Characterizing temporal genomic heterogeneity in pediatric low-grade gliomas

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Abstract

Recent discoveries have provided valuable insight into the genomic landscape of pediatric low-grade gliomas (LGGs) at diagnosis, facilitating molecularly targeted treatment. However, little is known about their temporal and therapy-related genomic heterogeneity. An adequate understanding of the evolution of pediatric LGGs' genomic profiles over time is critically important in guiding decisions about targeted therapeutics and diagnostic biopsy at recurrence. Fluorescence in situ hybridization, mutation-specific immunohistochemistry, and/or targeted sequencing were performed on paired tumor samples from primary diagnostic and subsequent surgeries. Ninety-four tumor samples from 45 patients (41 with two specimens, four with three specimens) from three institutions underwent testing. Conservation of BRAF fusion, BRAFV600E mutation, and FGFR1 rearrangement status was observed in 100%, 98%, and 96% of paired specimens, respectively. No loss or gain of IDH1 mutations or NTRK2, MYB, or MYBL1 rearrangements were detected over time. Histologic diagnosis remained the same in all tumors, with no acquired H3K27M mutations or malignant transformation. Changes in CDKN2A deletion status at recurrence occurred in 11 patients (42%), with acquisition of hemizygous CDKN2A deletion in seven and loss in four. Shorter time to progression and shorter time to subsequent surgery were observed among patients with acquired CDKN2A deletions compared to patients without acquisition of this alteration [median time to progression: 5.5 versus 16.0 months (p = 0.048); median time to next surgery: 17.0 months versus 29.0 months (p = 0.031)]. Most targetable genetic aberrations in pediatric LGGs, including BRAF alterations, are conserved at recurrence and following chemotherapy or irradiation. However, changes in CDKN2A deletion status over time were demonstrated. Acquisition of CDKN2A deletion may define a higher risk subgroup of pediatric LGGs with a poorer prognosis. Given the potential for targeted therapies for tumors harboring CDKN2A deletions, biopsy at recurrence may be indicated in certain patients, especially those with rapid progression.

Keywords: Pediatric low-grade gliomas, Genomics, Paired, Recurrence, Tumor evolution, BRAF, CDKN2A

Introduction

Genomically-driven therapy is increasingly being incorporated into the treatment of pediatric low-grade gliomas (LGGs), the most common type of brain tumor in children [1, 31, 44]. While prognosis is excellent when a gross total resection can be achieved, young patients with incompletely resected and/or progressive disease pose therapeutic challenges and may experience a chronic,
relapsing course, given relatively low durable response rates with standard chemotherapy and unacceptable long-term toxicity of irradiation [2, 14, 39]. Recent discoveries have provided valuable insight into the genomic landscape of pediatric LGGs at diagnosis, facilitating a shift in treatment strategy toward a molecularly targeted approach [31, 44]. An adequate understanding of the evolution of pediatric LGGs’ genomic profiles over time is critically important in guiding decisions about targeted therapeutics and diagnostic biopsy at recurrence.

Genetic aberrations within the mitogen-activated protein kinase (MAPK) pathway are prevalent in pediatric LGGs at diagnosis, resulting in activated downstream proliferation signaling and subsequent tumorigenesis [68]. Single driver genetic alterations have been consistently identified within specific histologic subtypes of pediatric LGGs, including BRAF-KIAA1549 fusions in pilocytic astrocytomas [29, 54], BRAFV600E point mutations in pleomorphic xanthoastrocytomas and gangliogliomas [17, 68], FGFRI duplications in diffuse astrocytomas [68], and MYB or MYBL1 rearrangements in diffuse astrocytomas and angiocentric gliomas [4, 49, 58]. Expanded knowledge of the genetic landscape of LGGs has supported the growing investigation and utilization of molecularly targeted agents, such as MEK or BRAF inhibitors, for tumors with MAPK pathway alterations, especially at relapse or progression [5, 8, 23, 50].

Despite this reliable understanding of the biology underlying LGGs in children at diagnosis, little is known about their temporal genomic heterogeneity and whether they undergo genetic evolution following therapy and/or at recurrence. Genomic analyses of 10 paired adult LGGs revealed significant genetic variation between diagnosis and recurrence, including transformation to high-grade gliomas (HGGs) following chemotherapy in some patients [27]; however, molecular differences between adult and pediatric LGGs are well-recognized [28, 30, 51, 68], with relatively low risk of malignant transformation in children [9, 40], limiting generalizability of these findings to the pediatric patient population. A landmark report describing whole-genome sequencing of pediatric LGGs included two pairs of primary and recurrent tumors, which demonstrated identical genomic profiles [68], but, to our knowledge, no other studies have evaluated genetic changes of LGGs in children over time.

Emerging data suggest variable temporal genomic heterogeneity across other pediatric central nervous system (CNS) tumors. In medulloblastoma, molecular subgroup is conserved [48], but there is significant divergence in targetable mutations between diagnosis and recurrence [41]. Transcriptomic changes between matched primary and recurrent pediatric posterior fossa ependymomas have been reported, yet with relative preservation of copy number alterations [24]. A study of temporal genomic heterogeneity across 16 paired pediatric HGGs demonstrated conservation of certain key driver mutations at recurrence, but acquisition or loss of others [51]. Successful incorporation of molecularly targeted therapy and consideration of repeat biopsy at recurrence in pediatric LGGs demands an adequate understanding of how their genomic profiles evolve over time and following prior treatment. In this study, we characterize the temporal genomic heterogeneity of pediatric LGGs by comparing fluorescence in situ hybridization (FISH), mutation-specific immunohistochemistry (IHC), and/or targeted sequencing in paired tumor samples from primary diagnostic and subsequent surgeries.

Materials and methods

Clinical cohort

This retrospective study was performed at Cincinnati Children’s Hospital Medical Center (CCHMC), Nationwide Children’s Hospital, and Akron Children’s Hospital. The patient cohort was chosen based on the availability of adequate tumor specimens for testing from both primary diagnostic and subsequent surgeries (biopsy, resection, or autopsy), with a confirmed histologic diagnosis of LGG by neuropathology review (CE, DB). Samples from subsequent surgeries were only included for analysis if they occurred at least 1 month following the previous surgery. Patient tumor samples were preserved either as fresh-frozen or formalin fixed paraffin embedded (FFPE) tissue. To ensure adequate tumor content, hematoxylin and eosin (H&E) slides were reviewed from each frozen specimen, the initial cut of each FFPE block, and an additional cut of FFPE block after scrolls were obtained for DNA extraction. Clinical data, including age, sex, surgery details, and prior treatments, were abstracted from the patients’ electronic health records and subsequently de-identified. All patient tumor samples and clinical data were collected after informed consent was provided by patients or legal guardians through institutional review board approved protocols at the respective institutions.

