Clinical characteristics and outcome of central nervous system tumors harboring NTRK gene fusions.

Running title: Central nervous system tumors with NTRK gene fusions.

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DO had a consultant activity for Bayer and Roche and is an Independent Data Monitoring Committee (IDMC) member for a Lilly product and is a consultant for Novartis Pharma France and Eusapharm.

JRH has consulted for Bayer Australia, Alexion Pharma and Boxer Capital. He sits on the scientific advisory board of Servier Pharma International.

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Key words: NTRK fusion, CNS tumor, TRK inhibitors

Key points:

- We report the largest cohort of patients with TRK fusion-driven primary CNS tumors.
- Young age and low-grade histology are associated with improved outcomes.
- TRKi appears to improve tumor control in a subset of patients, most notably for pediatric HGG.

Translational Relevance:

Neurotrophic tyrosine receptor kinase (NTRK) gene fusions have been reported at various frequencies in CNS tumors. These rare alterations are found in up to 2% of adult with glioma but the incidence reaches 40% in infants diagnosed with non-brainstem gliomas. New therapeutic approaches are being investigated but given its rarity there is limited data on the demographic and outcome of theses tumors. Our study describes the largest cohort of CNS tumors with NTRK fusion. We include 119 patients and describe key demographic and clinical characteristics of these rare tumors. We show that pediatric patients and those with tumors classified as LGG have better outcomes. Furthermore, there is also evidence that TRKi can provide better disease control when compared to previous therapies, especially in children. Data provided in our manuscript will help better understand expect evolution and potential efficacy of new treatments for CNS tumors with NTRK fusion.

Abstract

Purpose: TRK fusions are detected in less than 2% of central nervous system tumors. There are limited data on the clinical course of affected patients. **Experimental design**: We conducted an international retrospective cohort study of patients with TRK fusion-driven CNS tumors. **Results**: 119 patients were identified. The median age at time of diagnosis was 4.5 years. The majority were reported to have a histology consistent with a diagnosis of high-grade glioma (HGG) (57.1%) followed by low-grade glioma (LGG) (27.7%). Pediatric patients had a better prognosis with a median overall survival of 185.5 months compared to 24.8 months in adults (p<.0001). Patients with LGG also had a better outcome when compared to HGG (p=0.0012). The objective response was 68.8% with larotrectinib compared to 38.1% for non-targeted treatment. **Conclusions:** Children with LGG glioma had a favorable outcome compared to adult and HGG. TRK inhibitors appear to improve tumor control.

Introduction

NTRK1, *NTRK2*, and *NTRK3* genes code for the tropomyosin receptor kinase (TRK) family of receptors TRKA, TRKB, and TRKC, which are neurotrophic tyrosine receptor kinase (NTRK) proteins (1, 2). These receptors are expressed in neuronal tissue and play an essential role in the normal development and function of the nervous system (1, 3). *NTRK* gene fusions have been reported in a variety of pediatric and adult tumors and occur when the 3' region of the *NTRK* gene encoding the tyrosine kinase domain is joined in-frame with the 5' end of a fusion partner gene, either by intra- or inter-chromosomal rearrangement (4). The resulting fusion oncogene leads to the expression of a chimeric protein that retains the tyrosine kinase domain, is constitutively active, and drives downstream signaling (4).

NTRK gene fusions occur in up to 1% of all solid tumors and 2% of adult primary central nervous system (CNS) tumors (2, 5-7). In the pediatric population, *NTRK* gene fusions have been observed in up to 5.3% of high-grade gliomas (HGG) and 2.5% of low-grade gliomas (LGG) (7-10). Recently, a subset of gliomas with *NTRK gene* fusions have been grouped under the tumor type infant-type hemispheric glioma in the 2021 WHO Classification of Tumors of the CNS (11).

There are limited data on CNS tumors with NTRK fusion in the literature, mostly presented in case-reports and small case series or included in larger studies focused on molecular characterization of pediatric CNS tumors (12-22) (23). The largest cohort included 33 patients enrolled on two clinical trials and treated with larotrectinib, a selective TRK inhibitor (TRKi) (24). Larotrectinib and entrectinib have been FDA and EMA approved in a histology agnostic fashion for patients with NTRK fusion-positive solid tumors and are slowly starting to impact the management of CNS tumors with NTRK fusion: (25). However, the natural history and outcomes of patients with NTRK fusion-positive CNS tumors are not well described. A better understanding of these rare tumors will help interpret the efficacy and limitations associated with these new targeted therapy approaches. Herein we report the characteristics and outcomes of a large international cohort of pediatric and adult patients with CNS tumors harboring NTRK fusions.

Materials and Methods

Study Population

This is an international multicenter retrospective cohort study of patients with CNS tumor and NTRK fusion. All patients diagnosed between 2000 and 2021 with a confirmed TRK fusion were eligible. To identify patients, an invitation email was sent to oncologists and neuro-oncologists from international sites. Patients identified to have an *NTRK gene* fusion in the Children's Brain Tumor Network (CBTN) database were also included. The study was conducted in accordance with the Declaration of Helsinki and the study was approved by the institutional review board (IRB-CHU Sainte-Justine). Written informed consent was waived by the IRB giving the retrospective nature of the study and by the fact that data was coded and protected health information was removed.

