In 2016, with the publication of the novel guidelines for the classification of the central nervous system (CNS) tumours by the World Health Organization (WHO) (1), there was a paradigm shift in the classical diagnosis, mainly based in microscopy, by the integration of molecular markers in the novel stratification. Due to the intrinsic limitations of the collected CNS study material and the majority of the conservative procedures that ends in scarce material, and sometimes of difficult histology, this added value of the molecular data introduced a significant advance in tumour homogenous classification. The genetic classification is based on canonical events that have been collected throughout the recent years (2-5). Regarding the pivotal events that are used to stratify glioblastoma (GBM), we have the mutations in the isocitrate dehydrogenase 1 and 2 (IDH) genes (2,5) and in the promoter of the telomerase gene (TERTp) (5-10).

These alterations are used to aid in the diagnosis of up to 80% of GBM and can cluster patients with distinct prognostic features (5). With the molecular subgroups there is also an association for a particular mechanism used for telomere maintenance. Telomere maintenance can rely in mechanisms that are dependent in telomerase re-activation or re-expression, or in a telomerase non-dependent mechanism, the so-called alternative mechanism for telomere maintenance (ALT) (11). In a telomerase re-activation setting, TERTp mutations are so far recognized as the most frequent event and have been reported in several human cancers (6,8,12-15). In an ALT mechanism setting, the Alpha Thalassemia/Mental Retardation Syndrome X-Linked (ATRX) and Death Domain Associated Protein (DAXX) gene mutations were documented as the most frequently altered genes and were, until the study by Diplas et al. (16), the only known genes to be directly involved in ALT mechanism promotion. TERTp mutations are present in about 70% of IDH-wildtype GBM, associated with older patients that present a poorer prognosis with a shorter survival; this subgroup represents up to 90% of GBM (1,5). The remaining are IDH-mutant GBM, and present ATRX mutations in 70% of the cases (with ALT phenotype); this subgroup is composed of younger patients and presents a better outcome with increase overall survival (OS) when compared with the previous (1,4,5,8). In the study by Diplas et al. (16), the authors set to determine the genetic landscape of TERTp WT-IDH WT GBM. For this purpose, they identified a cohort of 16.9% of TERTp WT-IDH WT-1p/19q WT GBM from a series of 260 GBM that had been previously studied (5); of these, when 1p/19q co-deletion status was available it was negative, in this way this subset lack the three main glioma markers (TERTp WT-IDH WT-1p/19q WT)—triple negative tumours (17). The whole exome sequencing of this subgroup presented recurrent mutated genes in classical pathways as the RTK/RAS/PI3K (88%), P53 (40%), and RB (24%) pathways, as well as, copy number variations in PDGFRA (8%), MDM2
and MDM4 (12%) and CDKN2B (12%) genes. Analysis for glioma-associated driver alterations identified mutations in classical associated genes as PTEN (32%), NF1 (24%), EGFR (28%), TP53 (24%), ATRX (20%), and BRAF (20%), and in two novel candidate drivers that were not previously associated with GBM, SMARCAL1 (16%) and PPM1D (8%). The results obtained revealed several interesting findings. There was an enrichment of BRAF (V600E) mutations (20%) in the TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM, whereas BRAF alterations that were previously associated to low-grade pediatric gliomas, being rare in adult gliomas (18,19), had a high representation in this subgroup; with 80% of the patients being less or equal than 30 years old. The second novelty with this study was the detection of mutations in SMARCAL1 and PPM1D as novel gliomagenesis genes. The SMARCAL1 gene, that stands for the SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A Like 1 protein was the main focus of the study since it encodes an adenosine triphosphate (ATP)-dependent annealing helicase responsible for rewinding of Replication Protein A (RPA)-bound DNA at stalled replication forks for resolving telomere-associated replication stress (5); such similarity with ATRX function, also a member of the SWI/SNF family of chromatin remodelers, made it immediately an attractive candidate (16,20,21). All these facts led the authors to expand the cohort of TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM, and in 21% of these cases, SMARCAL1 mutations were detected. Given the similarities with ATRX, it was determined if SMARCAL1 inactivation was comparable to ATRX loss-of-function, and ALT initiation. The authors performed a battery of assays demonstrating classical ALT phenotypic features (16,20,21), such as the presence of ultrabright telomeric foci and C-circles and establishing a novel link of a gene to ALT development. Additionally, SMARCAL1-mutant GBM were mutually exclusive of GBM with ATRX loss of expression, reinforcing the independent contribution of each gene for the ALT mechanism. However, the authors noted that still other mechanisms should be involved because 61.5% of TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM remained without a telomere maintenance mechanism. To decipher further mechanisms, it was taken a whole genome sequencing approach in 8 TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM and structural variants (SV) of TERT were identified in 75% of the cases. This structural alteration consisted in half of the cases in translocations to other chromosomes and in the remaining cases in inversions within the same chromosome. In the expanded analysis of the TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM for TERT SV identification, the authors used break-apart FISH probes and found that half of the cases presented TERT structural rearrangements. Functionally, TERT-rearanged GBM expressed significantly higher levels of telomerase in comparison with the ALT-positive (ATRX- or SMARCAL1-mutated GBM) but with no striking differences in comparison with TERT<sup>MUT</sup> mutated GBM. All the previous data together allowed the creation of new genetic subtypes of gliomas defined in the study by Diplas et al. (16). TMM, telomere maintenance mechanism; WT, wild-type; MUT, mutations; AMP, amplification; SV, structural variation; DEL, deletion; GBM, glioblastoma.