Fluorescent in situ hybridization (FISH)

FISH for the following relevant genetic alterations was performed on tumor specimens by the Department of Molecular Genetics at CCHMC: BRAF duplications or rearrangements, FGFRI, MYB, MYBL1, or NTRK2 rearrangements, and CDKN2A deletions.

Mutation-specific immunohistochemistry (IHC)

IHC staining for H3K27M, BRAFV600E, and IDH1-R132H mutations as well as ATRX loss was performed on slides cut from FFPE blocks of pediatric LGG samples using conventional methods [67].
Targeted sequencing analysis
DNA extraction was carried out from frozen tissue using the Qiagen AllPrep DNA/RNA/miRNA Universal Kit following the manufacturer’s instructions. DNA from FFPE scrolls or core punches were isolated by suspending the paraffin scrolls in deparaffinization solution (Qiagen), followed by DNA extraction using the QIAamp DNA FFPE Tissue Kit. DNA quantification was conducted using the Quant-iT Picogreen or Qubit dsDNA assay (Thermo Fisher Scientific). Targeted DNA sequencing was performed on tumor specimens with adequate DNA for testing using the AmpliSeq 50 gene Focus Cancer Hotspot Panel V2 assay on the Illumina MiSeqDx instrument (Illumina, San Diego, CA). This panel, which requires 10–25 ng of genomic DNA and was validated on FFPE tissue, assessed mutations that include the following genes relevant to pediatric LGGs: \(BRAF, FGFR1, FGFR2, FGFR3, HRAS, KRAS, NRAS, PTPN11, IDH1, IDH2, \) and \(TP53\), among others. Additionally, Foundation Medicine next generation sequencing (Foundation Medicine, Cambridge, MA) was performed on select tumor specimens and when available, relevant results from this testing were also included in the analysis.

Statistical analysis
Continuous and categorical variables are described by median (range) and frequency (percent), respectively. The Wilcoxon Rank-Sum test and Fischer’s Exact test were used to assess for differences in age as well as interval systemic therapy and/or irradiation and World Health Organization (WHO) histologic grade, respectively, between patients whose tumors did or did not exhibit temporal genomic heterogeneity. Survival endpoints are described by the median time to event calculated using the Kaplan–Meier method. The Log-Rank test was used to evaluate potential associations between conservation or change in \(CDKN2A\) deletion status between diagnostic and recurrent tumor specimens with time to progression and time to subsequent surgery.

Results
Patient characteristics
A total of 94 primary diagnostic and subsequent surgical specimens from 45 pediatric patients with LGGs were included for analysis. Two tumor specimens were available for 41 patients, and three tumor specimens were available for four patients who underwent more than two surgeries (two of these four patients underwent four total surgeries, with identical histologic diagnoses confirmed on all four specimens for each patient, but only the latter three tumor specimens had adequate tissue for molecular testing). One other patient also underwent three total surgeries, but the time between his first two surgeries was less than 1 month (initial biopsy followed by gross total resection 1 week later), with identical histology and molecular testing on all specimens, so only specimens from the two surgeries more than 1 month apart were included for analysis. Two additional patients underwent a third neurosurgical resection due to radiographic or clinical concern for tumor progression, but pathology was not consistent with neoplasm (focal cortical dysplasia in one, reactive bone formation with dense fibrous tissue in the other), so these patients’ third specimens were not included in the analysis.

An overview of patient demographic and clinical characteristics as well as tumor histologic subtypes is shown in Table 1. Median time to second surgery was 19 months (range: 1.5–178 months), and median time from second to third surgery for those respective patients was 38.5 months (range: 1–118 months). Seventeen patients (38%) received systemic therapy; eight of these 17 patients received more than one successive systemic therapeutic regimen prior to second surgery due to recurrence/progression and/or treatment-related toxicity. Four patients (9%) received irradiation prior to their second surgery. Twenty-five patients (56%) did not undergo systemic therapy or irradiation prior to their second surgery; four of these patients did not have obvious clinical or radiographic concern for recurrence/progression, but a second surgery was undertaken to achieve maximal safe resection of residual tumor. Forty-two patients (93%) were alive at the time of last follow-up (median follow-up time of 87 months from diagnosis). Three patients passed away a median of 91 months (range: 71 to 128 months) from diagnosis [death was directly due to disease progression in one patient and due to unrelated causes in two patients (drug overdose, cardiogenic shock)].

Histologic subtype and grade
Histologic subtype and grade were conserved in 100% (45 of 45) patients, including at third surgery, with no evidence of malignant transformation to HGG.

Genomic findings
A comparison of the genomic profiles of individual patients’ matched tumor specimens, grouped by histopathologic diagnosis, is illustrated in Fig. 1. Figure 2 provides images of the most commonly identified histologic and molecular (FISH and mutation-specific IHC) findings at diagnosis and second surgery from four representative patients. A summary of temporal genomic heterogeneity in the overall cohort and within specific histopathologic subgroups, as well as a breakdown of the number of patients who had specific genetic testing performed on paired specimens, is shown in Table 2.
### Table 1 Overview of patient demographics and clinical characteristics

