ABSTRACT

Background. Pediatric brain tumors are the leading cause of death for children with cancer in the U.S. Incorporating next-generation sequencing data for both pediatric low-grade (pLGGs) and high-grade gliomas (pHGGs) can inform diagnostic, prognostic, and therapeutic decision-making.

Materials and Methods. We performed comprehensive genomic profiling on 282 pediatric gliomas (157 pHGGs, 125 pLGGs), sequencing 315 cancer-related genes and calculating the tumor mutational burden (TMB; mutations per megabase [Mb]).

Results. In pLGGs, we detected genomic alterations (GA) in 95.2% (119/125) of tumors. BRAF was most frequently altered (48%; 60/125), and FGFR1 missense (17.6%; 22/125), NF1 loss of function (8.8%; 11/125), and TP53 mutations (5.6%; 7/125) were also detected. Rearrangements were identified in 35% of pLGGs, including KIAA1549-BRAF, QKI-RAF1, FGFR3-TACC3, CEP85L-ROS1, and GOPC-ROS1 fusions. Among pHGGs, GA were identified in 96.8% (152/157). The genes most frequently mutated were TP53 (49%; 77/157), H3F3A (37.6%; 59/157), ATRX (24.2%; 38/157), NF1 (22.2%; 35/157), and PDGFRα (21.7%; 34/157). Interestingly, most H3F3A mutations (81.4%; 35/43) were the variant K28M. Midline tumor analysis revealed H3F3A mutations (40%; 40/100) consisted solely of the K28M variant. Pediatric high-grade gliomas harbored oncogenic EML4-ALK, DGKB-ETV1, ATG7-RAF1, and EWSR1-PATZ1 fusions. Six percent (9/157) of pHGGs were hypermutated (TMB >20 mutations per Mb; range 43–581 mutations per Mb), harboring mutations deleterious for DNA repair in MSH6, MSH2, MLH1, PMS2, POLE, and POLD1 genes (78% of cases).

Conclusion. Comprehensive genomic profiling of pediatric gliomas provides objective data that promote diagnostic accuracy and enhance clinical decision-making. Additionally, TMB could be a biomarker to identify pediatric glioblastoma (GBM) patients who may benefit from immunotherapy. The Oncologist 2017;22:1478–1490

Implications for Practice: By providing objective data to support diagnostic, prognostic, and therapeutic decision-making, comprehensive genomic profiling is necessary for advancing care for pediatric neuro-oncology patients. This article presents the largest cohort of pediatric low- and high-grade gliomas profiled by next-generation sequencing. Reportable alterations were detected in 95% of patients, including diagnostically relevant lesions as well as novel oncogenic fusions and mutations. Additionally, tumor mutational burden (TMB) is reported, which identifies a subpopulation of hypermutated glioblastomas that harbor deleterious mutations in DNA repair genes. This provides support for TMB as a potential biomarker to identify patients who may preferentially benefit from immune checkpoint inhibitors.
**INTRODUCTION**

Pediatric gliomas represent a diverse group of tumors of varying histologies, grades, and genetics. Broadly, these tumors can be divided into two major categories: pediatric low-grade (pLGG) and high-grade (pHGG) gliomas. Traditionally, gliomas have been diagnosed solely on histologic criteria; however, because morphologic overlap occurs frequently, there remains a need for objective data to promote diagnostic accuracy and support prognostic and therapeutic decision-making. With pediatric brain tumors surpassing leukemia as the leading cause of cancer-associated mortality among children, the need to identify new therapeutic strategies has never been greater [1].

The emergence of next-generation sequencing has helped identify key biomarkers, which in several instances are disease-defining alterations. Among pLGGs, BRAF alterations are most commonly observed in pilocytic astrocytomas, pleomorphic xanthoastrocytomas (PXA), gangliogliomas, and other glioneuronal lineage tumors [2–5]. Additionally, MYB alterations define subsets of pediatric diffuse gliomas, including MYB-QKI fusions for pediatric angiogenic gliomas [6, 7]. Mutations in histone proteins (H3F3A) reveal the importance and specificity of epigenome alterations in pHGGs [8–10].

Recently, checkpoint inhibitors (programmed death-ligand 1 [PD-L1] or programmed cell death protein 1 [PD-1] inhibitors) have emerged as potential therapeutic options for adult and pediatric high-grade gliomas [11–13]. Monoclonal antibodies that block PD-1 (pembrolizumab, nivolumab) or PD-L1 (atezolizumab) can boost the immune response against cancer cells and have shown promise in treating multiple cancer types [14]. Tumor mutational burden (TMB), defined as mutations per megabase (Mb), has proven to be a reliable biomarker for stratifying tumors (e.g., non-small cell lung cancer [NSCLC], melanoma) and is thought to serve as a proxy for overall neo-antigen burden [15, 16]. The value of TMB as an objective biomarker in the setting of pediatric gliomas remains largely unexplored; however, there is increasing optimism that high TMB may be a biomarker to define a subset of pHGGs most likely to respond to checkpoint inhibitors [11, 17].

