Comprehensive analysis of *MYB/MYBL1*-altered pediatric-type diffuse low-grade glioma

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ABSTRACT

Background: Pediatric-type diffuse low-grade gliomas (pLGG) harboring recurrent genetic alterations involving *MYB* or *MYBL1* are closely related tumors. Detailed treatment and outcome data of large cohorts are still limited. This study aimed to comprehensively evaluate pLGG with these alterations to define optimal therapeutic strategies.

Methods: We retrospectively reviewed details of pLGG with *MYB* or *MYBL1* alterations from patients treated or referred for pathologic review at St. Jude Children's Research Hospital. Tumor specimens were centrally reviewed, and clinical data were collated.

Results: Thirty-three patients (18 male; median age, 5 y) were identified. Two tumors had *MYBL1* alterations; 31 had *MYB* alterations, *MYB::QKI* fusion being the most common (n=10, 30%). Most tumors were in the cerebral hemispheres (n=22, 67%). Two patients (6%) had metastasis at diagnosis. The median follow-up was 6.1 years. The 5-year event-free survival (EFS) rate was 81.3±8.3%; the 5-year overall survival (OS) rate was 96.4±4.1%. Patients receiving a near-total or gross-total resection had a 5-year EFS of 100%; those receiving a biopsy or subtotal resection had a 5-year EFS rate of 56.6±15.2% ($p<0.01$). No difference in EFS was observed based on location, histology, or molecular alterations. However, the tumors that progressed or metastasized may have distinct methylation profiles with evidence of activation of the MAPK and PI3K/AKT/mTOR pathways.

Conclusions: pLGG with *MYB*/*MYBL1* alterations have good outcomes. Our findings suggest that surgical resectability is a crucial determinant of EFS. Further characterization is required to identify optimal treatment strategies for progressive tumors.

KEYWORDS

Pediatric diffuse low-grade glioma, *MYB*/*MYBL1* alterations, treatment, outcome, survival

KEY POINTS

- *MYB*/*MYBL1*-altered pediatric-type diffuse low-grade gliomas have favorable outcomes.
- Surgical resectability is a crucial determinant of long-term outcomes.
- Tumors that progressed or metastasized may have distinct methylation profiles with evidence of activation of the MAPK and PI3K/AKT/mTOR pathways.

IMPORTANCE OF THE STUDY

Pediatric-type diffuse low-grade gliomas (pLGGs) are known to harbor recurrent genetic alterations in *MYB* or *MYBL1*. Detailed treatment and outcome data from large cohorts to define optimal therapeutic strategies are lacking due to their rarity. A comprehensive evaluation of histopathologic, molecular, and clinical characteristics showed that *MYB*/*MYBL1*-altered pLGGs have favorable outcomes consistent with CNS WHO grade 1. No difference in EFS was observed based on the tumor's location, histology, or molecular alteration in our cohort. However, the tumors that progressed or metastasized may have distinct methylation profiles based on hierarchical cluster analysis, with evidence of activation of the MAPK and PI3K/AKT/mTOR pathways. Importantly, surgical resectability is a crucial determinant of long-term outcomes.

INTRODUCTION

Low-grade glioma (LGG) is the most common and varied group of primary central nervous system (CNS) tumors in children and adolescents, accounting for over 30% of such tumors.[1] Pediatric-type diffuse low-grade gliomas (pLGGs) are known to harbor recurrent genetic alterations, including those of *MYB* and *MYBL1*.[2-4] Tumors harboring these molecular alterations are classified as angiocentric glioma or diffuse astrocytoma according to their histology.[5] Based on the 2021 WHO CNS tumor classification, *MYB*- and *MYBL1*-altered diffuse astrocytoma and angiocentric glioma are considered grade 1 tumors.[6]

The management of pLGG depends on the patient's age, tumor location, resectability, and histopathologic diagnosis. Increasingly, molecular features play a part in treatment decisions. Specifically for tumors with *MYB*/*MYBL1* alterations, detailed treatment and outcome information is needed to define optimal therapeutic strategies because available outcome data are limited due to these tumors' rarity. The largest reported series of molecular and clinical annotated LGG included 20 tumors with *MYB*- or *MYBL1*-alterations showing overall good prognosis, but detailed treatment and correlation with outcomes were not included.[7] To this end, we comprehensively reviewed a multi-institutional cohort of 33 patients with *MYB*- or *MYBL1*-altered gliomas.