| Category                                                                 | Number of patients (%) |
|-------------------------------------------------------------------------|------------------------|
| **Gender**                                                              |                        |
| Female                                                                  | 27 (60%)               |
| Male                                                                    | 18 (40%)               |
| **Median age (years) at diagnosis (range)**                            | 5.8 (0.4–18.3)         |
| **Histologic diagnosis**                                               |                        |
| Pilocytic Astrocytoma                                                  | 26 (58%)               |
| Pilomyxoid Astrocytoma                                                 | 2 (4%)                 |
| Diffuse Astrocytoma                                                    | 7 (16%)                |
| Ganglioglioma                                                          | 5 (11%)                |
| Desmoplastic Infantile Ganglioglioma (DIG)                             | 1 (2%)                 |
| Pleomorphic Xanthroastrocytoma (PXA)                                    | 1 (2%)                 |
| Angiocentric glioma                                                    | 1 (2%)                 |
| Low grade glial or glioneuronal neoplasm, not otherwise specified (NOS)| 2 (4%)                 |
| **Extent of first surgical resection (at diagnosis)**                  |                        |
| Biopsy                                                                 | 10 (23%)               |
| Subtotal resection                                                     | 24 (50%)               |
| Gross total resection                                                  | 11 (27%)               |
| **Median time (months) to first progression**                          | 13 (1.5–178)           |
| **Median time (months) to second surgery**                             | 19 (1.5–178)           |
| **Extent of second surgical resection**                                |                        |
| Biopsy                                                                 | 2 (4%)                 |
| Subtotal resection                                                     | 17 (38%)               |
| Gross total resection                                                  | 22 (49%)               |
| Unknown                                                                | 4 (9%)                 |
| **Received systemic therapy prior to second surgery**                  | 17 (38%)               |
| **Received at least two systemic therapy regimens prior to second surgery** | 8 (17%)               |
| **Systemic therapy received (% of patients who received systemic therapy)** |                        |
| Carboplatin with or without Vincristine                                | 12 (71%)               |
| Vinblastine                                                            | 6 (35%)                |
| Bevacizumab with or without Irinotecan                                 | 3 (18%)                |
| Temozolomide                                                           | 2 (12%)                |
| Thioguanine, Procarbazine, Lomustine, and Vincristine                  | 2 (12%)                |
| Carboplatin or Cisplatin, Cyclophosphamide, and Etoposide              | 2 (12%)                |
| Trametinib                                                             | 1 (6%)                 |
| Everolimus                                                             | 1 (6%)                 |
| Lenalinomide                                                           | 1 (6%)                 |
| Vorinostat                                                             | 1 (6%)                 |
| Rapamycin                                                              | 1 (6%)                 |
| **Received irradiation prior to second surgery**                       | 4 (9%)                 |
| **Received no systemic therapy or irradiation prior to second surgery** | 25 (56%)               |
| **Underwent third surgery**                                            | 4 (9%)                 |
| **Median time (months) from second surgery to third surgery (range)**  | 38.5 (1–118)           |
| **Extent of third surgical resection**                                 |                        |
| Biopsy                                                                 | 1 (25%)                |
| Subtotal resection                                                     | 3 (75%)                |
| **Received systemic therapy between second and third surgeries**       | 2 (50%)                |
| **Systemic therapy received**                                          |                        |
| Temozolomide, then Vinblastine                                         | 1 (20%)                |
| Avastin and Irinotecan                                                  | 1 (20%)                |
and detailed herein. Due to inconsistent tumor specimen availability and adequacy, it was not possible to perform all molecular tests on all paired tumor samples; therefore, in cases with sparse tissue, focused panels of genomic alterations were selected based on relevance to tumor histopathologic classification (e.g., prioritizing \textit{IDH1} mutation testing for diffuse astrocytomas) and location (i.e., \textit{H3K27M} IHC testing for tumors of midline location). Targeted sequencing was performed on paired tumor samples from 17 patients and relevant findings are incorporated in Fig. 1 and Table 2 and described below. There were no significant differences in age at diagnosis, previous systemic therapy and/or irradiation, or histologic WHO grade (I versus II) between patients whose tumors exhibited temporal genomic heterogeneity (n = 12) and patients whose tumors had completely conserved genomic profiles (n = 33; Table 3).

**Pilocytic/pilomyxoid astrocytomas**

\textit{BRAF} fusion or duplication: Of 23 patients with pilocytic or pilomyxoid astrocytomas who had testing for \textit{BRAF}\textsuperscript{V600E} mutations performed on all paired surgical specimens, 27 (96%) tested negative at diagnosis and remained negative at recurrence (including 14 patients who received systemic therapy and two who received irradiation prior to subsequent surgery; Table 2). One patient's tumor tested positive by IHC at diagnosis, but lost this mutation at subsequent surgery 35 months after diagnosis (Patient #26, Fig. 1); this patient had not received previous systemic therapy or irradiation, and \textit{BRAF}\textsuperscript{V600E} sequencing was not available for either specimen. Of note, 35 tumor samples from the entire cohort (spanning all histopathologic diagnoses) had \textit{BRAF}\textsuperscript{V600E} testing performed by both IHC and targeted sequencing methods, and results were concordant in all but one [this patient with pilocytic astrocytoma had positive \textit{BRAF}\textsuperscript{V600E} testing by IHC, but negative by sequencing, so this was interpreted as negative for the reported analysis (this patient's subsequent tumor sample had negative \textit{BRAF}\textsuperscript{V600E} IHC)].

\textit{CDKN2A} deletion: Of 19 patients with pilocytic or pilomyxoid astrocytomas who had testing for \textit{BRAF}\textsuperscript{V600E} mutations performed on all paired surgical specimens, 27 (96%) tested negative at diagnosis and remained negative at recurrence (including 14 patients who received systemic therapy and two who received irradiation prior to subsequent surgery; Table 2). One patient's tumor tested positive by IHC at diagnosis, but lost this mutation at subsequent surgery 35 months after diagnosis (Patient #26, Fig. 1); this patient had not received previous systemic therapy or irradiation, and \textit{BRAF}\textsuperscript{V600E} sequencing was not available for either specimen. Of note, 35 tumor samples from the entire cohort (spanning all histopathologic diagnoses) had \textit{BRAF}\textsuperscript{V600E} testing performed by both IHC and targeted sequencing methods, and results were concordant in all but one [this patient with pilocytic astrocytoma had positive \textit{BRAF}\textsuperscript{V600E} testing by IHC, but negative by sequencing, so this was interpreted as negative for the reported analysis (this patient's subsequent tumor sample had negative \textit{BRAF}\textsuperscript{V600E} IHC)].

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patients with pilocytic or pilomyxoid astrocytomas, with four remaining positive and six remaining negative.

Three (16%) patients initially tested positive for CDKN2A deletion, then lost this genetic alteration on subsequent surgical resection. Two patients had not received systemic therapy or irradiation prior to subsequent surgery [Patients #18 and #26, Fig. 1 (Patient #26's subsequent tumor specimen also lost prior BRAF V600E mutation, as described above)]. The third patient had three tumor samples available for CDKN2A testing; CDKN2A deletions were conserved in the first two tumor samples (which were obtained 14 months apart and following chemotherapy), but no CDKN2A deletion was identified in the final specimen, obtained at autopsy approximately 7 years later and following further systemic therapy plus irradiation (Patient #5, Fig. 1).