In this study, we highlight the value of comprehensive genomic profiling (CGP) in the largest known cohort of pediatric gliomas and explore the most common alterations across diagnosis and anatomic location. Additionally, we explore TMB and associated genetic factors that may predispose patients to developing a hypermutator phenotype.

**MATERIALS AND METHODS**

Approval for this study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act of 1996 waiver of authorization, was obtained from the Western Institutional Review Board (IRB number 20152817). Comprehensive genomic profiling was performed in a Clinical Laboratory Improvement Amendment-certified, College of American Pathologists-accredited, New York State-regulated reference laboratory (Foundation Medicine, Inc.). All samples underwent central histopathologic review by a board-certified neuropathologist (S.H.R.) using World Health Organization (WHO) criteria. At least 50 ng of DNA per specimen was extracted from 282 clinical formalin-fixed paraffin-embedded tumor samples, and next-generation sequencing was performed on hybridization-captured, adaptor ligation-based libraries to high, uniform coverage (>500 read coverage depth) for all coding exons of 315 cancer-related genes and 28 genes commonly rearranged in cancer (FoundationOne, Foundation Medicine Inc., Cambridge, MA, https://www.foundationmedicine.com; supplemental online Table 1). Sequence data were analyzed for clinically relevant classes of genomic alterations, including base pair substitutions, insertions/deletions, copy number alterations, and rearrangements/fusions. All gene amplification events reported in this study represent high-level amplification. Although we recognize that there are differences for naming H3F3A mutations in the literature, in this study, H3F3A variants (H3F3A K28M or G34R/V) align with current Catalogue of Somatic Mutations in Cancer nomenclature, but are equivalent to K27M or G34R/V [18]. Tumor mutational burden was calculated over 1.1 Mb as the number of somatic, coding point mutations and indels per Mb of genome (low: 0–<6; intermediate: 6–19; high: ≥20 mutations per Mb).

**RESULTS**

**Cohort Demographics**

Comprehensive genomic profiling was performed on a cohort of 282 samples from pediatric patients diagnosed with low- and high-grade gliomas. The median age was 11 years and ranged from <1 to 18 years. Of the 282 patients, 50% (141/282) were male and 50% (141/282) were female (supplemental online Table 2). Tumors with noncategorical features were classified as low-grade glioma (LGG) not otherwise specified (NOS) or high-grade glioma (HGG) NOS. Integrated genomic analyses were then performed separately for pediatric HGGs (pHGGs) and LGGs (pLGGs). The pHGG cohort consisted of 125 patients (58 males and 67 females) with a median age of 9 years and ranging from <1 to 18 years, and included a diverse range of tumor types (supplemental online Table 3A). The mean tumor purity of samples was 30% and the mean coverage was 665X. The pHGGs consisted of 157 specimens of varying diagnoses (supplemental Table 3B) with a median age of 11 years (range: <1–18 years) and consisted of 83 males and 74 females. The mean tumor purity of samples was 65% and the mean coverage was 705X.

**Genomic Landscape of pLGGs**

Genomic alterations were identified in 95.2% (119/125) of pLGG specimens, with an average of 1.8 genomic alterations per specimen (Fig. 1A, 1B). BRAF was the most commonly altered gene, with variants detected in 48.0% (60/125) of cases including 26 base substitutions and 35 gene fusions. Other frequently altered genes included FGFR1, NF1, and TP53, which were reported in 17.6% (22/125), 8.8% (11/125), and 5.6% (7/125) of cases, respectively.

Among 46 pilocytic astrocytomas (PAs), 61% (n = 28) harbored KIAA1549-BRAF fusions, whereas 13% (n = 6) tumors harbored BRAF V600E mutations (supplemental online Fig. 1A). Interestingly, co-occurring alterations were detected in seven KIAA1549-BRAF PAs, including four with mutations in TP53, ARID1A, MDMA, or BRCA1, and three with CDKN2A/B deletions, and CDK4 or CDK6 amplifications. Of the 12 PAs in which BRAF alterations were not detected, 7 harbored FGFR1
alterations, including 1 with a duplication, three FGFR1 N546K, and three FGFR1 K656E variants (supplemental online Fig. 1A). The correlation between anatomic location and BRAF or FGFR1 alterations is illustrated in supplemental online Table 4. Five PAs without BRAF or FGFR1 alterations had mutations in other pediatric glioma-associated genes. One tumor harbored a previously reported QKI-RAF1 fusion co-occurring with a NOTCH1 V1575I mutation, whereas an uncharacterized EGFR R222C extracellular domain mutation was the only alteration detected in another PA [7]. NF1 loss-of-function mutations were detected in two PAs, with one tumor reporting a co-occurring PDGFRA A491T mutation. One other PA harbored GLI1 P535S and XRCC2 R258H mutations.

