

TERT promoter mutations: a novel independent prognostic factor in primary glioblastomas

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See the editorial by Huse, on pages 5–6.

Background. Activating somatic mutations in the promoter region of the telomerase reverse transcriptase gene (*TERT*) have been detected in several cancers. In this study we investigated the *TERT* promoter mutations and their impact on patient survival in World Health Organization grade IV glioblastoma multiforme (GBM).

Methods. The *TERT* core promoter region containing the previously described mutations and a common functional polymorphism (rs2853669) was sequenced in tumors and blood samples from 192 GBM patients. O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation status was assessed by pyrosequencing in 177 (92.2%) cases. Relevant clinical data were obtained from a prospectively maintained electronic database.

Results. We detected specific (–124 C>T and –146 C>T) *TERT* promoter mutations in 143/178 (80.3%) primary GBM and 4/14 (28.6%) secondary GBM ($P < .001$). The presence of *TERT* mutations was associated with poor overall survival, and the effect was confined to the patients who did not carry the variant G-allele for the rs2853669 polymorphism. An exploratory analysis suggested that *TERT* mutations might be prognostic only in patients who had incomplete resections and no temozolomide chemotherapy.

Conclusions. In this study, specific *TERT* promoter mutations were markers of primary GBM and predicted patient survival in conjunction with a common functional polymorphism. The prognostic impact of *TERT* mutations was absent in patients with complete resections and temozolomide chemotherapy. If confirmed in additional studies, these findings may have clinical implications, that is, *TERT* mutations appear to characterize tumors that require aggressive treatment.

Keywords: MGMT, primary glioblastoma multiforme, prognostic factor, *TERT* mutation.

Glioblastoma multiforme (GBM), World Health Organization (WHO) grade IV, is a highly malignant and regrettably frequent intracerebral tumor. Approximately 16% of primary brain and CNS tumors are GBM.¹ Clinically, about 5% of GBM cases develop from previously diagnosed low-grade or anaplastic gliomas. These tumors are termed secondary glioblastomas (secGBM).² Standard GBM treatment consists of a gross total resection whenever safely possible, conventional fractionated radiotherapy, and in most cases temozolomide (TMZ) chemotherapy.^{3,4} The role of subtotal resections is more controversial.⁵ Older patients may benefit from hypofractionated radiotherapy regimens.⁶ No specific treatment guidelines have been developed for secGBM. Adjuvant therapy options may be limited because patients may have already undergone

radiation or chemotherapy for the preexisting lower-grade glioma at the time of the GBM diagnosis.

Molecular genetic findings differ considerably between primary (pGBM) and secGBM. Isocitrate dehydrogenase 1 (*IDH1*) mutations are rare in pGBM but occur in >70% of clinically diagnosed secGBM. *TP53* mutations are more frequent in secGBM than pGBM (60%–90% vs 20%–35%). Primary GBM is characterized by frequent *EGFR* gene amplification (~40%), and deletions in *CDKN2A* (30%–50%) and *PTEN* (>20%). More recently, *ATRX* mutations have been observed in 60%–80% of secGBM but in only 5% of pGBM.² Telomerase activation is another molecular hallmark of GBM. Increased telomerase activity is seen in >60%–70% of GBM and has been associated with an adverse prognosis.^{7–9} In one study, secGBM displayed higher

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levels of telomerase activity than pGBM.¹⁰ Telomerase activation is also seen in many other cancers, including nonglial CNS tumors such as malignant and atypical meningiomas.^{9,11}

Telomerase counteracts physiological telomere shortening. The enzyme is constituted mainly by a catalytic reverse transcriptase and an RNA component encoded by the *TERT* and telomerase RNA component (*TERC*) genes, respectively. It is believed that the unlimited mitotic capacity of malignant tumors requires telomere maintenance through telomerase activation or alternative lengthening of telomeres.^{12,13} Support for the concept of mutation-driven telomerase activation during tumorigenesis came from the recent discovery of activating mutations in the promoter region of the *TERT* gene in melanomas and several other cancers, including gliomas. The -124 C>T (C228T) and -146 C>T (C250T) mutations account for a majority of the somatic *TERT* promoter alterations identified. Both result in increased gene expression through creation of de novo binding sites for Ets/ternary complex factor transcription factors.^{14–20} *TERT* promoter mutations have been associated with increased mRNA expression or telomerase activity in various tumors.^{16,18}

There are some data to suggest that in addition to somatic alterations, *TERT* germline variants may play a role in tumor biology. Genome-wide association studies have mapped susceptibility to glioma and other cancers to the *TERT* gene region.^{21–23} Interestingly, in gliomas the strength of this association appears to increase with the histological grade, that is, the *TERT* glioma susceptibility locus is a GBM rather than a general glioma locus.²⁴ A recent study has shown that *TERT* promoter mutations affect survival and disease recurrence in bladder cancer in conjunction with a common genetic polymorphism (rs2853669) located within a preexisting Ets2 binding site of the *TERT* promoter.²⁵ The variant G-allele disrupts the binding site, resulting in decreased telomerase activity.²⁶ Interestingly, *TERT* mutations conferred an adverse prognosis only in patients without the variant rs2853669 allele.²⁵

In conclusion, several lines of evidence suggest a prominent role for telomerase activation and the *TERT* gene in the biology and therefore possibly also the clinical course of GBM. In this study we have characterized a large cohort of diffuse gliomas (94.6% GBM) for *TERT* promoter mutations. *TERT* mutations were detected much more frequently in pGBM than in secGBM. *TERT* mutations in conjunction with the rs2853669 polymorphism were shown to predict patient survival. We also found that *TERT* mutations seemed to affect survival primarily in patients with residual tumor after surgery and no postoperative TMZ chemotherapy.

