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ORIGINAL ARTICLE

Diagnostic implications of *TERT* promoter mutation status in diffuse gliomas in a routine clinical setting

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Abstract IDH (isocitrate dehydrogenase) gene mutations are present in most diffuse low-grade gliomas and define the clinico-pathological core of the respective morphologically defined entities. Conversely, according to the 2016 WHO classification, the majority of glioblastomas belong to the IDH-wildtype category, which is defined by exclusion. *TERT* (telomerase reverse transcriptase gene) promoter mutations have been suggested as a molecular marker for primary glioblastomas. We analyzed molecular, histopathological, and clinical profiles of a series of 110 consecutive diffuse gliomas (WHO grades II-IV) diagnosed at our institution, in which *TERT* promoter mutation analysis had been performed as part of diagnostic work-up. A diagnostic algorithm based on IDH, *TERT*, ATRX, *H3F3A*, and 1p19q co-deletion status resulted in a consistent molecular classification with only 14 (13%) marker-negative tumors. *TERT* promoter mutations were present in 77% of IDH-wildtype tumors. The *TERT*/IDH-wildtype category was highly enriched for tumors with unconventional clinical or histological features. Molecular classes were associated with distinct rates of *MGMT* promoter methylation. We conclude that, in a routine diagnostic setting, *TERT* promoter mutations define a relatively homogeneous core group among IDH-wildtype diffuse gliomas that includes the majority of primary glioblastomas as well as their putative precursor lesions.

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Introduction

The 2016 revision of the WHO classification of tumors of the central nervous system has introduced a paradigm of "integrated diagnosis" that incorporates all tissue-based information, i.e., morphological and molecular features [17, 18]. This approach results in more narrowly defined and homogeneous core entities as compared to a purely morphological classification. For instance, the entity "astrocytoma, WHO grade II, IDH-mutant" (defined by the presence of *IDH1/IDH2,* i.e., isocitrate dehydrogenase 1 and 2 gene, mutations) encompasses the vast majority of tumors that were previously diagnosed as astrocytomas, WHO grade II on morphological grounds, but its clinico-pathological features are more homogeneous, as the genetic and clinical diversity of the remainder of cases has been transferred to a "wastebasket" category of "astrocytoma, WHO grade II, IDH-wildtype". In a similar way, the majority of morphologically defined anaplastic astrocytomas, WHO grade III as well as (anaplastic) oligodendrogliomas (WHO grades II and III) are transferred to molecularly defined core entities (Table 1). The WHO classification applies the attribute "IDH-wildtype" to diffuse gliomas lacking a defining molecular marker. These "IDH-wildtype" categories correspond essentially to the "not otherwise specified/NOS" or "unclassified" categories of other tumor classifications. In contrast, the 2016 WHO classification uses the modifier "NOS" to label cases in which molecular testing could not be performed or was inconclusive.

It may seem unsatisfactory that a vast majority of cases of the most common primary neuroepithelial tumor—glioblastoma—fall into a category defined by exclusion: the "wastebasket"

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Table 1 Splitting of the WHO 2007 morphologically defined entities into core entities and entities defined by exclusion according to the WHO 2016 classification

WHO 2007	WHO 2016		
	Core entity	Molecular negative	Inconclusive/not tested
Astrocytoma, WHO grade II	Astrocytoma, WHO grade II, IDH-mutant	Astrocytoma, WHO grade II, IDH-wildtype	Astrocytoma, WHO grade II, NOS
Anaplastic astrocytoma, WHO grade III	Anaplastic astrocytoma, WHO grade III, IDH-mutant	Anaplastic astrocytoma, WHO grade III, IDH-wildtype	Anaplastic astrocytoma, WHO grade III, NOS
Oligodendroglioma, WHO grade II	Oligodendroglioma, WHO grade II, IDH-mutant, and 1p/19q co-deleted	–	Oligodendroglioma, WHO grade II, NOS
Anaplastic oligodendroglioma, WHO grade III	Anaplastic oligodendroglioma, WHO grade III, IDH-mutant, and 1p/19q co-deleted	–	Oligodendroglioma, WHO grade III, NOS

category of IDH-wildtype glioblastomas accounts for most of this spectrum, as IDH-mutant glioblastomas and H3-K27M-mutant diffuse high-grade gliomas each represent only a small percentage of morphologically defined glioblastomas, especially in elderly patients [5, 30].

