


RESEARCH

Open Access



# Integrated genomic analysis reveals actionable targets in pediatric spinal cord low-grade gliomas

Adela Misove<sup>1,2</sup>, Ales Vicha<sup>1,2</sup>, Petr Broz<sup>2,3</sup>, Katerina Vanova<sup>1,2</sup>, David Sumerauer<sup>1,2</sup>, Lucie Stolova<sup>2</sup>, Lucie Sramkova<sup>2</sup>, Miroslav Koblizek<sup>1,3</sup>, Josef Zamecnik<sup>1,3</sup>, Martin Kyncl<sup>4</sup>, Zuzana Holubova<sup>4</sup>, Petr Liby<sup>5</sup>, Jakub Taborsky<sup>5</sup>, Vladimir Benes III<sup>5</sup>, Ivana Pernikova<sup>6</sup>, David T. W. Jones<sup>7,8</sup>, Martin Sill<sup>9</sup>, Terezia Stancokova<sup>10</sup>, Lenka Krskova<sup>1,3†</sup> and Michal Zapotocky<sup>1,2\*†</sup> 

## Abstract

Gliomas are the most common central nervous tumors in children and adolescents. However, spinal cord low-grade gliomas (sLGGs) are rare, with scarce information on tumor genomics and epigenomics. To define the molecular landscape of sLGGs, we integrated clinical data, histology, and multi-level genetic and epigenetic analyses on a consecutive cohort of 26 pediatric patients. Driver molecular alteration was found in 92% of patients (24/26). A novel variant of *KIAA1549:BRAF* fusion (*ex10:ex9*) was identified using RNA-seq in four cases. Importantly, only one-third of oncogenic drivers could be revealed using standard diagnostic methods, and two-thirds of pediatric patients with sLGGs required extensive molecular examination. The majority (23/24) of detected alterations were potentially druggable targets. Four patients in our cohort received targeted therapy with MEK or NTRK inhibitors. Three of those exhibited clinical improvement (two with trametinib, one with larotrectinib), and two patients achieved partial response. Methylation profiling was implemented to further refine the diagnosis and revealed intertumoral heterogeneity in sLGGs. Although 55% of tumors clustered with pilocytic astrocytoma, other rare entities were identified in this patient population. In particular, diffuse leptomeningeal glioneuronal tumors ( $n = 3$ ) and high-grade astrocytoma with piloid features ( $n = 1$ ) and pleomorphic xanthoastrocytoma ( $n = 1$ ) were present. A proportion of tumors (14%) had no match with the current version of the classifier. Complex molecular genetic sLGGs characterization was invaluable to refine diagnosis, which has proven to be essential in such a rare tumor entity. Moreover, identifying a high proportion of drugable targets in sLGGs opened an opportunity for new treatment modalities.

**Keywords:** Spinal cord, Low-grade glioma, *KIAA1549:BRAF* fusion, *NTRK* fusion, Methylation profiling

## Introduction

The majority of CNS tumors are located intracranially, and only 5% occur in the spinal cord [1]. Intramedullary spinal cord tumors have a glial origin and are biologically low-grade in 95%. Similarly, as in brain low-grade gliomas (LGGs), spinal cord (=intramedullary) low-grade gliomas in children (sLGGs) show a chronic course of the disease affecting the quality of life while the overall survival remains excellent [2]. Treatment of choice is maximal safe surgical resection under intraoperative

<sup>†</sup>Lenka Krskova and Michal Zapotocky contributed equally to this work

\*Correspondence: michal.zapotocky@fnmotol.cz

<sup>2</sup> Department of Pediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

Full list of author information is available at the end of the article



monitoring which must not endanger neurological function as chemotherapy can stabilize the progression of the disease in 40–50% [3, 4].

The urge is to predict the risk of progression and search for novel, effective treatment approaches. Molecular data are seldom, suggesting that the most prevalent alteration is *KIAA1549:BRAF* fusion [5]. Due to the rarity of the disease, genomic data are scarce or incomplete, and the use of targeted therapy in sLGGs was not demonstrated yet. Therefore, an institutional integrated clinical and comprehensive genetic study was conducted to reveal sLGG-associated molecular alterations and their therapeutic implications, assess intertumoral heterogeneity using methylation profiling, and demonstrate the effect of targeted therapy in this group of patients.

## Methods

### Patient cohort and clinical follow-up

Tumor samples and clinical data from patients with sLGGs diagnosed and treated at our center from 2000 to 2021 were retrieved for survival, radiologic data, and molecular evaluation. Patients were followed on an out-patient or in-patient basis with regular MRI imaging. All clinical data were collected retrospectively. The last patient was enrolled on 02/11/2020, and the disease status for all patients was updated on 01/07/2021. The institutional review board approved the study, and all patients obtained informed consent as per our routine procedure.

### Volumetric analysis

Volumetric analysis was implemented to measure tumor response to the targeted therapy. Lesions' volumes were estimated with open-source software 3D Slicer (version 4.10.2) using basic modules (Segment Editor, Segment Statistics) [6]. MRI sequences with the best spatial and contrast resolution were selected from available examinations for lesion volume evaluation.

### DNA and RNA extraction

The nucleic acids were extracted from formalin-fixed, paraffin-embedded tissue block using QIAamp DNA FFPE Tissue Kit (Quiagen, Germany) for genomic DNA and using high pure RNA paraffin kit (Roche Diagnostics, Mannheim, Germany) for total RNA. In the case of fresh frozen sections, extraction of genomic DNA and total RNA was manufactured using Trizol Reagent (Life Technologies, Merelbeke, Belgium). Neuropathologists selected the most representative tissue blocks containing the maximum percentage of tumor tissue before isolation of nucleic acid.

### cDNA synthesis and conventional RT-PCR

cDNA was prepared from RNA (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. cDNA was subjected to conventional RT-PCR amplification with primers specific for common *KIAA1549:BRAF* variants (*ex16:ex9*, *ex15:ex9*, *ex16:ex11*) as previously described [7].

### Sanger sequencing

PCR and Sanger sequencing were conducted to examine hotspot mutations at codons 27 and 34 of *H3F3A*, codon 600 of *BRAF* ex15, codons 546 and 656 of *FGFR1* ex12, and codon of *FGFR1* ex14 using previously described primer pairs. Amplification was performed using 2 × PCR BIO HS Taq Mix Red (PCR Biosystems Ltd., London, UK). The PCR products were electrophoresed in a 1.5% agarose gel and were recovered using the Gel DNA Fragments Extraction Kit (Geneaid, Taiwan). Sanger sequencing was performed using Big Dye Terminator v 3.1 chemistry (Life Technologies) and an ABI PRISM 3130 genetic analyzer Applied Biosystems. Results were analyzed using Chromas lite 2.01 (Technelysium, Pty Ltd, Brisbane, Australia).