Materials and Methods

Patients and Clinical Data

For the present study, 192 patients >18 years carrying a histological diagnosis of GBM were identified for whom appropriate tumor tissue had been deposited in the tumor bank of the Department of Neurosurgery at the University of Bonn. The cohort includes 178 pGBM as defined by clinical data (ie, regardless of *IDH1* mutational status; see below). All patients underwent surgery at the Department of Neurosurgery at the

University of Bonn between 1995 and 2012. All histopathological diagnoses were made at the Institute for Neuropathology/German Brain Tumor Reference Center at the University of Bonn Medical Center. Eighty-four cases (43.8%) were simultaneously enrolled in the German Glioma Network.²⁷

Clinical data were taken from a prospectively maintained electronic database. The clinical and demographic variables studied were age, preoperative Karnofsky performance index (KPI), histopathology (primary vs secondary glioblastoma), tumor location, extent of resection (biopsy vs partial vs gross total resection), radiotherapy (54–60 Gy vs early termination ≤54 Gy vs other radiotherapy vs none), and chemotherapy (TMZ radiochemotherapy according to the EORTC 26981–22981/NCIC CE3 trial protocol, intent to treat; TMZ radiochemotherapy including >3 adjuvant TMZ cycles vs other TMZ chemotherapy vs other chemotherapy vs none). Extent of resection was assessed on the first postoperative MRI scans; 47.4% of cases had early (<72 h) postoperative MRI scans. Patients were routinely followed as outpatients of the Department of Neurosurgery in Bonn, with MRI scans every 3–6 months. Some follow-up information was retrieved by contacting patients, their families, or their physicians directly. The study was approved by the University of Bonn Medical Center institutional review board for human research.

Molecular Analysis

Genomic DNA was isolated from tumor tissue and corresponding blood samples using standard methods. Tumor tissues were routinely assessed for tumor cell content using hematoxylin/eosin-stained frozen sections by an experienced neuropathologist (M.G.). Only samples containing >90% neoplastic cells were used for DNA isolation.

The core *TERT* promoter region was sequenced from the positions -278 to +65 from the ATG start site that included the site of recurrent somatic mutations and the rs2853669 polymorphism using PCR and Sanger sequencing as described.²⁵ O(6)-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation was assessed using pyrosequencing as previously described.²⁸ Mutational status of *IDH1* was investigated by direct sequencing.²⁹

Statistical Analysis

All statistical analyses were performed using commercially available software (SPSS version 21, IBM). Standard procedures (Fisher exact test, chi-square test, trend tests, Student *t*-test) were used for univariate analyses as indicated. Rates of overall survival (OS) and progression-free survival (PFS) were studied using univariate and multivariate Cox proportional hazards modeling (inclusion procedure). Survival endpoints were also analyzed with Kaplan–Meier estimates using the log-rank test for comparisons.

Results

TERT Mutations and Rs2853669 Genotypes in GBM

We investigated 192 GBM cases for mutations in the *TERT* promoter. Overall, somatic mutations were detected in 147

(76.6%) tumors with $-124\text{ C}>\text{T}$ in 108 and $-146\text{ C}>\text{T}$ in 39. The 2 mutations were mutually exclusive. The *TERT* promoter mutations were far more common in pGBM (80.3%) than in secGBM (28.6%) (chi-square test: $P < .001$; Table 1). Primary GBM patients with *TERT* mutations in tumors were significantly older than those harboring nonmutated tumors (mean age,

62.2 ± 11.4 y vs 55.5 ± 17.2 y; Student *t*-test: $P < .006$; see also Table 2).

The sequenced region of the *TERT* promoter also included the rs2853669 polymorphism. The overall frequencies for the AA, AG, and GG genotypes were 47.9%, 40.1%, and 12.0%, respectively (Table 1). Observed genotype frequencies did not

Table 1. *TERT* mutations and rs2853669 genotypes in pGBM and secGBM

	pGBM				secGBM			
	rs2853669				rs2853669			
	AA	AG	GG	All	AA	AG	GG	All
<i>TERT</i> mutation	66	60	17	143 (80.3%)	3	1	0	4 (28.6%)
<i>TERT</i> wild type	18	11	6	35 (19.7%)	5	5	0	10 (71.4%)
	84/178 (47.2%)	71/178 (39.9%)	23/178 (12.9%)	178 (100%)	8/14 (57.1%)	6/14 (42.9%)	0/14 (0%)	14 (100%)