Data collection

After institutional approval, centers received a standardized case report form which included patient's demographics, pathology characteristics, tumor location, treatments and outcome. Treatment regimens were collected and categorized as surgery, chemotherapy, radiation therapy, TRKi and other. For analysis purposes, "non-targeted therapy" included chemotherapy, radiation therapy and other, and excluded surgery only and TRKi. For CBTN subjects, these data were extracted from the CBTN database with additional queries to treating sites as necessary. When possible, response was assessed by the local investigator and categorized as CR (complete response), PR (partial response), MR (minor response), SD (stable disease), PD (progressive disease) and was considered N/E (not evaluable) if a gross total or near total resection was done prior to treatment (Supplementary Data S1). Centers were encouraged to use the RANO (Response Assessment in Neuro-Oncology) or Response Assessment in Pediatric Neuro-Oncology (RAPNO) criteria (26, 27). For patients enrolled in clinical trials, responses were not extracted to avoid interference and confidentiality breach. Clinically significant response was defined as CR, PR or MR.

Statistical analysis

Descriptive statistics were reported as counts and percentages for categorical variables and median and range for continuous variables. Outcomes including progression free survival (PFS) and overall survival (OS) were presented using Kaplan-Meier survival curves. If relevant, groups were compared using log rank test. Growth modulation index (GMI) was calculated based on the method described by Von Hoff et al (28). GMI is the

ratio of the progression-free survival (PFS) of a treatment compared to the time to progression (TTP) of the previous treatment ($PFS_{treatment}$ / $TTP_{previous\ therapy}$). Patients are censored at progression or last follow-up, the most recent report. It is usually accepted that a GMI ≥1.3 suggest a significant treatment benefit with a PFS which increases by 30% or more in the second-line therapy. The GMI has been used in several precision medicine trials including recent studies. (29-31). In our study, patients were included in the GMI analysis if they received a prior line of treatment (chemotherapy or radiation therapy) followed by progression and initiation of TRKi. Patients with a combination of TRKi and other systemic therapy or radiation were excluded. The Kaplan-Meier estimate was obtained using GMI calculated as the ratio of time to progression/censoring with TRKi to the time to progression with previous therapy (x-axis) and progression-free survival rate on TRKi (y-axis). At GMI=0, all patients treated with TRKi were at risk of progression and the survival probability was 1. The Kaplan-Meier estimate was used to describe the probability of progression-free surviving on TRKi past a specific value of GMI (1.3). The higher the probability of GMI>=1.3, the higher the benefit of TRKi compared to previous treatment. All statistical tests were two-sided and conducted at the 0.05 significance level. Statistical analyses were performed using SAS version 9.4.

Data availability

Patient data is not publicly available to protect patient privacy but is available upon reasonable request to the corresponding author (Sébastien Perreault, s.perreault@umontreal.ca)." Provided data will be de-identified.

Results

Study population

A total of 170 investigators from 41 countries were contacted by email. In total, 129 patients from 46 centers (21 countries) were identified. Ten patients with a reported NTRK alteration were excluded as it was not possible to confirm whether their tumor had an NTRK fusion; thus, the cohort included 119 patients. Thirty-one patients (26.1%) had previously been reported in the literature as part of a clinical trial, case series or case report (12-22) (23) Supplementary Figure S1.

The median follow-up of the entire cohort was 38.5 months (range 0.03-229.3 months) (Table 1). The median age at time of diagnosis was 4.5 years (range 0-78.4 years), and almost half of all patients were infants of less than 3 years of age (n= 53/117, 45.3%). The median age of the adult cohort was 50 years (range 18-78.4 years).

Tumor characteristics

The most frequent location for CNS tumors with an NTRK fusion was hemispheric (63.8%); 13 patients (13%) were found to have metastatic disease (Table 1). The majority were reported to have a histology consistent with a diagnosis of HGG (57.1%), followed by LGG (27.7%), embryonal tumors (4.2%) and others (10.9%) (Table 1). All 17 adult patients had a diagnosis of HGG. In children, LGG and HGG had a different distribution according to age, with HGG predominantly found in patients under three years of age (72%, p=0.0035) (Table 1). Most patients had an NTRK2 fusion (50.4%) (Figure 1). The specific *NTRK* gene (*NTRK1*, 2 or 3) was not associated with a specific histology (Supplementary Table S1). Sequencing was done at primary site using next generation sequencing for NTRK fusion and other alterations (Supplementary Table S2 and S3).

Treatment

For initial treatment, 40 patients (33.6%) had surgery only and then were observed without further treatment, 72 patients (60.5%) received either chemotherapy, radiation or other systemic therapy excluding TRKi, and 7 patients (5.9%) were treated upfront with TRKi (Table 2).