### MLPA

SALSA<sup>®</sup> MLPA<sup>®</sup> probemix P370 can be used to detect genomic duplications leading to the *KIAA1549:BRAF*, *SRGAP3:RAF1*, and *FGFR1:TACC1* fusion genes and for detection of copy number aberrations in the *BRAF*, *CDKN2A/2B*, *FGFR1*, *MYB*, and *MYBL1* genes. Furthermore, this probemix contains five specific probes detecting the BRAF p.V600E & four predominant IDH1 p.R132H and p.R132C and IDH2 p.R172M and p.R172K point mutations, which will only generate a signal when the mutation is present. MLPA was performed following the manufacturer's instructions. Data were analyzed using Coffalyser Software (MRC-Holland, Amsterdam, The Netherlands).

### RNA panel sequencing

Based on the quality of preserved nucleic acid in the samples, we were using one of Archer<sup>®</sup> FusionPlex<sup>®</sup> (Archer DX, Boulder, Colorado) commercially available panels—either Lung kit or Oncology Research kit. Despite different nucleic acid quality requirements resulting from the different number of analyzed genes, both panels are used for fusion and SNV identification with the advantage of identifying the fusion even with an unknown partner. Archer<sup>®</sup> FusionPlex<sup>®</sup> also offers robust performance even for FFPE samples. RNA extraction, library preparation, and parallel sequencing were performed as per the manufacturer's recommendation. Anchored Multiplex

polymerase chain reaction amplicons were sequenced on Illumina MiSeq, and the data were analyzed using the Archer and Arriba [8] (<https://github.com/suhrig/arriba/>) softwares. RT-PCR was performed to validate the fusion transcript identified by RNA sequencing (Additional file 1: Table S1).

### Methylation profiling

DNA methylation was performed using the Infinium Methylation EPICBeadChip Kit (Illumina, San Diego, CA, USA). A total of 250 ng of DNA from fresh frozen tumor tissue was treated with bisulfite conversion using the ZymoResearch EZ DNA Methylation kit (Zymo Research Corp, Irvine, CA, USA). In the case of FFPE samples, DNA restoration was performed as per the manufacturer's instructions (Infinium FFPE DNA Restoration kit, Illumina, San Diego, CA, USA). According to the manufacturer's explicit specifications, the Infinium HD Methylation Assay was performed at the laboratories of the Department of Pediatric Haematology and Oncology, Second Faculty of Medicine in Prague. The methylation class was established using web-based analysis via <https://www.molecularneuropathology.org/> using publicly available v11b4 and v12.5 versions of brain classifier. To compare spinal cord glioma samples with the DKFZ reference cohort, t-SNE analysis was performed with 10,000 most differentially methylated probes using Rtsne package v.0.13 as previously described [9].

### Statistical analysis

Progression-free survival and overall survival were analyzed by the Kaplan–Meier method, and p-values reported using the log-rank test in an open-source R statistical environment (v4.1.2), using R packages survival (v2.41–3), and ggplot2 (v2.2.1).

## Results

### Patient selection

During the study period, 42 pediatric patients with spinal cord tumors were diagnosed, excluding non-biopsied patients with known Neurofibromatosis type 2. Diagnosis of sLGGs was made in 23 patients; 12 patients were diagnosed with ependymoma, and four patients with ATRT or other entity (Fig. 1a). Retrospective evaluation of the spinal cord tumor cohort revealed discordance between

histology and the clinical course of the disease in three patients. Additional testing done in 2 ependymoma patients with atypical clinical course revealed rather glial tumors with ependymal features. Furthermore, one long-term surviving patient with an inoperable tumor initially described as anaplastic astrocytoma showed rather low-grade biology of the tumor with multiple progressions over many years of follow-up. In this particular patient, molecular studies revealed *NTRK2* fusion and confirmed a glial origin of the tumors with low-grade behavior. Therefore, these three patients were added to the sLGGs group in this article to a total number of 26 patients (Additional file 2: Table S2; sLGG\_01–sLGG\_26), representing 7% of all institutional pediatric LGGs (total number = 350). Median age at diagnosis for the sLGGs group was 4.55 years (range from 1.15 to 17.54 years). Histologically, the sLGGs group comprised predominantly of pilocytic astrocytoma, diffuse astrocytoma, and ganglioglioma.

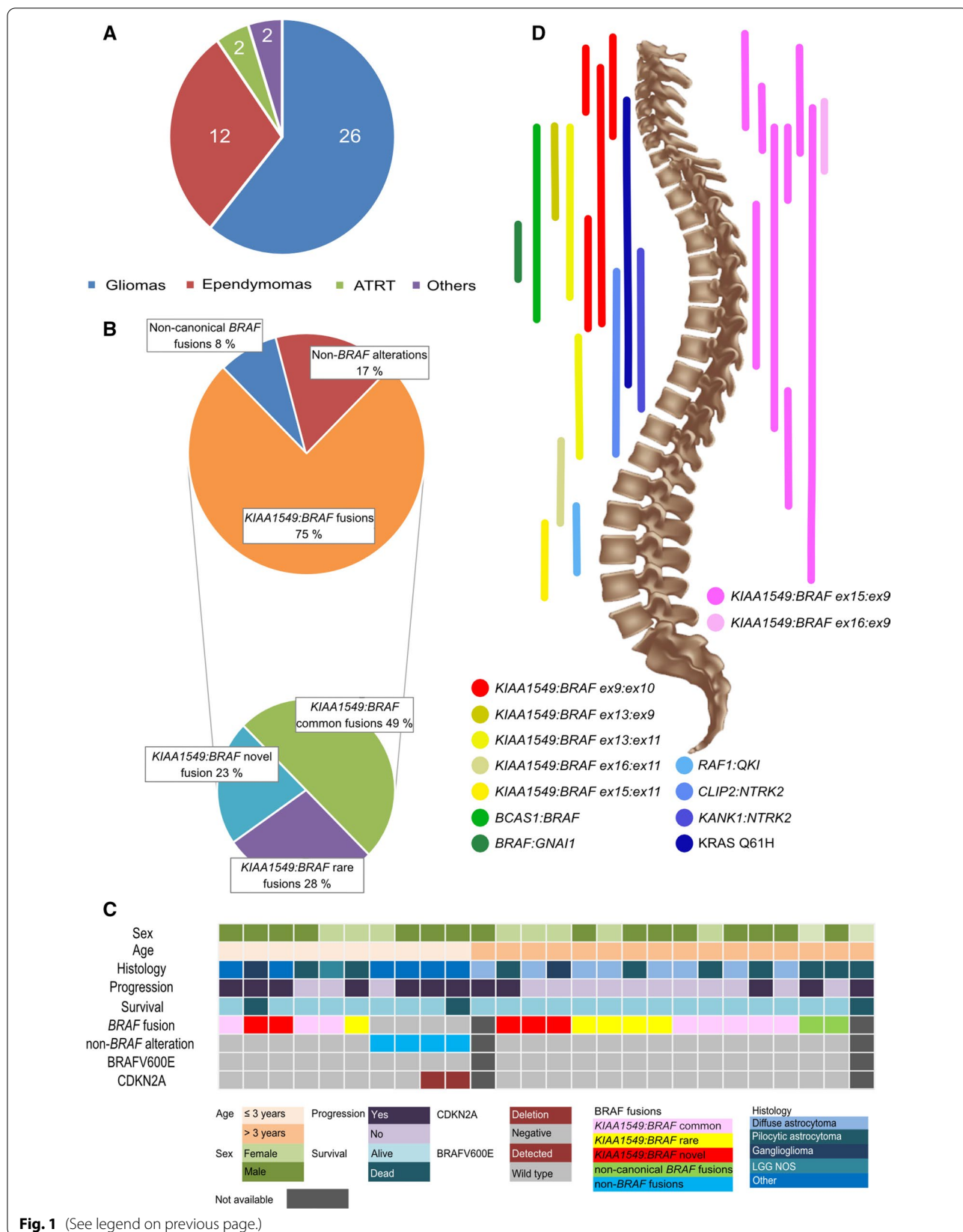
### Comprehensive genomic analysis uncovered a novel and rare alterations within the MAPK pathway

