Molecular Characterization Reveals *NF1* Deletions and *FGFR1* Activating Mutations in a Pediatric Spinal Oligodendroglioma

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Article Type: Brief Report

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/pbc.26346.

Word Count: Abstract: 90 words | Main Text: 1309 words

 # Tables: 0 | **# Figures**: 2 | **# Supplemental Files**: 3

Running Title: Molecular Profile of a Spinal Oligodendroglioma

Keywords: 1) Anaplastic oligodendroglioma; 2) Spinal cord neoplasms; 3) Molecular sequence data; 4) Precision Medicine; 5) Receptor, Fibroblast Growth Factor, Type 1; 6) Genes, NF1

Abbreviations Key:

AO	Anaplastic Oligodendroglioma
ATRX	Alpha Thalassemia/mental Retardation syndrome X-linked
ERK	Extracellular signal-Regulated Kinases
HGG	High-Grade Glioma
FGFR	Fibroblast Growth Factor Receptor
IDH	Isocitrate Dehydrogenase
MAPK	Mitogen-Activated Protein Kinase
MEK	MAPK/ERK Kinase
MGMT	O-6-Methylguanine-DNA Methyltransferase
MPNST	Malignant Peripheral Nerve Sheath Tumors
NF1 [non-italicized]	Neurofibromatosis type I

ABSTRACT

Pediatric spinal oligodendrogliomas are rare and aggressive tumors. They do not share the same molecular features of adult oligodendroglioma, and no previous reports have examined the molecular features of pediatric spinal oligodendroglioma. We present the case of a child with a recurrent spinal anaplastic oligodendroglioma. We performed whole exome (paired tumor and germline DNA) and transcriptome (tumor RNA) sequencing, which revealed somatic mutations in

NF1 and *FGFR1*. This data allowed us to explore potential personalized therapies for this patient and expose molecular drivers that may be involved in similar cases.

INTRODUCTION

Spinal cord oligodendrogliomas are very rare tumors, comprising less than five percent of spinal cord tumors in all ages.[1-5] In children, spinal tumors are even less common, with spinal gliomas accounting for only 1-3.5% of all pediatric CNS tumors; only a few cases and one case series of two spinal oligodendrogliomas have been reported in the pediatric population.[3,5,6] While standard treatment consists of surgical resection, alkylating chemotherapy, and radiation therapy, there is no consistently-followed regimen given the rarity of pediatric high-grade spinal oligodendrogliomas.

The field of neuro-oncology is increasingly moving toward personalized treatment options for patients. The molecular analysis of adult gliomas by numerous investigators has resulted in the identification of several cytogenetic markers that confer varying prognostic benefits and therapeutic responses.[1,7] However, these features (e.g., 1p/19q co-deletion) are less informative in the pediatric glioma setting.[8] Furthermore, the molecular features of pediatric spinal oligodendroglioma specifically have not previously been examined. We present a case of a child with a spinal anaplastic oligodendroglioma (AO) and discuss our investigation of potential targeted agents using tumor sequencing data.

RESULTS

A three-year-old female presented with a two-month history of neck pain, left leg weakness, asymmetric gait, and right head tilt. Spinal MRI revealed a 6 cm x 1 cm enhancing intramedullary mass extending from C4-T4 (**Fig. 1A**). She underwent uncomplicated subtotal resection of the

primary tumor. Total resection was avoided due to intimate involvement of the tumor with the spinal cord. Her gait had improved by one week after surgery. Pathology revealed a grade III AO with a Ki-67 tumor cell proliferative index of 10-25%. FISH was negative for 1p/19q co-deletion. She underwent adjuvant chemotherapy with 18 cycles of oral temozolomide, but when a three-month surveillance MRI suggested tumor progression, temozolomide was re-initiated for nine additional cycles. Surveillance MRIs remained stable for three years from discontinuation of chemotherapy, when she developed progressive neck pain and weakness in both legs. Repeat MRI revealed tumor progression as an enhancing lesion spanning C5-T2, with a nodular enhancing focus at C4, progression of edema to C2-C3, and a syrinx from the lower cervical spine to the conus (**Fig. 1B**).