When assessing treatments received during the observation period, 28 patients (23.5%) underwent surgery only without further treatment, most of these patients were pediatric

patients with LGG (60.7%) and 17.9% were children with HGG (p<0.0001). Forty-three patients (36.1%) received one line of treatment besides surgery and 48 patients (40.3%) received three or more lines of treatment (Table 2). A total of 51 patients (42.9%) were treated with TRKi at some point during their follow-up, including 39 patients (76.5%) with larotrectinib, 3 patients (5.9%) with entrectinib and 9 patients (17.6%) with other or non-specified TRKi (Table 2). The first patient that received a TRKi was treated in 2015. Four patients received two different TRKi, and one patient was reported to have received three different TRKi. Sixteen patients (13.4%) were enrolled on a therapeutic clinical trial of a TRKi; 8 with larotrectinib (two of whom subsequently received selitrectinib), three with entrectinib, and five with an “unspecified” TRKi.

Response to treatment

Non-targeted therapy was given 99 times in 65 patients and response was assessed for 63 of these 99 lines of treatment. Out of these 63 evaluable non-targeted therapy regimens, 24 (38.1%) resulted in an objective response (10 CR, 13 PR and 1 MR) (Supplementary Table S4). For 35 evaluable non-targeted regimens, the pediatric HGG objective response was 42.9% (6 CR, 8 PR, 1 MR). The infants (<3y) HGG objective response was 45.2% (6 CR, 7 PR, 1 MR) among 31 evaluable non-targeted regimens. The older children/adolescents HGG objective response was 25% (1 PR) among 4 evaluable non-targeted regimens.

Response to TRKi was evaluable for 33 separate instances of treatment overall, and for 21 instances of treatment for pediatric patients. The response rate was 42.4% overall (5 CR, 7 PR, 2 MR) and 61.9% for the pediatric patients (4 CR, 7 PR, 2 MR) (Supplementary Table S4). When restricted to patients with HGG, response to TRKi was evaluable for 22 separate treatment instances, with an overall response rate of 45.5% (5 CR, 4 PR, 1 MR) and a 90% response rate (4 CR, 4 PR, 1 MR) in children.

Larotrectinib was given 23 times in 22 pediatric patients and response was assessed following 16 of these 23 lines of treatment. Out of these 16 evaluable larotrectinib regimens, 11 (68.8%) demonstrated objective response (4 CR, 5 PR and 2 MR). When restricted to patient with pediatric HGG, response to larotrectinib was evaluable for eight patients with an overall response rate of 100% (4 CR, 3 PR, 1 MR) (Supplementary Table S4).

Survival Outcome

The median follow-up was 38.5 months (range: 0.03-229.3). At last follow-up, 88 patients (74%) were alive (Supplementary Table S5). The overall survival (OS) and the progression free survival (PFS) were analyzed for the entire cohort and according to patients' characteristics (Figure 2, Figure 3 and Supplementary Figure S2). The median overall survival of the entire cohort was 185.5 months (95% CI 99.5-229.3) (Figure 2A) and the median PFS was 25.5 months (95% CI 15.5-40.9) (Figure 3A). Gross total resection (GTR) and near total resection (NTR) were not associated with better OS (p=0.45) or PFS (p=0.40) when compared to partial resection or biopsy.

Pediatric patients had a better prognosis with a median OS of 185.5 months (95% CI 185.5-229.3) compared to 24.8 months in adults (95% CI 17.1-99.5)(p<.0001) (Figure 2B). However, the median PFS between pediatric and adult patients was not significantly different (25.8 months vs 11.1 months, respectively p=0.22) (Figure 3B). Patients with LGG also had a better prognosis with a median OS that was not reached compared to 99.5 months for HGG (95% CI 57.9-229.3) and 38.5 (95% CI 3.5-NE non-estimable) months for embryonal tumors (p=0.0012) (Figure 2C). This difference in OS by histology was also significant in the pediatric cohort (p=0.021). Pediatric patients with a HGG had a better outcome when compared to adults with HGG. The median OS with HGG was 185.5 months (95% CI 65.3- NE) for children compared to 24.8 (95% CI 17.1- NE) months for adults (p=0.0035). There was no difference in PFS between histologic subtype (p=0.30) (Figure 3C). Six patients with HGG underwent surgery only and two remained alive at last follow-up (range 28.7-43.7 months). NTRK gene fusion type (1,2, or 3) was not associated with a difference in OS (p=0.18) or (Figure 2D and Supplementary Figure S2A). We observed a tendency for an improved PFS in pediatric patients with NTRK1 or NTRK2 in the present cohort (p=0.0526 for the comparison NTRK1 vs. NTRK3 and p=0.0214 for the comparison NTRK2 vs. NTRK3)(Figure 3D and Supplementary Figure S2B). Patients with *CDKN2A/B* alteration had a median OS of 57.9 months compared to 229.3 months for patients without alteration (p=0.053) (Figure 2E). Most patients with *CDKN2A/B* alteration had a diagnosis of HGG (16/18-88.9%). There was no significant difference in PFS between patients with and without *CDKN2A/B* alteration (Figure 3E-F). Only one patient's tumor classified as LGG had a *CDKN2A/B* deletion. This infant had a cerebellar lesion that underwent two resections without systemic therapy or radiation therapy and was still alive

at a follow-up of 57 months. Finally, no difference was observed in OS of children with HGG that received radiation therapy or not (p=0.695) (Figure 2F).