Table 2. Demographic, clinical, and molecular findings in $n = 176$ primary glioblastomas vs *TERT* mutational status

Parameter		All	<i>TERT</i> Mutation	No <i>TERT</i> Mutation	<i>P</i>
Sex	Male	104/176 (59.1%)	85/141 (60.3%)	19/35 (54.3%)	NS
Age, y	Mean \pm SD	60.9 \pm 13.0	62.2 \pm 11.4	55.5 \pm 17.2	.006
	Median, range	64, 24–89	65, 29–84	59, 24–89	
Preoperative KPI	Mean \pm SD	79.3 \pm 11.9	79.4 \pm 11.9	79.0 \pm 11.9	NS
	Median, range	80, 40–100	80, 40–100	80, 50–100	
Tumor location	Right	91/176 (51.7%)	75/141 (53.2%)	16/35 (45.7%)	NS
	Left	76/176 (43.2%)	59/141 (41.8%)	17/35 (48.6%)	
	Bilateral	9/176 (5.1%)	7/141 (5.0%)	2/35 (5.7%)	
	Frontal	65/176 (36.9%)	51/141 (36.2%)	14/35 (40.0%)	
	Temporal	59/176 (33.5%)	47/141 (33.3%)	12/35 (34.3%)	
	Parieto-occipital	26/176 (14.8%)	22/141 (15.6%)	4/35 (11.4%)	
	Insula	13/176 (7.4%)	10/141 (7.1%)	3/35 (8.6%)	
	Multilobar	8/176 (4.5%)	8/141 (5.7%)	0	
	Other	5/176 (2.8%)	3/141 (2.1%)	2/35 (5.7%)	
	SVZ [§]	93/164 (56.7%)	74/131 (56.5%)	19/33 (57.6%)	
Extent of resection	Gross total	77/176 (43.8%)	60/141 (42.6%)	17/35 (48.6%)	NS
	Partial	96/176 (54.5%)	79/141 (56.0%)	17/35 (48.6%)	
	Biopsy	3/176 (1.7%)	2/141 (1.4%)	1/35 (2.9%)	
Radiation therapy	54–60 Gy	145/174 (83.3%)	115/139 (82.7%)	31/35 (88.6%)	NS
	<54 Gy	8/174 (4.6%)	7/139 (5.0%)	0	
	No radiotherapy	20/174 (11.4%)	17/139 (12.2%)	3/35 (8.6%)	
	Radiosurgery	1/174 (0.6%)	0	1/35 (2.9%)	
Chemotherapy	Radiochemotherapy (TMZ), intent to treat*	125/173 (72.3%)	101/138 (73.2%)	24/35 (68.6%)	NS
	Radiochemotherapy (TMZ) >3 adjuvant cycles*	77/173 (44.5%)	57/138 (41.3%)	20/35 (57.1%)	
	Any TMZ chemotherapy	129/173 (74.65%)	104/138 (75.4%)	25/35 (71.4%)	
	No chemotherapy	42/173 (24.3%)	33/138 (23.9%)	9/35 (25.7%)	
	Other chemotherapy	2/173 (1.2%)	0	2/35 (5.7%)	
MGMT	Promoter hypermethylation	80/162 (49.4%)	66/132 (50.0%)	14/30 (46.7%)	NS

Abbreviation: NS, nonsignificant.

*Radiochemotherapy with TMZ (EORTC-NCIC protocol).³ Intent to treat analysis.

[†]Radiochemotherapy with TMZ (EORTC-NCIC protocol)³ with >3 adjuvant TMZ cycles.

[§]Tumors involving the SVZ.

differ significantly from published data (see also http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2853669).²⁵ No associations were observed between *TERT* mutations (individually and any mutation vs no mutation) and the rs2853669 polymorphism (genotypes and AA genotype vs AG/GG = G-allele carrier status).

Correlations of *tert* Mutations with *mgmt* Promoter Methylation and *idh1* Mutations

MGMT promoter hypermethylation is a frequent finding in malignant gliomas and is an important prognostic and predictive biomarker. We therefore also investigated possible relations between *TERT* mutations, the rs2853669 polymorphism, and *MGMT* hypermethylation in 177 patients (92.2%) from this series. No significant correlations between *MGMT* status and *TERT* mutations or the rs2853669 polymorphism were observed in the unselected cohort and in the pGBM subset, respectively.

The high rate of *TERT* mutations in pGBM versus secGBM predicts an inverse correlation with *IDH1* mutations. *IDH1* mutation status was assessed in a subset of 97 cases from the present series. *IDH1* codon 132 mutations were found in 9/13 (69.2%) secGBM cases but in only 4/84 (4.8%) pGBM (chi-square test: $P < .001$). *IDH1* mutations were much more frequent in wild-type *TERT* cases (11/27 = 40.7%) than in *TERT*-mutated tumors (2/70 = 2.9%; Fisher exact test: $P < .001$). No correlation was seen between *IDH1* mutational status and the rs2853669 polymorphism.

