

A Unique Case Report of Infant-Type Hemispheric Glioma (Gliosarcoma Subtype) with *TPR-NTRK1* Fusion Treated with Larotrectinib

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Established Facts

- Infant-type hemispheric glioma harbor frequent tyrosine kinase gene fusions which results in constitutive activation of oncogenic pathways.

Novel Insights

- This is the first case of infant-type hemispheric glioma with gliosarcoma histology with *TPR-NTRK1* fusion, treated with larotrectinib for 8-month showing stable disease.

Keywords

Infantile gliosarcoma · NTRK fusion · Targeted therapy

Abstract

Herein, we present a rare case of a nine-month-old boy diagnosed with infant-type hemispheric glioma (gliosarcoma subtype) at the left frontal lobe. Following subtotal resection, the patient started chemotherapy with the BABY POG proto-

col. We describe the clinical diagnosis, histological characteristics, radiological features, molecular aspects, and management of this tumor. A comprehensive molecular analysis on the tumor tissue showed a *TPR-NTRK1* gene fusion. The patient was treated with a TRK inhibitor, larotrectinib, and exhibited a stable disease with residual lesion following 8 months of target therapy. The present study is the first report of an infantile gliosarcoma harboring *NTRK1* rearrangement treated with larotrectinib.

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Introduction

Gliosarcomas are a rare variant (2.4%) of *IDH* wild-type primary glioblastoma (GBM) [1]. These deadly primary malignant brain tumors are observed in adults but uncommon in children [1]. According to the newest 2021 WHO Classification of Tumors of the Central Nervous System, these tumors will now be designed as “infant-type hemispheric glioma” [2]. Recent studies have been shown that the outcome of infant gliomas is more closely related to the tumor location and genetic profile than histologic tumor grade [3]. Clinically, gliosarcoma is managed similarly to GBM, based on maximal safety surgical resection, concomitant radiotherapy, and temozolomide-based chemotherapy [4, 5]. Nevertheless, several clinical studies suggest that gliosarcoma patients have a slightly worse outcome, with median overall survival, ranging between 13 and 17.5 months and more responding to temozolomide [4, 5].

Gliosarcomas exhibit a biphasic tissue pattern, with regions of typical features of high-grade astrocytomas, such as microvascular proliferation, pseudopalisading necrosis, and high mitotic activity, that intermingle with regions, presenting a sarcomatous differentiation [1]. The first genetic profiling of gliosarcomas revealed a similar profile with primary GBM (*IDH* wild-type), such as *p16* homozygous deletions *PTEN* mutations and *TP53* mutations, yet no *EGFR* amplification was observed [6]. Further studies confirmed this profile and showed the absence of *IDH1/2* mutations and the presence of mutations in *TERT* promoter, *BRAF*, and *NF1* genes [7, 8]. A recent study performed an extensive molecular comparison of gliosarcomas and GBMs and identified novel differences between both entities, such as higher expression of PD-L1 and PD-1, higher markers of epithelial-to-mesenchymal transition, copy number amplification of *LYL1*, mutations in *PTPN11* gene, and *NTRK1* fusions in gliosarcomas [9].

Few reports of infantile gliosarcoma have been reported, and due to its rarity, there are no available data regarding its etiology and genetics. Compared to adults, the prognosis of infantile gliosarcoma is dismal [10, 11], and the few cases with better outcomes may suffer severe neurological and neurocognitive deficits as a consequence of surgery and chemotherapy [12]. Therefore, more effective treatment strategies for infantile gliosarcoma are urgently needed.

Identifying agnostic biomarkers has raised great expectations for cancer patients [13]. Remarkably, identifying *NTRK* gene fusions, which are frequently reported in

pediatric brain gliomas, has shown promising, safe, durable, and effective antitumor activities with anti-TRK inhibitors larotrectinib and entrectinib [14–19].

In the present study, we report a case of a 9-month-old baby diagnosed with gliosarcoma. The extensive molecular profile showed an *NTRK* fusion detection, leading to the treatment with larotrectinib at the Barretos Cancer Hospital (BCH), Brazil. We described the clinical course from diagnosis to therapy with larotrectinib and comprehensively evaluated this unique pediatric brain tumor.