METHODS

Study Population

Thirty-three patients were identified between September 1999 and December 2022: 22 were treated at St. Jude Children's Research Hospital (St. Jude), and 11 were treated at other institutions in the USA, South Africa, or Brazil. Comprehensive clinical, imaging, and histopathologic data were centrally reviewed as available. Institutional review board approval was obtained at St. Jude (IRB #19-0061) and outside institutions based on local guidelines.

Histopathologic and molecular studies

The histopathology of cases with available tissue (n = 33) was centrally reviewed by a board-certified neuropathologist (JC). The tumors were broadly classified as diffuse astrocytoma (n = 10) or angiocentric glioma (n = 23) based on their dominant histologic patterns. Molecular alterations were determined by performing immunohistochemistry (n = 33)[8-12], DNA methylation array (n= 32)[8-12], RNA sequencing (n = 29) [8-12], interphase fluorescence *in situ* hybridization (n = 24)[8-12], or PCR-based sequencing (n = 11)[8,11], as described previously.

Statistical analysis

Descriptive statistics were used to analyze all parameters. Characteristics are presented as percentages, means ± SD, or medians for skewed distributions. The follow-up duration was calculated as the time between the date of diagnosis and the date of death, event, or last follow-up. The date of diagnosis was defined as the date of the initial tissue acquisition (date of first biopsy or resection). Progressive disease was defined by radiologic progression, together with clinical deterioration and/or a need for intervention. The Kaplan–Meier method was used to summarize the event-free survival (EFS) and overall survival (OS) rates, and these were compared among patient subgroups by performing the log-rank test. The duration of OS was determined as the time between diagnosis and either death from any cause or the last follow-up, whichever was earlier. The duration of EFS was determined as the time between diagnosis and either the first detection of disease progression, death from any cause, or last follow-up, whichever was earlier. Response Assessment in Pediatric Neuro-Oncology (RAPNO) criteria were used to classify radiologic response to chemotherapy.[13] For all analyses, a p-value of less than 0.05 was considered statistically significant. Statistical analysis was performed with GraphPad Prism, v9.1.

RESULTS

Demographic and presenting characteristics

Thirty-three patients were included in this study. Detailed descriptions of patient-level data for the cohort are included in Figure 1. Eighteen (55%) were males, and the median age at diagnosis was 5.4 years (range, 1.8-40.0). Four patients were older than 18 years of age. The cerebral hemisphere was the most common location of tumors (n=22, 66%). Two patients (6%) had metastatic disease at diagnosis: a patient with a brainstem tumor had cerebellar metastasis, and a patient with a diencephalic tumor had ventricular metastasis. Seizures were the most common presenting sign, found among 16 (48%). Other common signs were ataxia (15%), headaches (12%), and cranial nerve palsies (12%). All patients with seizures had cerebral hemispheric tumors, and all patients presenting with ataxia had brainstem tumors. Three tumors (9%) were incidentally found: one each in the frontal lobe, temporal lobe, and thalamus.

Surgical interventions

For all patients, a surgical intervention was performed. A biopsy was performed in 10 patients (30%), and a gross-total resection of the tumor was achieved in 15 patients (45%). Degree of resection was associated with tumor location: 14 patients (93%) of those with GTR had tumors in the cerebral hemisphere, and all tumors where only a biopsy was performed were deep or midline tumors. Of 22 patients with tumors in the cerebral hemispheres, 17 (77%) had a GTR or NTR. Three patients (9%) presented with hydrocephalus and required cerebrospinal fluid diversion.