Impact of *TERT* Mutations on Patient Survival

In order to identify a potential impact of *TERT* mutations on patient survival, clinical data from 176 patients with pGBM were analyzed after exclusion of 2 cases for which the *TERT* mutational status was assessed only in recurrent rather than the primary tumor. Demographic data for this cohort and key clinical findings versus *TERT* mutational status are detailed in Table 2. There was no association between tumor location and the *TERT* mutational status (Table 2). Specifically, there was no

correlation between *TERT* mutations and a presumed tumor origin in the subventricular zone (SVZ). This latter location is considered a stem cell niche, and GBM contacting the SVZ may carry a particularly adverse prognosis.³⁰

One hundred fifty-five patients (88.1%) were followed until tumor recurrence; 153 (86.9%) cases until death. Mean follow-up was 16.5 ± 15.3 months (median: 12.0; range: 1–97). Overall survival at 1 year was 52.9% (median: 13.0 ± 0.5 ; 95% CI: 11.0–15.0). Progression-free survival at 1 year was 30.6%, and median PFS was 7.0 ± 0.5 months (95% CI: 5.9–8.1). *TERT* mutations were seen in 141/176 (80.1%) cases. The secGBM subset of our cohort was deemed too small ($n = 14$) and too heterogeneous to allow for a meaningful survival analysis.

In view of the recent finding that *TERT* mutations have a powerful impact on patient outcome in bladder cancer cases without the variant G-allele of the rs2853669 polymorphism, we primarily investigated a potential role of *TERT* mutations as a prognostic parameter against the background of the patients' rs2853669 status. Kaplan–Meier estimates showed that the presence of a *TERT* mutation was a significant negative predictor of OS in the overall series (median OS with vs without *TERT* mutation = 11.0 ± 1.3 vs 16.0 ± 4.3 mo; log-rank test: $P = .038$; Fig. 1A). Interestingly, the impact of *TERT* mutational status on OS was indeed profoundly influenced by the rs2853669 genotype. The prognostic influence of the *TERT* promoter mutations was largely restricted to patients without the variant G-allele of the polymorphism (rs2853669 AA, median OS: *TERT* mutation vs no $\sim 9.0 \pm 0.7$ vs 18.0 ± 6.1 mo; log-rank test: $P = .04$; rs2853669 AG/GG, *TERT* mutation vs no $\sim 14.0 \pm 0.7$ vs 16.0 ± 1.3 mo; log-rank test: $P = \text{NS}$; Fig. 1B and C). We observed particularly poor survival in patients with *TERT* promoter mutations who did not carry the variant G-allele for the rs2853669 polymorphism (Kaplan–Meier estimates, median OS: no *TERT* mutation/rs2853669 AG/GG = 16.0 ± 1.2 mo; no *TERT* mutation/rs2853669 AA = 18.0 ± 6.1 mo; *TERT* mutation/rs2853669 AG/GG = 14.0 ± 6.7 mo; *TERT* mutation/rs2853669 AA = 9.0 ± 1.0 mo; overall log-rank test: $P = .015$; pairwise comparisons revealed significant survival differences only between the latter and all other patient subgroups).

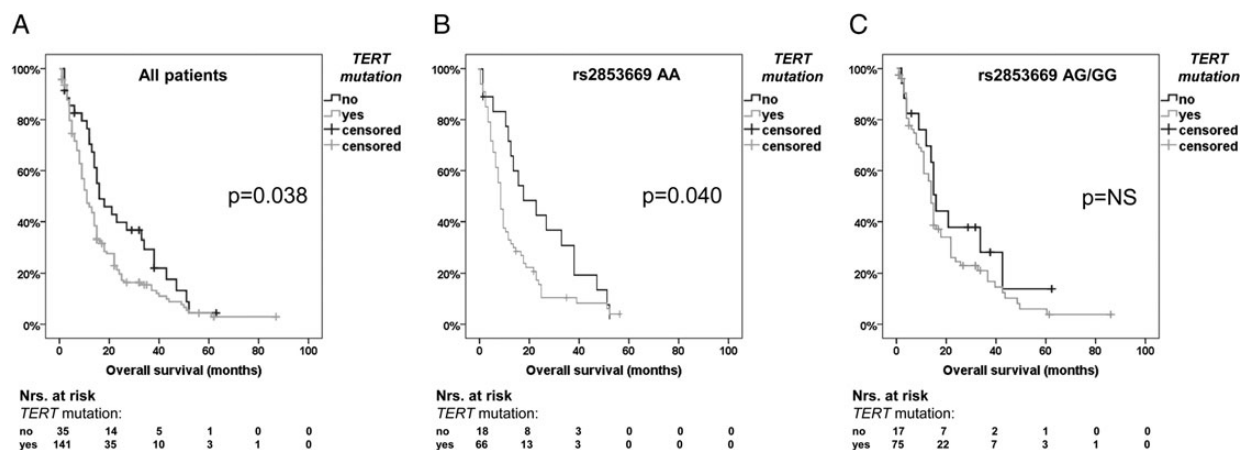


Fig. 1. Kaplan–Meier estimates of overall survival (OS) stratified for *TERT* mutational status for (A) all patients with primary glioblastomas (pGBM; log rank test: $P = .038$), (B) pGBM patients with the rs2853669 AA genotype (AA; log rank test: $P = .04$), and (C) pGBM patients with the rs2853669 AG/GG (variant) genotypes (log rank test: $P = \text{NS}$).