Comprehensive genetic analysis consisting of Sanger sequencing, MLPA, and RNA sequencing was employed to determine the genetic landscape of pediatric LGGs. Oncogenic driver alterations were detected in 93% (n = 24) of patients; no alterations were detected in two patients where analysis failed due to insufficient tissue quality. Alterations found across sLGGs could be classified into two groups (Fig. 1b: 1) *BRAF* alteration consisting of *KIAA1549:BRAF* fusions (75%) with the high occurrence of rare and novel fusion variants, and non-*KIAA1549:BRAF* fusions (8%), 2) non-*BRAF* alterations (17%). Surprisingly, no case harboring BRAFV600E or *H3F3A/HIST1H3B* mutation was identified. We also evaluated the presence of the secondary alterations and found two cases of *CDKN2A* homozygous deletion in non-*BRAF* tumors. (Fig. 1c) Furthermore, pathogenic *MET* and *EGFR* variants were detected in two *KIAA1549:BRAF* cases.

A novel variant of *KIAA1549:BRAF* fusion (*ex10:ex9*) was identified using RNA sequencing in four cases (Fig. 2a). Rare types of *KIAA1549:BRAF* fusions (*ex13:ex9*, *ex13:ex11*, *ex16:ex11*, *ex15:ex11*) were identified in further 21% sLGG patients. Non-canonical

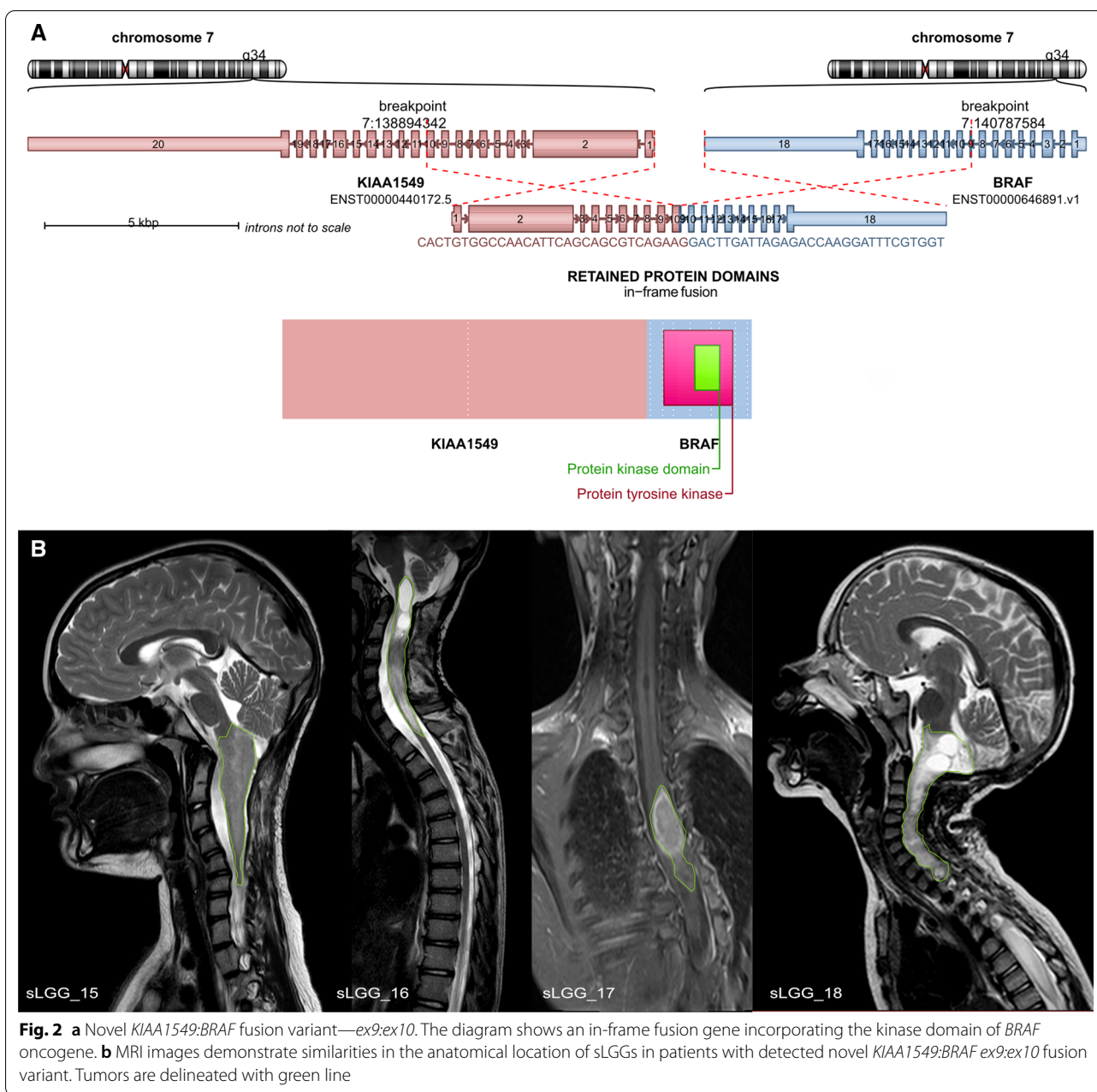
(See figure on next page.)

**Fig. 1** **a** Overview of the total number of 42 spinal cord tumors diagnosed within 2000–2021. **b** Pie of pie demonstrates three molecular alteration groups in sLGGs; tumors driven with canonical *BRAF* fusions, non-canonical *BRAF* fusions, and non-*BRAF* alterations. **c** Oncoplot summarizes the relation of demographic (sex, age), clinical (progression, survival), and molecular-pathology data (histology, driver alteration, *CDKN2A* status), **d** Comparison of the molecular alterations, anatomical location, and extent of the tumors. The position of vertical lines shows the anatomical location of the tumor and the length of vertical lines outlines the levels of the spinal cord affected by each tumor sample. Molecular subtypes are shown in colors. On the left side are displayed common *KIAA1549:BRAF* fusions (pink) in contrast with the right side where are rare *KIAA1549:BRAF* fusions (yellow), a novel type of *KIAA1549:BRAF* fusion (red), non-*KIAA1549:BRAF* fusions (green) and non-*BRAF* alterations (blue)



**Fig. 1** (See legend on previous page.)





*BRAF* fusions were detected in two patients; accounting for *BCAS1:BRAF* and *GNAI1:BRAF*. Two independent methods verified those rare and novel *BRAF* fusions using RT-PCR with specific primers and chromosome 7q34 duplication using MLPA.

