NTRK Fusions Identified in Pediatric Tumors: The Frequency, Fusion Partners, and Clinical Outcome

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PURPOSE Neurotrophic tyrosine receptor kinase (NTRK) fusions have been described as oncogenic drivers in a variety of tumors. However, little is known about the overall frequency of NTRK fusion in unselected pediatric tumors. Here, we assessed the frequency, fusion partners, and clinical course in pediatric patients with NTRK fusion–positive tumors.

PATIENTS AND METHODS We studied 1,347 consecutive pediatric tumors from 1,217 patients who underwent tumor genomic profiling using custom-designed DNA and RNA next-generation sequencing panels. NTRK fusions identified were orthogonally confirmed.

RESULTS AND DISCUSSION NTRK fusions were identified in 29 tumors from 27 patients with a positive yield of 2.22% for all patients and 3.08% for solid tumors. Although NTRK2 fusions were found exclusively in CNS tumors and NTRK1 fusions were highly enriched in papillary thyroid carcinomas, NTRK3 fusions were identified in all tumor categories. The most canonical fusion was ETV6-NTRK3 observed in 10 patients with diverse types of tumors. Several novel NTRK fusions were observed in rare tumor types, including KCTD16-NTRK1 in ganglioglioma and IRF2BP2-NTRK3 in papillary thyroid carcinomas. The detection of an NTRK fusion confirmed the morphologic diagnosis including five cases where the final tumor diagnosis was largely based on the discovery of an NTRK fusion. In one patient, the diagnosis was changed because of the identification of an ETV6-NTRK3 fusion. One patient with infantile fibrosarcoma was treated with larotrectinib and achieved complete pathologic remission.

CONCLUSION NTRK fusions are more frequently seen in pediatric tumors than in adult tumors and involve a broader panel of fusion partners and a wider range of tumors than previously recognized. These results highlight the importance of screening for NTRK fusions as part of the tumor genomic profiling for patients with pediatric cancer.

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INTRODUCTION

Rearrangements involving the neurotrophic tyrosine receptor kinase (NTRK) genes NTRK1, NTRK2, and NTRK3 encode fusion proteins containing the intact NTRK kinase domain that are oncogenic drivers of a histologically diverse group of tumors. NTRK rearrangements involving dozens of genes have been described and lead to constitutively unregulated activation of TRK kinases and downstream pathways.1

In children, the incidence of NTRK fusions is high (> 90%) in certain tumors such as infantile fibrosarcoma (IF), congenital mesoblastic nephroma, and secretory carcinoma, and lower (5%-26%) in pediatric papillary thyroid carcinomas (PTCs)2 and in a subset of pediatric gliomas.3-5 By contrast, NTRK fusions are rarely identified in GI stromal tumors, melanoma, lung adenocarcinoma, acute leukemia, and soft-tissue sarcomas with a range of histologic morphologies.5-8

The dramatic and durable objective responses in cancers harboring NTRK fusions have led the (US) Food and Drug Administration to approve first-generation oral NTRK inhibitors, larotrectinib and entrectinib, for use in patients of various ages with advanced solid tumors regardless of tumor histology.9-11 Other NTRK inhibitors are in clinical development including selitrectinib (LOXO-195), talactrectinib (DS-6051b),12 and repotrectinib (TPX-005).13,14 Clinical trials are ongoing to determine the optimal use of these drugs in children (Children’s Oncology Group Trial ADVL1823, Clinical-Trials.gov identifier: NCT03834961). We conducted a single institution retrospective review of tumors with NTRK fusions identified by next-generation sequencing at the Children’s Hospital of Philadelphia (CHOP) to assess the frequency, fusion partners, and clinical course in infants, children, and adolescents with NTRK fusion–positive tumors to highlight the importance of...
NTRK Fusions in Pediatric Tumors: Frequency, Partner, and Outcome

**CONTENT**

**Key Objective**
NTRK fusions are tumor-agnostic or age-independent biomarkers that identify patients suitable for treatment with the US Food and Drug Administration–approved TRK inhibitors. The spectrum of NTRK fusions has been described in adult cancers, but is incompletely known for pediatric cancers. This single institutional study examined the frequency, fusion partner, and clinical outcome of NTRK fusions in a large cohort of unselected patients with pediatric cancer.