TERT (telomerase reverse transcriptase gene) promoter mutations are present in about 80% of glioblastomas and less frequently in other types of gliomas [1–3, 6, 14, 15, 22, 28]. They occur in a non-random pattern as related to other genetic denominators of glioma subgroups [5, 16, 29]. Specifically, they appear to be mutually exclusive with *ATRX* (alpha-thalassemia/mental retardation syndrome, x-linked gene) mutations, which are a prevalent in astrocytomas with IDH or histone gene mutations and can be detected through loss of ATRX immunostaining [21]. Conversely, among IDH-mutant gliomas, *TERT* promoter mutations are common in 1p/19q-co-deleted oligodendrogliomas. It has been suggested that tumors which derive from tissues with a low rate of self-renewal (including the CNS) need to acquire mechanisms counteracting telomere shortening in order to evade cellular senescence [14]. Indeed, *TERT* promoter mutations lead to increased telomerase activity while *ATRX* mutations are associated with the alternative lengthening of telomeres (ALT) phenotype. The latter is believed to be mediated by homologous recombination and is independent of telomerase activity. A "triple test" combining *TERT*, IDH, and 1p/19q status has been shown to be a strong prognosticator in morphologically diagnosed diffuse gliomas WHO grade II/III [9]. This prognostic impact relates at least in part to the fact that *TERT*-mutant, IDH-wildtype anaplastic astrocytomas, WHO grade III, may represent early or underdiagnosed glioblastomas.

At our institution, we have therefore implemented *TERT* promoter mutation analysis in our routine integrative diagnostic approach to diffuse gliomas. We reasoned that *TERT* promoter status might allow to reallocate tumors from the "IDH-wildtype" to biologically and clinically meaningful "*TERT*-mutant" categories. To test whether this concept leads to a conclusive classification in a routine diagnostic setting, we analyzed the results of our integrated diagnostic approach in a series of 110 diffuse gliomas.

Material and methods

Patient cohort

We identified a total of 110 patients with diffuse gliomas in which *TERT* promoter mutation analysis had been performed as part of a comprehensive morphological and molecular work-up in the study period. *TERT* promoter mutation analysis was introduced in our department in March 2015 and used on a routine basis since May 2015. In the present study, cases diagnosed until July 2016 were included. Seven tumors were recurrences of previously diagnosed gliomas. All patients had given informed consent. For the purpose of this study, we assigned tumors to molecular classes and diagnostic subgroups defined according to Table 2.

Immunohistochemistry

Immunostaining for R132H-mutant IDH1 and ATRX had been performed in all cases as previously described [13]. R132H-IDH1 immunostaining was classified as positive or negative depending on the presence or absence of staining in neoplastic cells. ATRX immunostaining was classified as deficient, when there was complete lack of staining in neoplastic cells with non-neoplastic cells being present as internal positive control, or as proficient if staining of neoplastic cells was retained.

Molecular studies

MGMT (methylguanine-DNA methyltransferase) promoter methylation status was assessed by primer extension-based

Table 2 Definition of molecular classes and diagnostic subgroups for the purpose of the present study. Items in parentheses are not considered part of the definitions, but expected to be present in most cases of the respective group

Molecular class	Diagnostic subgroup	*TERT*	IDH	1p/19q co-del	ATRX	H3F3A	Histology
IDH-mut	Astrocytoma, IDH-mut	WT	mut	neg	(def)	WT	AII / AAIII
	Glioblastoma, IDH-mut	WT	mut	neg	(def)	WT	GBMIV
	Oligodendroglioma, IDH-mut, and 1p/19q co-deleted	(mut)	mut	pos	prof	WT	OII / AOIII
TERT-mut/ IDH-WT	preGBM, *TERT*-mut	Mut	WT	(neg)	prof	WT	AII / AAIII
	GBM, *TERT*-mut	Mut	WT	(neg)	prof	WT	GBMIV
Histone-related	DMG, H3K27-mut	WT	WT	(neg)	(def)	K27M-mut	HGG
	HGG, H3G34-mut	WT	WT	(neg)	(def)	G34-mut	HGG
	Putatively histone-mut	WT	WT	(neg)	def	n.i.	HGG
Unclassified		WT	WT	(neg)	prof	WT	Any diffuse glioma
Inconclusive		*Any other combination of findings*					