Anatomical distribution of the genetic alterations (Fig. 1d) interestingly showed *KIAA1549:BRAF ex10:ex9*-positive tumors located in the upper half of the spinal cord with partial medulla oblongata involvement in two cases (two cases in the cervical spine (C1–C7), one case in the cervical and upper thoracic spine (C2–T2), and

one in the upper thoracic spine (T2–T7) (Fig. 2b). To evaluate the frequency of the *KIAA1549:BRAF ex10:ex9* fusion variant, the cohort was expanded using 205 institutional cases of intracranial pediatric LGGs of various locations with known molecular drivers (Additional file 3: Fig. S1). Among more than 50 cases with detectable *KIAA1549:BRAF*, no case harbored an *ex10:ex9* variant suggesting exclusive occurrence in the spinal cord.

Non-*BRAF* alterations were detected in four tumor samples consisting of *CLIP2:NTRK2*, *KANK1:NTRK2*, *RAF1:QKI*, and *KRAS Q61H*. The young age of three

years and under at diagnosis characterized this group of patients. This group's histological appearance was not typical for LGG, and molecular testing helped refine the diagnosis. *CLIP2:NTRK2* case was diagnosed as an anaplastic astrocytoma grade 3. Despite HGG histology, the presence of *CDKN2A* homozygous deletion, and multiple progressions, the patient was alive 15 years from the time of diagnosis. Pathologists reported two cases (*KANK1:NTRK2* and *RAF1:QKI*) as ependymomas, but these tumors were reclassified as low-grade glioma due to the clinical course, underlying molecular alteration, and methylation profile. Based on the molecular profile, the case with *KANK1:NTRK2* that also harbored *CDKN2A* homozygous deletion was treated with a radiation-sparing approach using chemotherapy only as first-line therapy. Patient with sLGG harboring *RAF1:QKI* underwent subtotal resection followed by careful observation. *KRAS* Q61H mutated case had histology of low-grade glioneuronal tumor (LGNT), and the patient was observed only after partial resection.

#### Methylation profiling revealed significant intertumoral heterogeneity among sLGGs

The current version (v12.5) of the Heidelberg methylation classifier does not provide any methylation class specific for spinal cord gliomas in contrast to spinal cord ependymomas. Therefore, we performed methylation profiling to evaluate how would sLGGs be classified based on the epigenetic features and to discern intertumoral heterogeneity. The analysis was performed using publicly available Heidelberg classifier v12.5. Out of 22 patients (91.6%) with sufficient tissue available, 12 tumors (55%) were predicted as pilocytic astrocytoma, subclass posterior fossa (PA-PF) despite variable calibrated scores (calibrated scores (CS) 0.35–0.99). Three tumors (14%) with 1p deletion matched with diffuse leptomeningeal glioneuronal tumor (DLGNT), methylation class 1 (DLGNT – MC1) (two CS 0.99, one CS 0.25). One anaplastic astrocytoma with *CLIP2:NTRK2* fusion was classified as anaplastic pilocytic astrocytoma (CS 0.62), currently also known as high-grade astrocytoma with piloid features (HGAP). The other *NTRK2* fused glioma (*KANK1:NTRK2*), originally diagnosed as ependymoma, was classified as pleomorphic xanthoastrocytoma (PXA) (CS 0.90). One case (*QKI:RAF1*) was clustered with a subtype A of glioneuronal tumors (CS 0.99). One case (*KIAA1549:BRAF ex10:ex9*) was classified as desmoplastic infantile ganglioglioma / desmoplastic infantile astrocytoma (CS 0.59). The remaining three tumors (14%) matched with control tissue most probably due to low tumor tissue content. Moreover, t-SNE analysis using a previously published reference cohort was performed to

further refine the methylation class prediction. As classified by v12.5, significant proportion of the samples clustered nearby PA-PF cluster. They seemed to be forming a separate cluster suggesting a possible distinction from posterior fossa pilocytic astrocytoma. Remaining samples clustered with DLGNT, GNT, PXA, and other clusters as predicted with the classifier (Fig. 3).