Six (21%) patients acquired a CDKN2A deletion at recurrence or progression, including two patients who
Fig. 2  Histologic and molecular findings in paired samples from representative patients.  

a The pilocytic astrocytoma from Patient #18 retained a \(BRAF\) fusion. Note that the partial duplication of \(3'\)BRAF (7q34) b is most commonly associated with the \(BRAF-KIAA1549\) fusion product. c The ganglioglioma from Patient #40 retained a \(BRAF^{V600E}\) mutation (d \(BRAF\) V600E IHC, 400x). e The pilocytic astrocytoma from Patient #2 acquired a hemizygous deletion (loss of one copy) of CDKN2A (f), while the diffuse astrocytoma (g) from Patient #33 lost this alteration (h). (2A, E, and G, H&E x100; 2C, x200)
Table 2  Overview of temporal genomic heterogeneity

|          | BRAF fusion | BRAFV600E | CDKN2A deletion | H3K27M mut. | IDH1 mut. | FGFR1 fusion | NTRK2 fusion | MYB fusion | MYBL1 fusion | ATRX loss |
|----------|-------------|-----------|-----------------|-------------|-----------|--------------|--------------|------------|--------------|-----------|
| Entire Cohort (all diagnoses, n = 45) |             |           |                 |             |           |              |              |            |              |           |
| Patients with paired tumor samples tested | 34          | 44        | 26              | 21          | 28        | 28           | 17           | 16         | 12           | 18        |
| Conserved |             |           |                 |             |           |              |              |            |              |           |
| Remained positive (S, I) | 17 (S = 9, I = 1) | 2 (S = 0, I = 0) | 4 (S = 1, I = 0) | 0          | 2* (S = 0, I = 1) | 0            | 0           | 0          | 0           | 1* (S = 0, I = 1) |
| Remained negative (S, I) | 17 (S = 4, I = 3) | 41 (S = 17, I = 5) | 11 (S = 4, I = 1) | 21 (S = 9, I = 3) | 26 (S = 10, I = 2) | 27 (S = 10, I = 4) | 17 (S = 5, I = 3) | 16 (S = 5, I = 3) | 12 (S = 4, I = 2) | 17 (S = 7, I = 3) |
| Changed |             |           |                 |             |           |              |              |            |              |           |
| Acquired (S, I) | 0          | 0        | 7 (S = 3, I = 1) | 0          | 0       | 1 (S = 0, I = 0) | 0           | 0         | 0           | 0        |
| Lost (S, I) | 0          | 1 (S = 0, I = 0) | 4* (S = 1, I = 2) | 0          | 0       | 0           | 0           | 0         | 0           | 0        |
| By Histopathologic diagnosis: |             |           |                 |             |           |              |              |            |              |           |
| Pilocytic or Pilomyxoid Astrocytoma (n = 28) |             |           |                 |             |           |              |              |            |              |           |
| Patients with paired tumor samples tested | 23          | 28        | 19              | 13          | 15        | 19           | 9            | 10         | 7           | 10        |
| Conserved |             |           |                 |             |           |              |              |            |              |           |
| Remained positive (S, I) | 15 (S = 9, I = 1) | 0        | 4 (S = 2, I = 0) | 0          | 0       | 0           | 0            | 0         | 0           | 0        |
| Remained negative (S, I) | 8 (S = 2, I = 1) | 27 (S = 14, I = 2) | 6 (S = 2, I = 0) | 13 (S = 6, I = 1) | 15 (S = 7, I = 1) | 18 (S = 8, I = 2) | 9 (S = 3, I = 1) | 10 (S = 3, I = 1) | 7 (S = 2, I = 1) | 10 (S = 5, I = 1) |
| Changed |             |           |                 |             |           |              |              |            |              |           |
| Acquired (S, I) | 0          | 0        | 6 (S = 2, I = 1) | 0          | 0       | 1 (S = 0, I = 0) | 0           | 0         | 0           | 0        |
| Lost (S, I) | 0          | 1 (S = 0, I = 0) | 3* (S = 1, I = 1) | 0          | 0       | 0           | 0            | 0         | 0           | 0        |
| Diffuse Astrocytoma (n = 7) |             |           |                 |             |           |              |              |            |              |           |
| Patients with paired tumor samples tested | 3          | 6         | 2               | 5           | 7        | 5            | 4            | 2          | 1           | 2        |
| Conserved |             |           |                 |             |           |              |              |            |              |           |
| Remained positive (S, I) | 0          | 0        | 0               | 0           | 2* (S = 0, I = 1) | 0            | 0           | 0         | 0           | 1* (S = 0, I = 0) |
Results for the entire cohort (all histologic diagnoses) are shown together at the top, followed by results for each histologic diagnosis subgroup individually. The number of patients who had testing for a given genetic alteration performed on paired tumor specimens is shown in the top row of each category. Subsequent rows illustrate the number of patients with conversed or changed (acquired or lost) status for given genetic alterations, specifying the number of patients who received systemic therapy (“S”) or irradiation (“I”) between surgeries.

* One of these patients had conserved IDH1 R132H mutations (Patient #33) and the other had conserved IDH1 R132G mutations (Patient #32).

* Although this patient had conserved ATRX loss demonstrated on IHC, sequencing revealed different ATRX mutations in the diagnostic and recurrent tumor samples, as described in the text.

* One of these patients had CDKN2A testing performed on three tumor specimens, with CDKN2A deletions identified (conserved) on the first two tumor samples, and subsequently lost on the third (post-mortem) tumor sample.
received cytotoxic chemotherapy (both with carboplatin and temozolomide, one with additional vincristine, vinblastine, and trametinib) and one who received photon irradiation prior to subsequent surgery (Table 4). Small (<5%) increases in the Ki67 proliferative index were detected in three of the recurrent tumors which acquired CDKN2A deletions, compared to their respective diagnostic specimens lacking this alteration; no other concurrent unfavorable morphologic changes were identified with acquisition of CDKN2A deletion (i.e., no change in mitotic activity or WHO grade, as noted above).

H3K27M mutation: Testing for the H3K27M mutation was performed on paired tumor samples from 13 patients with pilocytic or pilomyxoid astrocytomas (mostly with midline tumor locations) and was negative in all, including six patients who received systemic therapy and one who received irradiation prior to subsequent surgery (Table 2).

FGFR1 rearrangement: An FGFR1 rearrangement was detected in one of 19 (5%) patients with pilocytic or pilomyxoid astrocytomas who had this testing performed on all paired surgical specimens. This patient acquired an FGFR1-TACC1 rearrangement at recurrence (initially tested negative), with no prior systemic therapy or irradiation (Patient #22, Fig. 1). The remaining 18 patients had conserved negative FGFR1 rearrangement status on both primary and subsequent surgical specimens (including eight who received systemic therapy and two who received irradiation; Table 2).