Growth Modulation Index

The growth modulation index (GMI) was calculated in 31 patients (20 pediatric and 11 adults). Twenty patients were excluded from the analysis (11 received TRKi plus another treatment, five did not received a treatment prior to a TRKi, two had no available data, two were treated with TRKi but without prior progression). The median GMI was 1.1 (range 0.09-11.51) and 15/31 (48.4%) had GMI≥1.3. For the pediatric population, the median GMI was 1.36 (range 0.15-11.51) and 10/20 (50.0%) had GMI≥1.3. Eight patients (40%) remained on TRKi at the last reported timepoint. The average time on TRKi was 26 months and no patient without progression discontinued treatment. For the pediatric subgroup with HGG (N=12), the median GMI was 0.6 (range 0.15-7.58) and 4/12 (33.3%) had GMI≥1.3.

The Kaplan-Meier estimate for the probability of having GMI ≥ 1.3 for TRKi was 0.62 (95% CI = 0.36, 0.80) in pediatric patients (this probability was 0.44 (95% CI = 0.15, 0.70) in the pediatric subgroup with HGG (N=12)) and 0.45 (95% CI = 0.17, 0.71) in adult patients (N=11). The PFS for pediatric patients treated with TRKi was therefore better compared to previous therapy (Figure 4A). However, this difference was not observed in the pediatric HGG subgroup nor in adult patients (Figure 4B).

Specifically for larotrectinib, GMI was calculated as a ratio of PFS with larotrectinib to TTP of the prior line of therapy in 23 patients (14 pediatric and 9 adults). The median GMI was 1.66 (range 0.09-11.51) and 14/23 (60.9%) had GMI≥1.3. For the pediatric population, the median GMI was 2.11 (range 0.15-11.51) and 10/14 (71.4%) had GMI≥1.3. Specific median GMI to compare pediatric LGG (N=2) to HGG (N=8) was not possible due to small number of patients in pediatric subgroup with LGG available for this analysis. For the pediatric subgroup with HGG (N=8), the median GMI was 1.3 (range 0.15-7.58) and 4/8 (50.0%) had GMI≥1.3.

Using the Kaplan-Meier estimate, the probability of having GMI≥1.3 for larotrectinib was 0.85 (95% CI = 0.52, 0.96) in pediatric patients (this probability was 0.73 (95% CI = 0.28, 0.93) in the pediatric subgroup with HGG (N=8)) and 0.44 (95% CI = 0.14, 0.72) in adult patients (N=9) (Figure 4C and 4D). The PFS with larotrectinib was superior when

compared to previous therapies for pediatric patients (including the pediatric HGG subgroup) but this difference was not observed in adult patients (Figure 4C and 4D).

Discussion

To our knowledge, we here present the largest cohort of patients with CNS tumors and confirmed NTRK fusions. We show that pediatric patients and those with tumors classified as LGG have better overall survival. Furthermore, there is also evidence that TRKi can provide better overall response and PFS when compared to previous therapies, especially in children.

The outcome of pediatric patients is significantly better when compared to adults, with a median OS of more than 15 years compared to two years for adults. However, the pediatric cohort included a mixture of histology while adult cohort were all HGG. Amongst HGG, adults had a worse outcome. Our observations are in line with what as been reported in the SCOUT/NAVIGATE trials. When treated with larotrectinib, no adult reached a partial response compared to 40% of children (24, 32). This difference in outcome will be important to account for in ongoing and future clinical trials. Within the present study, additional risk factors that could explain this difference were not identified. Additional molecular alterations that would differentiate pediatric from adult tumors were not reported in this dataset, as central testing was not performed. However, NTRK fusions may be late events in the pathophysiology of adult CNS tumors or serve as one of multiple oncogenic mutations in adult tumors, in contrast to children where NTRK fusions are thought to act as the primary driver mutation. To answer this question, next generation sequencing data and DNA methylation profiles will need to be collected and correlated with outcome.

While we acknowledge that glioma grading can be challenging, especially in young children, our data shows that grading based on histology appears to remain an important predictor of outcome in CNS tumor with NTRK fusions. This concept will also need to be validated by central review of histology, molecular features and methylation analysis.

We observed a tendency for an improved PFS in pediatric patients with NTRK1 in the present cohort. This observation has not been reported in other cancers and might not be clinically significant. However, this observation should be explored in future cohort studies and clinical trials. Our study showed no difference in fusion partner, but also will need prospective follow up to confirm the fusion partner is not of significance.