Material and Methods

Immunohistochemistry Analysis

Immunohistochemical staining was carried out on formalin-fixed paraffin-embedded (FFPE) tissue sections (4- μ m thick). An automated staining system (BenchMark Ventana ULTRA™), and the following primary antibodies were used: anti-GFAP (EP672Y, Rabbit Monoclonal; Roche), anti-EMA (E29, Mouse Monoclonal; Roche), anti-p53 (Bp53-11, Mouse Monoclonal; Roche), anti-Ki-67 (30-9, Rabbit Monoclonal; Roche), anti-synaptophysin (MRQ-40, Rabbit Monoclonal; Roche), anti-INI-1 (MRQ-27, Mouse Monoclonal; Roche), anti-ALK1 (ALK01, Mouse Monoclonal; Roche), anti-somatostatin receptor type 2 (SSRT2) (Clone: EP149; Cell Marque), and Olig2 (Clone: EP112, Mouse Monoclonal; Medaysis).

The UltraView DAB IHC Detection Kit was used for visualization of the antibody reaction, except for the INI-1, ALK1, and SSRT2, which were detected using the OptiView DAB IHC Detection Kit. The slides were counterstained with hematoxylin, and controls were used to verify appropriate staining. The reticulin staining was performed using Reticulin/Nuclear Fast Red Stain Kit (Artisan) on Artisan PRO, Dako Agilent platform.

Molecular Profiling

Nucleic Acid Isolation

DNA and RNA isolation was performed from FFPE tumor tissue, sectioned on slides with a thickness of 10 μ m (minimum of 60% tumor area) using the QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany) and RNeasy FFPE Mini Kit (QIAGEN). Measurement of RNA and DNA quantity was done with the Qubit Fluorometric Quantification system (Life Technologies) as previously reported [20]. For fusion analysis, RNA quantity was assessed using 4150 TapeStation (Agilent Technologies) following the manufacturer's instructions.

Mutation, Copy Number Variation, and Fusion Analysis – OncoPrint Childhood Cancer Research Assay

The somatic mutations, copy number variation (CNV), and fusions were analyzed via next-generation sequencing (NGS) using the OncoPrint Childhood Cancer Research Assay – OCCRA (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000521253) (Thermo Fisher Scientific, Pleasanton, CA, USA), a pediatric panel covering mutations in hot spot regions of 82 genes, a complete exon coverage for 44 genes, CNV in 24 genes, and >1,700 isoform variants for fusion analysis.

DNA and RNA libraries were constructed using the OncoPrint™ Childhood Cancer Research Assay DNA/RNA and Ion AmpliSeq™ Kit for the Chef DL8 instrument on the Ion Chef™ instrument (Thermo Fisher Scientific). The purified libraries at 50 pM were pooled at an 80:20 ratio (DNA: RNA). Emulsion PCR, bead enrichment, and chip loading were automatically performed on an Ion Chef™ instrument (Thermo Fisher Scientific) using the Ion 540™ Kit-Chef (Life Technologies, Carlsbad, CA, USA) and Chip 540 (Thermo Fisher Scientific). Sequencing was conducted in an Ion S5™ System (Life Technologies). Torrent Suite Software and Variant Caller Plugin Reporter™ Software (version 5.16.0.2) and OncoPrint™ Childhood Cancer Research - v2.3 - DNA and Fusions workflow (version 5.16) were used to call the call SNVs/MNVs, CNV, and fusions.

Mutation analysis involved several steps in filtering variants: (i) variants with read depth <200× and <5% of variant allele frequency were filtered out; (ii) variants in coding regions and promoter regions or splice variants were retained; and besides, (iii) synonymous and variants with an incidence >1% in gnomAD and Brazilian genomic variants database were filtered out. Pathogenicity of variants was assigned by curated databases COSMIC, ClinVar, and the pathogenicity predictor FATHMM (available in COSMIC). In addition, variants were classified according to the AMP Classification using VarSome platform [21].

CNV detection was evaluated using Thermo Fisher's proprietary algorithm and based on an internal baseline created using FFPE normal samples. An MAPD >0.5 was considered a failure QC. Samples were considered positive for amplification when 5% confidence (minimum ploidy gain: 5% CI) bound was ≥ normal ploidy plus 2 (5% CI ≥ 4.0) and positive for copy loss when minimum ploidy loss (95% CI) < 2 will (95% CI < 2).

