonk, NY, USA).

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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