She underwent subtotal resection of the recurrent tumor and syrinx decompression via syringo-subarachnoid shunt placement. Lower extremity weakness improved post-operatively with no new deficits. Recurrent tumor pathology remained AO, with microvascular proliferation and a Ki-67 of 4%, IDH mutation negative, O-6-methylguanine-DNA methyltransferase (MGMT) promoter unmethylated, ATRX preserved, and p53 not overexpressed (**Figs. 1C and 1D**). Restaging studies with brain MRI and CSF cytologic analysis were unremarkable. Total spine MRI two weeks after surgery showed decreased size of the syrinx and stable residual tumor. She underwent conformal proton radiotherapy with 50.4 GyE to the resection bed and vertebral bodies, but she subsequently developed acute radiation necrosis and back pain, which has responded well to steroids and bevacizumab.

At the time of recurrence, the patient was enrolled on PEDS-MIONCOSEQ, a precision oncology study involving whole exome (paired tumor and germline DNA) and transcriptome (tumor RNA) sequencing. Clinically-integrated sequencing was performed according to previous published methodology.[9] Nucleic acid preparation, high-throughput sequencing, and computational analysis were performed using standard protocols in our sequencing laboratory in the Michigan Center for Translational Pathology, which adheres to the Clinical Laboratory Improvement Amendments (CLIA).

Sequencing revealed a somatic point mutation and three small deletions in *NF1*, consistent with biallelic inactivation (**Supplementary Table S1**). There was no evidence of germline *NF1* alterations, skin lesions, or family history suggestive of Neurofibromatosis type I (NF1). Sequencing also revealed two somatic activating missense mutations in *FGFR1* (**Supplementary Table S2**). No mutations in *BRAF* were observed, which are recurrently found in some oligodendrogliomas.[10] Sequencing results were otherwise unremarkable (**Supplementary Fig. S1**). Results of her tumor sequencing were discussed in our multidisciplinary CNS precision medicine tumor board – teleconferenced with clinicians at multiple children's hospitals – which recommended adjuvant therapy with a fibroblast growth factor receptor (FGFR) inhibitor and/or a MAPK/ERK Kinase (MEK) inhibitor, although she was not eligible for any clinical trials using these agents at that time. Therapy with the FGFR inhibitor ponatinib (off-study) is planned as a maintenance therapy upon clinical improvement.

DISCUSSION:

The prognosis for pediatric high-grade gliomas (HGG) remains dismal despite multimodal treatment approaches, and the few reports on outcomes for spinal cord HGG's are also discouraging.[5,11,12] High-grade oligodendrogliomas in any location are uncommon in children and have unfavorable outcomes as well.[4,11,12] Only four AO's were confirmed among 250 HGG patients treated on the CCG-945 Children's Cancer Study Group clinical trial, and four non-glioblastoma/anaplastic astrocytomas were observed among 107 patients with HGG treated on the ACNS0126 Children's Oncology Group trial.[11,12] Lundar *et al.* followed 35 children with cerebral oligodendrogliomas and found five- and 10-year overall survival of only 27% and 18%, respectively, for the anaplastic tumors.[4] The prognosis of the very few reported pediatric spinal AO is also poor; Merchant *et al.* reported 2 patients with AO among 11 high-grade pediatric spinal tumor patients who survived only 29 and 39 months, respectively.[5]

The molecular features of adult oligodendroglioma are well described. 1p/19q co-deletion is found in 50-70% of adult gliomas and confers an improved response to chemotherapy and overall survival.[1,7] However, this high frequency and survival benefit for co-deletion has not been shown in pediatric gliomas.[4,8,12,13] Similarly, methylation of the MGMT promoter and *IDH1* mutations are only rarely seen in pediatric HGG.[14] While these markers are helpful in guiding adult oligodendroglioma therapy, new molecular markers are needed for management of pediatric oligodendroglioma.