Table 3. Univariate predictors of survival in patients with primary glioblastomas

Parameter		<i>P</i>	HR	95% CI
Age ≥ 64 y	88/176 (50%)	.002	1.66	1.20–2.29
Sex (male)	104/176 (59.1%)	.979	1.00	0.73–1.39
KPI, 90–100	69/176 (39.2%)	.009	0.64	0.46–0.89
GTR	77/176 (43.8%)	<.001	0.44	0.32–0.62
Tumor location: SVZ	93/164 (56.7%)	.119	1.31	0.93–1.82
Radiotherapy 54–60 Gy	146/174 (83.9%)	<.001	0.27	0.17–0.42
Radiochemotherapy (TMZ), intent to treat ^a	125/173 (72.3%)	<.001	0.49	0.34–0.70
Radiochemotherapy (TMZ), >3 cycles ^b	77/173 (44.5%)	<.001	0.39	0.28–0.54
Any TMZ chemotherapy	129/173 (73.3%)	<.001	0.38	0.26–0.55
MGMT promoter hypermethylation	80/162 (49.4%)	<.001	0.45	0.32–0.64
IDH1 codon 132 mutation	4/84 (2.3%)	.690	0.81	0.30–2.24
<i>TERT</i> mutation	141/176 (80.1%)	.046	1.51	1.01–2.27
rs2853669 AA	84/176 (47.7%)	.047	1.38	1.01–1.90
<i>TERT</i> mutation + rs2853669 AA	66/176 (37.5%)	.003	1.63	1.18–2.26

Abbreviation: GTR, gross total resection.

^aRadiochemotherapy with TMZ (EORTC-NCIC protocol),³ intent to treat analysis.

^bRadiochemotherapy with TMZ (EORTC-NCIC protocol)³ with >3 adjuvant TMZ cycles.

Table 4. Cox proportional hazards modeling (inclusion procedure) of OS in *n* = 158 patients with primary glioblastoma

Parameter	<i>P</i>	HR	95% CI
Age ≥ 64 y	.016	1.56	1.09–2.23
KPI, 90–100	.309	0.82	0.56–1.20
GTR	<.001	0.51	0.35–0.74
Radiotherapy 54–60 Gy	.001	0.66	0.51–0.85
TMZ chemotherapy	.001	0.48	0.31–0.74
MGMT promoter hypermethylation	<.001	0.37	0.26–0.54
<i>TERT</i> mutation	.002	2.05	1.30–3.23
rs2853669 AA	.456	1.14	0.80–1.63

Abbreviation: GTR, gross total resection.

Additional exploratory univariate regression analyses identified age, preoperative KPI, extent of resection, postoperative radiation and chemotherapy, rs2853669 G-allele carrier status, and MGMT methylation status as additional factors associated with prognosis (Table 3). Confirming the presence of *TERT* mutations as an independent negative prognostic parameter were multivariate Cox regression models of OS using *TERT* mutational status, rs2853669 carrier status, age (<64 vs ≥ 64 y, median: 64), preoperative KPI (median: ≤ 80 vs >80), extent of resection (gross total vs residual tumor), radiotherapy (54–60 Gy vs all others), chemotherapy (any TMZ vs all others), and MGMT status as covariates (Table 4). The presence of *TERT* mutations was associated with a 2-fold increase of the risk of death.

Finally we also explored whether the impact of the *TERT* mutations on patient survival might vary with therapeutic variables. *TERT* mutations correlated with patient survival only in the presence of residual tumor (Kaplan–Meier estimates, median OS: complete resection, *TERT* mutation vs no \sim 18.0 \pm 2.9

vs 21.0 \pm 7.5 mo, log-rank test: *P* = NS; residual tumor, *TERT* mutation vs no \sim 8.0 \pm 1.2 vs 14.0 \pm 1.9 mo, log-rank test: *P* = .004; Fig. 2A and B). Comparing patients receiving any TMZ chemotherapy vs no or other chemotherapy, *TERT* mutational status was significantly predictive of OS only in the latter group (Kaplan–Meier estimates, median OS: any TMZ chemotherapy, *TERT* mutation vs no \sim 14.0 \pm 1.0 vs 18.0 \pm 4.8 mo, log-rank test: *P* = NS; no TMZ chemotherapy, *TERT* mutation vs no \sim 5.0 \pm 1.1 vs 9.0 \pm 8.7 mo, log-rank test: *P* = .016; Fig. 2C and D). In summary, the prognostic impact of *TERT* mutations was lost whenever patients had complete resections and postoperative TMZ chemotherapy.