For the RNA library, 250,000 mapped reads and a minimal read length of 60 pb were required for successful sequencing. At least 40 reads supporting a fusion were required to call it present, and only target fusions were considered. Mutation analysis of *H3F3A* was also performed by bidirectional Sanger sequencing as reported [22].

Fusion Detection: Archer VR Fusion Plex VR

Archer VR Fusion Plex Solid Tumor Panel with Anchored Multiplex PCR (ArcherDX, Inc.) was used to detect gene fusions (online suppl. Table 2). Briefly, the target-enriched cDNA library was prepared with the Archer Fusion Plex Solid Tumor Panel (ArcherDX) as per the manufacturer's description. In short, reverse-transcription of RNA was followed by real-time quantitative PCR to determine the sample quality. Hereafter, end-repair, adenylation, and universal half-functional adapter ligation of double-stranded cDNA fragments were followed by 2 rounds of PCR with universal primers and gene-specific primers, covering 53 target genes that rendered the library fully functional for clonal amplification and sequencing using the MiSeq (Illumina). With the Archer Analysis software version 6.2 (ArcherDX), the produced libraries were analyzed for the presence of relevant fusions. In their absence, the sequencing quality was assessed by the following criteria: quantification cycle of real-time quantitative PCR (QC score) <30, a minimal total read number of 3 million with, 10,000 or more total unique RNA reads, and ten or more unique RNA starting-site reads per GSP control. The criteria for calling a positive fusion were 5 or more unique supporting RNA reads and 3 or more unique starting sites among the reads.

Fluorescence in situ Hybridization

The presence of *NTRK1* fusion was validated by fluorescence in situ hybridization (FISH) using 5-µm thick sections. Normal lung cells were used as negative controls of all the reactions. In brief, slides with tissue sections were subjected to deparaffinization by an overnight incubation (~12 h) at 60°C. Subsequent enzymatic digestion was performed in pepsin with 0.01 N HCL for 37°C/1 min. The slides were then submitted to dehydration by sequential washes in ethyl alcohol (70%, 85%, and 100%) and a hybridization step with the ZytoLight SPEC *NTRK1* Break Apart Probe (ZytoVision) probe for 22 h. Washes with a solution of 2× SSC with 0.3% IGEPAL (Sigma) and with 2X SSC alone were performed at 64°C and room temperature, respectively, for 4 min. Finally, the nuclei were then visualized with DAPI (blue) (ZytoVision). A total of 60 neoplastic cells were counted under a Nikon Eclipse 50i microscope at ×100 magnification using DAPI, FITC, and red filters.

Results

Case Description

A nine-month-old boy with no family history of cancer presented increased brain perimeter, bulging of the anterior fontanelle, and absence crisis. Brain magnetic resonance imaging (MRI) was performed identifying a solid-cyst lesion located at the left frontal lobe that was 5.0 × 4.6 × 3.5 cm in size (Fig. 1a, b). The patient was submitted to subtotal surgical resection (90% of the tumor volume), with a residual lesion of 2.1 × 1.2 cm in size (Fig. 1c, d).

Two months after diagnosis, the patient was transferred to the BCH and submitted to a second-look surgery for drainage of extensive subdural. The pathological anatomy exam review was performed in the FFPE of the first surgery, and altogether with the clinical and MRI findings, a final diagnosis of gliosarcoma (WHO grade IV) was made. Following the multidisciplinary pediatric neuro-oncology team discussion at the BCH, the patient started the chemotherapy treatment according to the BABY POG protocol (cyclophosphamide and vincristine, changing with cisplatin, and etoposide) for 8 months (5 February 2020 to 14 October 2020/15–23 months old), maintaining a stable lesion (Fig. 1i).