Of the 12 diffuse astrocytomas, IDH1 and TP53 mutations were the most frequent alterations, co-occurring in 41.6% (5/12) of cases (supplemental online Fig. 1B). These five IDH1-mutated tumors were from mid-to-late adolescence (ages 15–17) patients with genetics similar to adult diffuse astrocytomas,
which frequently harbor co-occurring TP53 and IDH1 mutations [19, 20]. Three of these TP53/IDH1-mutated tumors also harbored other mutations, including one tumor with PIK3CA D350H, ACVR1B, and ATRX frame-shift mutations, another tumor with a KRAS G13D, and the third tumor with FGFR1 K656E and ALK R1212H mutations. Of the seven DA2s without IDH1 and TP53 lesions, we detected an FGFR3-TACC3 fusion co-occurring with a PIK3CG D238N mutation in one tumor and another tumor with a CEP85L-ROSI fusion (supplemental online Fig. 1B). Other tumors harbored KRAS Q61H and PIK3R2 Q494* mutations, a KDMSC R179H mutation co-occurring with homozygous TSC2 deletion, NF1 frame-shift mutations, or EGFR A289V and PIK3CA H1047R mutations. Furthermore, our cohort included a hypothyhalamic DA2 with an H3F3A K28M mutation, which raises the possibility that the tumor may follow a more aggressive clinical course as supported by recent studies. These showed that H3F3A K28M mutations are a negative prognostic factor among midline pLGGs. Additionally, in light of this finding, midline infiltrating LGGs harboring H3F3A K28M mutations may be best classified as the newly recognized entity, diffuse midline glioma H3F3A-K28M mutant (WHO grade IV) [10, 21, 22].

Glioneuronal tumors including gangliogliomas (n = 8), two rosette forming glioneuronal tumor (RFGNT) and one PKA, were enriched for BRAF alterations, specifically BRAF V600E mutations, which occurred in 63.6% (7/11) of cases (supplemental online Fig. 1C). In two cases, one GG and the PKA, the BRAF V600E mutation co-occurred with CDKN2A/B deletions. Interestingly, another ganglioglioma (GG) harbored a TMEM106B-BRAF fusion as well as loss of CDKN2A/B. We also identified a GG with an H3F3A K28M mutation co-occurring with an APC I307K and NF1 frame-shift mutations. Both RFGNTs harbored PIK3CA H1047R mutations, with one tumor harboring co-occurring FGFR1 N546K and BRIPl A453T mutations (supplemental online Fig. 1C).

Among pLG G LOS tumors, the most common alterations involved BRAF, FGFR, and NF1, which occurred in 31.6% (12/38), 21.1% (8/38), and 15.8% (6/38) of cases, respectively (supplemental online Fig. 1D). Of the 12 cases with BRAF lesions, 7 harbored BRAF V600E mutations, with 5 tumors harboring co-occurring mutations including CDKN2A/B deletions, RUNX1 deletion, KEI amplification, ARIDIA Q1835* or PRKDCh H1613R mutations. We also detected KIAA1549-BRAF fusions in three pLG G LOS tumors. Two other tumors harbored a BRAF G466V mutation or T599_V600 insertion. FGFR altered tumors (n = 8) included an FGFR3-TACC3 fusion with a co-occurring PIK3CA M1043V mutation, FGFR2-INA fusion, and another tumor with an FGFR1 duplication (supplemental online Fig. 1D). The other five tumors reported FGFR1 point mutations, including two with FGFR1 K656E variants and three with FGFR1 N546K variants; four of these tumors harbored co-occurring mutations including PIK3CA, NF1, orPTPN11 mutations. Of the six NF1 mutated pLG G LOS tumors, three harbored co-occurring FGFR1 mutations, and the other three tumors harbored co-occurring MLL3, PTPN11, or ARIDIA and CDKN2 mutations.

Nine LGG LOS tumors harbored alterations in a variety of other glioma-associated genes. For example, in one tumor, we identified a TPM3-NTRK1 fusion co-occurring with CDKN2A/B deletions. Another tumor harbored a GOPC-ROSI fusion with TP53 and PTPN11 mutations. In the other seven tumors, we detected DNM1T3A and KDMSC, PIK3R1, BRCA2, KRAS, SLIT2, VHL, or ATM and CTTNB1 mutations. Taken together, these findings highlight a subset of LGG LOS tumors, which are difficult to classify solely on histologic features, but can be categorized by their genomic signatures for use in refining pathologic diagnoses and supporting clinical decision-making.

Of the nine PMA s in our cohort, five harbored FGFR1 alterations, including three with K656E mutations and two with duplications. The other four PMA s harbored BRAF alterations wherein three tumors were V600E positive and the other had a KIAA1549-BRAF fusion co-occurring with EPHB1 V322I mutation.

The less prevalent histologic subtypes included four oligodendrogliomas grade 2 (G2), two dysembryoplastic neuroepithelial tumour (DNET), two RGNTs and one of each diffuse intrinsic pontine glioma (grade II) (DIPG G2), papillary glioneuronal tumor (PGNT), PXA (G2) and disseminated oligodendroglial-like leptomeningeal tumor (DOLT) (Fig. 2). Three of the four oligodendrogliomas harbored fusions including a QKI-RAFF, FGFR2-PASD1, or FGFR3-TACC3. The fourth tumor harbored IDH1 R132H and CIC p15025*12 mutations, which is genomically similar to adult oligodendrogliomas [19]. In the two DNETs, a DNTM3A splice site mutation was detected in one tumor and an FGFR1 K656E mutation with SOX2 amplification in the other. Both RGNTs harbored PIK3CA H1047R mutations, with one of the tumors reporting co-occurring FGFR1 N546K and BRIPl A453T mutations. In the DIPG (G2), PGNT, PXA (G2), and DOLT a KIAA1549-BRAF fusion, FGFR1 N546K and PIK3R1 F456_Q547del, BRAF V600E, CDKN2A/B deletions, or KIAA1549-BRAF and SMDAP4 P295I alterations, were identified, respectively (Fig. 2).