Mut mutant; *WT* wildtype; *neg* negative; *pos* positive; *prof* proficient, *def* deficient; *AII* astrocyoma, WHO grade II; *AAIII* anaplastic astrocytoma, WHO grade III; *OII* oligodendroglioma, WHO grade II; *AOIII* anaplastic oligodendroglioma, WHO grade III;*GBMIV* glioblastoma, WHO grade IV

quantitative polymerase chain reaction [31] and results were available for all cases but two. *IDH1/IDH2* mutation analysis was performed in 51 cases as previously described [11]. Specifically, *IDH1/IDH2* mutation analysis was performed in all of the following circumstances if R132H-mutant IDH1 immunostaining was negative: tumors in younger adults (with an age of 50 as a laboratory-internal cut-off before introduction of the current WHO classification); ATRX-deficient tumors; non-glioblastoma histology. *IDH1/IDH2* mutation analysis confirmed the presence of an *IDH1* R132H mutation detected immunohistochemically in 7 cases and additionally identified an R132G mutation in one case. 1p/19q co-deletion status was assessed by microsatellite analysis [13] in 40 cases, including 22 of 23 IDH-mutant tumors. *TERT* promoter mutation analysis was performed by Sanger sequencing as previously reported [6]. Tumors that were both IDH- and *TERT*-wildtypes underwent *H3F3A* mutation analysis by Sanger sequencing as previously published [19].

Statistical analysis

Statistical analysis was performed with GraphPad Prism, Version 6 (GraphPad Software Inc., La Jolla, CA, USA). For statistical analysis of age distributions, Kruskal-Wallis test was performed. Rate of *MGMT* promoter methylation across classes was compared by chi-square test. For Kaplan-Meier survival analysis, log-rank (Mantel-Cox) test was utilized.

Results

An overview of molecular findings and age at diagnosis is given in Fig. 1 and Table 3. IDH gene mutations were present in 23 tumors (22 of these being *IDH1* R132H mutations). Thirteen of these (corresponding to oligodendrogliomas) harbored concomitant 1p/19q co-deletion, all but one of which were also *TERT* promoter mutant. Of the remaining 10 IDH-mutant tumors (corresponding to astrocytomas) 8 were ATRX deficient. ATRX deficiency and 1p/19q co-deletion were mutually exclusive, while 2 tumors displayed neither loss of ATRX immunoreactivity nor 1p/19q co-deletion.

Among 87 IDH-wildtype tumors, 67 (77%) were *TERT*-mutant. Histologically, 59 were diagnosed as glioblastoma (WHO grade IV), 6 as anaplastic astrocytoma (WHO grade III), and 2 as astrocytoma (WHO grade II). Out of 20 IDH/*TERT*-wildtype tumors, 6 were ATRX-deficient, indicating the possibility of underlying histone gene mutations. Among a total of 79 *TERT* mutations, there were 58 (73%) C288T and 21 C250T transitions. A single *TERT*-mutant/IDH-wildtype tumor showed loss of heterozygosity for all informative markers for 1p19q, which was considered a non-specific finding, likely related to small subtelomeric deletions [8]. *TERT* promoter mutations were mutually exclusive with ATRX deficiency. Within the IDH-wildtype/*TERT*-mutant group, the specific type of mutation (C228T vs. C250T transitions) was not statistically associated with age at diagnosis, sex, or overall survival (data not shown).

Four tumors (3%) displayed *H3F3A* hotspot mutations, all but one of which showed loss of ATRX. An additional 3 tumors were ATRX-deficient, but did not show *H3F3A* hotspot mutations.

Thirteen tumors (12%) lacked any of the above genetic alterations and were attributed to the "unclassified" group. These were highly enriched for cases with unconventional histological and/or clinical features (Table 4).