Pathological and Immunohistochemistry Findings

Histological sections of tumor FFPE tissue of the first surgery showed immature neoplasia characterized by proliferation of cells with intense pleomorphism and nuclear hyperchromatism, karyomegaly, and mitotic index of 23 mitoses/10 HPF. Areas of geographic necrosis and microvascular proliferation typical of GBM are noted. In certain areas, a neoplasm assumes a fascicular arrangement with spindle cells (Fig. 2a). Immunohistochemical

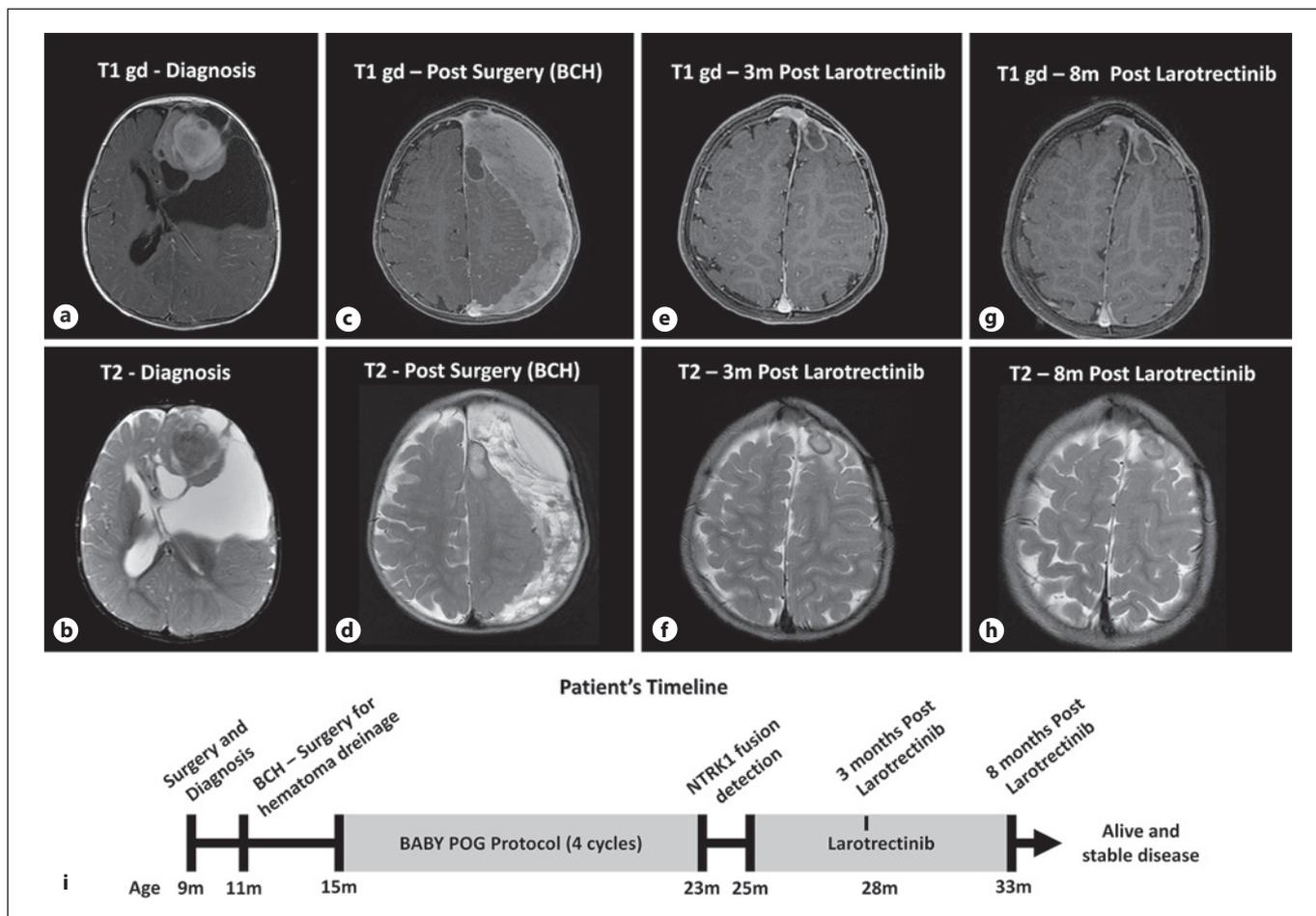


Fig. 1. MRI and patient's timeline. **a** Axial T1 MRI with gd contrast at diagnosis. **b** Axial T2 at diagnosis. **c** Axial T1 MRI with gd contrast after partial surgical resection. **d** Axial T2 after partial surgical resection. **e** Axial T1 MRI with gd after 3 months treatment with larotrectinib. **f** Axial T2 after 3 months treatment with larotrectinib. **g** Axial T1 MRI with gd after 8 months of treatment with larotrectinib. **h** Axial T2 after 8 months of treatment with larotrectinib. **i** Timeline of intervention. gd, gadolinium.