With growing evidence for the role of targeted therapies in pediatric neuro-oncology, the value of CGP is eminently clear. In the case of an 11-year-old male diagnosed with neurofibromatosis type 1 and a cerebellar PA, multiply recurrent and treated with surgery alone, CGP identified NFI (splice site 2251 + 1G>A, splice site 3708 + 1G>A) and PDGFRa A491T alterations. Subsequent combination therapy with everolimus (mTOR inhibitor) and trametinib (MEK inhibitor) yielded a robust antitumor response. As demonstrated by T1 post-gadolinium coronal magnetic resonance imaging images (Fig. 2H), 3 months of dual inhibitor therapy resulted in a marked decrease in the enhancing component of this patient’s tumor.

Genomic Landscape of pHGGS
We detected genomic alterations in 96.8% (152/157) of HGG specimens, with an average of 6.5 genomic alterations per sample (Fig. 3A, 3B). The most frequently mutated gene in this cohort was TP53, which was altered in 49% (77/157) of samples. Other frequently altered genes included H3F3A mutations in 37.6% (59/157), NF1 loss of function mutations in 24.2% (38/157), ATRX loss of function mutations in 22.2% (35/157), and PDGFRa alterations in 21.7% (34/157) of pHGGS (Fig. 3A, 3B).

In our cohort of 90 GBMs, TP53 was mutated in 62.2% (56/90) of cases, and H3F3A alterations were detected in 47.8% (43/90) of cases (Fig. 4A). Interestingly, the vast majority (81.4%, 35/43) of H3F3A mutations were the K28M variant, compared with 18.6% (8/43) being G35R variants. Although frequent in pLGGS, only five pediatric glioblastoma (pGBM)s harbored BRAF V600E mutations, and of these tumors, four harbored co-occurring H3F3A K28M mutations. Unlike adult
GBM, alterations in EGFR, PTEN, CDKN2A, and CDKN2B were only observed in 7.8% (7/90), 15.6% (14/90), 20% (18/90), and 15.6% (14/90) of pediatric GBMs, respectively [23, 24]. However, PDGFRA alterations, including point mutations and amplifications, were observed in 25.6% (23/90) of cases, which is nearly twice the rate observed in adult GBMs (15%; Fig. 4A) [25].

Anaplastic astrocytomas showed frequent EGFR alterations (mutations and amplifications), TP53 mutations, and PIK3CA mutations at rates of 40.7% (11/27), 33.3% (9/27), and 18.5% (5/27), respectively (Fig. 4B). Mutations in the TERT promoter were also frequent in AA3 tumors, with 25.9% (7/27) of tumors harboring the −124C>T variant. Four anaplastic astrocytomas (AAs) also harbored H3F3A K28M mutations that co-occurred

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Figure 2. Histologic subtypes and associated genomic alterations. Hematoxylin and eosin (H&E) staining and corresponding genomic alteration for four oligodendrogliomas (A), two DNETs (B), two RFGNTs (C), one DIPG G2 (D), one PGNT (E), one PXA G2 (F), and one DOLT (G). (H): H&E from a pilocytic astrocytoma harboring NF1 splice site and PDGFRA missense mutations. Postcontrast magnetic resonance imaging shows decrease in tumor size following 3 months of treatment with everolimus and trametinib.

Abbreviations: NF1, neurofibromatosis 1; PDGFRA, platelet derived growth factor receptor A.
with PIK3R1, FGFR1 and ATRX, PIK3CA and NF1, or TP53 mutations.

In the pHGG NOS cohort, H3F3A was altered in 23.1% (6/26) of cases, all of which were the K28M variant (Fig. 4C). NF1 and TP53 alterations were also frequent in pHGG NOS; they were detected in 23.1% (6/26) of tumors, co-occurring in five of the six cases. NF1 alterations were predominately loss of function mutations (5/6 cases), with one tumor harboring a homozygous NF1 deletion. FGFR1 (19.2%, 5/26) and PDGFRA (19.2%, 5/26) alterations were also prevalent in pHGG NOS tumors. Three of five FGFR1 mutated tumors harbored N546K variants co-occurring with other mutations including CDKN2A/B deletions, PIK3CA H1047R or H3F3A K28M mutations. Of the five PDGFRA altered tumors, four reported amplifications and

Figure 3. Genomic landscape of pHGG. (A): Tile plot illustrating the type of recurrent genomic alterations (n ≥ 2) and corresponding histologic diagnosis in 157 pHGGs. (B): Long-tail distribution highlighting the top 10 altered genes in the pHGG cohort. Types of alterations are color-coded using key at bottom of bar graph.