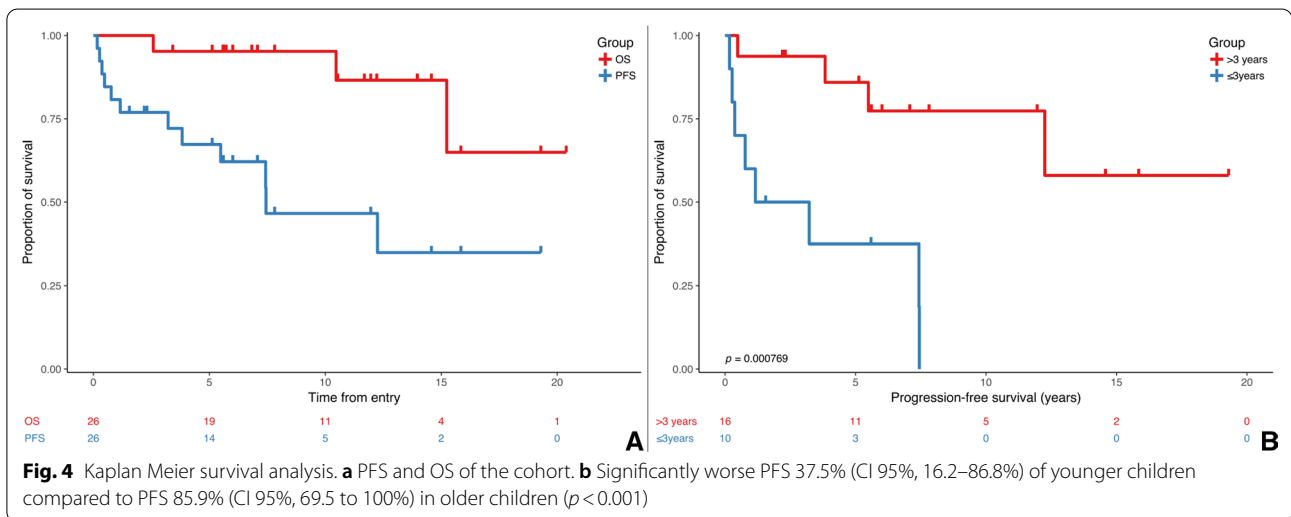
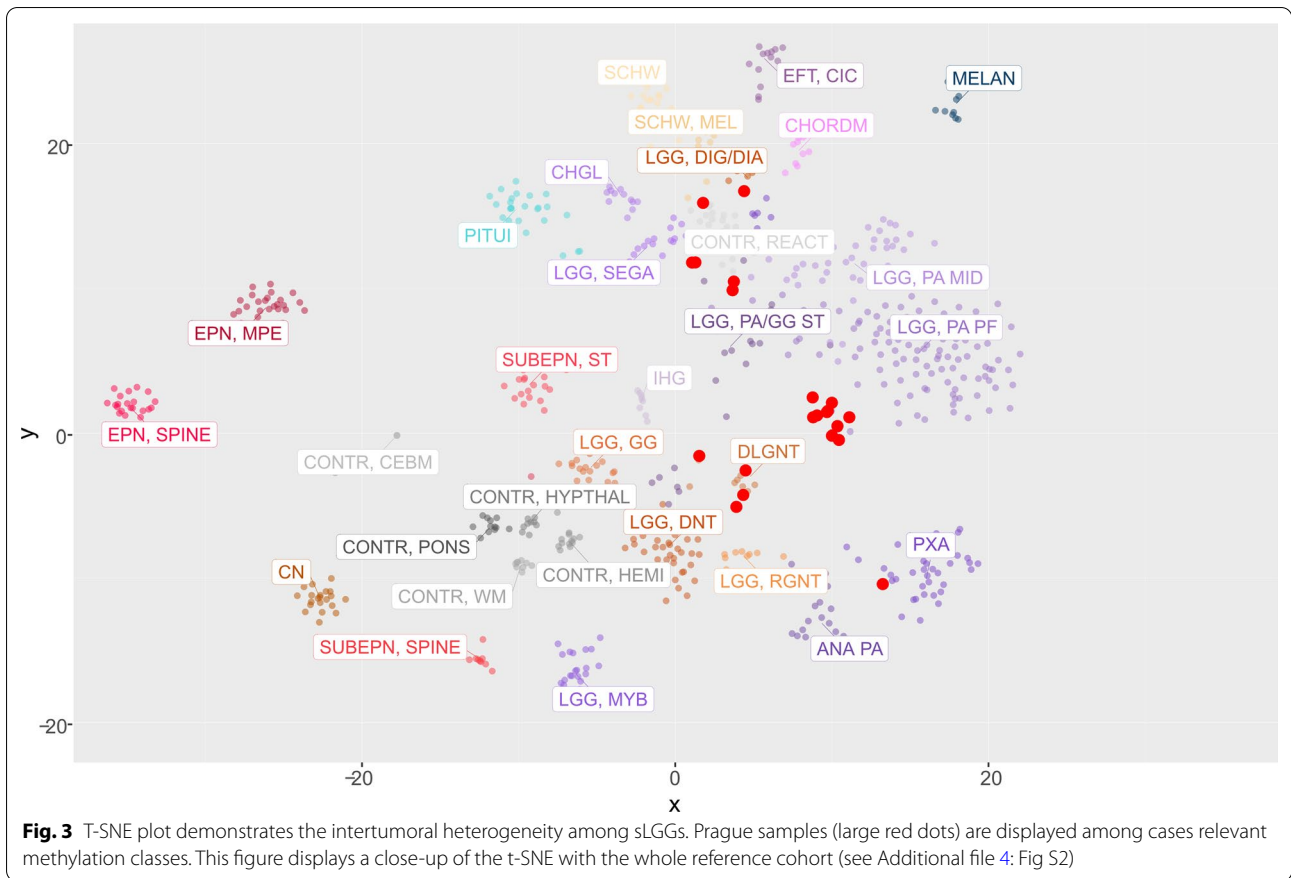
#### Clinical outcome and exploitation of molecular targets

At a median follow-up of 6.96 years (IQR: 3.42–12.22), 5-year progression-free survival was 67.3% (95% confidence interval [CI], 50.8–89.1%). Overall survival rate at 5 years was 95.2% (95% [CI], 86.6–100.0%) (Fig. 4a). Infants of three years and younger fared significantly worse compared to those older than three with 5-year PFS 37.5% (CI 95%, 16.2–86.8%) and 85.9% (CI 95%, 69.5–100%), respectively ( $p < 0.001$ ) (Fig. 4b) Three patients died of the disease 15, 10, and two years after diagnosis respectively due to the progressive disease, regardless of the histological grade or molecular alteration (one with *CLIP2:NTRK2*, one without known driver alteration, and one with *KIAA1549:BRAF ex10:ex9* variant).

Based on molecularly identified targets, four patients received targeted therapy using MAPK pathway inhibitors. Three patients with *KIAA1549:BRAF* fusion were treated with MEK-inhibitor trametinib. According to the volumetric measurements, one of the patients (sLGG\_15) responded with stable disease, and two patients (sLGG\_06 and sLGG\_07) exhibited partial responses (reduction of 51% and 61%) that were achieved after 5 and 8 months, respectively. Patient (sLGG\_22) with *CLIP2:NTRK2* fusion was treated with TRK inhibitor larotrectinib with quickly induced volume reduction, not meeting partial response (40%), detectable on magnetic resonance 54 days after the therapy initiation (Fig. 5). The radiological volume reduction was accompanied by significant clinical improvement with no remarkable drug-related toxicity. Unfortunately, tumor progression accompanied by clinical decline was detected after 22 months of targeted therapy. After another five months, the patient died due to the tumor progression to the brainstem. DNA sequencing from autopsy material did not reveal any point mutations in the *NTRK1/2/3* kinase domain; thus, the mechanism of acquired resistance remained unknown [10].