We report that almost a quarter of patients, including two with HGG, underwent surgery only and were long-term survivors. Based on this observation, it is possible that select patients who undergo a gross total resection could be cautiously observed before initiating systemic therapy, including those with pediatric HGG, given their outstanding OS (median OS 185.5 months). In our study, we did not observe a better outcome in children with HGG that received radiation therapy compared to those who did not. The decision to use radiation therapy should therefore be balanced against the potential side effects especially in young children. In addition, given the observed GMI and excellent response rates to TRKi, it may be reasonable to consider limited resection in specific cases of pediatric HGG, and offer a TRKi as an initial treatment. We did not observe a significant difference between TRKi and systemic therapy for pediatric HGG but giving the toxicity profile the use of TRKi are a new interesting avenue. Response rate and GMI of HGG treated with larotrectinib were particularly high and significantly more efficacious when compared to systemic treatment. Upfront therapy using TRKi is under investigation (NCT04655404).

Not surprisingly, more than half of pediatric LGG underwent surgery only. LGG with NTRK fusions do not appear to be at higher risk of requiring additional lines of treatment when compared to historical LGG data. However, given the fact that some of these lesions might be difficult to resect, surgery with high risk of neurological deficits should be avoided. In these specific cases of LGG, standard chemotherapy should be considered and TRKi might offer an interesting alternative especially in the context of recurrent disease. Given the small number of LGG available for response analysis and GMI, no conclusion on efficacy of TRKi compared to chemotherapy can be drawn.

Assessing the efficacy of treatment in a retrospective cohort is challenging. The evaluation of response was not centralized but rather based on local investigator evaluation. Clinical practice does not always follow formal RANO/RAPNO response criteria. Regardless, we observed that pediatric patients had a better response rate to larotrectinib when compared to non-targeted therapy, suggesting that this treatment approach might yield a clinical benefit. The efficacy of other TRKi could not be evaluated given the small number of patients.

Comparing response rate at different time points is also a significant limitation. A patient facing multiple relapses may be less likely to respond to treatment. The GMI is an

innovative measure which is useful for relapsed and refractory cancers. Its clinical application has been accepted to evaluate the efficacy of treatment using an intra-patient control. In a given patient who had progression, an effective therapy should increase the next time-to-progression. In our study we demonstrated a GMI of 2.1 for the pediatric patients treated with larotrectinib, which is substantially higher than the 1.33 GMI cut-off associated with a probable efficacy. Although arbitrary, this threshold of 33% improvement seems appropriate, as PFS tends to decrease with subsequent lines of systemic therapy in other solid tumor (33). A number of the patients included remained on treatment as of the data cut-off, suggesting that the median GMI may increase further. We suggest that GMI could be integrated in the ongoing studies as a secondary objective to evaluate the efficacy of TRKi. One limitation of the GMI is that it selects against patients who succumb after first line of therapy, since these critical patients are removed from the analysis. Another limitation is the small sample size of patients without HGG evaluable for analysis; thus, limiting our ability to generalize these observations to other histologies.

While we broadly reached out to providers by email to identify potential patients, it is likely that we gathered more responses from centers with whom we had previously established collaborations through other clinical studies. Both the fact that two of the principal authors have been involved in clinical trials involving larotrectinib, and the young age of this cohort (larotrectinib is the only FDA approved TRK inhibitor for patients below 12 years of age) might explain why we collected more patients treated with larotrectinib as compared to other TRKi. Another potential limitation of this study is that only patients locally identified to have NTRK fusions were included. This may bias toward patients with worse outcomes, as testing may have been performed more frequently in patients with difficult to treat or relapsed disease.

Finally, despite the fact we reported the largest cohort of patients with CNS tumors and NTRK fusion, the study includes a small number of patients with embryonal tumors and even LGG. We are planning to continue data collection and increase the number of patients but there is an urgent need for future prospective clinical trials addressing the current limitations of our data analysis.

Conclusion

In summary, we describe a large cohort of patients with CNS tumors and NTRK fusion. We identified that young age and low-grade histology are associated with improved outcomes. TRKi appears to improve tumor control in a subset of patients, most notably for pediatric HGG. Additional prospective study and clinical trials are needed to improve management of patients with CNS tumors and NTRK fusion. Standard treatments such as chemotherapy and radiotherapy could be compared to upfront treatment with TRKi. Minimally invasive surgery followed by treatment with TRKi and second look surgery could also be investigated within a clinical trial.

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Table 1

Clinical characteristics of the entire cohort of patients with CNS tumor and NTRK fusion.