Among 73 tumors histologically diagnosed as glioblastoma, 59 (81%) were *TERT*-mutant/IDH-wildtype, 2 (3%) were IDH-mutant, and 12 (16%) were *TERT*-/IDH-wildtype. Conversely, among 37 tumors with WHO grade

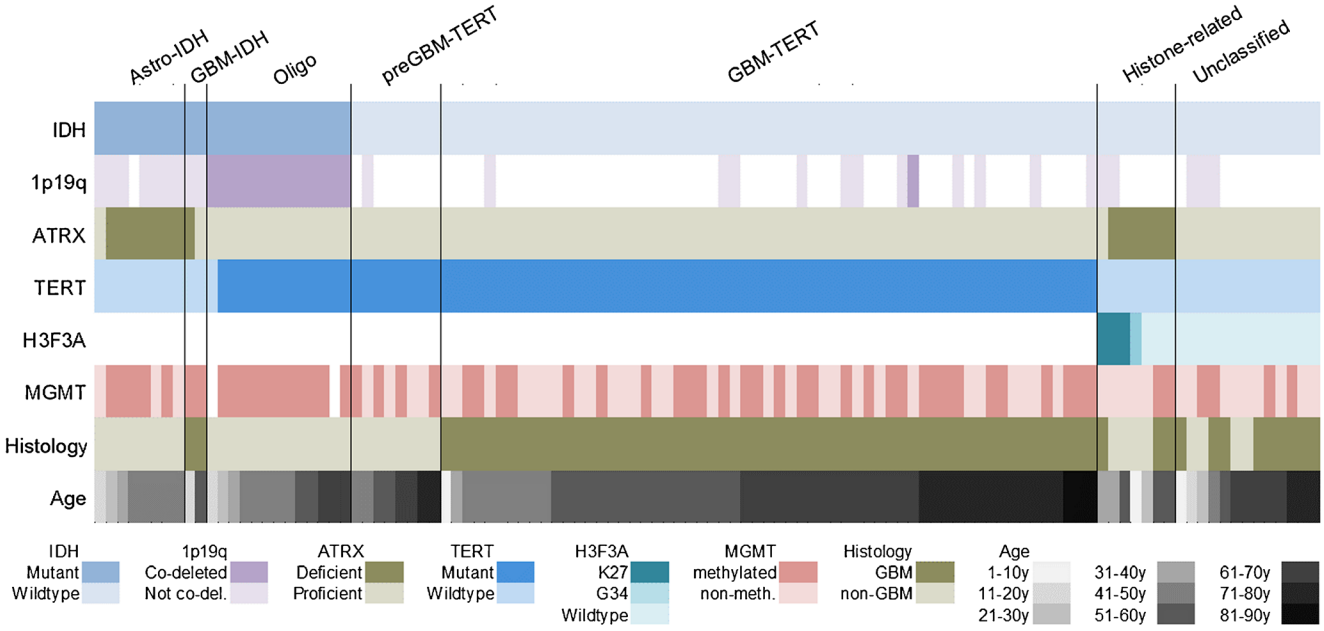

Fig. 1 Overview of molecular and demographic data for the 110 cases included in the present study. (white fields indicate "not available")

II/III histology, 8 (22%) were *TERT*-mutant/IDH-wildtype, 21 (57%) were IDH-mutant, and 8 (22%) were *TERT*-/IDH-wildtype.

MGMT promoter methylation analysis had been performed in 108 cases. There was promoter methylation in 18/21 (86%) IDH-mutant tumors, in 34/67 (51%) *TERT*-mutant/IDH-wildtype tumors, and in 6/20 (30%) *TERT*/IDH-wildtype tumors.

Median age at diagnosis was 63 years (range 9–88 years) for *TERT*-mutant/IDH-wildtype, 47 years (17–63 years) for IDH-mutant, and 55 years (range 1–9 years) for *TERT*-/IDH-wildtype tumors.

The attribution of tumors to the respective molecular classes and diagnostic categories is summarized in Table 3. Age at diagnosis was similar for diagnostic subgroups within each molecular class, but differed statistically significantly by Kruskal-Wallis test over all molecular classes (approximate p value < 0.0001). Rate of *MGMT* promoter methylation was statistically significantly different ($p = 0.0038$ by chi-square) between molecular classes (Figs. 2 and 3), too.