Histopathologic and molecular features

All tumors had diagnostic samples for pathology review. All were classified as diffuse low-grade glioma that demonstrated extensive infiltration of the involved CNS parenchyma with numerous entrapped neurofilament-positive axons (Fig. 2). Twenty-three (70%) were diagnosed as angiocentric glioma (AG) based on their dominant growth patterns (Fig. 2A). Five tumors (15%)

were diagnosed as diffuse astrocytoma (DA, Fig. 2B), and 5 (15%) as isomorphic diffuse glioma (IDA, Fig. 2C). All AGs showed areas resembling DA or IDA around the infiltrating edges where the tumor cell densities were lower. The DAs frequently showed variable degrees of angiocentric growth around small vessels. The tumor cells showed bland histology and were positive for GFAP (Fig. 2E). Tumor cells showing angiocentric growth were negative for Olig2 (Fig. 2F) and Sox10 (Fig. 2G), but the diffusely infiltrating single tumor cells were frequently positive for these markers. However, MAP2 immunoreactivity was observed in the angiocentric tumor cells but not in the infiltrating ones (Fig. 2H). Perinuclear dot-like EMA immunoreactivity was a characteristic finding in all tumors (Fig. 2I). No histologic evidence of transformation was noted in any subsequent or recurrent samples; all demonstrated low-grade histology with low levels of mitotic activity, similar to their primary tumors. Two tumors had *MYBL1* alterations; 31 had *MYB* alterations, most commonly *MYB::QKI* fusion (n=10). The distribution of *MYB* and *MYBL1* alterations is included in Figure 1. No other genetic alterations were identified in any of the tumors.

Thirty-two tumors had sufficient tissue for genome-wide DNA methylation profiling via the Illumina Infinium MethylationEPIC array. As shown in Figure 3A, the methylation profiles of these tumors formed a single cluster on the t-SNE plot. Nonetheless, the tumors that were metastatic at diagnosis or underwent progression form subclusters, suggesting the presence of distinct underlying epigenetic features. Furthermore, when performing unsupervised clustering of the cohort, the dendrogram (Figure 3B) shows that tumors that were either metastatic or had progression form a distinct group. While the small sample size of rare progressive and metastatic tumors precluded detailed DNA methylome or transcriptome analysis, the immunopositivity for phospho-ERK1/2 (Figure 3C) and phospho-S6 (Figure 3D) suggested the activation of the MAPK and PI3K/AKT/mTOR pathways in these tumors.

Adjuvant treatment and outcomes

Twenty-one patients required no further treatment for the tumor after a surgical intervention, mostly gross-total resection (GTR). In terms of adjuvant therapy, 7 patients (21%) received cytotoxic chemotherapy, and 6 patients (18%) received radiotherapy (RT). Carboplatin/vincristine was the most used chemotherapy regimen (n=5). No patient received any form of targeted therapy. In all 7 patients receiving cytotoxic chemotherapy after subtotal resection (STR, n=1) or biopsy (n=6), radiologically stable disease was seen based on the RAPNO criteria. All patients receiving RT were given focal radiation; the highest dose was 54.0 Gy (n=5). Of the 6 patients who received RT, 4 had disease control, and 2 experienced subsequent disease progression. Patient-level information on the treatments received is included in Supplement 1.

Five patients had events, and 4 patients had progressive disease. One patient who had severe developmental delay died suddenly due to a cause unrelated to the tumor without documented tumor progression. One patient's thalamic tumor progressed after subtotal resection (STR), and the disease was subsequently controlled after a second resection (near-total resection, NTR). A patient with a diencephalic tumor had a slowly progressive disease that required 2 debulking surgeries and 2 chemotherapy regimens. One patient had a large thalamic tumor that was biopsied and progressed after focal RT. Finally, one patient had a pontine tumor with metastasis that progressed after carboplatin/vincristine and then after RT. Treatment-related acute myelogenous leukemia developed in this patient after 9 years, and the patient died. A swimmer plot depicts the disease trajectory of patients in whom a biopsy or STR was performed at diagnosis, capturing all the patients with events (Figure 4).