	Entire cohort n (%)	Ped cohort n (%)	pedLGG n (%)	pedHGG n (%)
Age category (n=118)	n=118[#]	n=101	n=33	n=50
Pediatric (<18y)	101 (85.6%)			
Adults (>=18y)	17 (14.4%)	NA	NA	NA
	n=117	n=100[#]	n=32	n=50
Infants and toddler (<3y)	53 (45.3%)	53 (53.0%)	11 (34.4%)*	36 (72%)*
Children (>=3y to <12y)	32 (27.4%)	32 (32.0%)	15 (46.9%)	10 (20%)
Adolescent (>=12y to <18y)	15 (12.8%)	15 (15.0%)	6 (18.8%)	4 (8%)
Adults (>=18y)	17 (14.5%)	NA	NA	NA
Gender (n=119)	n=119	n=101	n=33	n=50
Female	53 (44.5%)	46 (45.5%)	13 (39.4%)	28 (56%)
Male	66 (55.5%)	55 (54.5%)	20 (60.6%)	22 (44%)
Tumor location (n=116)	n=116	n=99	n=32	n=50
Hemispheric	74 (63.8%)	58 (58.6%)	18 (56.3%)	28 (56%)
Spine	10 (8.6%)	9 (9.1%)	0 (0%)	7 (14%)
Suprasellar	8 (6.9%)	8 (8.1%)	4 (12.5%)	4 (8%)
Diencephalic	5 (4.3%)	5 (5.1%)	3 (9.4%)	1 (2%)
Brainstem	11 (9.5%)	11 (11.1%)	5 (15.6%)	6 (12%)
Cerebellum	8 (6.9%)	8 (8.1%)	2 (6.3%)	4 (8%)
Metastic status (n=100)	n=100	n=82	n=27	n=38
localized	87 (87.0%)	71 (86.6%)	25 (92.6%)	32 (84.2%)
metastatic	13 (13.0%)	11 (13.4%)	2 (7.4%)	6 (15.8%)
Histological_diagnosis_(n=119)	n=119	n=101	n=33	n=50
LGG	33 (27.7%)	33 (32.7%)		NA
HGG	68 (57.1%)	50 (49.5%)	NA	
Embryonal	5 (4.2%)	5 (5.0%)	NA	NA
Other	13 (10.9%)	13 (12.9%)	NA	NA
NTRK fusion (n=119)	n=119	n=101	n=33	n=50
NTRK1	29 (24.4%)	24 (23.8%)	6 (18.2%)	12 (24%)
NTRK2	60 (50.4%)	51 (50.5%)	23 (69.7%)	22 (44%)
NTRK3	30 (25.2%)	26 (25.7%)	4 (12.1%)	16 (32%)

LGG: Low-grade glioma, HGG: High-grade glioma, Others: High-grade neuro-epithelial tumor (n=5), low grade neuroepithelial tumor (n=3), ependymoma (n=3), extracutanenous juvenile xanthogranuloma, ganglioneuroblastoma

[#]For two patients the exact age was unknown but one patient was identified as a pediatric patient.

* p-value from chi-square test =0.0035

Table 2

Treatment modalities

	Entire cohort n (%)	Ped cohort n (%)	pedLGG n (%)	pedHGG n (%)
Extent of Surgery at tumor diagnosis	n=119	n=101	n=33	n=50
Unknown	11 (9.2)	10 (9.9%)	1 (3.0%)	7 (14%))
BX	19 (16.0)	18 (17.8%)	5 (15.2%)	13 (26%)
GTR	37 (31.1)	28 (27.7%)	13 (39.4%)	9 (18%)
NTR	11 (9.2)	9 (8.9%)	2 (6.1%)	5 (10%)
STR	41 (34.5)	36 (35.6%)	12 (36.4%)	16 (32%)
Treatment at diagnosis (non-mutually exclusive)	n=119	n=101	n=33	n=50
Surgery only	40 (33.6)	39 (38.6%)	23 (69.7%)*	9 (18%)*
Radiation therapy	42 (35.3)	25 (24.8%)	3 (9.1%)	14 (28%)
Chemotherapy	66 (55.4)	50 (49.5%)	6 (18.2%)	37 (74%)
Other systemic treatment	1 (0.01)	0 (0%)	0 (0%)	0 (0%)
TRKi	7 (5.9)	6 (5.9%)	1 (3%)	5 (10%)
Treatment received overall	n=119	n=101	n=33	n=50
Surgery only	28 (23.5)	27 (26.7%)	17 (51.5%)*	5 (10%)*
Number of lines of treatment received[#]				
1	43 (36.1)	38(37.6%)	11 (33.3%)	20 (40%)
2	20 (16.8)	16 (15.8%)	3 (9.1%)	12 (24%)
3	12 (10.1)	10 (9.9%)	1 (3%)	9 (18%)
4	11 (9.2)	7 (6.9%)	1 (3%)	2 (4%)
5	3 (2.5)	2 (2.0%)	0 (0%)	1 (2%)
6	2 (1.7)	1 (1.0%)	0 (0%)	1 (2%)
Number of patients receiving TRKi	n=51	n=39	n=7	n=27
First line	7 (13.7)	6 (15.4%)	1 (14.3%)	5 (18.5%)
Second line	26 (51.0)	20 (51.3%)	3 (42.9%)	16 (59.3%)
Third line	13 (25.5)	8 (20.5%)	2 (28.6%)	3 (11.1%)
Fourth line	3 (5.9)	4 (10.3%)	1 (14.3%)	3 (11.1%)
Fifth line	2 (3.9)	1 (2.6%)	0 (0%)	0 (0%)
Initial TRKi type	n=51	n=39	n=7	n=27
Larotrectinib	39 (76.5)	29 (74.4%)	5 (71.4%)	20 (74.1%)
Entrectinib	3 (5.9)	2 (5.1%)	1 (14.3%)	0 (0%)
Not specified	7 (13.7)	7 (18.0%)	1 (14.3%)	6 (22.2%)
Other	2 (3.9)	1 (2.6%)	0 (0%)	1 (3.7%)

BX: biopsy, GTR: Gross total resection, NTR: near total resection, STR: subtotal resection. See Supplementary Data S1 for definition.