**Knowledge Generated**
Our study demonstrated that NTRK fusions are more frequent in pediatric tumors and involve a broader panel of fusion partners and a wider range of tumors than previously recognized and highlights pediatric cancers in which NTRK fusions are more or less likely to occur.

**Relevance**
This study provided important findings regarding NTRK fusion–positive pediatric cancers and cancers where NTRK fusions are rarely seen. The data can help prioritize pediatric tumors for NTRK fusion testing to enable implementation of targeted treatment in children and adolescents with cancer.

NTRK fusion screening in patients with pediatric cancer for personalized tumor stratification and treatment.

**PATIENTS AND METHODS**
We evaluated 1,347 consecutive tumors from 1,217 pediatric patients including 604 male and 613 female patients from CHOP for NTRK fusions as part of routine comprehensive clinical sequencing from March 2016 to September 2019. For patients whose tumor harbored an NTRK fusion, chart review was conducted to determine treatment received and clinical outcome. Length of follow-up time was recorded. Histology results were reviewed and tabulated. Immunohistochemistry was performed in a subset of cases using pan-TRK antibody (Abcam, Cambridge, UK, 1:100 dilution) (Table 1).

Fusion gene detection was performed using the CHOP Cancer Fusion Panel as previously described. Briefly, target-specific primers covering 673 exons were custom designed to identify known fusion genes and potential novel fusion genes associated with 110 cancer genes using anchored multiplex polymerase chain reaction (PCR) technology (ArcherDX, Inc, Boulder, CO). Total RNA (or total nucleic acid from formalin-fixed paraffin-embedded (FFPE) samples) was extracted from the tumor samples and reverse-transcribed into cDNA. Libraries were constructed using Archer Universal RNA Agent Kit v2 for Illumina. Barcoded libraries were pooled and sequenced on Illumina HiSeq platform using 150 bp paired-end sequencing. Sequence data were analyzed using the home brew software ConcordS v2 (for SNVs and indels) and NextGENe v2 Next-Generation Sequencing Analysis Software (for CNAs; SoftGenetics, LLC, State College, PA). Clinically significant variants including SNVs, indels, and CNAs were confirmed by Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA), real-time PCR, or droplet digital PCR (ddPCR) when necessary. All genomic coordinates are based on Genome Reference Consortium Human Build 37 (GRCh37).

**RESULTS AND DISCUSSION**

The Spectrum of NTRK Fusions in Pediatric Cancers
We performed fusion gene studies of 1,347 consecutive tumors from 1,217 pediatric patients to evaluate the spectrum of NTRK fusions in the largest cohort of pediatric tumors to date. NTRK fusions were identified in 29 tumors from 27 patients with a positive yield of 2.22% for all tumors and 3.08% for solid tumors (Tables 1 and 2, Fig. 1). Of the 27 NTRK fusions, five were novel (not previously published) at the time of discovery (Table 1). The age of NTRK-positive patients ranged from 0.1 to 17 years with 17 females and 10 males. NTRK fusions were detected in 13% of PTCs (10 of 76), 1.9% of CNS tumors (7 of 364), 1.8% of non-CNS, non-PTC solid tumors (8 of 435), and 0.4% of hematologic malignancies (2 of 472) (Table 2). Specifically, NTRK2 fusions were found exclusively in CNS tumors, and NTRK1 fusions were highly enriched in PTCs, whereas NTRK3 fusions were identified in all tumor categories (Table 2 and Fig. 1). Some fusions were recurrently identified in historically distinct tumor types, such as ETV6-NTRK3 in six PTCs, two IFs, one secretory carcinoma of salivary glands, and one congenital glioblastoma; as well as...
## TABLE 1. Summary of NTRK Fusions Identified in the Cohort With Treatment and Follow-Up

