Molecular and clinical heterogeneity of adult diffuse lower-grade IDH wildtype gliomas: assessment of TERT promoter mutation and chromosome 7 and 10 copy number status allows superior prognostic stratification

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With the 2016 revision of the World Health Organization classification of tumors of the central nervous system (WHO 2016) testing for the presence of mutations in isocitrate dehydrogenase 1 and 2 (*IDH*) and chromosome 1p/19q status is the cornerstone of glioma classification.[5] Approximately 80% of diffuse lower-grade (grade II & III) gliomas (DLGG) are *IDH* mutated and have a relatively favorable prognosis compared to their *IDH* wildtype (*IDHwt*) counterparts.[2] The prognosis of *IDHwt* DLGG is almost similar to primary glioblastoma and genetic aberrations that are seen in primary glioblastoma are also reported in *IDHwt* DLGG: the combination of trisomy of whole chromosome 7 and loss of chromosomal arm 10q (+7/-10q), and telomerase reverse transcriptase gene promoter (*TERTp*) mutations.[2, 6, 7] However, +7/-10q or *TERTp* mutations are not part of the WHO 2016 criteria and not all *IDHwt* DLGG have these specific molecular aberrations.[2, 5] Although clinical trials have not been performed, in view of their poor prognosis aggressive treatment regimens for *IDHwt* DLGG has been suggested. However, as this is not a well-defined separate entity, the question remains whether *IDHwt* DLGG classified according to current WHO classification qualifies as a single entity, with sufficient information to estimate prognosis adequately and therefore guide treatment, or if the assessment of additional markers is necessary and if so which. A recent study by Aibaidula et al. showed that *IDHwt* DLGG are prognostically heterogeneous and that markers like *TERTp*, *EGFR* amplification and *H3F3A* mutation could be of additional value.[1] The prognostic role of +7/-10q and its relationship with *TERTp* mutations were not reported however. In this study we report on the impact of additional molecular markers, including +7/-10q and *TERTp*, on overall survival in adult *IDHwt* DLGG.

In our institute targeted Next-Generation Sequencing is part of routine diagnostics for DLGG. We assess copy number changes of chromosome 1, 7 (including *EGFR* amplification), 9p, 10, 12, 19, and mutational status of genes *IDH1/2*, *TP53, ATRX, CIC, FUBP1, EGFR, PIK3CA, CDKN2A, PTEN, H3F3A, BRAF, NOTCH1, TERT*p. In our routine diagnostics we use gain of whole chromosome 7 and loss of whole chromosomal arm 10q as criterion for +7/-10q status. Our sequencing protocol has been described previously.[3, 4] Between January 2003 and January 2017 we sequenced a total of 639 tumors as part of daily diagnostic routine (samples since 2013) and as part of a project on extent of resection in DLGG (samples since 2003).[8] Of these, 510 tumors were histologically classified as DLGG and on sequencing 74 were *IDHwt*. We collected Karnofsky Performance Status (KPS) at diagnosis, age, gender and overall survival which was defined as time between date of diagnostic imaging and date of death. Patients that were alive at the time of analysis were censored.

Further stratification of these *IDHwt* DLGG showed a molecularly heterogeneous group of tumors. Only 52,7% of patients (n=39) showed a +7/-10q phenotype that is presumed to be a molecular characteristic of glioblastoma. Of these, all but one also

Table 1. Patient characteristics

	All patients	IDH 7+/10-	Only *TERTp*	*BRAF*	*H3F3A*	Unclassified
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Characteristic						
Number of patients	74	39	14	3	3	15
Gender						
Male	49 (66.2%)	24 (61.5%)	11 (78.6%)	3 (100.0%)	2 (66.7%)	9 (60.0%)
Female	25 (33.8%)	15 (38.5%)	3 (21.4%)	0 (0.0%)	1 (33.3%)	6 (40.0%)
KPS at diagnosis						
100	29 (39.2%)	14 (35.9%)	1 (7.1%)	3 (100.0%)	2 (66.7%)	9 (60.0%)
90	21 (28.4%)	12 (30.8%)	4 (28.6%)	0 (0.0%)	1 (33.3%)	4 (26.7%)
80	19 (25.7%)	10 (25.6%)	7 (50.0%)	0 (0.0%)	0 (0.0%)	2 (13.3%)
70	3 (4.1%)	2 (5.1%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
60	2 (3.7%)	1 (2.6%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Age at diagnosis						
median (IQR)	56 (47-63)	57 (52-64)	60 (51-68)	47 (32-49)	25 (24-32)	52 (42-62)
age <40	11 (14.9%)	2 (5.1%)	1 (7.1%)	1 (33.3%)	3 (100.0%)	4 (26.7%)
age 40 - 60	34 (45.9%)	21 (53.8%)	6 (42.9%)	2 (66.7%)	0 (0.0%)	5 (33.3%)
age >60	29 (39.2%)	16 (41.0%)	7 (50.0%)	0 (0.0%)	0 (0.0%)	6 (40.0%)