TRKi tyrosine kinase inhibitor Other repotrectinib, selitrectinib.

Number of line of treatment[#]: including systemic and radiation therapy and excluding surgery only.

*: p-value from chi-square test <0.0001

Figure 1 NTRK fusion partners

Figure 1: CIRCOS schematic representation of *NTRK* genes and fusion partners.

Figure 2 - Overall Survival outcome

Figure 2 A) Overall survival of the entire cohort. B) Overall survival of adult compared to pediatric patients (p<0.0001), C) Overall survival according to histology (p=0.012) LGG: Low-grade glioma, HGG: High-grade glioma D) Overall survival according to NTRK fusion type (p=0.180). E) Overall survival according to CDKN2A/B alteration (p=0.053) F) Overall survival in pediatric HGG that received or not radiation therapy at one point during their treatment (p=0.695). p-value from Log-Rank test.

Figure 3 - Progression-free survival outcome

Figure 3 A) Progression-free survival of the entire cohort. B) Progression-free survival of adult compared to pediatric patients (p=0.219), C) Progression-free survival according to histology (p=0.303) LGG: Low-grade glioma, HGG: High-grade glioma D) Progression-free survival according to NTRK fusion type for the pediatric cohort (p=0.042) E) Progression-free survival according to CDKN2A/B alteration (p=0.066) F) Progression-free survival according to CDKN2A/B alteration for patients with HGG (p=0.452). p-value from Log-Rank test.

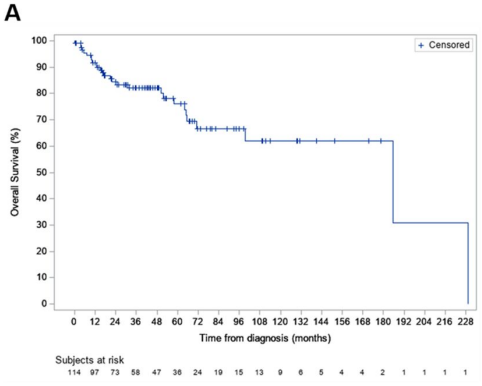
Figure 4

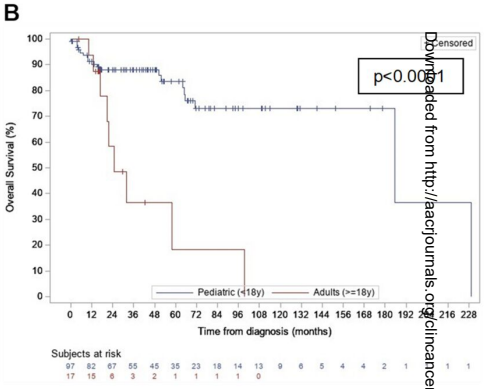
Kaplan-Meier plot of Progression-Free Survival on TRKi or larotrectinib specifically and Time to Progression on the previous line of therapy

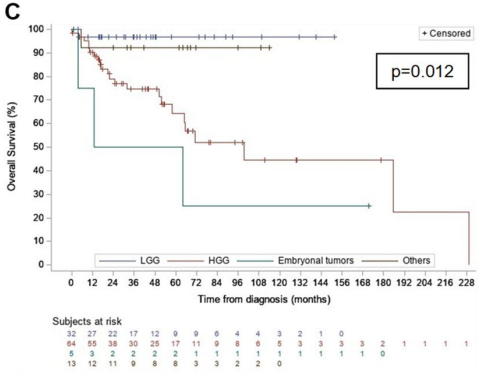
Figure 4 A) The 31 patients for whom GMI was calculated (regardless of histology) - Progression-Free Survival on TRKi and their Time to Progression on the previous line of therapy B) The 23 HGG patients for whom GMI was calculated -Progression- Free Survival on TRKi and their Time to Progression on the previous line of therapy C) The 23 patients treated with larotrectinib for whom GMI was calculated (regardless of histology) - Progression-Free Survival on larotrectinib and their Time to Progression on the previous line of therapy D) The 17 HGG patients treated with larotrectinib for whom GMI was calculated -Progression- Free Survival on larotrectinib and their Time to Progression on the previous line of therapy