IDH1 mutations, NTRK2, MYB, and MYBL1 rearrangements, and ATRX loss: Among patients with pilocytic or pilomyxoid astrocytomas who had testing for IDH1 mutations (n = 15), rearrangements of NTRK2 (n = 9), MYB (n = 10), or MYBL1 (n = 7), and ATRX loss (n = 10) performed on both paired surgical specimens, results remained negative in all paired samples, with no acquisitions or losses, including after systemic therapy or irradiation (Table 2).

Additional targeted sequencing results: Targeted sequencing was performed on paired tumor specimens of 10 patients with pilocytic or pilomyxoid astrocytomas. No loss or acquisition of alterations in the following genes were identified over time or following treatment (including five patients who received systemic therapy prior to subsequent surgery): AKT1, ALK, ATM, CDH1, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFR, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, and STK11. A mutation in EGFR (N115K c.345T > A) was identified in one patient (Patient #19) and conserved at second surgery.

Diffuse astrocytomas

BRAF fusion or duplication and BRAFV600E mutation: Among patients with diffuse astrocytomas who had testing for BRAF fusion/duplication (n = 3) or BRAFV600E mutation (n = 6) performed on all paired surgical specimens, testing remained negative in all, with no acquisition or loss (including in one patient who received systemic therapy and one patient who received irradiation prior to subsequent surgery; Table 2).

IDH1 mutation and ATRX loss: Among seven patients with diffuse astrocytomas who had testing for IDH1 mutations performed on paired surgical specimens, two (29%) patients tested positive at diagnosis and remained positive at recurrence. One patient (who received irradiation) had conserved IDH1 R132H mutations identified by IHC (Patient #33, Fig. 1) and one patient had conserved IDH1 R132G mutations identified on targeted sequencing (Patient #32, Fig. 1). IDH1 mutation testing for the other five patients remained negative, with no acquisition or loss (Table 2).

Other temporal genomic changes (CDKN2A deletion, ATRX loss, TP53 mutations) in one patient with an IDH1-mutant diffuse astrocytoma: The aforementioned

| Table 3 | Comparison of patients whose tumors exhibited temporal genomic heterogeneity (n = 12) and patients whose tumors had completely conserved genomic profiles (n = 33) |
|---------|--------------------------------------------------------------------------------------------------|
| | Patients whose tumors had temporal genomic heterogeneity | Patients whose tumors did not have temporal genomic heterogeneity | p value |
| n | 12 | 33 | – |
| Age at diagnosis [median (Range) in years] | 5.8 (0.4–15.7) | 6.1 (0.8–18.3) | 0.70 |
| Received systemic therapy and/or irradiation between surgeries [n (%)] | 6 (50%) | 14 (42%) | 0.30 |
| WHO grade I [n (%)] | 10 (91%) | 24 (73%) | 0.66 |

Statistics were performed using the Wilcoxon Rank-Sum test for differences in age, and using Fischer’s Exact test for differences in the proportions who received interval systemic therapy and/or irradiation and for WHO grade (I versus II) histology, with p-value <0.05 considered significant

* Excluding patients with tumors of indeterminate WHO grading [pilocytic astrocytoma or low grade neoplasm (unclear if WHO grade I versus II)]
| Patient ID # | Age at diagnosis (years) | Sex | Tumor Pathologic classification | Tumor location | Metastatic disease at diagnosis | Extent of surgery #1 | Time to first progression (months) | Time to surgery #2 (months) | Treatment received between diagnosis and gain of CDKN2A deletion | Current status (months from diagnosis to last follow-up) |
|--------------|--------------------------|-----|--------------------------------|----------------|-------------------------------|---------------------|-------------------------------|-------------------------------|---------------------------------------------------------------|----------------------------------------------------------|
| 1            | 5                        | F   | Pilocytic astrocytoma          | Diencephalic (hypothalamic/optic pathway) | No               | STR                | 20                            | 26                            | Chemotherapy (carboplatin, temozolomide)                        | Alive (104)                                               |
| 2            | 12                       | M   | Pilocytic astrocytoma          | Diencephalic (hypothalamic/optic pathway) | No               | STR                | 2                             | 27                            | Irradiation photon (50.4 Gy)                                   | Alive (116)                                               |
| 4            | 0.8                      | F   | Pilomyxoid astrocytoma         | Diencephalic (hypothalamic)                 | No               | Biopsy             | 3.5                           | 7                             | Chemotherapy (carboplatin/vincristine, vinblastine, temozolomide, trametinib), additional debulking surgery | Alive (58)                                               |
| 15           | 1                        | M   | Pilocytic astrocytoma          | Posterior fossa                             | No               | STR                | 19                            | 19                            | Surgery only                                                   | Alive (93)                                               |
| 18           | 5                        | M   | Pilocytic astrocytoma          | Posterior fossa                             | No               | STR                | 17                            | 17                            | Surgery only                                                   | Alive (182)                                               |
| 26           | 15                       | M   | Pilocytic astrocytoma          | Cerebrum (Right temporal-parietal)          | No               | STR (biopsy, then STR 1 week later) | 5.5                            | 55                            | Surgery only                                                   | Deceased (91; Drug overdose, unrelated to disease)         |
| 41           | 0.4                      | F   | Desmoplastic infantile ganglioglioma | Cerebrum (Right frontal) | No               | STR                | 4                             | 8                             | Chemotherapy (carboplatin/vincristine)                        | Alive (94)                                               |
patient with an IDH1-mutant (R132H) diffuse astrocytoma (Patient #33, Fig. 1) was found to have several genetic changes at recurrence and following radiotherapy, including loss of a hemizygous CDKN2A deletion (initially tested positive). Although conserved ATRX loss was demonstrated by IHC in both tumor samples, targeted sequencing revealed different ATRX mutations (R1426* in the diagnostic specimen and R1302fs*44 in the recurrent, post-irradiation specimen). Additionally, two somatic mutations in TP53 (E258G, R267W) were detected at diagnosis and conserved at recurrence; however, additional unique TP53 alterations were identified in this patient’s tumor samples, which were not shared (del exons 2–4, K132Q, N131del, R248W in the primary diagnostic sample, and R273C and E285* [both subclonal] in the recurrent sample), indicating possible loss and acquisition of these aberrations, respectively. H3K27M mutation: H3K27M mutation testing was performed on paired tumor samples from five patients with diffuse astrocytomas and was negative in all, including one patient who received systemic therapy prior to subsequent surgery (Table 2).