Of note, a conclusive molecular classification was possible for all tumors. Specifically, *TERT* promoter mutations and ATRX deficiency were mutually exclusive and ATRX immunostaining could conclusively be interpreted in all cases. Furthermore, all IDH-mutant and 1p/19q co-deleted tumors were ATRX-proficient. Conversely, all IDH-/*TERT*-double mutant tumors were 1p/19q co-deleted.

Results of Kaplan-Meier survival analysis are shown in Fig. 4. Despite the relatively short follow-up survival differed not only in a statistically significant way across all classes (p = 0.0003 by Mantel-Cox Log-rank test), but also by pairwise comparison between the following combinations of classes: IDH-mutant vs. IDH-wildtype/TERT-mutant (p = 0.0002); IDH-mutant vs. histone-related (p = 0.0004); IDH-wildtype/TERT-mutant vs. unclassified (p = 0.0468);

Table 3 Prevalence of molecular classes and diagnostic subgroups and associated age at diagnosis and *MGMT* promoter status

Molecular class	Diagnostic subgroup	*N*	Age (median)	Age (range)	% *MGMT* promoter methylated
IDH-mut	Astrocytoma, IDH-mut	8	41	17–49	63%
	Glioblastoma, IDH-mut	2	38	19–57	100%
	Oligodendroglioma, IDH-mut, and 1p/19q co-deleted	13	49	18–63	100%
TERT-mut/ IDH-WT	preGBM, *TERT*-mut	8	63	43–72	50%
	GBM, *TERT*-mut	59	63	9–88	51%
Histone-related		7	39	6–55	29%
Unclassified		13	65	1–79	31%
Inconclusive		0			

Table 4 Clinical and histopathological features of tumors attributed to unclassified and histone-related classes according to Table 2

	Histology	Clinico-pathological features	
Unclassified			Age, sex
1	Glioblastoma, WHO grade IV	Posterior fossa tumor	1, F
2	Astrocytoma, WHO grade II	Right temporal, non-enhancing tumor "isomoporphic" histology	18, F
3	"Massively calcified low-grade glioma"	Cerebellar tumor, reported previously [12]	25, M
4	Radiation-induced high-grade glioma	Cerebellar tumor History of CNS irradiation in childhood *TP53* mutation identified	43, F
5	Gliosarcoma, WHO grade IV	Right temporal tumor	55, M
6	Glioblastoma, WHO grade IV	Left frontal tumor	61, M
7	Low-grade astrocytoma	Recurrent pineal tumor 7 years after initial surgery	65, M
8	Glioblastoma, WHO grade IV	Tumor in corpus callosum	66, F
9	Glioblastoma, WHO grade IV	Bifrontal tumor Biopsy, subtotally necrotic tissue	67, M
10	Glioblastoma, WHO grade IV	Left parietal tumor	69, M
11	Glioblastoma, WHO grade IV	Left frontal tumor	72, M
12	Glioblastoma, WHO grade IV	Cerebellar tumor *TP53* mutation identified	78, M
13	Glioblastoma, WHO grade IV	Right frontal tumor	79, M
Histone-related			
14	Glioblastoma, WHO grade IV	Solid and cystic temporal tumor *H3F3A* G34 mutation	6, M
15	Anaplastic astrocytoma, WHO grade III	Frontal, bihemispheric, non-enhancing tumor invovling corpus callosum and basal ganglia *H3F3A*-wildtype, ATRX-deficient	28, F
16	Glioblastoma, WHO grade IV	Contrast-enhancing left thalamic tumor *H3F3A* K27 mutation	31, F
17	Anaplastic astrocytoma, WHO grade III	Bilateral thalamic tumor *H3F3A* K27 mutation	39, M
18	Diffuse midline glioma, H3 K27M-mutant	Brainstem glioma *H3F3A* K27 mutation	52, M
19	Glioblastoma, WHO grade IV	Right parieto-occipital tumor *H3F3A*-wildtype, ATRX-deficient	54, F
20	Giant cell glioblastoma, WHO grade IV	Left parieto-occipital tumor *H3F3A*-wildtype, ATRX-deficient	55, F

histone-related and unclassified (p = 0.0374). Conversely, there were no significant differences between IDH-mutant vs. unclassified or between IDH-/*TERT*-wildtype vs. histone-related tumors.