#### Discussion

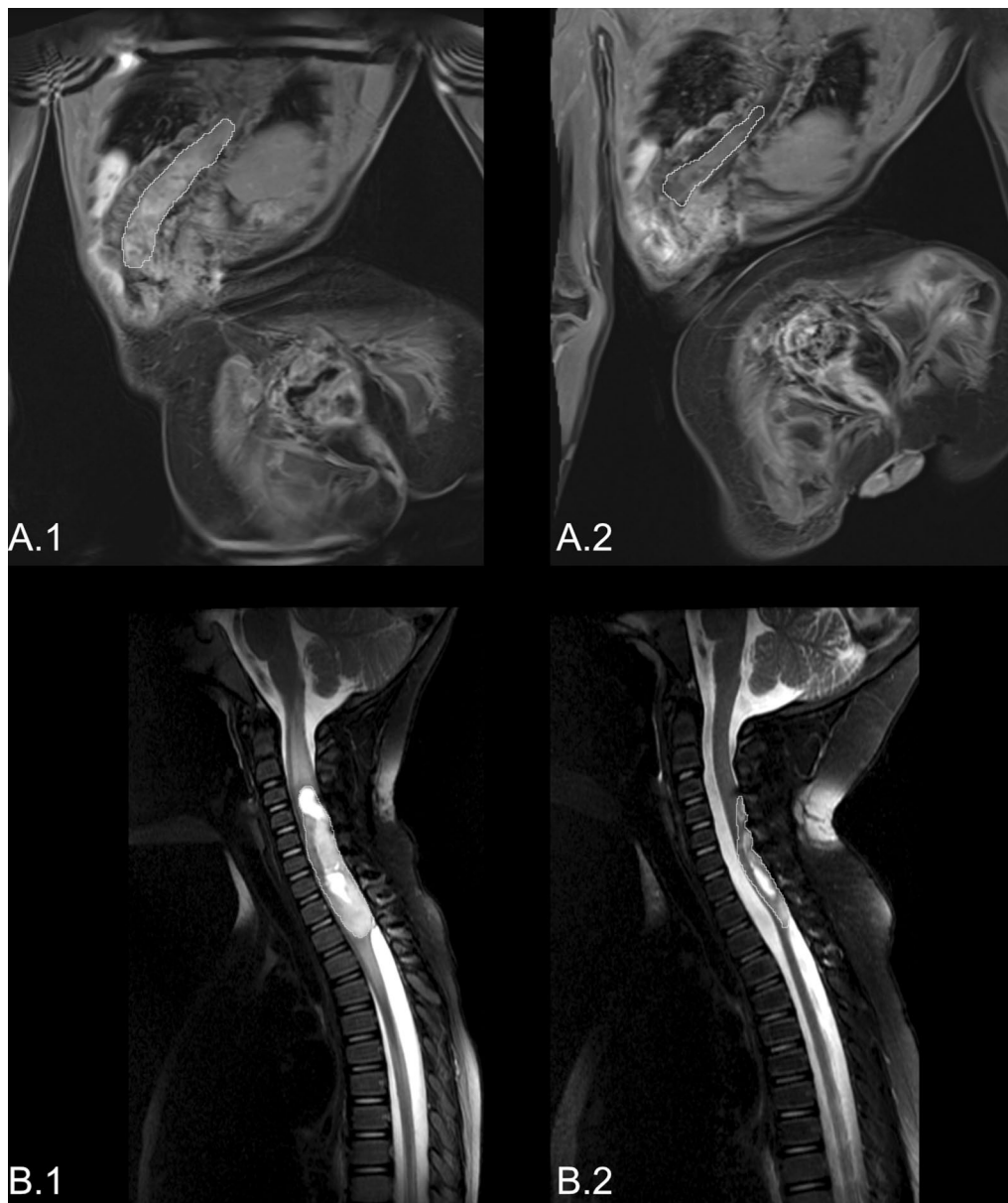
Here we present a study with comprehensive genomic and epigenomic analysis in a 20-year retrospective single institutional non-selected cohort of 26 consecutive sLGG patients in the context of clinical course,



including quantitative imaging analysis and employment of novel treatment modalities. New findings regarding significant epigenetic heterogeneity were for the first time reported in pediatric sLGG population. Furthermore, identification of fusion landscape

including novel fusion variants highlighted the impact of our data on diagnostics and new therapeutic opportunities.

We confirmed that the majority of pediatric sLGGs harbored *KIAA1549:BRAF* fusions, but unlike intracranial



**Fig. 5** Radiological response to the targeted therapies. The white line (**a.1**) indicates tumor volume before initiation of NTRK inhibitor in patients sLGG\_22, and (**a.2**) shows a 40% tumor volume reduction after 54 days of therapy. Likewise, the white line (**b.1**) shows tumor volume before initiation of MEK inhibitor in patient sLGG\_07, and (**b.2**) shows a 61% tumor volume reduction after eight months of therapy

location, rare and novel variants were predominantly present. In particular, the novel *KIAA1549:BRAF ex10:ex9* was uncovered in our study. Importantly, this fusion variant was not found in any of our institutional pediatric intracranial LGGs cases, making this variant specific for the spinal cord compartment. Moreover, to our knowledge, this fusion variant has not been reported yet in the literature. As reported in other *KIAA1549:BRAF*

variants, the variant *ex10:ex9* also lacked the autoinhibitory domain and caused MAPK activation in the same manner [11, 12] (Fig. 2a).

Non-*BRAF* fusions were detected in children younger than three years and consisted of tumors with *NTRK2* and *RAF1* fusions and *KRAS* mutation. The clinical course of the disease in non-*BRAF* fusion patients tended to progress requiring multiple treatment



modalities. Furthermore, two patients with non-canonical *BRAF* fusions (*BCAS1:BRAF*, *GNAI1:BRAF*) were revealed and confirmed that non-canonical *BRAF* fusions could occur in the sLGGs. Interestingly, there was no spinal cord glioblastoma characterized by histone *H3F3A* mutation in the presented cohort of spinal cord tumors [13]. No *BRAF* V600E mutation was detected in our cohort, probably due to a limited number of patients and a low prevalence of *BRAF* V600E mutation in sLGG [5, 14].

Survival in regards to 5-year PFS (67.3%) and OS (95.2%) was comparable with more extensive series with predominantly intracranial pediatric LGGs or sLGGs [5, 14]. Importantly, significantly worse PFS in younger children compared to the older ones stood out in the presented cohort. The extent of resection did not influence our PFS data as only two patients had their sLGGs completely removed. Therefore, the poorer outcome of younger children might have been related to the presence of non-*BRAF* alterations in combination with more frequent occurrence of cases with atypical histology. Our observation of a poorer prognosis was consistent with previously published clinical trials where young children fared significantly worse [15, 16]. Frequent non-*BRAF* fusions in younger children with sLGGs and histology not typical for true pediatric LGGs resembled cases of infant hemispheric gliomas, [17, 18] underlying the necessity of multi-layer diagnosis integrating histopathology and molecular genetics analysis. Some of our very young children with sLGGs were diagnosed with "ependymoma-like" histology, and further molecular investigation helped to refine the diagnosis.

Previously published studies evaluated the genomic landscape of spinal cord gliomas. More extensive series usually presented limited information on molecular alterations restricted to data from molecular biology methods such as FISH, NanoString platform, or RT-PCR [5], most probably due to poor tumor tissue availability. Grob et al. showed that 42% of sLGGs tested positive for *KIAA1549:BRAF* fusion [5]. It is essential to underline that the most frequent alterations in our cohort were rare and novel *KIAA1549:BRAF* fusion variants. The mechanism of *BRAF* activation remains the same, but commonly used diagnostic methods (RT-PCR with specific primers/NanoString) will not detect rare fusion variants, potentially compromising an opportunity for targeted therapy for those patients. The rare *KIAA1549:BRAF* *ex13:ex11* fusion variant was only described in one case of spinal glioneuronal tumor [19]. *RAF:QKII* is known to activate MAPK/ERK and PI3K/mTOR signaling and was already described in a single case report of an adult diagnosed with spinal pleomorphic xanthoastrocytoma [20]. Moreover, some other larger studies focused on single

nucleotide variants rather than gene fusions in the adult population and therefore were unable to capture the whole landscape of fusions related to pediatric sLGG [21, 22].