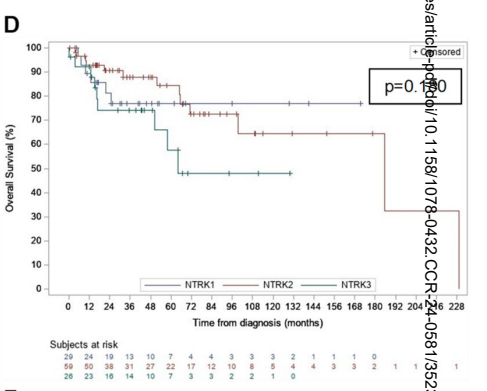
Figure 1

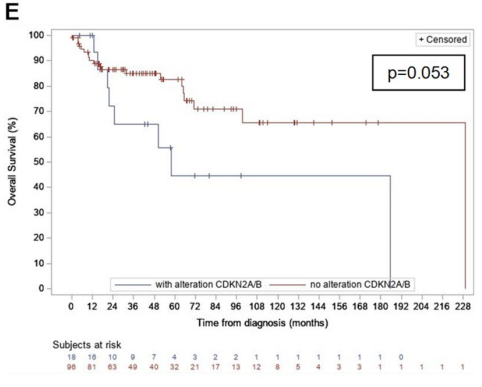
Figure 2











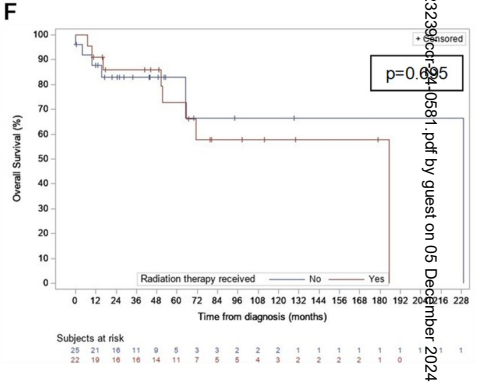


Figure 3

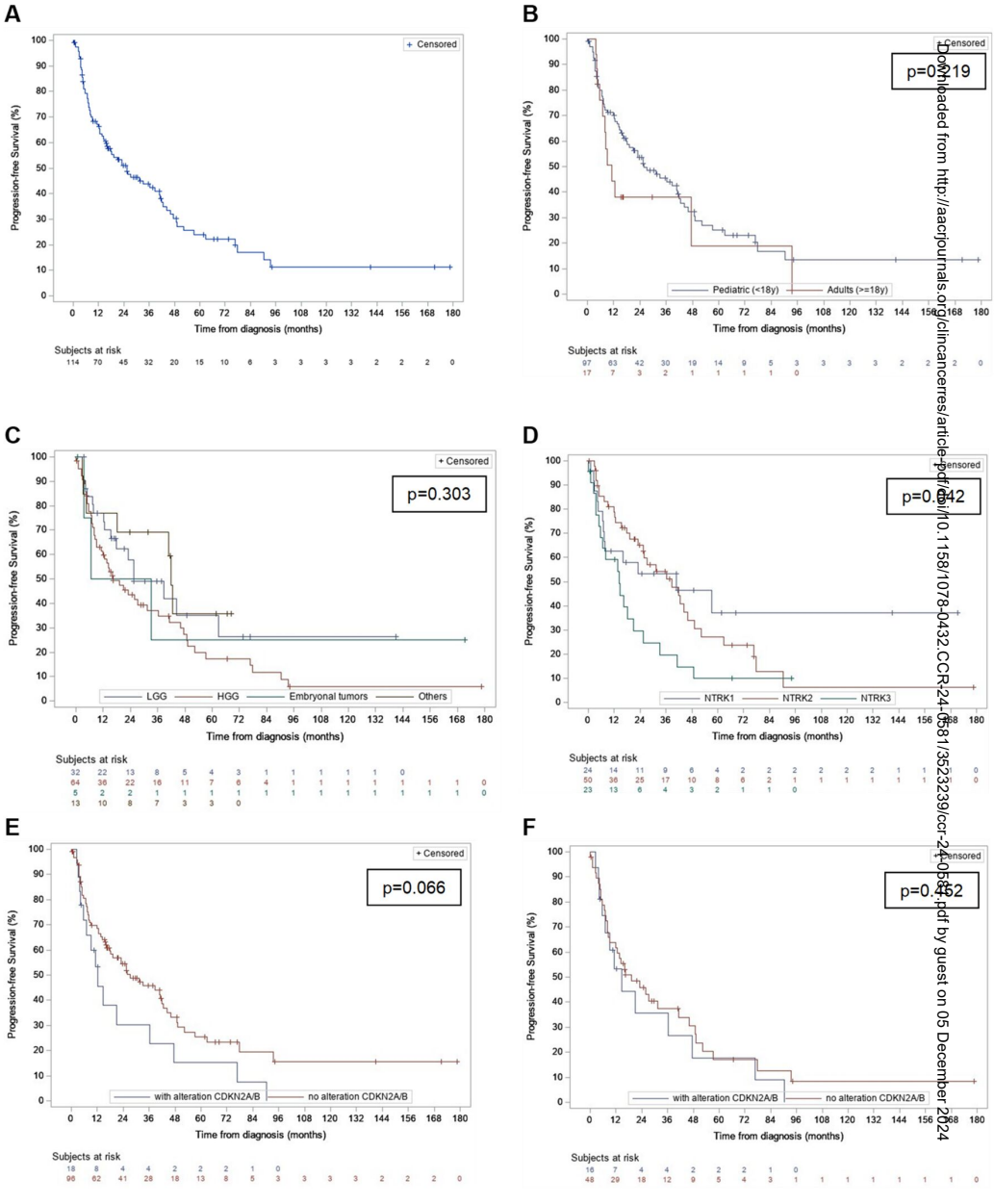


Figure 4