Discussion

The present study demonstrates that—in a routine diagnostic setting—the addition of *TERT* promoter mutation status to

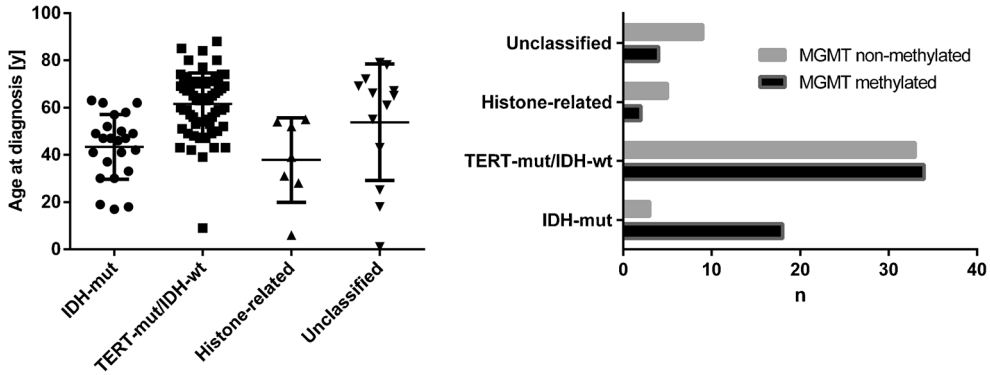

Fig. 2 Age distribution and rate of *MGMT* promoter methylation across molecular classes

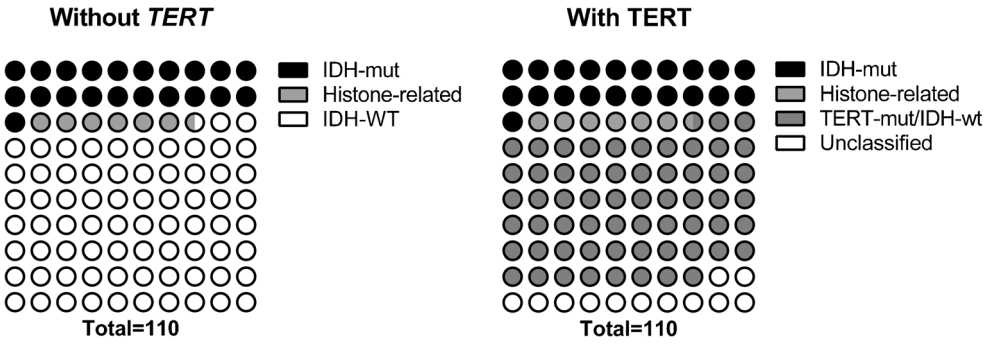

Fig. 3 Rate of marker-positive and marker-negative tumors without vs. with *TERT* mutational status as part of classification

standard markers results in a conclusive molecular-informed classification, with IDH-wildtype/*TERT*-mutant tumors representing a relatively homogeneous core group of primary glioblastomas. Additionally, *TERT*-mutant tumors with non-glioblastoma histology are identified whose clinical course according to previous studies is similarly aggressive as that of glioblastomas [9]. These likely represent precursor lesions of primary glioblastoma or in some cases may result from sampling error. The 67 tumors of this entire group corresponded to 61% of all diffuse gliomas and 77% of all *IDH*-wildtype tumors. The prevalence of *TERT* promoter mutations associated with various histological categories resembles that reported in other studies [5, 15, 32].

Seven IDH/*TERT*-wildtype tumors were *H3F3A*-mutant and/or ATRX-deficient. For the purpose of this study, they were subsumed in the "histone-related" class, given that loss of ATRX in and IDH-wildtype tumor suggests [7] indicating the possibility of histone gene mutations. Their clinico-pathological features are summarized in Table 3.