Discussion

TERT promoter mutations, described initially in melanoma, have now been shown to occur frequently in a variety of cancers. The histological spectrum encompasses various carcinomas (in particular bladder and thyroid cancers), sarcomas, tumors of the CNS, and other neoplasms.^{16–20} In the present study we found that the *TERT* promoter mutations are highly frequent (80.3%) in pGBM compared with secGBM and gliomas of WHO grades II and III. We also observed an inverse association between the *TERT* promoter mutations and *IDH1* mutations as reported earlier.^{16–18,20} These data provide additional support for the concept of separating pGBM from other diffuse gliomas. The principal novel finding of our study with potential clinical relevance is an association of the *TERT* promoter mutations with poor overall patient survival and modification of the effect by a common polymorphism within the core *TERT* promoter. We also show that the mutations are associated with increased patient age.

Based on the prevalence in different cancer types, it has been hypothesized that the *TERT* promoter mutations occur mainly in tumors that are derived from tissues with low rates of self-renewal.¹⁷ Even though exceptions to that hypothesis

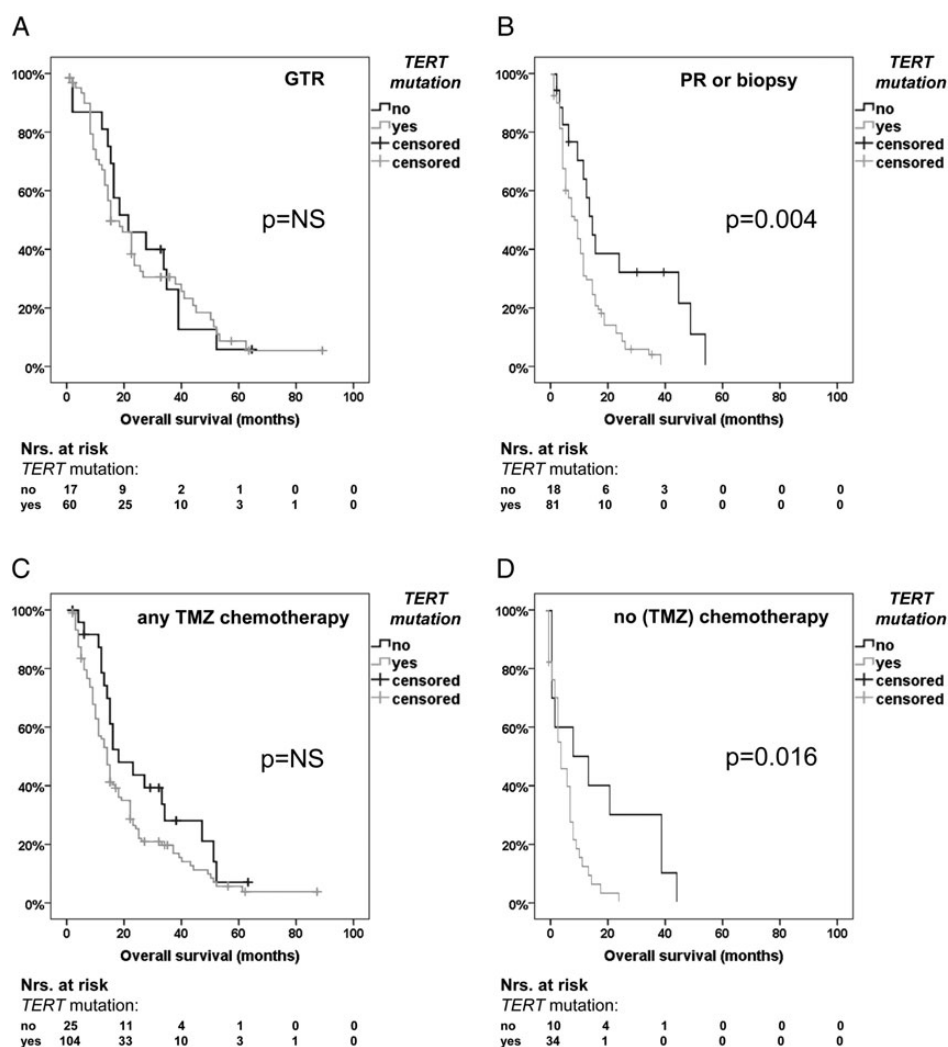


Fig. 2. Kaplan–Meier estimates of overall survival (OS) of patients with primary glioblastomas (pGBM) stratified for *TERT* mutational status and (A) gross total resection ($P = NS$, log rank test) vs (B) residual tumor (log rank test: $P = .004$), (C) temozolomide (TMZ) chemotherapy (log rank test: $P = NS$) vs (D) no or other chemotherapy (log rank test: $P = .016$).

have been observed, the high rate of *TERT* mutations in pGBM fits well within the concept. Self-renewal is not a prominent feature of CNS tissues and glial cells. The relatively low frequency of *TERT* mutations in secGBM and other gliomas has been explained by the activity of an alternative mechanism of telomere lengthening in those tumors, possibly due to mutations of the *ATRX* gene.^{17,31}