The median follow-up was 6.1 years. The 5-year EFS rate was 81.3±8.3%; the 5-year OS rate was 96.4±4.1% (Figure 5A and 5B). Patients with an NTR or GTR had a 5-year EFS rate of 100% (Figure 5C), and those with a biopsy or STR had a 5-year EFS rate of 56.6±15.2% ($p<0.01$). No statistical difference in EFS rate was observed based on the tumor's location, histologic group, or molecular alteration (Figure 5D, 5E, 5F).

DISCUSSION

MYB/*MYBL1*-altered pLGGs are rare and have only recently been recognized in the WHO classification.[6] Therefore, detailed annotations of historical treatment approaches and outcome data are essential to determine the optimal approach to patient management. This study describes the largest cohort of *MYB*-/*MYBL1*-altered pLGGs with detailed clinical annotation.[7,14] Although tumors with these alterations present with varying clinical, histologic, and molecular characteristics, they portend good outcomes, especially when substantial resection is achieved. Furthermore, different *MYB* or *MYBL1* alterations do not show divergent outcomes. Our data are consistent with prior reports of the overall good prognosis, high frequency of cerebral hemispheric tumors, and high fraction of tumors with *MYB* alterations representing AG histologically.[6,15] The outcomes of this cohort support the notion that pLGGs with *MYB* and *MYBL1* alterations are consistent with CNS WHO grade 1.

Regarding the treatment approach, these tumors have a low likelihood of recurrence when a gross-total or near-total resection is performed. Given that the most common anatomic location of these tumors is in the cerebral hemispheres, a substantial resection seems possible in a high fraction of patients. Despite no tumor shrinkage was seen in patients in whom cytotoxic chemotherapy was used, prolonged progression-free intervals were seen even with residual tumor. Although only a few patients received RT in this cohort, it appears that response to RT can be sustained. These two responses to adjuvant therapy are consistent with those in larger series of other pediatric LGGs, where radiotherapy has more disease control than cytotoxic chemotherapy does.[16,17] As with other low-grade glioma, the optimal approach to the use of RT needs to consider the risk of progression, efficacy of other therapeutic options, and the anticipated risk profile.[18] There was some clinical heterogeneity in our cohort, with a subset of patients having

multiple progressions despite the use of chemotherapy and radiotherapy. Although the degree of resection was not described for the cohort, in a study of 26 patients with isomorphic diffuse glioma, no patients received adjuvant therapy.[14] In this context, additional analyses are needed to clarify the role of adjuvant therapy and the optimal approach for patients where gross-total or near-total resections are not possible.

Based on DNA methylation profiling and unsupervised clustering analyses, some biological heterogeneity in the tumors can be observed. Although this is the largest cohort of *MYB/MYBL1*-altered tumors, an even larger cohort would be necessary to characterize the rare tumors that have the potential to metastasize or progress after adjuvant treatment, thus conferring higher clinical risk. Metastatic and progressive *MYB*-altered tumors showed increased levels of pERK1/2 and pS6 (maker of MAPK and PI3K/AKT/mTOR pathway activation, respectively), suggesting that single-agent or combinatorial therapy against these pathways may have the potential for treating metastatic or progressive tumors. Simultaneous activation of the MAPK and PI3K/AKT/mTOR pathways has been observed in other pLGGs, including progressive hypothalamic/optic pathway pilocytic astrocytoma[8] and tectal glioma[19], further supporting the continued development of more brain-penetrant inhibitors against these pathways for difficult-to-treat pLGGs.

Our study does have limitations. First, although patients included in this study spanned a greater than 20-year period, the median follow-up was 6.1 years, which may not capture the complete history of these tumors, especially if disease progression could be very protracted. Further maturation of this cohort and expanding the number of included patients could address this shortcoming. Moreover, a larger cohort could uncover additional details on factors associated with disease progression. In addition, only a fraction of patients received radiation or cytotoxic

chemotherapy; hence, a definitive conclusion on these therapies' roles in *MYB/MYBL1*-altered tumors cannot be reached.