| Patient ID | Histologic Diagnosis | Age at Pathologic Diagnosis (y) | Sex | Fusion Gene | Impact of Identified NTRK Fusions on Diagnosis<sup><i>a</i></sup> | Stage/Anatomic Location At Diagnosis | Surgical Resection | Radiation Therapy/Chemotherapy | Outcome |
|------------|----------------------|-------------------------------|-----|-------------|-------------------------------------------------|-----------------------------------|------------------|-------------------------------|---------|
| CNS        |                      |                               |     |             |                    |                                  |                   |                               |         |
| 1          | Pilocytic astrocytoma, anaplastic | 2 F                        |     | KAN1K2- NTRK3<sup>a</sup> | 1 Local, cerebellum | Gross total; repeat gross total 3 y later | No | Recurrence: No; Progressive: No at 33 mo |         |
| 2          | Astrocytoma, diffuse | 12 F                        |     | C2orf44- NTRK3<sup>a</sup> | 1 Local, temporal lobe | Gross total | No | No recurrence at 23 mo |         |
| 3          | Astrocytoma, diffuse | 16 F                        |     | QKI-NTRK2   | 1 Local, temporal lobe | Gross total | No | No recurrence at 21 mo |         |
| 4          | Ganggliolipoma | 7 M                        |     | ACTO1K1- NTRK3<sup>a</sup> | 1 Local, parietal lobe | Gross total | No | No recurrence at 11 mo |         |
| 5          | Ganggliolipoma | 9 M                        |     | TRED4A- NTRK2<sup>a</sup> | 1 Local, parietooccipital lobes | Gross total | No | No recurrence at 10 mo |         |
| 6          | Medul nevous glial tumor NOS | 11 M                      |     | SPECC1L- NTRK3<sup>a</sup> | 1 Local, frontal lobe | Gross total | No | No recurrence at 34 mo |         |
| 7          | Congenital glioblastoma | 0.3 F                     |     | ETV6- NTRK3 | 1 Local, posterior cerebral hemispheric | No | Death (multisystem failure) |         |
| Non-CNS non-PCT solid tumors |                      |                               |     |             |                    |                                  |                   |                               |         |
| 8          | T-lymphoblastic lymphoma | 5 F                        |     | RBPMS1- NTRK3<sup>a</sup> | 1 Mediastinal mass, CNS 1, BM MRD negative | No | AALL231-LIKE (arm A) | No recurrence at 21 mo |         |
| 9          | Myeloid Sarcoma | 16 F                        |     | TPM3-NTRK2 | 1 Liver, CNS unknown, BM MRD-positive | N/A | AIDE with GTMZ induction | Death during induction |         |
| Thyroid tumors |                      |                               |     |             |                    |                                  |                   |                               |         |
| 10         | Secretory carcinoma | 3 M                        |     | ETV6- NTRK3 | 2 Local, right parotid, lymph node-negative | No recurred focal proton radiation therapy | No recurrence at 44 mo |         |
| 11         | Myofibroblastic sarcoma, intermediate grade | 13 F                   |     | TFG-NTRK3  | 1 Local, thigh | Gross total | No | No recurrence at 46 mo |         |
| 12         | Malignant spindle cell tumor<sup>c</sup> | 10 F                     |     | RBPMS1- NTRK3<sup>a</sup> | 3 Local, Liver (porta hepatitis) | Gross total (liver transplant); 2 cycles ifos/doxo; 2 cycles VDC | No recurrence at 39 mo |         |
| 13         | IF | 0.2 M                        |     | ETV6- NTRK3 | 3 Local, tongue | Near total (positive margins) | No | No recurrence at 38 mo |         |
| 14         | IF | 0.1 M                        |     | ETV6- NTRK3 | 3 Local, orbit | Near total (positive margins) | No | No recurrence at 27 mo |         |
| 15         | IF | 0.5 M                        |     | SPECC1L- NTRK3<sup>a</sup> | 3 Local, triceps | Gross total | Lantarctinib | No recurrence at 6 mo |         |
| 16         | Epithelial melanocytic tumor of uncertain malignant potential (cutaneous melanocytoma) | 12 F                     |     | PDRK1-NTRK2 | 1 Local, ankle | Near total (positive margins) | No | No recurrence at 12 mo |         |
| 17         | Spindle cell tumor, low grade<sup>d</sup> | 0.5 M                     |     | STRK5- NTRK3<sup>a</sup> | 3 Local, tongue | Near total (positive margins) | No | No recurrence at 9 mo |         |
| Abbreviations: ADE, chemotherapy regimen including cytarabine, daunorubicin, and etoposide; BM, bone marrow; CNS 1, level 1 CNS involvement; GTMZ, gemtuzumab; M, minimal residual disease; RAI, radioactive iodine; VDC, chemotherapy regimen including vincristine, doxorubicin, and cyclophosphamide.  
<sup>a</sup>Novel fusions (not previously published) at the time of discovery. 
<sup>b</sup>Impact of identified NTRK fusions on diagnosis: 1—the NTRK fusions identified confirmed the clinical and/or pathological diagnoses; 2—the diagnosis was changed because of the identification of the NTRK fusion; 3—tumor diagnosis was largely based on the discovery of the NTRK fusion. 
<sup>c</sup>These tumors now meet criteria for the diagnosis of NTRK-rearranged spindle cell neoplasm according to the Fifth edition of the Soft Tissue and Bone Tumors WHO Classification (2020).
RBPMSS-NTRK3 in a spindle cell sarcoma and a T-lymphoblastic lymphoma. By contrast, different NTRK fusions were present in tumors with the same histological diagnosis, such as KCTD16-NTRK2 and TRIM24-NTRK2 in two male patients of similar age with ganglioglioma, WHO grade I (Table 1). Several novel NTRK fusions were observed in rare tumor types, such as KANK1-NTRK2 in malignant anaplastic pilocytic astrocytoma,18 C2orf44-NTRK2 in diffuse astrocytoma, IRF2BP2-NTRK1 in PTC, KCTD16-NTRK2 in ganglioglioma, and SPECC1L-NTRK2 in glioneuronal tumor19 (Fig. 1). These data demonstrate that NTRK fusions are far more frequently seen in pediatric tumors than in adult tumors and involve a broader panel of fusion partners and a wider range of pediatric tumors than previously recognized.20,21 With the recent (US) Food and Drug Administration approval of larotrectinib and entrectinib for the treatment of adult and pediatric NTRK-positive, unresectable solid tumors, identification of these fusions directly affects patient care.22