The most important finding of this study is the association of *TERT* mutations with an adverse prognosis of pGBM patients in the absence of the variant G-allele of the rs2853669 polymorphism. In this study, in conformity with a previous report on bladder cancer,²⁵ we found that patients with somatic *TERT* promoter mutations who were noncarriers of the variant G-allele for the polymorphism carried the worst overall prognosis. On the other hand, the effect of the mutations was not statistically significant in patients who carried the variant allele of the polymorphism either in homozygous or heterozygous form. The rs2853669 polymorphism located at -245 bp upstream of the ATG site results in abrogation of a preexisting Ets2 site in the

proximal region of the *TERT* promoter. Mutations at the Ets2 binding site have been shown to inhibit c-Myc binding to the E-box.³² The variant allele of the polymorphism has been previously shown to be associated with low telomerase activity in non-small cell lung carcinoma.²⁶

Rs2853669 is located 8.8 kb and 6.8 kb apart from rs2736100 and rs2853676, respectively. These latter single nucleotide polymorphisms (SNPs) have been shown to predispose to malignant glioma in a large genome-wide association study.²¹ We found no associations between the rs2736100 genotypes and patient outcome in a subset of patients who could be analyzed for clinical correlates.²⁴ The rs2853669 polymorphism has also been reported to be associated, though inconsistently, with an increased susceptibility to breast cancer.³³ Of note, the SNP rs2736098, which has also been associated with cancer susceptibility, is located only 1.2 kb away from rs2853669. The linkage disequilibrium between rs2736098 and both rs2736100 and rs2853676 is poor. In summary, it remains unclear at this point whether the (functional)

polymorphism rs2853669 is behind any of the 5p15 associations with glioma and/or cancer susceptibility.²¹

An exploratory analysis investigating the prognostic impact of *TERT* mutations versus all generally accepted prognostic parameters, both clinical (age, KPI, extent of resection, adjuvant therapy, tumor location) and molecular (*IDH1*, *MGMT* promoter methylation), revealed *TERT* mutational status as an independent predictor of the patients' prognosis. This is the first study showing that *TERT* mutations impact on GBM patient survival in a large patient cohort for which detailed clinical data were made available. A correlation between patient survival and *TERT* mutations has also been described by Killela et al,¹⁷ albeit in a much smaller patient cohort. Most recently, Nonoguchi and coworkers¹⁹ investigated a relatively large GBM cohort for *TERT* mutations. In this study, *TERT* mutations could be linked to patient survival only in the univariate analysis.¹⁹ However, this study lacked the necessary detailed clinical data for determining any robust association.

The identification of a prognostic biomarker may have profound implications. While the overall prognosis of many patients with malignant gliomas remains dismal, the concept of individualized (or rather biomarker-based) therapy has improved survival in a selected patient subset. The efficacy of TMZ chemotherapy seems closely related if not restricted to tumors characterized by methylation of the *MGMT* promoter.³⁴ Among elderly patients, TMZ chemotherapy only is an option in patients with *MGMT* methylation, which spares some from adverse side effects of radiotherapy.^{6,35} Having this background in mind, we explored whether the prognostic role of *TERT* mutations might vary with therapeutic variables. Our data appear to suggest a prognostic impact of *TERT* mutations in particular in patients with residual tumor who do not receive TMZ chemotherapy. However, these findings clearly require validation in an independent cohort because of the exploratory character of the analysis and the borderline statistical significance if one takes into account the issue of multiple testing. One also has to bear in mind that the patient subsets characterized by different treatment regimes (eg, partial vs gross total resection, TMZ chemotherapy yes vs no) were likely not balanced. More aggressive tumors might have been less often amenable to complete resections and chemotherapy—for instance, because of the respective patients' poor clinical condition. Some patients were treated in the pre-TMZ era. It is nevertheless tempting to speculate that surgery and TMZ chemotherapy in contrast to radiotherapy are effective against tumor cells responsible for the adverse prognosis associated with the *TERT* promoter mutations. Conversely the tumors with the *TERT* promoter mutations can be hypothesized to require a more aggressive surgical and chemotherapeutic strategy.

In conclusion, in this study we describe frequent *TERT* –124 C>T and –146 C>T mutations in pGBM but not in secGBM or gliomas of WHO grades II/III. These data provide further support for the concept of pGBM as a separate tumor entity fundamentally different from other diffuse gliomas. Furthermore, we show an association of the *TERT* promoter mutations with adverse outcome that is modified by the variant allele of a common polymorphism. *TERT* mutational status proved to be an independent predictor of patient survival. In addition, *TERT* mutations may have potential as a biomarker for treatment stratification.