Molecular analysis of our patient's tumor displayed *NF1* loss-of-function mutations *FGFR1* activating mutations. Mutations in the tumor suppressor gene *NF1* are classically associated with NF1, a germline disorder characterized by neurofibromas, malignant peripheral nerve sheath tumors (MPNST's), optic pathway gliomas, and cutaneous findings.[15] *NF1* codes for neurofibromin, which promotes inactivation of Ras, downregulating the Ras-Mitogen-activated protein kinase (MAPK) pathway.[16] Loss-of-function mutations in neurofibromin result in up-regulation of the Ras-MAPK pathway and increased cellular proliferation. Sporadic, *NF1* somatic mutations have been observed

by sequencing in many tumor types.[17] Interestingly, patients with a diagnosis of NF1 (germline *NF1* loss) have a slightly higher incidence of intramedullary spinal tumors.[18] This raises the question of whether glial precursor cells in the spine are predisposed to malignant transformation with *NF1* loss, whether somatic or germline.

The FGFR family is comprised of five receptor tyrosine kinases that function in wound healing, angiogenesis, and cellular proliferation through their interaction with fibroblast growth factors.[19] FGFR1 interfaces with several pathways, including Ras-MAPK.[19] Sporadic *FGFR1* mutations have been observed in many tumor histologies, including two medulloblastoma, one low-grade glioma, and three glioblastoma cases.[17]

Given the lack of known effective therapies for the recurrent spinal AO in this child and the molecular leads to the oncogenesis of her tumor from the sequencing, targeted therapies are a consideration. In particular, inhibition of the Ras-MAPK pathway could be accomplished at the sites of both observed somatic mutations (**Fig. 2**). This could be done upstream via FGFR1 inhibition (e.g., ponatinib), or downstream via MEK inhibition (e.g., trametinib, which is actively being studied in other *NF1*-deficient tumors).[20]

Given that 64% of the sequenced tumor fraction expressed an *FGFR1* mutation and only 20% expressed alterations in *NF1*, we will initiate ponatinib for this patient. Ponatinib displays moderate CNS penetration and has published use in the pediatric population.[21] Future combination therapy with trametinib will be considered if the patient tolerates ponatinib therapy and does not display objective response. Response will be assessed by determining if the patient shows reduction in tumor size on surveillance brain imaging (a decrease of <25% of the largest diameters of measurable lesions and no evidence of new lesions) and remains without worsening tumor-related symptoms.

In conclusion, we report genomic data for a case of pediatric spinal AO, a rare and aggressive tumor. The targetable mutations discovered in this child's tumor point to potential personalized therapies for her, and suggest molecular drivers that may be involved in similar cases.

CONFLICT OF INTEREST STATEMENT:

The authors report no disclosures or conflicts of interest.

ACKNOWLEDGMENTS:

The authors thank the patient and her family. C.J.K. is supported by NIH/NICHD K12 funding to the University of Michigan Department of Pediatrics (PI: Opipari), and the University of Michigan Janette Ferrantino Investigator Award. The University of Michigan PEDS-MIONCOSEQ study was supported by grant 1UM1HG006508 from the National Institutes of Health Clinical Sequencing Exploratory Research Award (PI: Arul Chinnaiyan, Co-I: Rajen Mody). Additionally, the authors thank the Michigan Center for Translational Pathology for whole exome and transcriptome tumor sequencing analysis through the PEDS-MIONCOSEQ program.

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LEGENDS:

Figure 1: Spinal Oligodendroglioma Imaging and Pathology

1A) Spinal MRI, sagittal image, T1 series post-contrast, illustrating tumor after initial resection. **1B)** Spinal MRI, sagittal image, T1 series post-contrast, showing recurrent disease in the cervical spine, primarily in the C4-T2 region. **1C)** Tumor parenchyma shows histologic findings on

hematoxylin and eosin stain consistent with grade III anaplastic oligodendroglioma: focal high density of round to spindled cells; many cells with perinuclear halos; scattered dark and angulated anaplastic nuclei; two large areas of microvascular proliferation at the left side of the figure; and microcysts filled with mucin are prominent near the lower right corner of the figure. **1D)** ATRX protein is preserved in nuclei of vascular cells and neoplastic cells (ATRX loss is frequently seen in *ATRX*-mutated astrocytoma).