NTRK Fusions in Pediatric Carcinomas

NTRK fusions were detected in 10 pediatric PTCs (13%) including 6 patients with ETV6-NTRK3 fusions, one diffuse sclerosing variant (Figs. 2E-F), two mixed follicular, papillary, and solid growth patterns, one widely invasive follicular variant (Fig. 2D), and two classic variants; one with TPR-NTRK1 fusions, both tall cell variants; one with SQSTM1-NTRK1 fusion showing a mixed classic and micropapillary growth patterns; and one patient with a novel IRF2BP2-NTRK1 fusion at the time of discovery, encompassing a diffuse sclerosing PTC (Table 1). Finally, there was a single case of secretory carcinoma of salivary glands with the canonical ETV6-NTRK3 fusion identified (Table 1). In PTC, tumorigenesis is associated with constitutive activation of the MAPK and PI3K signaling pathways secondary to somatic point mutations in BRAF, PTEN, DICER1, and RAS as well as fusions involving RET, ALK, and NTRK.2,23 Gene fusions have been reported in both sporadic and radiation-induced PTCs and are more common in pediatric (50%-60%) compared with adult tumors (approximately 15%).2,23 RET and NTRK fusions are the most common, reported in 25%-30% and approximately 10% (range, 0%-26%) of pediatric PTCs, respectively.2,23 NTRK3 fusions are usually observed more commonly in PTCs than NTRK1 fusions,2,23 which is similar to what we observed in our patient cohort. All the PTCs in our cohort were sporadic, none associated with

TABLE 2. Distribution of Neurotrophic Tyr receptor kinase Fusion–Positive Cases in the Cohort

| Histologic Diagnosis         | Patients With NTRK1 Fusions | Patients With NTRK2 Fusions | Patients With NTRK3 Fusions | Total No. of Patients With NTRK-Positive Fusions | Total No. of Patients (Tumors) Tested | Positive Rate (%) |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-------------------------------------------------|--------------------------------------|------------------|
| CNS                         | 0                           | 6                           | 1                           | 7                                               | 338 (364)                           | 2.07             |
| Hematologic malignancy      | 1                           | 0                           | 1                           | 2                                               | 405 (472)                           | 0.49             |
| Non-CNS nonpapillary thyroid carcinoma solid tumors | 1                           | 0                           | 7                           | 8                                               | 401 (435)                           | 2.00             |
| Thyroid tumors              | 4                           | 0                           | 6                           | 10                                              | 73 (76)                             | 13.70            |