Abbreviations: AA(G3), anaplastic astrocytoma grade III; AGG, anaplastic ganglioglioma; APXA, anaplastic pleomorphic xanthoastrocytoma grade III; G, grade; HGG, high-grade glioma; NOS, not otherwise specified; OG, oligodendrogliial; pHGG, pediatric high-grade glioma.
three of these tumors harbored co-occurring CDKN2A/B deletions (Fig. 4C).

Among high-grade DIPGs, H3F3A mutations were detected in 66.7% (6/9) of cases, with these cases reporting only the K28M variant (Fig. 4D). In the H3F3A mutated DIPGs, four harbored co-occurring TP53 mutations and the other two tumors harbored PTPN11 and TERT mutations or CDKN2C deletion and PIK3R1 mutations. The three cases without H3F3A alterations reported only a TERT promoter – 124C>T, PDGFR A D842del, or no reportable mutations, respectively.

The six anaplastic PXAs in our cohort reported a spectrum of mutations: one tumor harbored a BRAF V600E mutation with CDKN2A/B deletions and a TERT promoter mutation, whereas in the other PXAs, only TP53 S183*, PIK3CA G118D, TSC2 Y1033fs*1, RB1 E672fs*5, or RB1 T140fs*5 and TP53 F109fs*14 mutations were detected.

Contrary to adult oligodendrogliomas, the one anaplastic oligodendroglioma in our cohort did not harbor IDH1/2 mutations or 1p/19q co-deletion [19, 22, 26]. We detected KRA S G12V, EGFR R222C, and TERT promoter – 124C>T mutations, a
genomic signature that better aligns with an astrocytic lineage glioma. Of the less prevalent histologic subtypes, one anaplastic ganglioglioma harbored a BRAF V600E mutation with a co-occurring CDKN2A/B deletion.

**Genomic Analysis of Midline, Brainstem, and Spinal Cord Gliomas**

The strongest clinical prognostic factors in pediatric gliomas include histologic grade and extent of resection, which is largely dependent on tumor location. We divided our cohort into midline and nonmidline tumors and examined the most frequently mutated genes in each group. Midline tumor locations included thalamus (n = 37), brainstem (n = 31), spinal cord (n = 12), hypothalamus (n = 7), basal ganglia (n = 2), and other (pineal, third and fourth ventricles; n = 11). Of the 282 samples, 35.5% (n = 100) were classified as midline, including 46 pLGGs and 54 pHGGs. The most common diagnoses among midline pLGGs were LGG NOS (n = 17), PA (n = 16), and DA2 (n = 5), and those among pHGGs were GBM (n = 29), AA3 (n = 9), DIPG (n = 8), and HGG NOS (n = 8).

**Figure 5.** Long-tail distribution highlighting the top 10 altered genes in pLGG (A) and pHGG (B) midline tumors; distribution of most commonly mutated genes in pHGG thalamic (C), brainstem (D), and spinal cord (E) tumors. Long-tail distribution of the top 10 altered genes in pLGG (F) and pHGG (G) nonmidline tumors.

Abbreviations: EGFR, epidermal growth factor receptor; pHGG, pediatric high-grade glioma; pLGG, pediatric low-grade glioma.
Overall, H3F3A was the most commonly altered gene in midline tumors, altered in 40% of cases, with the K28M variant detected in all 40 mutated cases (supplemental online Figure 2A). TP53 mutations were detected in 28% (28/100) of cases, and among these, 20 harbored co-occurring H3F3A K28M mutations. Interestingly, BRAF alterations occurred in 25% (25/100) of midline gliomas, with 12 tumors harboring KIAA1549-BRAF fusions and 11 tumors with BRAF V600E mutations.

Among midline pLGGs, BRAF (43.5%), NF1 (17.4%), FGFR1 (17.4%), and PIK3CA (10.9%) were the most commonly altered genes (Fig. 5A). Among low-grade tumors, H3F3A was mutated in only 6.5% of cases. In contrast, the genes most commonly altered in midline pHGGs were H3F3A (68.5%), TP53 (51.9%), PDGFA (25.9%), and NF1 (24.1%; Fig. 5B). Among 27 thalamic pHGGs, H3F3A mutations were detected in 66.7% (n = 18) of tumors and entirely consisted of the K28M variant (Fig. 5C). Similarly, 66.7% (10/15) of high-grade brainstem gliomas and 77.8% (7/9) of spinal cord HGGs harbored H3F3A (K28M) mutations (Fig. 5D, 5E).

Of the 182 nonmidline gliomas, the most frequently mutated genes were TP53 (30.8%, 56/182), BRAF (27.5%, 50/182), and CDKN2A (15.4%, 28/182; Fig. 5F, 5G, and supplemental online Fig. 2B). Twenty-four of the BRAF-altered nonmidline gliomas harbored fusions, 24 others had BRAF V600E mutations, and 2 tumors had BRAF S605G or D594G mutations. In nonmidline tumors, H3F3A mutations were detected in 12.1% (22/182) of cases, including 14 K28M and 8 G35R/V variants.