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**Figure 1** Novel genetic subtypes of gliomas defined in the study by Diplas et al. (16). TMM, telomere maintenance mechanism; WT, wild-type; MUT, mutations; AMP, amplification; SV, structural variation; DEL, deletion; GBM, glioblastoma.

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<th>IDH profile</th>
<th>Diffuse glioma</th>
<th>Mutant</th>
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and MDM4 (12%) and CDKN2B (12%) genes. Analysis for glioma-associated driver alterations identified mutations in classical associated genes as PTEN (32%), NF1 (24%), EGFR (28%), TP53 (24%), ATRX (20%), and BRAF (20%), and in two novel candidate drivers that were not previously associated with GBM, SMARCAL1 (16%) and PPM1D (8%). The results obtained revealed several interesting findings. There was an enrichment of BRAF (V600E) mutations (20%) in the TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM, whereas BRAF alterations that were previously associated to low-grade pediatric gliomas, being rare in adult gliomas (18,19), had a high representation in this subgroup; with 80% of the patients being less or equal than 30 years old. The second novelty with this study was the detection of mutations in SMARCAL1 and PPM1D as novel gliomagenesis genes. The SMARCAL1 gene, that stands for the SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A Like 1 protein was the main focus of the study since it encodes an adenosine triphosphate (ATP)-dependent annealing helicase responsible for rewinding of Replication Protein A (RPA)-bound DNA at stalled replication forks for resolving telomere-associated replication stress (5); such similarity with ATRX function, also a member of the SWI/SNF family of chromatin remodelers, made it immediately an attractive candidate (16,20,21). All these facts led the authors to expand the cohort of TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM, and in 21% of these cases, SMARCAL1 mutations were detected. Given the similarities with ATRX, it was determined if SMARCAL1 inactivation was comparable to ATRX loss-of-function, and ALT initiation. The authors performed a battery of assays demonstrating classical ALT phenotypic features (16,20,21), such as the presence of ultrabright telomeric foci and C-circles and establishing a novel link of a gene to ALT development. Additionally, SMARCAL1-mutant GBM were mutually exclusive of GBM with ATRX loss of expression, reinforcing the independent contribution of each gene for the ALT mechanism. However, the authors noted that still other mechanisms should be involved because 61.5% of TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM remained without a telomere maintenance mechanism. To decipher further mechanisms, it was taken a whole genome sequencing approach in 8 TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM and structural variants (SV) of TERT were identified in 75% of the cases. This structural alteration consisted in half of the cases in translocations to other chromosomes and in the remaining cases in inversions within the same chromosome. In the expanded analysis of the TERT<sup>WT</sup>-IDH<sup>WT</sup> GBM for TERT SV identification, the authors used break-apart FISH probes and found that half of the cases presented TERT structural rearrangements. Functionally, TERT-rearanged GBM expressed significantly higher levels of telomerase in comparison with the ALT-positive (ATRX- or SMARCAL1-mutated GBM) but with no striking differences in comparison with TERT<sup>MUT</sup> mutated GBM. All the previous data together allowed the creation of new genetic subtypes of gliomas defined in the study by Diplas et al. (16). TMM, telomere maintenance mechanism; WT, wild-type; MUT, mutations; AMP, amplification; SV, structural variation; DEL, deletion; GBM, glioblastoma.
that presented SMARCAL1 mutations (38.5%), a novel mechanism with phenotypic markers compatible with ALT. The SMARCAL1-mutant GBM contrary to the ATRX-mutated GBM often presented mutations in TP53 as well as in PTEN and NF1; such co-occurrences may be necessary for gliomagenesis. In addition to the subgroups with ALT, we have the IDHWT GBM subgroup that rely on telomerase re-expression due to TERT SVs. Altogether, this novel genetic events account for more than 80% of the TERTpWT-IDHWT GBM. In the study by Diplas et al. (16), it was also evaluated how this subgroup genetic integration impacts patients’ survival. Both IDHWT GBM, either with ALT or with TERT SVs exhibited the poorest OS (14.9 and 19.7 months, respectively), similarly to the IDHWT GBM with TERTp mutations (OS: 14.7 months); contrasting with the better outcome of GBM patients with IDH mutations and absence of TERTp alterations (OS: 37.1 months).