FIG 1. Summary of molecular findings of NTRK fusion–positive patients identified in the cohort at CHOP.
previous exposure to radiation, and NTRK fusions were found in both prepubertal and pubertal patients across the spectrum of histological variance. In thyroid cancers, NTRK fusions were 100% specific for PTC and, of the 10 patients with PTC with NTRK fusions, the majority presented with invasive disease, characterized by lymphovascular permeation throughout the thyroid, extrathyroidal extension, lateral neck lymph node metastases (8 of 10 with N1b, 80%), extranodal extension, and pulmonary metastasis (5 of 10 with M1, 50%) (Table 1). Within PTC tumors harboring an NTRK fusion and pulmonary metastasis, three were found to harbor an NTRK1 fusion and two with an NTRK3 fusion (Table 1 and Fig. 1). The data are limited but suggest that the presence of an NTRK fusion may have diagnostic, prognostic, and therapeutic significance in PTCs and may hold clinical utility for stratifying surgical and medical care. Standard therapy for advanced PTCs in children involves surgical resection of gross disease followed by radioactive-iodide (RAI) therapy. In a recent multicenter, open-label phase I or II study of larotrectinib for the treatment of pediatric patients with solid tumors, two children with advanced PTCs were treated for > 7 months and remained progression-free, although, unfortunately, the objective response to treatment was not reported.

NTRK Fusions in Pediatric CNS Tumors

All the CNS tumors identified with NTRK fusions were either gliomas or mixed neuronal glial tumors. Most of the CNS tumors contained NTRK2 fusions with different 5′ partners, except the single congenital glioblastoma with ETV6-NTRK3 (Table 1 and Fig. 1). Four of the six NTRK2 fusions were novel fusions at the time of discovery with different 5′-partner genes including KANK1, C2orf4, KCNTD16, and SPECC1L. NTRK fusion genes have been described in pediatric low- and high-grade gliomas at a low prevalence, although one study reported a finding of 40% of nonbrainstem high-grade gliomas in children younger than 3 years old containing an NTRK fusion gene (n = four of the 10 samples). Six of the 7 NTRK fusion–positive CNS tumors were low-grade gliomas (LGG), which may be partially due to the higher frequency of LGG in our unselected pediatric cohort (approximately 45% of all CNS tumors). Our cohort demonstrates an increased prevalence of NTRK2-associated fusions within CNS tumors compared with those occurring extracranially (Table 1 and Fig. 1), which has been observed in prior studies. The therapeutic implication of these fusions in pediatric brain tumor is at this time unclear based on limited available publications. Although the duration of follow-up in our study is limited, the majority of patients underwent standard of care for their CNS tumor subtype with resection of the primary tumor without recurrence. Specifically, five of the seven patients in our cohort have been managed surgically without adjuvant treatment, consistent with the natural history of these tumors without NTRK fusions. However, although the patient cohort has not required adjuvant therapy to date, given the potential for recurrence and the potential morbidity of repeat surgical resection, medical management targeting TRK may need to
TABLE 3. Morphologic and Immunohistochemical Features of Soft-Tissue NTRK-Fused Tumors

| Patient ID | Initial Diagnosis | Age | Fusion | Distinctive Morphologic Features | Mitotic Rate (4/10 HPF) | Immunohistochemistry |
|------------|------------------|-----|--------|---------------------------------|-------------------------|----------------------|
| 11         | Myofibroblastic sarcoma, intermediate grade* | 13  | IFG-NTRK3 | Fascicular and/or herringbone with infiltrative growth | 6                      | Patchy + – Patchy + NP |
| 12         | Malignant spindle cell tumor* | 10  | RBPMS-NTRK3 | Nuclear palisading and fascicular growth | 6                      | Patchy + Rare weak + +, Cytoplasmic and nuclear |
| 13         | IF               | 0.2 | ETV6-NTRK3 | IMT-like | 34                     | Patchy + Rare + +, Cytoplasmic and nuclear |
| 14         | IF               | 0.1 | ETV6-NTRK3 | Fascicles-like, IMT-like, and focal fascicular growth | 7                      | + – – +, Cytoplasmic and nuclear |
| 15         | IF               | 0.5 | SPECC1L-NTRK3 | Infiltrative primitive cells, fibrosarcoma-like | 25                     | + + – +, Cytoplasmic and membrane |
| 17         | Spindle cell tumor, low grade* | 0.5 | STRN3-NTRK3 | Mild to moderate cellularity with vascular proliferation and scattered plasma cells | 2                      | – + – Patchy +, Cytoplasmic and membrane |

Abbreviations: HPF, high power field; IMT, inflammatory myofibroblastic tumor; NP, not performed.