FGFR1, NTRK2, MYB, and MYBL1 rearrangements: Among patients with diffuse astrocytomas who had testing for rearrangements of FGFR1 (n = 5), NTRK2 (n = 4), MYB (n = 2), or MYBL1 (n = 1) performed on both paired surgical specimens, results remained negative in all pairs, with no acquisition or loss (Table 2).

**Gangliogliomas**

BRAF fusion or duplication: Testing for BRAF fusions or duplications was performed on paired surgical specimens in four patients with gangliogliomas, with conserved findings in all. Testing remained positive in two (50%) patients (including one who received systemic therapy prior to subsequent surgery) and remained negative in two (50%) patients (Table 2).

BRAF^{V600E} mutation: All five patients with gangliogliomas had BRAF^{V600E} mutation testing performed on paired surgical specimens, with no acquisition or loss detected. Two patients (40%) tested positive at diagnosis and remained positive at recurrence, and three patients (60%) remained negative (Table 2).

**Other LGGs**

Paired tumor samples from five additional patients with LGGs with other histologic diagnoses were included for analysis [desmoplastic infantile ganglioglioma (n = 1), pleomorphic xanthoastrocytoma (n = 1), angiocentric glioma (n = 1), and low-grade glial or glioneuronal neoplasms, not otherwise specified (n = 2)]. Acquisition of a hemizygous CDKN2A deletion was identified in the patient with a desmoplastic infantile ganglioglioma following systemic therapy (Patient #41, Fig. 1 and Table 4). No other loss or gain of genetic alterations were detected in this patient or the other four patients by FISH, mutation-specific IHC, or targeted sequencing. Mutations in CDH1 (A298T) and FGFR1 (K656_ T658> MTP) were identified by targeted sequencing in one patient with a low-grade glioneuronal neoplasm (dysembryoplastic neuroepithelial tumor [DNET]-like) and were conserved at recurrence (Patient #45, Fig. 1).

**Prognostic impact of temporal changes in CDKN2A deletion status**

Among 24 patients in the entire cohort who had CDKN2A deletion testing performed on paired tumor specimens, shorter time to progression (defined as time from diagnosis to a new medical or surgical intervention in response to clinical and/or radiographic concern for progression) and shorter time to subsequent surgery were observed among the seven patients with acquired CDKN2A deletions compared to patients without acquisition of this genetic alteration (median time to progression: 5.5 months versus 16.0 months (p = 0.048); median time to next surgery: 17.0 months versus 29.0 months (p = 0.031), Fig. 3; note: patients without clinical or radiographic progression were excluded from this analysis). Additionally, patients whose tumors acquired CDKN2A deletions had shorter time to progression and shorter time to subsequent surgery compared to patients with conserved CDKN2A deletions on primary and recurrent tumor samples (median time to progression: 5.5 versus 41.0 months (p = 0.009); median time to next surgery: 17.0 versus 41.0 months (p = 0.043), Fig. 3). Acquisition of CDKN2A deletion was also associated with shorter time to subsequent surgery when compared to loss of CDKN2A deletion (median: 17.0 months versus 46.5 months (p = 0.037)).

**Genomic conservation in small sample of metastatic lesions**

Four of 45 (9%) patients had metastatic disease at diagnosis and/or recurrence/progression. Two of these patients developed metastases at the time of recurrence, and surgical resection of these new metastatic lesions revealed identical genomic profiles to the respective primary tumors. One patient with a hypothalamic pilomyxoid astrocytoma developed tumor recurrence along a previous left frontal biopsy tract, which was subsequently biopsied (20 months after diagnostic surgery and following successive treatment with chemotherapy, a MEK inhibitor (trametinib), and an mTOR inhibitor); both primary and recurrent metastatic specimens remained positive for BRAF fusion and negative for BRAF^{V600E} (Patient #7, Fig. 1).
patient with a periventricular low-grade glioneuronal neoplasm (DNET-like) later developed a new, non-continuous right temporal lobe mass, which was subsequently resected (13 months after diagnostic surgery and following treatment with craniospinal irradiation); both primary and recurrent metastatic specimens were found to have the same aforementioned mutations in CDH1 (A298T) and FGFR1 (K656_T658 > MTP) on sequencing and were otherwise negative for BRAF, FGFR1, NTRK2, MYB, or MYBL1 rearrangements, CDKN2A deletions, or BRAF^{V600E} or IDH1 mutations (Patient #45, Fig. 1).

Discussion
To our knowledge, this is the first study to evaluate temporal and therapy-related genomic heterogeneity of pediatric LGGs through paired FISH, mutation-specific IHC, and/or targeted sequencing of a cohort of 94 total primary diagnostic and subsequent surgical tumor specimens. A direct comparison of the genomic profiles...
of paired samples reveals conservation of most genetic alterations over time and after therapy, but with possible changes in CDKN2A deletion status, including acquired CDKN2A deletions in a potentially higher risk subset of patients.

Most targetable genetic aberrations in pediatric LGGs, including BRAF alterations, are conserved over time, at recurrence, and following treatment with chemotherapy, other systemic therapy, or irradiation. These results are consistent with and significantly expand upon whole-genome sequencing data reported by Zhang et al. showing identical genetic profiles of two pairs of primary and recurrent pediatric LGGs, including preserved FGFR1 duplications [68]. Importantly, BRAF fusion or duplication status was conserved in 100% of patients in our pediatric LGG cohort, with no acquisition or loss over time (including at third surgery) or after therapy. As BRAF fusions are identified in a large portion of pilocytic astrocytomas [29, 54] and there is growing evidence supporting the efficacy and tolerability of MEK inhibitors for pediatric LGGs harboring BRAF fusions [5, 8, 50], our findings have important therapeutic implications. Clinicians should feel confident that pediatric LGGs with BRAF fusions detected at diagnosis will retain this genetic alteration, such that targeted therapy with MEK inhibitors can be implemented at relapse without requiring genetic confirmation with repeat biopsy, in agreement with most providers’ current practice as well as previous clinical trials of these agents in the recurrent, refractory setting not mandating repeat molecular testing [5, 8, 50]. Additionally, BRAF fusions were conserved in recurrent tumor samples from three patients who progressed despite prior single-agent MEK inhibitor treatment, indicating likely preservation of this alteration following failed MEK inhibitor monotherapy. Although limited by a small sample size and deserving further exploration, this finding supports prior reports implicating alternative escape mechanisms (PI3K-AKT-mTOR signaling cascade), rather than loss of this BRAF alteration, in fusion-positive pediatric LGGs which develop resistance to MEK inhibitors, suggesting a potential role for future combination therapy in these patients [26].