reactions showed strong GFAP expression of the gliomatous component (Fig. 2b) and reticulin staining in the sarcomatous areas (Fig. 2c). Ki-67 expression was present in 30% of neoplastic cells (Fig. 2d). There was no immunostaining for Olig2, SSRT2, EMA, and synaptophysin (data not shown), and it depicted positive expression of INI1 (online suppl. Fig. 1) and focal p53 expression. Therefore, a final diagnosis of infant-type hemispheric glioma (gliosarcoma subtype) was made.

Molecular Studies

We further performed a comprehensive molecular characterization in the tumor FFPE tissue of the first surgery. The analysis of the Archer FusionPlex Solid Tumor Panel revealed a *TPR* (*NM_003292.2*) *exon 33:NTRK1*

(*NM_002529.3*) *exon 12* gene fusion (Fig. 3a). The number of unique reads supporting the event was 334 reads (79.9%). The presence of a translocation involving the *NTRK1* gene was observed in 23.3% of the analyzed tumor cells by FISH (Fig. 3b, c).

The OCCRA panel analysis revealed 148 variants with >200× depth and variant allele frequency >5%, of which none of them were considered pathogenic/likely pathogenic after further filtering (online suppl. Table 3). Furthermore, no CNVs or fusions were detected in the examined regions of this panel. Notably, the breakpoint of the *TPR-NTRK1* fusion identified by the Archer FusionPlex Solid Tumor Panel is not present in the OCCRA panel. In addition, no mutations in *H3F3A* were detected using Sanger sequencing analysis.