Apart from the definition of these novel subgroups of TERTpWT-IDHWT GBM, there was also a novel finding, the discovery of a new gene associated to ALT. The authors created a set of experiments to elucidate SMARCAL1 contribution for this process. The first approach was to start with cancer cell lines that presented mutations in this gene and to evaluate if these cell lines recapitulated the findings detected in the human GBM samples. Both SMARCAL1-mutant cell lines had abolished expression of the SMARCAL1 protein and maintained intact expression of ATRX and DAXX. Still, ALT phenotypical markers were present and included ALT-associated promyelocytic leukemia (PML) bodies (APBs), DNA C-circles, and the classical ultrabright telomere DNA foci by FISH (22); exogenous expression of SMARCAL1 was able to restore, or partially restore, the previous changes. The second approach went the opposite way and was the evaluation of GBM cell lines after CRISPR/Cas9 gene removal of the SMARCAL1 gene. Under this approach, isogenic SMARCAL1−/− GBM cell lines were assessed for the ALT markers, and it was observed that there was a significant increase of C-circles, ultrabright telomere foci formation and APBs presence; this increase was more evident when the alterations that targeted SMARCAL1 helicase domains, pointing its domain important role.

Overall, in the study by Diplas et al. (16), two novel subgroups of TERTpWT-IDHWT GBM were identified. They represent two genetically defined GBM subgroups, IDHWT-ALT and IDHWT-TERTWT that present similarities with the established IDHmut and TERTmUT, both subgroups relying in novel genetic alterations result in ALT-mediated or telomerase-mediated mechanisms for telomere maintenance with novel alterations (Figure 1). Within the gliomas, SMARCAL1 mutations seem to be rare in lower-grade gliomas (WHO grade II–III) and only present in high grade gliomas (WHO grade 4); also, TERT SV were only detected in GBM (WHO grade 4). The detection of a high frequency of BRAF mutations was a novelty that opens a new therapeutic option for this subgroup of patients younger than 30 years old, since this is an alteration that is drug-targetable. It remains now to be understood what is the true expression of this novel gene associated to ALT, the SMARCAL1 and its prevalence within cancer. In a short overview of the cancer genomic data accessed in cBioPortal (23,24) for cancer genomics (Figure 2) we
detected that, besides GBM, several cancers present mutations in SMARCAL1 and despite the prevalence of these alterations to be inferior to 3% it may be useful to select patients with particular clinicopathological features.

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None.

**Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

**References**


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