Methylation profiling using Heidelberg classification [9] was demonstrated as a powerful research tool in pediatric CNS tumors. We employed the whole-genome DNA methylation array to uncover epigenetic features of sLGGs. A dominant cluster with the methylation class "pilocytic astrocytoma, posterior fossa" suggested a possibility of a common cell of origin with the most frequent intracranial group of LGGs. Nevertheless, the current classifier scored 55% of tumors in our sLGGs group between 0.3 and 0.97. This was in keeping with t-SNE analysis demonstrating these tumors form a cluster nearby the PA-PF cluster. This data could suggest distinct epigenetic features of sLGGs compared to PA-PF. Moreover, tumors clustering with rare methylation classes were detected, in particular DLGNT, HGAP, and PXA. Three DLGNT cases were circumscribed as spinal cord lesions without any evidence of dissemination through the neuroaxis. HGAP and PXA were represented by cases with uncharacteristic histology (not clear LGGs), either anaplastic astrocytoma or anaplastic ependymoma, presence of *NTRK2* fusions, and long-term survival. Several cases ( $n=3$ ) did not match any methylation classes suggesting either high normal tissue content or a rare entity yet to be defined [23]. Previous study attempted to characterize spinal cord gliomas using methylation profiling. The cohort consisted of 19 adults and seven children with high-grade and low-grade gliomas, and therefore a portion of cases clustered with Diffuse Midline Glioma *H3FA3*-positive and glioblastoma *IDH* wild-type. They also described two adult cases with HGAP and very short survival, two cases with *IDH* mutant glioma, and one DLGNT. Eight cases matched with pilocytic astrocytoma, but the tissue was not available to perform RNA sequencing, and therefore authors were not able to demonstrate the presence of characteristic fusions [24].

Comprehensive genomic analysis was critical not only to uncover the molecular landscape of pediatric sLGGs but also to identify high-priority targets for novel therapies. In particular, targeted therapy was used in four sLGGs patients with progressive disease who presented with neurological decline. Previously, MEK-inhibitor trametinib was shown to benefit a proportion of patients with progressive NF1 or *BRAF*-driven LGGs [25, 26], and our series of cases suggest clinical benefit with objective responses documented on magnetic resonance imaging in sLGGs. In addition, NTRK inhibitors have shown high efficacy in multiple NTRK-driven cancers [27], and our case demonstrated significant clinical benefit of such therapy in the patient with *NTRK2* fused sLGG.

A relatively small cohort size, short follow-up, and retrospective data collection did not allow a more comprehensive prognostic marker evaluation. Furthermore, volumetry was used as a method for response evaluation considering bidimensional measurement less feasible [28], especially in our sLGG patients frequently suffering from significant spinal deformities. Therefore, response assessment in Pediatric Neuro-Oncology (RAPNO) was not implemented in this study [29].

Nevertheless, we have assembled a coherent group of solely pediatric patients with extensive molecular analysis, and our data demonstrated the importance of integrative molecular-pathological diagnosis and enlightening the potential of targeted treatment for sLGGs. Future large prospective studies evaluating prognostic markers, the efficacy of targeted therapies, and volumetric response assessment in sLGG are needed.

## Conclusion

This study provides essential data on the molecular background of purely pediatric cohort of anatomically defined low-grade gliomas confined to the spinal cord. Methylation profiling revealed epigenetic landscape of sLGG demonstrating that 55% of cases cluster with posterior fossa pilocytic astrocytoma samples with the remaining 45% being very heterogeneous. Despite this epigenetic heterogeneity, sLGGs harbor driver alterations within MAPK pathway. In contrast to the intracranial LGGs, non-*BRAF* fusions (including *NTRK* fusions) and rare *KIAA1549:BRAF* variants, including novel variant *ex9:ex10*, represent frequent molecular drivers in sLGG. Our data clearly demonstrated the presence of druggable targets, and our case series demonstrated promising results in disease control with targeted therapy. However, further research and prospective clinical trials are required to evaluate the role of targeted therapy in sLGG patients.

## Abbreviations

ATRT: Atypical teratoid rhabdoid tumor; cDNA: Complementary DNA; CI: Confidence interval; CS: Calibrated score; DLGNT: Diffuse leptomeningeal glioneuronal tumor; DNA: Deoxyribonucleic acid; Ex: Exon; FGFR: Fibroblast growth factor receptors; GNT: Glioneuronal tumor; HGAP: High-grade astrocytoma with piloid features; IDH: Isocitrate dehydrogenase; IQR: Interquartile range; LGG: Low-grade glioma; LGNT: Low-grade glioneuronal tumor; MAPK: Mitogen-activated protein kinase; MC: Methylation class; MEK: Mitogen-activated protein kinase kinase; MLPA: Multiplex ligation-dependent probe amplification; MRI: Magnetic resonance imaging; NTRK: Neurotrophic tyrosine receptor kinase; PA-PF: Pilocytic astrocytoma–posterior fossa; PCR: Polymerase chain reaction; PXA: Piloxyoid astrocytoma; RAPNO: Response assessment in Pediatric Neuro-Oncology; RNA: Ribonucleic acid; RT-PCR: Reverse transcription polymerase chain reaction; sLGGs: Spinal low-grade gliomas.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40478-022-01446-0>.

**Additional file 1: Table S1.** Table of primers used to validate the fusion transcripts identified by RNA sequencing.

**Additional file 2: Table S2.** Table showing the complete sLGGs cohort emphasizing the original histology before molecular pathology reevaluation, anatomical location, and molecular-biology data.

**Additional file 3: Fig. S1.** A total number of pediatric intracranial LGG patients with known genetic alteration is divided by anatomical location. Importantly, *KIAA1549:BRAF ex9:ex10* variant fusion was detected solely in the upperspine.

**Additional file 4: Fig. S2.** T-SNE analysis displaying Prague samples (large red dots) among reference cohort samples.

## Acknowledgements

Not applicable.

## Author contributions

Study design: AM, LK, MZ. Data collection: AM, KV, PL, JT, IP, TS. Molecular-genetic experiments: AM, AV, LS, LK. Imaging data analysis: MK, ZH. Histology revision: MK, JZ. Interpretation of the data: AM, MZ, LK, AV, PB, DJ, MS. Critical revision of the article: MZ, DS, LS, VB. All authors read and approved the final manuscript.

## Funding

This study was supported by Ministry of Health of the Czech Republic, Grant No. NU21-07-00419 (DS, AV, MZ, JS), PRIMUS/19/MED/06 Charles University Grant Agency, Prague, Czech Republic (MZ, LK, AM, KV), GAUK No. 204220, MH CZ–DRO, University Hospital Motol, Prague, Czech Republic (00064203) (DS, AV, MZ, JS), The project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102)—Funded by the European Union—Next Generation EU. Foundation Národ dětem and Foundation 1000 statečných.

## Availability of data and materials

The datasets are publically available at Mendeley Data <https://doi.org/10.17632/xzkg4jvm2.1>.

## Declarations

### Ethics approval and consent to participate

Ethics approval and consent were waived.

### Consent for publication

Consent for publication was obtained from parent or legal guardian. Institutional consent form was used.

### Competing interests

The authors declare that they have no competing interests.

### Author details

<sup>1</sup>Prague Brain Tumor Research Group, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic. <sup>2</sup>Department of Pediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic. <sup>3</sup>Department of Pathology and Molecular Medicine, Second Faculty of Medicine, Charles University Prague and Faculty Hospital Motol, Prague, Czech Republic. <sup>4</sup>Department of Radiology, Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic. <sup>5</sup>Department of Neurosurgery, Second Faculty of Medicine, Charles University

and University Hospital Motol, Prague, Czech Republic. <sup>6</sup>Department of Neurology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic. <sup>7</sup>Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany. <sup>8</sup>Pediatric Glioma Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany. <sup>9</sup>Division of Biostatistics, German Cancer Research Center (DKFZ), Heidelberg, Germany. <sup>10</sup>Department of Pediatric Oncology and Hematology, Children's University Hospital, Banska Bystrica, Slovakia.