BRAFV600E mutations have been identified in certain subtypes of pediatric LGGs [17, 68] and also represent a promising therapeutic target, given emerging efficacy and safety data of BRAF inhibitors in children with recurrent LGGs harboring this alteration [6, 23, 34]. In our paired LGG cohort, BRAFV600E mutation status (mostly negative) was conserved over time and following treatment in 98% of patients. Loss of BRAFV600E was observed in one patient by IHC testing, though confirmatory sequencing was not available; while mutant-specific IHC for BRAFV600E generally correlates well with BRAF sequencing, it may on occasion yield false positive or false negative results [18]. Furthermore, this patient did not receive previous systemic therapy or irradiation, and there was no obvious radiographic evidence of progression at the time of second surgery [performed due to clinical concern (increased seizures)], so this genetic change should be interpreted cautiously and may be the result of sampling bias. Our findings overall suggest conservation of BRAFV600E mutation status in pediatric LGGs, including after previous therapy, yet with possible rare risk of loss that deserves further investigation.

Similarly, no tumors acquired NTRK2, MYB, or MYBL1 fusions or IDH1 mutations, suggesting preserved negative status of these genetic alterations can also likely be presumed at recurrence, including after prior treatment, without the need to obtain confirmatory biopsy tissue. Although acquisition of FGFR1 rearrangement occurred in one patient (without preceding systemic therapy or irradiation), the majority (96%) of tumors with available FGFR1 testing remained negative at recurrence and following therapy, supporting conserved status of this alteration commonly as well.

Histologic diagnosis and tumor grade were conserved in 100% of pediatric LGGs at recurrence or progression in our cohort, with no evidence of malignant transformation to HGG. Additionally, IHC testing for the H3K27M mutation, a genetic alteration which confers a dismal prognosis [32, 37] and is now sufficient criteria alone for WHO histologic grade IV diagnosis in patients with diffuse midline glioma [36], was negative in all tested paired tumor specimens, with no acquisition identified at time of relapse. These findings are consistent with prior studies suggesting evolution to HGG is extremely rare among pediatric LGGs even after prior systemic therapy or irradiation [9, 40].

A comparison of the molecular biology of primary and metastatic disease in pediatric LGGs is almost entirely absent in the literature to date [12]. Metastases at diagnosis and/or progression were seen in four (9%) patients in our cohort, consistent with a relatively low frequency of metastatic disease in pediatric LGGs [12]. Two of these metastatic lesions were biopsied or resected at the time of progression, and their genomic profiles were identical to the respective primary tumors, following chemotherapy in one patient and irradiation in the other patient. These results suggest genetic alterations may be conserved in metastases over time and after prior treatment; however, given the small number of patients with metastatic disease available for genomic testing, further study assessing spatial genomic heterogeneity in pediatric LGGs is needed to draw more definitive conclusions.

Although most genetic alterations were conserved over time in our pediatric LGG cohort, changes in
CDKN2A deletion status at recurrence or progression were observed in 11 patients (42% of patients who had this testing performed on paired tumor specimens), with acquisition of hemizygous CDKN2A deletions in seven patients and loss in four. It is possible this discordance in CDKN2A deletion results over time is due to differences in sampling locations within tumor specimens and/or between tumor and closely surrounding or contaminated normal brain tissue. While we cannot definitively rule out sampling bias as an explanation for these findings, we believe this is less likely, given the lack of spatial heterogeneity of other genetic alterations analyzed, conservation of negative CDKN2A deletion status in one patient who had this testing performed on paired metastatic tumor samples, and evidence of spatial preservation of CDKN2A deletions from intratumoral genomic studies in glioblastoma [42, 56]. This genetic alteration has potential implications for treatment, as CDKN2A deletion or inactivation, which results in unrestricted progression through the G1-S cell cycle checkpoint, can be targeted with CDK4/6 inhibition. There is emerging evidence supporting the efficacy of CDK4/6 inhibitors in various solid tumors which harbor CDKN2A deletions or inactivation, as demonstrated in pediatric HGGs following targeted therapy [51], potentially as an acquired treatment resistance mechanism. Among the four patients in our cohort whose tumors exhibited loss of CDKN2A deletions, two had undergone previous irradiation. One of these patients was also treated with a MEK inhibitor, mTOR inhibitor, and lenalidomide in the time between previous surgery and autopsy; given the close interplay between the Ras/Raf/MEK/ERK, PI3K/Akt/mTOR, and CDK4/6/Rb pathways [45], with the former two acting upstream of the latter, it is possible that loss of CDKN2A deletion in this patient represented acquired resistance to prior MEK and mTOR inhibitor therapy. However, the other two patients with loss of CDKN2A deletions at recurrence had not been treated with prior systemic therapy or irradiation. Further research is therefore necessary to determine which tumors are at highest risk of acquiring or losing CDKN2A deletions, including investigating the role of prior treatment and whether temporal changes in CDKN2A deletion status contribute to acquired resistance to targeted agents.

Notably, shorter time to progression and subsequent surgery was observed in patients in our cohort whose tumors acquired hemizygous CDKN2A deletions, suggesting tumors that gain this genetic alteration may represent a unique subset of pediatric LGGs with a poorer prognosis. Previous studies have shown that the presence of CDKN2A deletions at diagnosis defines a higher risk group with worse outcomes [10, 35, 47, 53, 65]. Our novel findings indicate this genetic alteration can be acquired at relapse and may confer a worse prognosis. While conserved CDKN2A deletion status was not associated with poorer outcomes in our cohort (although interpretation is limited by the small sample size), patients with acquisition of hemizygous CDKN2A deletion at recurrence exhibited shorter time to progression and shorter time to next surgery, both when compared to patients without acquisition of this genetic alteration and when directly compared with patients whose tumors had preserved CDKN2A deletions from diagnosis [of note, there were no differences in other factors known to impact prognosis, including extent of first surgical resection (majority underwent subtotal resection) or proportion with diencephalic tumor location, between patients with gain of CDKN2A deletion versus those with conserved positive CDKN2A deletion status]. Acquired CDKN2A inactivation (gene deletion or hypermethylation resulting in decreased expression) at time of progression has been reported in studies of paired tumor analyses of other malignancies, including lymphoma, cervical cancer, and prostate cancer [19, 22, 46, 63]. Additionally, in two studies of pediatric LGGs which underwent malignant
transformation, while CDKN2A loss was identified in a majority of tumors at diagnosis and conserved over time, two of 16 paired tumor specimens gained CDKN2A deletions at the time of evolution to HGG (one of eight from each report) [9, 40]. To our knowledge, this is the first study to suggest acquisition of CDKN2A deletion can occur and potentially contribute to progression in pediatric LGGs, even in the absence of malignant transformation.