had a *TERTp* mutation. In contrast, 18.9% of patients (n=14) were *TERTp* mutated, but showed no +7/-10q pattern. 4.1% (n=3) were classified as *BRAF* mutated glioma, and 4.1% (n=3) as *H3F3A* mutated glioma. Age at diagnosis of *BRAF* and *H3F3A* mutant patients was generally younger compared to the other groups (table 1). A substantial part of samples (20.3%; n=15) could not be further stratified (either no known classifying variants or no variants found at all). The clinical importance of this heterogeneity becomes clear by overall survival analysis(Figure 1). The few *BRAF* mutant patients (median overall survival not reached) and the unclassified patients had better outcome compared to +7/-10q, *TERTp* mutated only and *H3F3A* mutated patients. The latter three all have a very poor prognosis. The longer overall survival of unclassified patients is remarkable. An explanation might be that these unclassified *IDHwt* DLGG belong to a specific, not yet identified, molecular subset with better prognosis. Additional immunohistochemistry with *IDH1* R132H antibody was negative in 12 unclassified patients. For the 3 other unclassified patients there was no available tissue anymore for immunohistochemistry. However, in each of these 3 samples we found somatic variants or copy number alterations suggestive of tumor, although not classifying. In these samples there was sufficient coverage of both *IDH1* and *IDH2*. Therefore, it is less likely that these tumors are actually true low-grade gliomas of oligodendroglial or astrocytic lineage wherein the corresponding molecular aberrations escaped detection due to technical limitations or unexpected low tumor cell percentage. Another

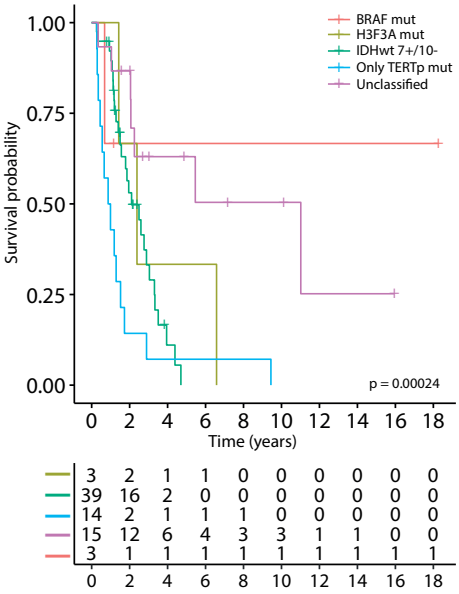

Figure 1. Overall survival of all 74 patients stratified by molecular subgroup.

interesting observation is the survival difference between the *IDH*wt +7/-10q (also *TERTp* mutated, except for one patient) patients and the patients with only a *TERTp* mutation; the patients with only a *TERTp* mutation have a significant shorter overall survival (Log-Rank test: p = 0.024). To confirm our findings we analyzed the 56 *IDHwt* DLGG in the publically available Cancer Genome Atlas (TCGA) and found that 20 samples showed a +7/-10q phenotype (all but two also *TERTp* mutated) and 18 samples were only *TERTp* mutated. Unfortunately the small sample size and limited follow-up do not allow to adequately give conclusions about prognostic differences between these groups. Therefore our findings require confirmation in an independent dataset to determine whether the *TERTp* mutated subgroup without +7/-10q is a biological distinct group.

In conclusion, we showed that adult *IDHwt* DLGG is a molecularly heterogeneous group of tumors with a widespread variation in prognosis. Consequently, assessment of only *IDH* and 1p19q status is not accurate enough to label *IDHwt* DLGG as prognostically poor and therefore to guide treatment decisions of these tumors. According to our results at least assessment of *TERTp* mutational status or +7/-10q status is necessary in *IDHwt* glioma to confirm whether a patient with DLGG has poor survival. However, when validated in independent series, assessment of both +7/-10q status and *TERTp* status seems preferable since *TERTp* mutation without +7/-10q shows shorter overall survival in our dataset. When *TERTp* is wildtype or +7/-10q is not present, further testing for other markers (at least *H3F3A* and *BRAF*) is necessary to adequately inform patients about prognosis and to decide on treatment.

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