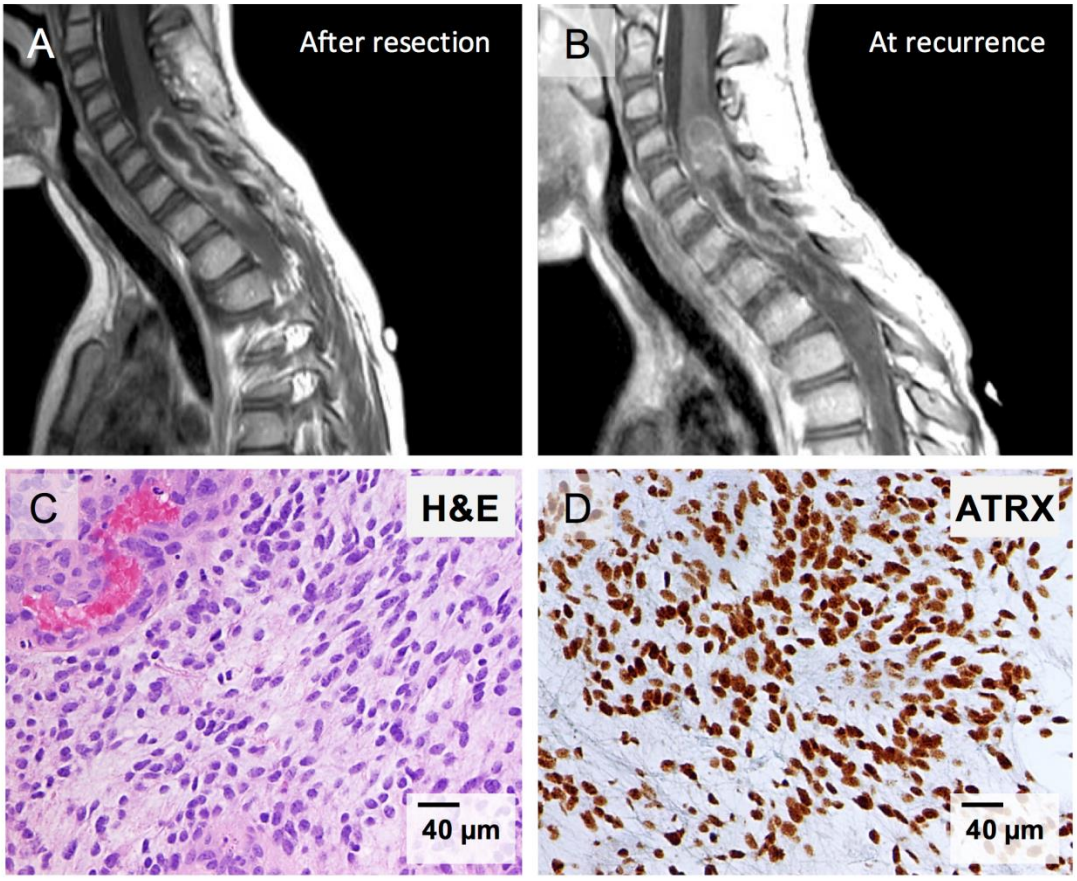
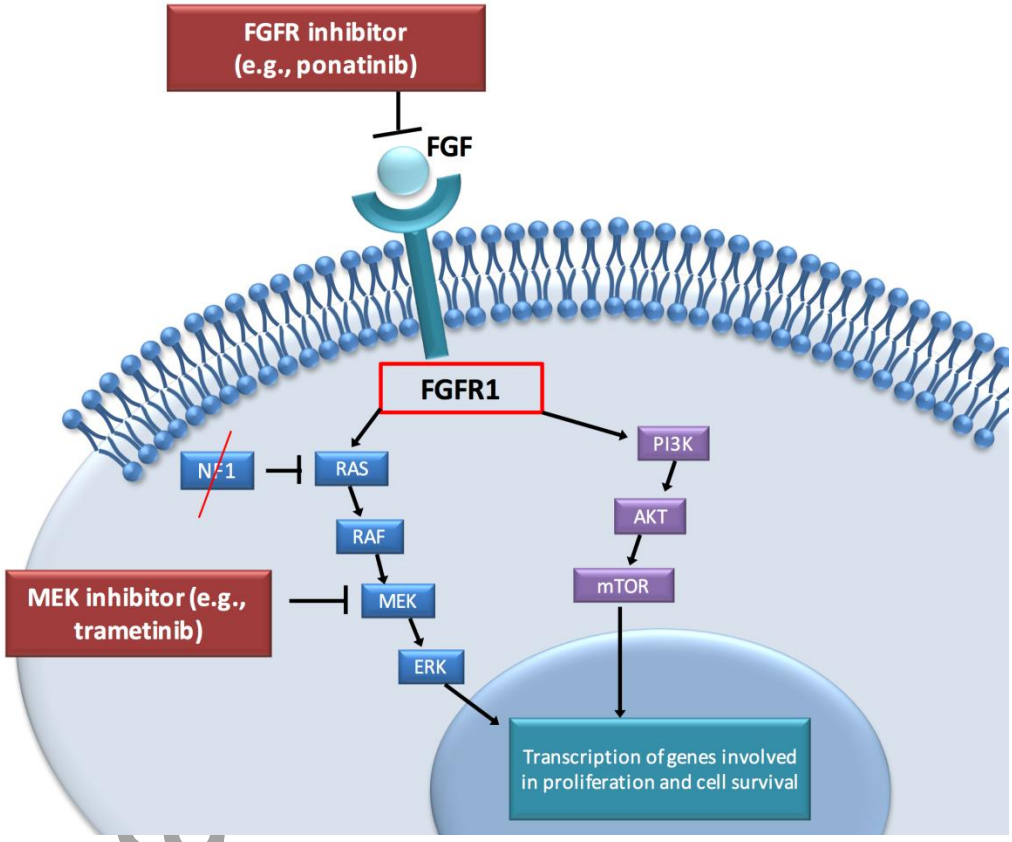