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References

- Dolecek TA, Propp JM, Stroup NE, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005–2009. *Neuro Oncol.* 2012;14(Suppl 5):v1–v49.
- Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. *Clin Cancer Res.* 2013;19(4):764–772.
- Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352(10):987–996.
- Stummer W, Pichlmeier U, Meinel T, et al. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol.* 2006;7(5):392–401.
- Sanai N, Polley MY, McDermott MW, et al. An extent of resection threshold for newly diagnosed glioblastomas. *J Neurosurg.* 2011;115(1):3–8.
- Malmstrom A, Gronberg BH, Marosi C, et al. Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. *Lancet Oncol.* 2012;13(9):916–926.
- Boldrini L, Pistolesi S, Gisfredi S, et al. Telomerase activity and hTERT mRNA expression in glial tumors. *Int J Oncol.* 2006;28(6):1555–1560.
- Lotsch D, Ghanim B, Laaber M, et al. Prognostic significance of telomerase-associated parameters in glioblastoma: effect of patient age. *Neuro Oncol.* 2013;15(4):423–432.
- Langford LA, Piatyszek MA, Xu R, et al. Telomerase activity in human brain tumours. *Lancet.* 1995;346(8985):1267–1268.
- Harada K, Kurisu K, Tahara H, et al. Telomerase activity in primary and secondary glioblastoma multiforme as a novel molecular tumor marker. *J Neurosurg.* 2000;93(4):618–625.
- Simon M, Park TW, Leuenroth S, et al. Telomerase activity and expression of the telomerase catalytic subunit, hTERT, in meningioma progression. *J Neurosurg.* 2000;92(5):832–840.
- Aubert G, Lansdorp PM. Telomeres and aging. *Physiol Rev.* 2008;88(2):557–579.
- Gunes C, Rudolph KL. The role of telomeres in stem cells and cancer. *Cell.* 2013;152(3):390–393.
- Huang FW, Hodis E, Xu MJ, et al. Highly recurrent *TERT* promoter mutations in human melanoma. *Science.* 2013;339(6122):957–959.

15. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. *Science*. 2013;339(6122):959–961.
16. Arita H, Narita Y, Fukushima S, et al. Upregulating mutations in the TERT promoter commonly occur in adult malignant gliomas and are strongly associated with total 1p19q loss. *Acta Neuropathol*. 2013;126(6):267–276.
17. Killela PJ, Reitman ZJ, Jiao Y, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci U S A*. 2013;110(15):6021–6026.
18. Vinagre J, Almeida A, Populo H, et al. Frequency of TERT promoter mutations in human cancers. *Nat Commun*. 2013;4:2185.
19. Nonoguchi N, Ohta T, Oh JE, et al. TERT promoter mutations in primary and secondary glioblastomas. *Acta Neuropathol*. 2013;126(6):931–937.
20. Koelsche C, Sahm F, Capper D, et al. Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system. *Acta Neuropathol*. 2013;126(6):907–915.
21. Shete S, Hosking FJ, Robertson LB, et al. Genome-wide association study identifies five susceptibility loci for glioma. *Nat Genet*. 2009;41(8):899–904.
22. Rajaraman P, Melin BS, Wang Z, et al. Genome-wide association study of glioma and meta-analysis. *Hum Genet*. 2012;131(12):1877–1888.
23. Sanson M, Hosking FJ, Shete S, et al. Chromosome 7p11.2 (EGFR) variation influences glioma risk. *Hum Mol Genet*. 2011;20(14):2897–2904.
24. Simon M, Hosking FJ, Marie Y, et al. Genetic risk profiles identify different molecular etiologies for glioma. *Clin Cancer Res*. 2010;16(21):5252–5259.
25. Rachakonda PS, Hosen I, de Verdier PJ, et al. TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. *Proc Natl Acad Sci U S A*. 2013;110(43):17426–17431.
26. Hsu CP, Hsu NY, Lee LW, et al. Ets2 binding site single nucleotide polymorphism at the hTERT gene promoter—effect on telomerase expression and telomere length maintenance in non-small cell lung cancer. *Eur J Cancer*. 2006;42(10):1466–1474.
27. Hartmann C, Hentschel B, Simon M, et al. Long-term survival in primary glioblastoma with versus without isocitrate dehydrogenase mutations. *Clin Cancer Res*. 2013;19(18):5146–5157.
28. Mikeska T, Bock C, El-Maarri O, et al. Optimization of quantitative MGMT promoter methylation analysis using pyrosequencing and combined bisulfite restriction analysis. *J Mol Diagn*. 2007;9(3):368–381.
29. Muller T, Gessi M, Waha A, et al. Nuclear exclusion of TET1 is associated with loss of 5-hydroxymethylcytosine in IDH1 wild-type gliomas. *Am J Pathol*. 2012;181(2):675–683.
30. Jafri NF, Clarke JL, Weinberg V, et al. Relationship of glioblastoma multiforme to the subventricular zone is associated with survival. *Neuro Oncol*. 2013;15(1):91–96.
31. Liu XY, Gerges N, Korshunov A, et al. Frequent ATRX mutations and loss of expression in adult diffuse astrocytic tumors carrying IDH1/IDH2 and TP53 mutations. *Acta Neuropathol*. 2012;124(5):615–625.
32. Xu D, Dwyer J, Li H, et al. Ets2 maintains hTERT gene expression and breast cancer cell proliferation by interacting with c-Myc. *J Biol Chem*. 2008;283(35):23567–23580.
33. Beesley J, Pickett HA, Johnatty SE, et al. Functional polymorphisms in the TERT promoter are associated with risk of serous epithelial ovarian and breast cancers. *PLoS One*. 2011;6(9):e24987.
34. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005;352(10):997–1003.
35. Wick W, Platten M, Meisner C, et al. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. *Lancet Oncol*. 2012;13(7):707–715.