It is important to acknowledge that all CDKN2A deletions identified in our cohort (with acquisition, loss, or conservation over time) were hemizygous, not homozygous. Although the presence of homozygous CDKN2A deletions at diagnosis in pediatric LGGs is well-recognized as an independent driver of cellular proliferation, with associated worse outcomes as described above [47, 53, 65], research into the biological consequences and prognostic impact of hemizygous deletions is lacking. Given reports demonstrating poorer prognosis in pediatric LGGs with decreased expression of p16<sup>INF4a</sup> (protein encoded by CDKN2A) [25, 47], significant reduction in p16<sup>INF4a</sup> expression among tumors with either hemizygous or homozygous CDKN2A deletions in at least one study [43], and aggressive clinical behavior of LGGs harboring hemizygous CDKN2A deletions [66], it is possible that the hemizygous CDKN2A deletions acquired in our cohort resulted in sufficiently low protein expression to drive cell cycle progression. However, another study conversely did not observe a clinically meaningful decrease in p16<sup>INF4a</sup> expression among tumors with either hemizygous or homozygous CDKN2A deletions in at least one study [20]; therefore, the extent to which hemizygous CDKN2A deletions contribute to tumorigenesis, either independently or in combination with other genetic alterations, remains uncertain and critically deserving of further investigation. Given reported simultaneous acquisition of CDKN2A inactivation with other oncogenic mutations in solid tumors at progression [22] as well as genetic/epigenetic alterations in oncogenes or tumor suppressor genes commonly co-occurring with CDKN2A inactivation across various malignancies [57, 69], it is possible that pediatric LGGs which acquire hemizygous CDKN2A deletions have developed additional co-driver mutations, which were not tested for here, but should be investigated in future study. Additionally, there is emerging pre-clinical and clinical data explaining potential mechanisms by which CDKN2A deletions, perhaps in combination with other concurrent genetic aberrations, contribute to tumor progression in pediatric LGGs. Specifically, the role of CDKN2A inactivation in allowing escape from tumor senescence has been documented in pediatric LGGs [25, 35], and results from a pediatric LGG xenograft murine model suggests that CDKN2A deletion, in combination with BRF<sup>V600E</sup> mutation, is a key molecular change that mediates tumor progression, invasion, and migration [33]. Of the seven patients in our cohort whose tumors acquired hemizygous CDKN2A deletions, none had concurrent BRF<sup>V600E</sup> mutations at diagnosis or progression, but four had BRF fusions which were conserved over time; further research is thus needed to determine whether hemizygous CDKN2A loss and BRF fusions may act synergistically in facilitating tumor growth.

Lastly, in addition to loss of CDKN2A deletion, temporal changes in the TP53 and ATRX genetic landscape of one adolescent patient with an IDH1-mutant diffuse astrocytoma, treated initially with radiotherapy alone, were identified at recurrence. Although some TP53 mutations were conserved, both loss and acquisition of other likely pathogenic TP53 genetic alterations occurred. Additionally, sequencing of the diagnostic and recurrent tumor specimens revealed different ATRX mutations, though both resulted in conserved loss of ATRX function. Irradiation-induced mutagenesis of TP53 and ATRX has been reported, at least in secondary gliomas which developed following therapeutic irradiation for a prior malignancy [38]. Furthermore, in the aforementioned studies of paired pediatric HGGs and malignantly-transformed adult LGGs, heterogeneous genetic alterations of TP53 and/or ATRX were similarly observed within the IDH1-mutant tumor pairs [27, 51], with conservation of IDH1-R132H mutations, similar to our patient. These findings deserve further exploration and may be more applicable to the adolescent and young adult neuro-oncology population, given the relatively low frequency of IDH1-mutations in pediatric LGGs [3, 64], which was re-demonstrated in our cohort.

Our study was limited by a small sample size and insufficient tumor tissue to perform all relevant molecular tests on all paired specimens, further decreasing the number of analyzable cases. Additional valuable genomic analyses such as whole genome and/or whole exome sequencing, RNA sequencing, and methylation testing were not available, and should therefore be incorporated into future studies in order to expand the molecular knowledge gained from FISH, mutation-specific IHC, and targeted sequencing performed here. Further research assessing spatial genomic heterogeneity in pediatric LGGs will also be critical, both to explore on a larger scale the above findings suggesting preservation of genetic alterations in metastases and to investigate sampling bias as a potential explanation for the discordance observed in CDKN2A deletion status, which we cannot definitely rule out. Finally, future investigation into potential mechanisms underlying acquisition or loss of CDKN2A deletions, risk factors for these temporal changes, and the biological consequences and prognostic
impact of hemizygous (as opposed to homozygous) CDKN2A deletions, is essential.

Despite these limitations, this report characterizes temporal genomic heterogeneity in a pediatric LGG cohort and offers novel findings with important therapeutic implications. We demonstrate that most actionable genetic alterations in pediatric LGGs, including BRAF fusions or mutations, are conserved at recurrence, after prior systemic therapy or irradiation treatment, and in a small number of tumors with metastases. Repeat biopsy therefore is likely not necessary to confirm preservation of BRAF alteration status. Histologic diagnosis and grade remained the same in all tumors, with no acquisition of H3K27M mutations or evidence of malignant transformation. However, changes in CDKN2A deletion status over time were demonstrated, and acquisition of hemizygous CDKN2A deletion may define a higher risk subgroup of pediatric LGGs with a poorer prognosis. Given the potential for targeted therapies for tumors harboring CDKN2A deletions, performing a biopsy at recurrence may be indicated in certain patients, especially those with rapid progression.

Acknowledgements

We thank all the patients and families who have supported this project. We also thank the PLGA Fund at the Pediatric Brain Tumor Foundation (PBTF) and CancerFree KIDS for their support of this study.

Funding

PLGA Fund at Pediatric Brain Tumor Foundation (PBTF) and CancerFree KIDS.

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Received: 25 August 2020 Accepted: 10 October 2020
Published online: 05 November 2020

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