Figure 2: Proposed Targeted Inhibitors of the Ras-MAPK Pathway

Neurofibromin loss-of-function and FGFR1 activation in this tumor are the two identified potential drivers of oncogenesis, and inhibitors of either pathway are attractive targeted therapy agents. Upstream inhibition of the Ras-MAPK pathway at the level of FGFR1 can be achieved with the tyrosine kinase inhibitor, ponatinib. Downstream inhibition of the Ras-MAPK pathway can be achieved with the MEK inhibitor, trametinib.



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Supplementary Figure S1: Tumor Copy Number Profile and LOH Plot

Copy number profile revealed no remarkable change in the genome, with absence of copy gains, copy losses, and copy neutral loss of heterozygosity (LOH).

Supplementary Table S1: Sequence Analysis—Observed Mutations and Indels

Somatic mutations and insertions/deletions observed for *FGFR1* and *NF1* on chromosomes 8 and 17, respectively, are presented. 32% of the tumor variant fraction contained a TTT→CTA base change in the *FGFR1* gene, and 10% of the tumor variant fraction contained a C→G base change in the *NF1* gene. Additionally, in *NF1*, a frameshift deletion and two non-frameshift deletions were observed at 3%, 2%, and 3% tumor variant frequency, respectively.

Abbreviations: AA: Amino Acid; FPKM: Fragments Per Kilobase of transcript per Million mapped reads; COSMIC: Catalogue of Somatic Mutations in Cancer; fs: frameshift, del: deletion.

Supplementary Table S2: Sequence Analysis Summary

Notable mutations were observed in *FGFR1* and *NF1*, amounting to probable biallelic inactivation of *NF1* and activation of the FGFR1 protein, but otherwise no remarkable genomic changes or germline findings were observed.