The 13 "unclassified" (i.e., IDH/*TERT*-wildtype, ATRX-proficient) tumors were remarkably enriched for unconventional clinical and/or morphological features. These included tumors with infratentorial or deep hemispheric localization, history of CNS irradiation, pediatric age, or unconventional histological features (Table 4). The heterogeneity of "unclassified" tumors is also underlined by the wide age distribution which contrasts with both the IDH-mutant and the IDH-wildtype/*TERT*-mutant categories. As expected, IDH-mutant tumors were prevalent predominantly in younger adults, while apart from a single pediatric tumor, all IDH-wildtype/*TERT*-mutant tumors occurred in adults.

With regard to markers considered non-defining (i.e., those in parenthesis in Table 2), the vast majority of tumors showed the expected marker patterns. The exceptions were a single ATRX-proficient non-co-deleted IDH-mutant astrocytoma and IDH-mutant glioblastoma each, one *TERT*-wildtype oligodendroglioma, one IDH-wildtype glioblastoma that tested positive for 1p19q co-deletion (likely a non-specific finding, see above).

The high-grade "unclassified" tumors were enriched for "outliers" as opposed to the more classical features of IDH-wildtype/*TERT*-mutant tumors, even though it included a subset of glioblastomas with typical clinical and pathological presentation. Nevertheless, our findings are consistent with the latter to corresponding to a clinico-pathological core of primary glioblastoma. The "unclassified" category also included a biopsy with minimal viable tissue in which no *MGMT* promoter methylation was identified either, raising the possibility of a false-negative result due to subtotally necrotic tissue.

Low-grade tumors from the "unclassified" group may be expected to deviate from IDH-mutant low-grade tumors in either of two directions: they likely include both more aggressive and more indolent tumors as compared to the clinical course associated with IDH mutations. This is suggested by the survival curve of "triple negative" gliomas [9], which after a steep decline (corresponding to a subset of aggressive tumors) stabilizes with little mortality occurring later than 3 years after diagnosis (related to a subgroup of tumors with long-term survival). Of note, these marker-negative gliomas showed a similarly shaped survival curve (green line in Fig. 4) in the present study despite limited follow-up. Gliomas with *MYB/MYBL1* rearrangements [25] may account for at least a fraction of IDH-wildtype diffuse gliomas with indolent clinical course, especially in younger patients. Indeed, one tumor showed an "isomorphic astrocytoma" [4] histology, i.e. minimal histological atypia and low cellularity, which is similar to the findings reported for gliomas with *MYB/MYBL1* rearrangements. One previously reported case [12] of "massively calcified low-grade glioma" also fell into the "unclassified" category.

The routine diagnostic setting from which the present study originates reduces the potential bias related to highly selected study population. It also allows to confirm the practical feasibility of the diagnostic approach presented herein, as it included cases with limited or qualitatively suboptimal tissue availability.

With respect to *H3F3A* mutation status, our findings were in accordance with previous studies [5, 26] which found histone gene mutations to be present in the majority of ATRX-deficient IDH-/*TERT*-wildtype tumors and uncommon in ATRX-proficient tumors. Given that we did not assess other histone H3 genes than *H3F3A*, there is a possibility that the

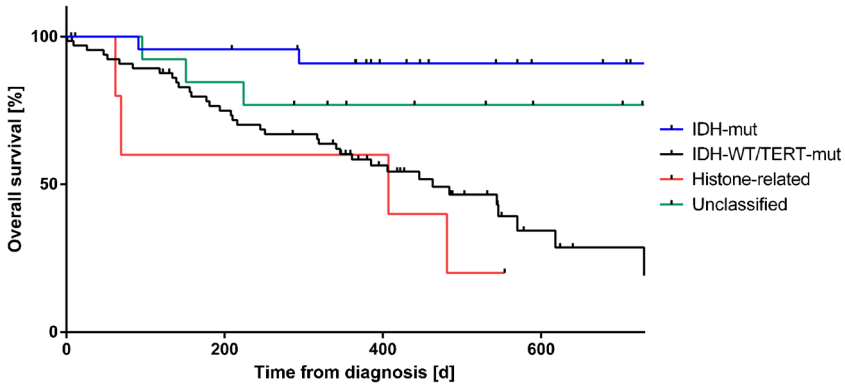

Fig. 4 Overall survival by molecular class

single ATRX-deficient *H3F3A*-wildtype tumor harbors an alternative mutation.