In summary, our study shows that pLGGs with *MYB*/*MYBL1* alterations present with varying histologic, molecular, and clinical characteristics but have good outcomes. In addition, our findings show that surgical resectability is a primary factor for long-term tumor control. Further characterization is required to identify the subgroup of tumors with the highest propensity for progression.

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Conflict of Interest

The authors declare no conflicts of interest.

Authorship

Conceptualization: DCM, JC; Methodology: DCM, JC; Investigation: IQ, SS, TWB, AD, NS-S, DVS, RT, ASW, MW, PK, XL, AG, GWR, JC; Visualization: DCM, JC; Funding acquisition: DCM, JC; Project administration: DCM, JC; Supervision: DCM, JC; Writing—original draft: DCM, JC; Writing—review and editing: All authors. All authors have read and approved the final version.

Data Availability

Data will be provided upon reasonable request to the corresponding authors.

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FIGURE LEGENDS

Figure 1: Genetic alteration and clinical characteristics among 33 *MYB/MYBL1*-altered pediatric-type diffuse low-grade glioma. Samples are arranged in columns, with categories labeled along the rows.

Figure 2: Histopathology of *MYB/MYBL1*-altered pediatric-type diffuse low-grade glioma. A) Tumors with the angiocentric glioma pattern showed apparent angiocentric growth with areas resembling diffuse astrocytoma. Entrapped neurons could be seen. B) Tumors with the diffuse astrocytoma pattern frequently showed variable degrees of angiocentric growth around small vessels. C) Isomorphic diffuse glioma showed subtle changes in cellularity. D) The tumors demonstrated extensive infiltration of the involved CNS parenchyma with numerous entrapped neurofilament-positive axons. E) The tumor cells were positive for GFAP. F-G) Tumor cells showing angiocentric growth were negative for Olig2 and Sox10. H) MAP2 immunoreactivity was observed in the angiocentric tumor cells. I) Perinuclear dot-like EMA immunoreactivity was a characteristic finding. Scale bar: 100μm

Figure 3: DNA methylation–based characterization of *MYB/MYBL1*-altered pediatric-type diffuse low-grade glioma. A) t-SNE plot of the cohort. B) Unsupervised hierarchical clustering. C) Metastasized and progressive *MYB*-altered tumors showed increased immunoreactivity for phospho-ERK1/2 (pERK1/2), a marker of activated MAPK pathway. D) Metastasized and progressive *MYB*-altered tumors showed increased immunoreactivity for phospho-S6 (pS6), a marker of activated PI3K/AKT/mTOR pathway. ATRT: atypical teratoid rhabdoid tumor; BCOR: CNS tumor with *BCOR* internal tandem duplication; CBPA: cerebellar pilocytic astrocytoma; CIC: *CIC*-fused sarcoma; DNET: dysembryoplastic neuroepithelial tumor; ETMR: embryonal tumor with multilayered rosettes; FOXR2: *FOXR2*-activated CNS neuroblastoma; G3/G4: group 3/group 4 medulloblastoma; G34: H3 G34-altered diffuse hemispheric glioma; HTPA: hypothalamic

pilocytic astrocytoma; IDH-A/O: IDH-mutant astrocytoma and oligodendroglioma; IHG: infant-type hemispheric glioma; K27: H3 K27-altered diffuse midline glioma; MGNT: myxoid glioneuronal tumor; MN1: *MN1*-altered astroblastoma; MYB-M: *MYB*-altered tumors with metastasis; MYB-P: progressive *MYB*-altered tumors; PB: pineoblastoma; RGNT: rosette-forming glioneuronal tumor; SEGA: subependymal giant cell astrocytoma; SHH-CHL/AD: childhood/adult SHH-activated medulloblastoma; WNT-WNT-activated medulloblastoma; YAP: *YAP1*-fused ependymoma; ZFTA: *ZFTA*-fused ependymoma. Scale bar: 100 μm

Figure 4: Swimmer plot of patients with biopsy or STR. Bars for each patient are colored based on the initial surgical procedure. Events and interventions are described in the figure legend.