Received: 22 July 2022 Accepted: 13 September 2022

Published online: 26 September 2022

## References

- Barker DJP, Weller RO, Garfield JS (1976) Epidemiology of primary tumours of the brain and spinal cord: a regional survey in southern England. *J Neurol Neurosurg Psychiatry* 39:290–296
- Constantini S, Miller DC, Allen JC, Rorke LB, Freed D, Epstein FJ (2000) Radical excision of intramedullary spinal cord tumors: surgical morbidity and long-term follow-up evaluation in 164 children and young adults. *J Neurosurg Spine* 93:183–193
- Gnekow AK, Kandels D, Van TC, Azizi AA, Opocher E, Stokland T et al (2019) SIOP-E-BTG and GPOH guidelines for diagnosis and treatment of children and adolescents with low grade glioma. *Klin Padiatr* 231:107–135
- Beneš V, Barsa P, Beneš V, Suchomel P (2009) Prognostic factors in intramedullary astrocytomas: a literature review. *Eur Spine J* 18:1397–1422
- Grob ST, Nobre L, Campbell KR, Davies KD, Ryall S, Aisner DL et al (2020) Clinical and molecular characterization of a multi-institutional cohort of pediatric spinal cord low-grade gliomas. *Neuro-Oncol Adv* 2:1–9
- Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin JC, Pujol S et al (2012) 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magn Reson Imaging* 30:1323–1341
- Tian Y, Rich BE, Vena N, Craig JM, MacConaill LE, Rajaram V et al (2011) Detection of KIAA1549-BRAF fusion transcripts in formalin-fixed paraffin-embedded pediatric low-grade gliomas. *J Mol Diagn* 13:669–677
- Uhrig S, Ellermann J, Walther T, Burkhardt P, Frohlich M, Hutter B et al (2021) Accurate and efficient detection of gene fusions from RNA sequencing data. *Genome Res* 31:448–460
- Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D et al (2018) DNA methylation-based classification of central nervous system tumours. *Nature* 555:469–474
- Cocco E, Schram AM, Kulick A, Misale S, Won HH, Yaeger R et al (2019) Resistance to TRK inhibition mediated by convergent MAPK pathway activation. *Nat Med* 25:1422–1427
- Jones DTW, Kocalkowski S, Liu L, Pearson DM, Bäcklund LM, Ichimura K et al (2008) Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. *Cancer Res* 68:8673–8677
- Pfister S, Janzarik W, Remke M, Ernst A, Werft W, Becker N et al (2008) BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. *J Clin Invest* 118(5):1739–1749
- Konar SK, Bir SC, Maiti TK, Nanda A (2017) A systematic review of overall survival in pediatric primary glioblastoma multiforme of the spinal cord. *J Neurosurg Pediatr* 19:239–248
- Ryall S, Zapotocky M, Fukuoka K, Nobre L, Guerreiro Stucklin A, Bennett J et al (2020) Integrated molecular and clinical analysis of 1000 pediatric low-grade gliomas. *Cancer Cell* 37:569–583.e5
- Ater JL, Zhou T, Holmes E, Mazewski CM, Booth TN, Freyer DR et al (2012) Randomized study of two chemotherapy regimens for treatment of low-grade glioma in young children: a report from the Children's Oncology Group. *J Clin Oncol* 30:2641–2647
- Gnekow AK, Walker DA, Kandels D, Picton S, Perilongo G, Grill J et al (2017) A European randomised controlled trial of the addition of etoposide to standard vincristine and carboplatin induction as part of an 18-month treatment programme for childhood ( $\leq 16$  years) low grade glioma: a final report. *Eur J Cancer* 81:206–225
- Clarke M, Mackay A, Ismer B, Pickles JC, Tatevossian RG, Newman S et al (2020) Infant high-grade gliomas comprise multiple subgroups characterized by novel targetable gene fusions and favorable outcomes. *Cancer Discov* 10:942–963
- Guerreiro Stucklin AS, Ryall S, Fukuoka K, Zapotocky M, Lassaletta A, Li C et al (2019) Alterations in ALK/ROS1/NTRK/MET drive a group of infantile hemispheric gliomas. *Nat Commun* 10:1–13
- Chiang JCH, Harrelld JH, Orr BA, Sharma S, Ismail A, Segura AD et al (2017) Low-grade spinal glioneuronal tumors with BRAF gene fusion and 1p deletion but without leptomeningeal dissemination. *Acta Neuropathol* 134:159–162
- Daoud EV, Wachsmann M, Richardson TE, Mella D, Pan E, Schwarzbach A et al (2019) Spinal pleomorphic xanthoastrocytoma with a QKI-RAF1 fusion. *J Neuropathol Exp Neurol* 78:10–14
- Chai RC, Zhang YW, Liu YQ, Chang YZ, Pang B, Jiang T et al (2020) The molecular characteristics of spinal cord gliomas with or without H3 K27M mutation. *Acta Neuropathol Commun* 8:1–11
- Zhang M, Iyer RR, Azad TD, Wang Q, Garzon-Muvdi T, Wang J et al (2019) Genomic landscape of intramedullary spinal cord gliomas. *Sci Rep* 9:1–8
- Lebrun L, Bizet M, Melendez B, Alexiou B, Absil L, Van Campenhout C et al (2021) Analyses of DNA methylation profiling in the diagnosis of intramedullary astrocytomas. *J Neuropathol Exp Neurol* 80:663–673
- Biczok A, Strübing FL, Eder JM, Egensperger R, Schnell O, Zausinger S et al (2021) Molecular diagnostics helps to identify distinct subgroups of spinal astrocytomas. *Acta Neuropathol Commun* 9:119
- Selt F, Van Tilburg CM, Bison B, Sievers P, Harting I, Ecker J (2020) Response to trametinib treatment in progressive pediatric low-grade glioma patients. *J Neurooncol* 149:499–510
- Kondyli M, Larouche V, Saint-Martin C, Ellezam B, Pouliot L, Sinnett D et al (2018) Trametinib for progressive pediatric low-grade gliomas. *J Neurooncol* 140:435–444
- Drilon A, Laetsch TW, Kummar S, DuBois SG, Lassen UN, Demetri GD et al (2018) Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. *N Engl J Med* 378:731–739
- D'Arco F, O'Hare P, Dashti F, Lassaletta A, Loka T, Tabori U et al (2018) Volumetric assessment of tumor size changes in pediatric low-grade gliomas: feasibility and comparison with linear measurements. *Neuroradiology* 60:427–436
- Fangusaro J, Witt O, Hernáiz Driever P, Bag AK, de Blank P, Kadom N et al (2020) Response assessment in paediatric low-grade glioma: recommendations from the Response Assessment in Pediatric Neuro-Oncology (RAPNO) working group. *Lancet Oncol* 21:e305–e316

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

### Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