In sum, our findings are consistent with a concept that IDH-wildtype/*TERT*-mutant diffuse gliomas represent a clinico-pathological core of primary glioblastomas and their precursors. This would be expected to translate into distinct gene expression and epigenetic profiles as well as clinical and histomorphological features [20]. In any event, inclusion of *TERT* promoter mutation analysis into a diagnostic molecular panel shifts the vast majority of otherwise marker-negative diffuse gliomas into a biologically plausible category (Fig. 3). Furthermore, increasing evidence suggests that *TERT* promoter mutation status is of prognostic significance not only in diffuse gliomas with non-glioblastoma histology [9], but also in glioblastoma [3, 24]. Specifically, *TERT* promoter mutations appear to indicate a poor outcome in IDH-wildtype glioblastomas. Additionally, *TERT* promoter mutation analysis may have a role in establishing the neoplastic nature of glial proliferation in contradistinction to gliosis in otherwise equivocal cases. Furthermore, the presence of a *TERT* promoter mutation in an IDH-mutant glioma supports a diagnosis of oligodendroglioma.

A molecularly refined classification may also form a foundation for critical reappraisal of established prognostic and predictive markers. A precedent for this need is the observation that the WHO grading may not be prognostically relevant in IDH-mutant gliomas [27] other than glioblastoma—or at least not with the established cut-off between grade II and grade III. Conversely, "dilution" of diagnostic categories by outliers may also have led to false-negative statistical findings in the past. In particular, the significant variation in prevalence of *MGMT* promoter methylation between the different genetic groups may have been a confounding factor in previous studies which assessed the prognostic and predictive value of *MGMT* promoter methylation in diffuse gliomas.

On the basis of our and others' findings [5, 26], a step-wise molecular diagnostic approach appears to be efficient and feasible (Fig. 5), wherein ATRX immunostaining and IDH (immunostaining or mutation analysis, as indicated) results direct further molecular testing. In this setting, histone gene mutations are absent or at least very infrequent in IDH- or *TERT*-mutant tumors. Conversely, it may be advisable to search for them by both mutation analysis and H3-K27M immunostaining in IDH-/*TERT*-wildtype tumors (especially if ATRX-deficient), given the less than optimal sensitivity of each assay alone [7].

Further studies are required to validate (or disprove) the concept that *TERT*-mutant/IDH-wildtype diffuse gliomas represent a clinico-pathological core group of primary glioblastomas and their precursors. Eventually, however, another update of the WHO classification may include "*TERT*-mutant/IDH-wildtype" and other integrated diagnostic categories analogous to the "IDH-mutant" ones introduced in the 2016 revision. The currently evolving multifactorial morphological, clinical, and molecular classification of acute myeloid leukemia [23] may provide a model for future developments in the understanding of glioma biology. In a long-term perspective, such a comprehensive diagnostic approach to diffuse gliomas will likely form a basis for outcome prediction and improved personalized therapies, which may ultimately help to overcome the still dismal prognosis of patients diagnosed with diffuse high-grade gliomas today [10].

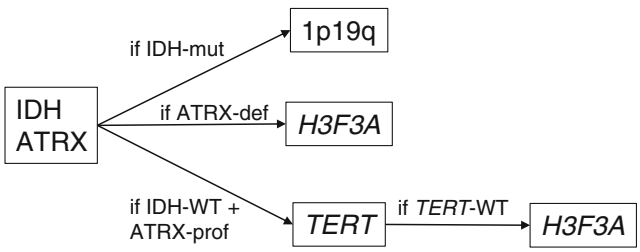

Fig. 5 Proposed step-wise molecular diagnostic algorithm for diffuse gliomas. *IDH* R132H-IDH1 immunostaining and/or *IDH1/IDH2* mutation analysis, as indicated. *ATRX* ATRX immunostaining. *1p19q* analysis for 1p19q co-deletion. *TERT TERT* promoter mutation analysis. *H3F3A H3F3A* mutation analysis +/− H3K27M immunostaining

Compliance with ethical standards Research was performed in accordance with ethical standards and legislation. Patients gave their informed consent.

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Conflict of interest The authors declare that they have no conflict of interest.

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