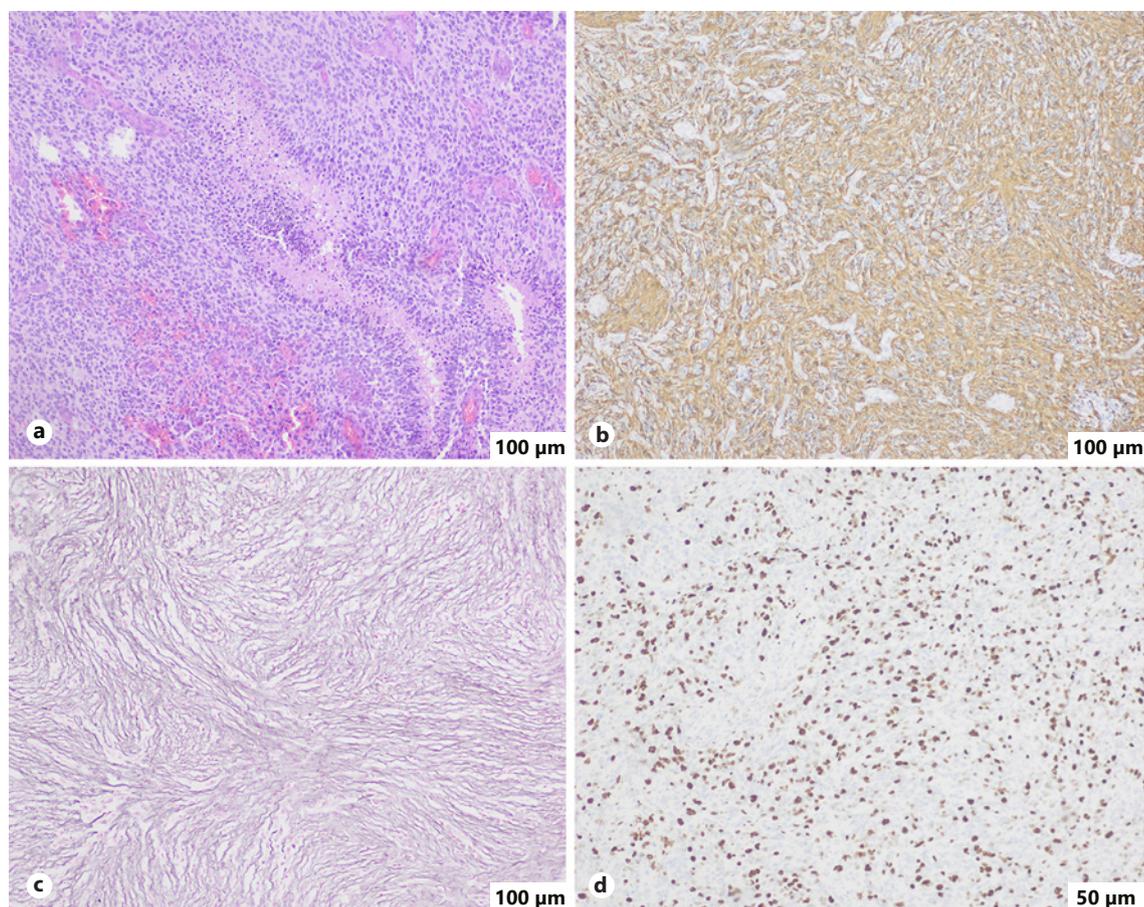


Fig. 2. Histological features of infantile gliosarcoma section of the initial surgery. **a** HE staining, Immunohistochemical staining of GFAP (**b**), reticulin staining (**c**), Ki67 proliferation index (**d**). HE, hematoxylin-eosin; GFAP, glial fibrillary acidic protein.

Targeted Therapy

Recently, the ESMO and Canadian guidelines for molecular pathology of pediatric high-grade gliomas were implemented at the BCH, which involves assessment of *NTRK* fusions [23, 24]. Due to the long-term detrimental effects of the BABY POG protocol, such as neurocognitive impairment, development delay, and endocrine alterations, and the identification of the actionable *TPR-NTRK1* gene fusion on 12 December 2020 (age of 21 months), the patient initiated the treatment with an oral anti-TRK inhibitor, larotrectinib (Bayer).

The patient was treated with larotrectinib at 100 mg/m²/day twice a day, acquired by the BCH. The 3-month MRI exams evaluation showed stable residual disease (Fig. 1e, f), and at 8-month evaluation, the lesion presented with the same size, yet, exhibiting a contrast hypo-uptake (Fig. 1g, h), suggesting that the characteristics of the lesion have changed, with putative tumor necrosis. More-

over, in the last clinical update (8 months), the patient presents 100% Lansky performance with good clinical response, improved quality of life, age-appropriate neuro-psychomotor development, and no side effects. A timeline of the clinical interventions is represented in Figure 1i.

Discussion

This study represents the first report of a *bonafide* infant-type hemispheric glioma, with gliosarcoma histology, harboring an *NTRK1* gene fusion treated with larotrectinib. Infantile (<3 years old) gliosarcomas are extremely rare. To the best of our knowledge, only 13 cases have been reported in the literature [10, 11]. Although very few cases exhibited longer outcomes, infantile gliosarcomas share a gloomy prognosis with adult gliosarcomas [10, 11].