Figure 5: Kaplan–Meier plots of cohort outcomes. A) Overall survival of the whole cohort. B) Event-free survival of the whole cohort. C) Event-free survival based on the degree of surgical resection. D) Event-free survival separated by anatomic location. E) Event-free survival separated by histology. F) Event-free survival based on molecular alterations. p-value calculated by the log-rank test.

Figure 1

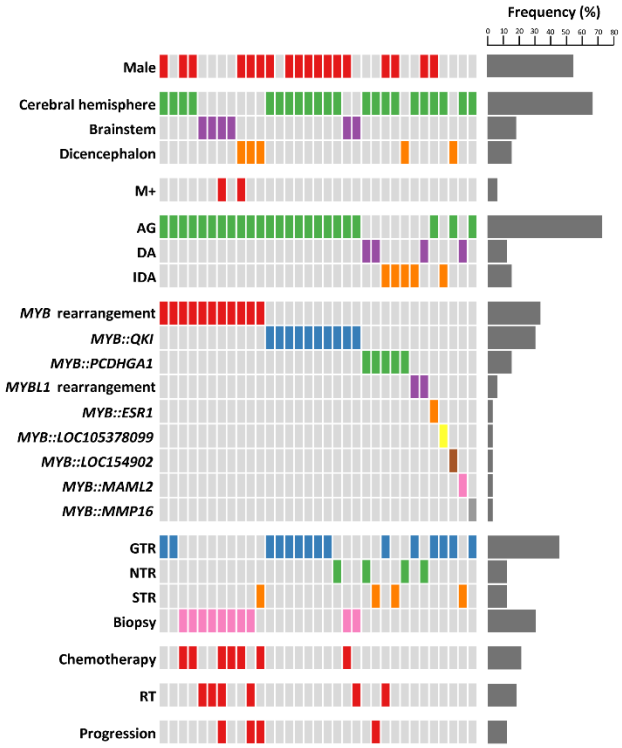

Figure 2

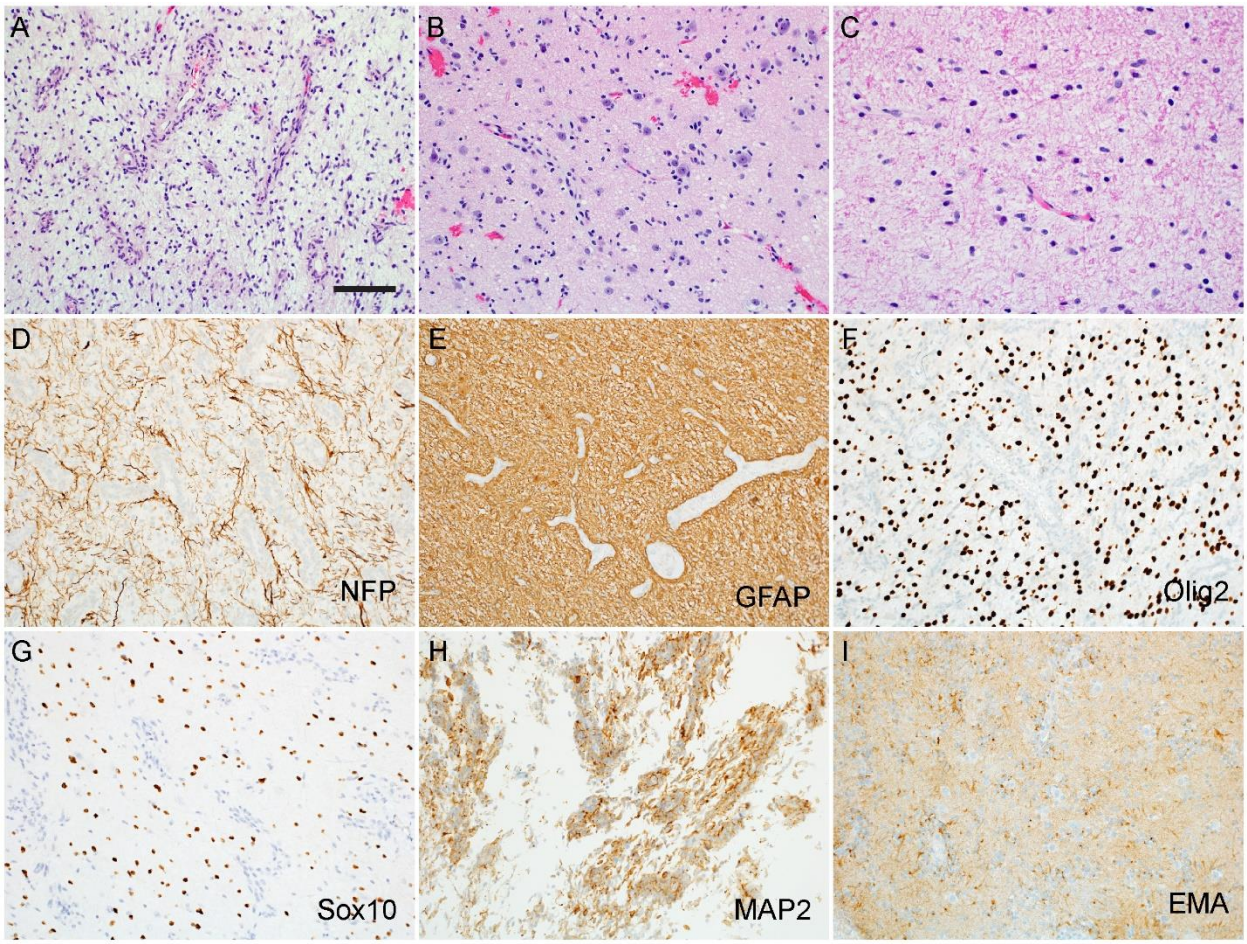

Figure 3

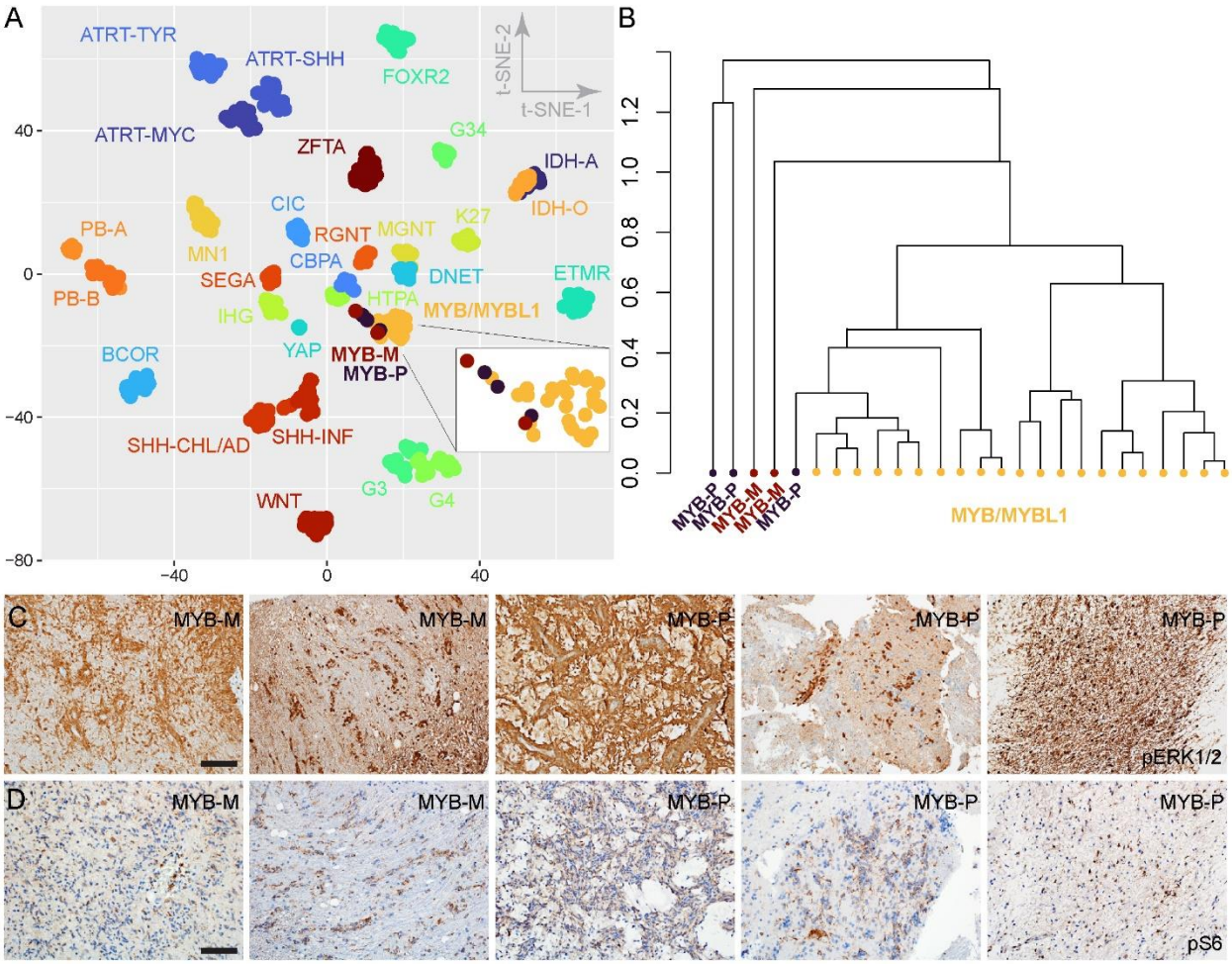

Figure 4

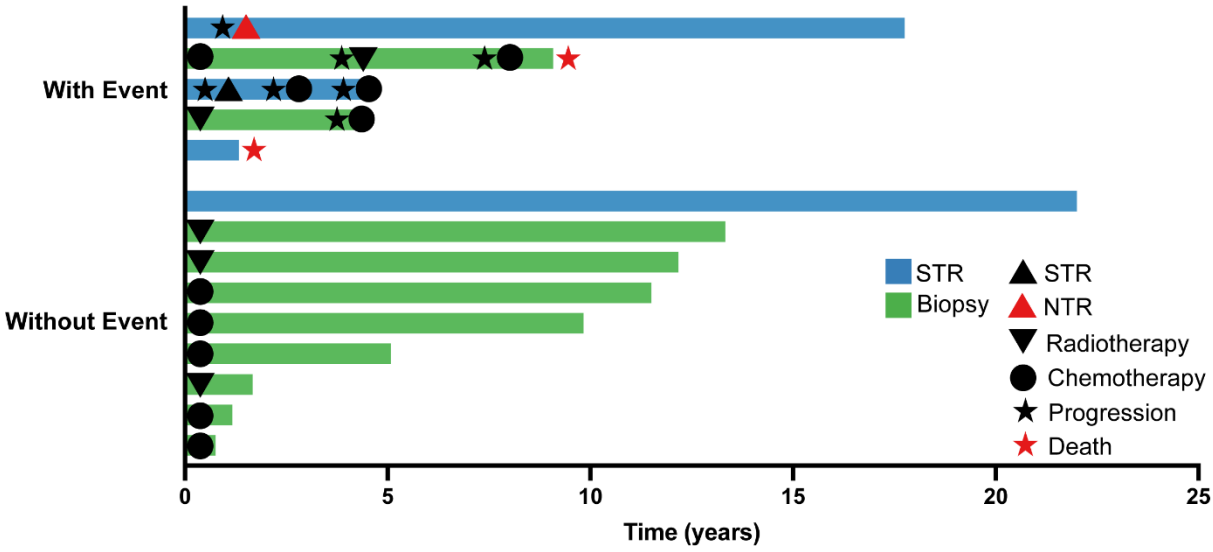

Figure 5