**Gene Fusions Are Critical Driver Events in pLGGs and pHGGs**

Gene fusions were most common among pLGGs, with 35.2% (44/125) of tumors harboring an in-frame fusion. Thirty-four of these fusions involved BRAF, and of these, 33 were KIAA1549-BRAF.
BRAF fusions. All the KIAA1549-BRAF fusions were in-frame and contained the BRAF kinase domain (Fig. 6A). Fourteen of these contained the first 15 exons of KIAA1549 fused to exons 9–18 of BRAF, 6 involved the first 16 exons of KIAA1549 fused to exons 9–18 of BRAF, 2 with exons 1–16 of KIAA1549 fused to exons 11–18 of BRAF, 9 consisted of exons 1–6 of KIAA1549 fused to exons 9–18 of BRAF, 1 consisted of exons 1–12 of KIAA1549 fused to exons 10–18 of BRAF, and 1 contained exons 1–13 of KIAA1549 fused to exons 11–18 of BRAF. The other BRAF fusion was detected in a GG and consisted of the first 4 exons of TMEM106B fused to exons 8–18 of BRAF. This fusion was also in-frame and contained the BRAF kinase domain. Three FGFR3-TACC3 fusions were detected among pLGGs, including one diffuse astrocytoma, WHO grade II (DA), one oligodendroglioma, WHO Grade II (OG), and one pLGG NOS (Fig. 6B). All three of these fusions were in-frame, contained the FGFR3 kinase domain, and consisted of the first 17 exons of FGFR3 fused to exons 10–16 of TACC3. Two FGFR2 in-frame

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**Figure 7.** Tumor mutational burden in pHGGs and associated gene mutation frequencies. (A): Distribution of tumor mutational burden in cohort of pLGG (blue) and pHGG (red). (B): Summary of TMB statistics in pLGG and pHGG. Long tail distribution of the most commonly altered genes in pHGG with low (C), intermediate (D), and high (E) TMB.

Abbreviations: TMB, tumor mutational burden; HGG, high-grade glioma; Mb, megabase; pHGG, pediatric high-grade glioma; pLGG, pediatric low-grade glioma.
fusions that contained the kinase domain were also detected in the pLGG subgroup (Fig. 6B). The first was an FGFR2-PASD1 fusion detected in an OG that consisted of exons 1–17 of FGFR2 fused to exons 10–16 of PASD1, and the second was an FGFR2-INA fusion detected in a pLGG NOS that consisted of exons 1–17 of FGFR2 fused to exons 2–3 of INA. Additionally, two in-frame QKI-RAF1 fusions were detected in one OG and a recurrent PA (Fig. 6C). These fusions consisted of the first 3 exons of QKI fused to exons 8–17 of RAF1. We also identified ROS1 fusions, including a CEP85L-ROS1 detected in a recurrent DA2 and a GOPC-ROS1 in a pLGG NOS. Although the fusion partner differed, both consisted of exons 35–43 of ROS1 (Fig. 6D). Finally, a TPM3-NTRK1 fusion was detected in a pLGG NOS that consisted of exons 1–10 of TPM3 fused in-frame to exons 12–17 of NTRK1, which includes the NTRK1 kinase domain (Fig. 6E).

Gene fusions were rare among pHGGs but were detected in six unique pHGG specimens (Fig. 6F). The first pHGG NOS harbored an in-frame EML4-ALK fusion, containing the first 2 exons of EML4 fused to exons 20–29 of ALK. This variant, which has been previously described, comprises the components necessary and sufficient for cell transformation [27, 28]. In the second case, an in-frame DGKB-ETV1 fusion was identified in a GBM and consisted of the first 22 DGKB exons fused to exons 7–14 of ETV1. In the two other cases, an in-frame KIAA1549-BRAF fusion was detected in a pHGG NOS and an AA3, wherein the first 15 exons of KIAA1549 were fused to exons 9–18 of BRAF and included the kinase domain of BRAF. In a separate pHGG NOS, an ATG7-RAF1 fusion was detected and consisted of exons 1–18 of ATG fused to exons 8–17 of RAF1. Lastly, an EWSR1-PATZ1 fusion was identified in a pHGG NOS, which comprised exons 1–9 of EWSR1 fused to exons 1–5 of PATZ1.