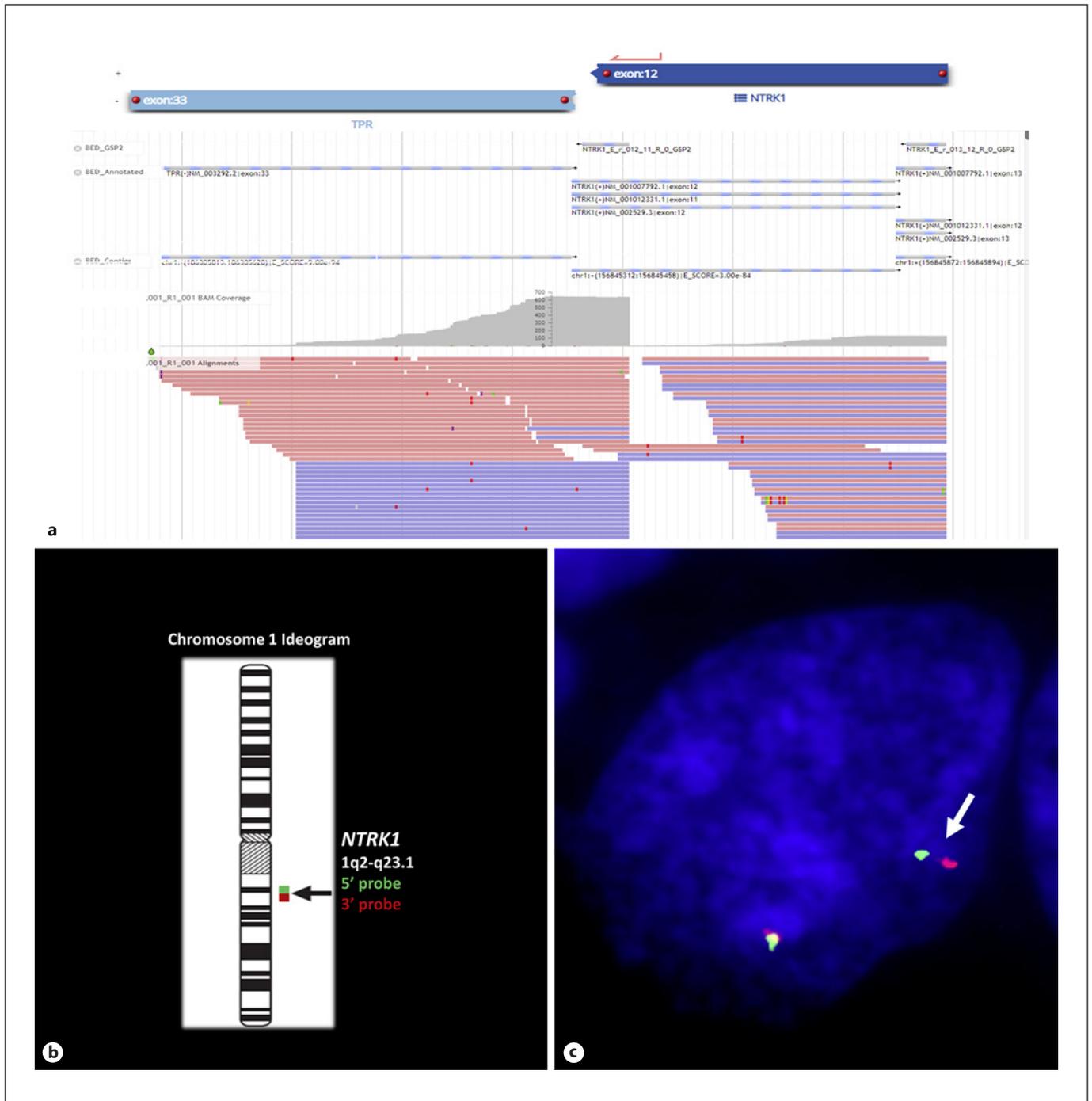


Fig. 3. NGS and FISH analysis showing the presence of *NTRK1* gene fusion in the sample from the patient's first surgery. **a** Visualization of sequenced reads using Archer VR FusionPlex VR (JBrowse 1.11.6) of *TPR* and *NTRK1* genes. The red and blue lines represent the different reads supporting the fusion event involving exons 33 and 12 of *TPR* and *NTRK1*, respectively. **b** Representation of the FISH assay for *NTRK1* translocation detection at the 1q22-q23.1 region. In the absence of translocation, the green (5') and the red (3') probes should be identified together. **c** In the presence of translocation, the green and the red signals are shown separately, indicating the disruption of 5' and 3' regions of the *NTRK1* gene (magnification $\times 100$).

Different from adults and children, infant-type gliomas do not show clear association of histologic tumor grade and clinical outcome. Infant low-grade gliomas are usually more aggressive compared to high-grade tumors, and the patient's outcome is more related to the tumor location and specific genetic alterations. According, 3 different groups were described: group 1 are hemispheric tumors harboring genetic alterations in *ALK*, *ROS1*, *NTRK*, and *MET*, and patients show an intermediate outcome; group 2 are hemispheric tumors with RAS/MAPK mutations and shows an excellent outcome; while group 3 are tumors that arise in the midline, also harbor mutations in the RAS/MAPK pathway, and shows the worse outcome [3]. This new division can better stratify patients and determine more accurate treatment strategies, especially for cases with genetic alterations such as fusions involving *NTRK* that are targeted for therapy [3]. In this context, recent ESMO and Canadian guidelines suggested the routine *NTRK* evaluation in pediatric high-grade gliomas using molecular approaches, such as NGS [23, 24].

NTRK fusions are observed in approximately 3% of solid tumors [25]. The incidence of these genetic alterations varies considerably between different tumor types. In brain tumors, *NTRK* fusions' frequencies also vary according to the patient's age and tumor location. It was reported to be more frequent in infant hemispheric high-grade gliomas [17–19, 26]. Moreover, the tyrosine kinase domain of the *NTRK* gene showed a wide range of different fusions partners in pediatric and infant brain tumors [14], yet with a predominance of *ETV6* as the partner of the *NTRK3* gene [18, 19]. Usually, *NTRK1/3* fusions are found in hemispheric high-grade, and *NTRK2* fusions were found in midline gliomas and low-grade gliomas [14].

In the present study, we report the presence of a *TPR-NTRK1* fusion. The most frequent fusion partners of *NTRK1* in infantile and pediatric gliomas are *ARHGEF11*, *BCAN*, *LMNA*, and *TPM3* genes [14, 27]. In our study, we found the presence of a fusion with the *TPR* gene, which, interestingly, was also reported in a 2-year-old glioma with anaplastic features [27]. Similar to our case, Torre et al. [27] also did not observe other driver mutations in his *TPR-NTRK1* glioma. It is worth mentioning that due to differences in the design of the OCCRA and Archer NGS panel, the *TPR-NTRK1* fusion was only detected in the latter as the partner *TPR* region was not covered by the OCCRA assay. These important technical differences of commercially available assays should be considered when selecting the NGS panel for clinical diagnostic purposes.

Our patient showed stable disease and the absence of any adverse effect following 8 months of larotrectinib

treatment. The presence of *NTRK1* fusion in a subset (23%) of neoplastic cells by FISH could justify the lack of complete response. We continue the treatment with larotrectinib until disease progression or significant toxicity since it is still unknown the duration of anti-TRK treatment in malignant gliomas. Moreover, the experience with other targeted therapies suggests that drug cessation can lead to disease progression [28]. Similar good responses without adverse effects were reported in 2 other infant HGG with *ETV6-NTRK3* fusion treated with larotrectinib [18, 19]. Interestingly, the phase 1/2 STAR-TRK-NG trial (NCT02650401) reported a complete response to entrectinib, another TRK inhibitor in a central nervous system tumor harboring a *TPR-NTRK1* fusion [29]. On the other hand, a 9-year-old child diagnosed with gliosarcoma, with cerebrospinal fluid penetration, harboring *EML4-NTRK3* fusion, after unsuccessful treatment with radio- and chemotherapy, showed initial response to entrectinib, yet the case progressed and disseminated, leading to a 6-month survival upon treatment [17].

In conclusion, this is the first report of an infant-type hemispheric glioma (gliosarcoma subtype), harboring *NTRK1* rearrangement treated with larotrectinib with the stable disease following 8 months of treatment. This case also demonstrates how patients can benefit from molecular profiling and the discovery of druggable specific molecular alteration.

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Statement of Ethics

The local Ethics Committee approved this study (BCH IRB/project no. 1785/2019). The methodology was performed following the relevant guidelines and regulations (Declaration of Helsinki – revised in 2013). The written informed consent had been obtained from the parents of the patient for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

The authors have declared no competing interests. R.M.R. is a member of the journal editorial board as an associate editor.

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Author Contributions

B.M.M., M.B.R., and D.A.M. drafted the manuscript. M.B.R. and F.E.P. designed, performed molecular experiments, and analyzed the data. D.A.M. contributed to the interpretation of the results. B.M.M. and C.R.A.J. conducted the diagnosis, clinical guid-

ance, and patient's treatment. C.E.B.C. performed the MRI processing. M.F.Z. performed the FISH analysis. I.V.V.S. and M.M.M. performed the pathologic examination and made the patient's diagnosis. B.M.M. and R.M.R. conceived the original idea, supervised the project, and edited and revised the manuscript critically for important intellectual content. All the authors critically reviewed and approved the final manuscript.

Data Availability Statement

The data are available and will be shared upon reasonable request to the corresponding authors.

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