### Table 1. Hypermutated pediatric GBMs harbor mutations in MMR pathway genes.

| Sample ID | Age (years) | Sex | Diagnosis | Location | Newly diagnosed or recurrent | TMB | MSH2 | MSH6 | MLH1 | POLE | PMS2 | POLD1 |
|-----------|-------------|-----|-----------|----------|-------------------------------|-----|------|------|------|------|-------|-------|
| 1         | 14          | M   | GBM       | Temporal | Recurrent                     | 43  | P622S| E484K, T1219I, G1218D, S998F | —    | —    | —    | —    |
| 2         | 4           | M   | GBM       | Frontal  | Newly diagnosed               | 50  | M485T, V63G | D439G, S1094P | —    | —    | —    | —    |
| 3         | 13          | M   | GBM       | Frontal  | Recurrent                     | 118 | —    | A159D, P1055, R243H, R988H | E34*  | A1349V, M444K | —    | K706fs* |
| 4         | 13          | F   | GBM       | Frontal  | Newly diagnosed               | 160 | R406Q | R1035*, K476N, R1034W | L426I | V1270G | S46I, A520V | Q369H |
| 5         | 15          | F   | GBM       | Temporal | Recurrent                     | 176 | —    | G1138E | —    | A2180V | —    | G705C |
| 6         | 7           | M   | GBM       | Temporal | Recurrent                     | 392 | R406Q | R298*, 3647–6, 3647–1deltaacag, D268Y, S219I | R100* | S297F | —    | —    |
| 7         | 7           | F   | GBM       | Temporal | Recurrent                     | 448 | —    | —    | S461T, A1967V, P1432T, S780I | R315* | R652W, R689W |
| 8         | 16          | F   | GBM       | Spinal cord | Recurrent                     | 581 | T796N, V367I | R248fs*8, F115I, G1157C, P320S | Y245C | V411L, A288V, A789T | R421*, R169H |
| 9         | 8           | M   | HGG (NOS) | Frontal  | Newly diagnosed               | 95  | K347E | R988H | E102D | E978G, F1366V, N251fs*12 | —    | T473M |

Abbreviations: —, no data; F, female; GBM, glioblastoma; HGG, high-grade glioma; M, male; NOS, not otherwise specified; TMB, tumor mutational burden.

### Hypermutant GBMs Enriched for Mutations in DNA Mismatch Repair Genes

Tumor mutational burden was calculated for all pLGGs and pHGGs and classified as low (0–<6), intermediate (6–20), or high (≥20). In pLGGs, TMBs ranged from <1 to 758, with a median of 0.9 mutations per Mb (Fig. 7A, 7B). All but one pLGG reported a low or intermediate TMB, with the single outlier harboring a TMB of 758 mutations per Mb, which was a recurrent cerebellar pLGG NOS in a late adolescent male.

In the pHGG group, the TMB ranged from <1 to 758, with a median of 1.8 mutations per Mb. The majority of tumors demonstrated a low TMB (82.2%, 129/157) compared with those in TMB-intermediate (12.1%, 19/157) and TMB-high groups (5.7%, 9/157; Fig. 7A, 7B). Although TP53 mutations were the most frequent alteration across all pLGGs, H3F3A mutations were exclusive to tumors with low and intermediate TMBs (Fig. 7C, 7D). The average TMB of H3F3A mutated pLGGs was 1.8 mutations per Mb and ranged from <1 to 17.1, highlighting that these mutations are mutually exclusive from hypermutated pLGGs in our dataset. The remaining 6% (9/157) of pLGGs were classified as hypermutated, with TMBs ranging from 43–575 mutations per Mb, and all harbored mutations in TP53 (100%, 9/9), NFI (88.9%, 8/9), SETD2 (66.7%, 6/9), and ATRX (66.7%, 6/9; Fig. 7E). Such high mutation rates raised the possibility of biallelic mismatch repair disease (bMMRD). Among the nine pHGGs (four newly diagnosed, five recurrent) with a high TMB, six harbored functional mutations in mismatch repair (MMR) or proofreading genes MSH2, MSH6, MLH1, POLE, PMS2, or POLD1 (Table 1), whereas the other three tumors harbored variants of...
unknown significance in these MMR genes. Interestingly, functional mutations in bMMRD genes were not detected in pHGG tumors with low or intermediate TMB.

**Discussion**

In this study, 282 pediatric gliomas were analyzed by CGP and genomic alterations were identified in over 95% of cases. Among pLGGs, the most common alterations were BRAF alterations, followed by FGFR1 lesions, whereas in pHGGs, TP53 and H3F3A mutations dominated the genomic landscape. Surprisingly, in our pHGG cohort, the H3F3A K28M variant (n = 51) was most prevalent, with only 8 of 59 pGBM cases with H3F3A mutations reporting a G35R variant; midline tumors also enriched for K28M variant. Additionally, only the K28M variant was detected in pLGGs harboring H3F3A mutations. The presence of H3F3A mutations in pLGGs has significant clinical impact because previous reports demonstrated that the prognosis for K28M mutated gliomas is worse compared with G35R and wild-type H3F3A cases [10, 21, 22].

We also identified recurrent and novel fusions in both pLGGs and pHGGs. We report EML4-ALK, DGKB-ETV1, KIAA1549-BRAF, ATG7-RAF1, and EWSR1-PATZ1 rearrangements in pLGGs. Because these fusions have been previously reported, we hypothesize that they are the main oncogenic drivers. Furthermore, these patients may benefit from currently available small molecule inhibitors that disrupt the oncogenic fusion activity, as is the case for EML4-ALK fusions in NSCLCs, which respond to the ALK inhibitors [29].

Although surgical resection remains the most effective treatment option for pLGGs, tumors located in eloquent areas not amenable to surgical resection (e.g., motor cortex) require alternative therapeutic strategies. Recent reports highlight tumor regression of BRAF mutated pLGGs in response to dabrafenib, a BRAF inhibitor, and several clinical trials of BRAF inhibitors are ongoing for pediatric patients with BRAF-mutated gliomas [30]. Furthermore, a clinical trial (NCT01734512) open for recurrent pLGGs aims to evaluate the efficacy of everolimus, an mTOR inhibitor. We report a multiply recurrent NF1 mutated PA previously treated with surgery alone that now shows a remarkable response to dual inhibitor therapy (everolimus and trametinib) following 3 months of treatment. Ongoing H3F3A K28M peptide vaccine trials (NCT02960230) represent another active area of investigation for DIPG patients.

**Conclusion**

With increasing reports of high TMB in gliomas, immunotherapy has emerged as a novel therapeutic strategy for adult and pediatric brain tumors. More recently, interrogation of sequencing data has revealed TMB as a critical biomarker for predicting responses to therapy [31, 32]. In the adult population, hypermutated or TMB-high cancers have been reported to respond to immune checkpoint inhibitors (ICPI) [33]. As previously reported, bMMRD pediatric glioma patients experienced durable responses to ICPI at tumor recurrence following standard of care [11]. We identified nine patients with hypermutated gliomas characterized by TMBs ranging from 43 to 575 mutations per Mb, and representing a genomically distinct group of pHGGs. Given that these patients may preferentially benefit from ICPI, these findings demonstrate how CGP can reliably and routinely identify hypermutated gliomas.

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**Author Contributions**

**Conception/design:** Adrienne Johnson, Eric Severson, Siraj Ali, John Crawford, Shakti Ramkissoon

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**Provision of study material or patients:** John Crawford

**Collection and/or assembly of data:** Adrienne Johnson, Eric Severson, Laurie Gay, Mandy Covert, Garrett Frampton, Sigmund Hsu, Glenn J. Lesser, Kimberly Stogner-Underwood, Ryan T. Mott, Sarah Z. Rush, Jennifer J. Stanke, Sonika Dahiya, James Sun, Prasanth Reddy, John Crawford, Shakti Ramkissoon

**Data analysis and interpretation:** Adrienne Johnson, Eric Severson, Laurie Gay, Mandy Covert, Garrett Frampton, Zach Chalmers, Rachel Erlich, Yakov Chudnovsky, David Fabrizio, Alex B. Schrock, Siraj Ali, John Crawford, Shakti Ramkissoon

**Manuscript writing:** Adrienne Johnson, Eric Severson, Laurie Gay, Mandy Covert, Siraj Ali, John R. Crawford, Shakti H. Ramkissoon

**Final approval of manuscript:** Adrienne Johnson, Eric Severson, Laurie Gay, Jo-Anne Vergilio, Julia Elvin, James Suh, Sugganth Daniel, Mandy Covert, Garrett M. Frampton, Sigmund Hsu, Glenn J. Lesser, Kimberly Stogner-Underwood, Ryan T. Mott, Sarah Z. Rush, Jennifer J. Stanke, Sonika Dahiya, James Sun, Prasanth Reddy, Zachary R. Chalmers, Rachel Erlich, Yakov Chudnovsky, David Fabrizio, Alex B. Schrock, Siraj Ali, Vincent Miller, Philip J. Stephens, Jeffrey Ross, John R. Crawford, Shakti H. Ramkissoon

**Disclosures**

Adrienne Johnson: Foundation Medicine, Inc. (E, OI); Eric Severson: Foundation Medicine, Inc. (E, OI); Laurie Gay: Foundation Medicine, Inc. (E, OI); Jo-Anne Vergilio: Foundation Medicine, Inc. (E, OI); Julia Elvin: Foundation Medicine, Inc. (E, OI); James Suh: Foundation Medicine, Inc. (E, OI); Sugganth Daniel: Foundation Medicine, Inc. (E, OI); Mandy Covert: Foundation Medicine, Inc. (E, OI); Garrett M. Frampton: Foundation Medicine, Inc. (E, OI); Sigmund Hsu: AbbVie, Cortice Pharmaceutical (RF), Foundation Medicine, Inc. (H); Glenn J. Lesser: Insys Therapeutics (C/A), Incyte, New Link Genetics, Novartis, Pfizer, Vascular Biogenics (RF), Stemline Therapeutics (SAB); James Sun: Foundation Medicine, Inc. (E, OI); Prasanth Reddy: Foundation Medicine, Inc. (E, OI); Zach Chalmers: Foundation Medicine, Inc. (C/A, OI); Rachel Erlich: Foundation Medicine, Inc. (E, OI); Yakov Chudnovsky: Foundation Medicine, Inc. (E, OI); David Fabrizio: Foundation Medicine, Inc. (E, OI); Alexa B. Schrock: Foundation Medicine, Inc. (E, OI); Siraj Ali: Foundation Medicine, Inc. (E, IP, OI); Jeffrey Ross: Foundation Medicine, Inc. (E, RF, OI); Shakti H. Ramkissson: Foundation Medicine, Inc. (E, OI).

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