*These tumors now meet criteria for the diagnosis of NTRK-rearranged spindle cell neoplasm according to the Fifth Edition of the Soft Tissue and Bone Tumors WHO Classification (2020).

be considered. Larotrectinib and entrectinib have shown antitumor effect for both primary brain tumors and solid tumors with brain metastases with systemic administration suggesting adequate penetration of the blood-brain barrier. In a study of nine patients with primary CNS tumors treated with NTRK inhibitors, disease control was observed in all evaluable patients with stable disease in seven patients. Of note, there is overlap of three fusion genes between our CNS patient cohort and the study cohort (KANK-NTRK2, SPECC1L-NTRK2, and ETV6-NTRK3), suggesting that these patients may also derive benefit from larotrectinib in the setting of disease recurrence and/or progression.14

NTRK Fusions in Pediatric Soft-Tissue Tumors

Multiple soft-tissue tumors were identified with NTRK3 rearrangements (Table 1) (Figs. 1 and 2A-C). There was a single case of cutaneous melanocytoma with epithelioid morphology containing PRDX1-NTRK1 fusion (case 16). All other soft-tissue cases were spindle cell tumors with key morphologic and immunohistochemical findings described in Table 3. Some tumors exhibited typical age, morphology, and ETV6-NTRK3 fusion compatible with IF, showing densely cellular fascicular growth of primitive ovoid cells and infiltration of surrounding tissue (cases 13 and 14). Other spindle tumors in the infantile age range (cases 15 and 17) showed similar morphology to IF, but contained variant NTRK3 fusions (Table 3 and Fig. 2C). Notably, case 17 was initially not diagnosed as sarcoma because the lower cellularity and mitotic rate of that tumor did not suggest malignancy (Fig. 2C). Two spindle cell tumors in older children (cases 11 and 12) showed morphologic and immunohistochemical features of myofibroblastic sarcoma, one with marked nuclear palisading reminiscent of schwannoma (Table 3 and Fig. 2A). In all cases where performed, pan-NTRK immunohistochemistry was positive (Table 3). Clinical classification and treatment based on histology only, particularly in soft-tissue tumors with spindle morphology, have proven to be challenging in some cases because of variable histologic features and immunohistochemical patterns.30 As more tumors are being studied for fusions, the morphologic spectrum is expanding, such that the Fifth Edition of the Soft Tissue and Bone Tumors WHO Classification now includes an emerging entity titled “NTRK-rearranged spindle cell neoplasm” to encompass spindle cell soft-tissue tumors with NTRK gene rearrangements (other than IF). It remains to be seen if soft-tissue histologic classification, molecular classification, or some combination of both will provide clinicians with the most accurate information for personalized treatment. Pathology diagnostic criteria will continue to shift as the field evolves to determine which features of a tumor are most prognostically significant for accurate classification. Despite the challenges and naming ambiguity, the detection of NTRK fusions is critical to providing targeted therapy in cases where resection would be morbid or impossible.32 In our cohort, one IF (case 15) with a SPECC1L-NTRK3 fusion was treated with larotrectinib and achieved complete tumor regression (Fig. 3). In a phase I or II study, two children with locally advanced IF displayed sufficient tumor shrinkage during larotrectinib treatment to allow for limb-sparing surgery and both remained progression-free without larotrectinib treatment after the follow-up of 4.8 and 6.0 months.11 In another study of five children with locally advanced TRK fusion sarcomas, a median of six cycles of larotrectinib was given and all patients achieved partial response prior to surgical resection.32 These studies and our experience showed that the use of TRK inhibitor can prevent pediatric patients from disfiguring surgery or amputation, reduce recurrence rate, and improve the quality of life.
NTRK Fusions Facilitate Precision Diagnosis, Prognosis, and Therapy

Follow-up information is available for all patients, and follow-up times ranged from 6 to 46 months. In almost all cases, the detection of an NTRK fusion confirmed the morphologic diagnosis, and in five cases, the final tumor diagnosis was largely based on the discovery of an NTRK fusion (excluding other differential diagnostic considerations). One exception was the case of secretory carcinoma, which was initially diagnosed as mucoepidermoid carcinoma, but later changed to secretory carcinoma following the detection of the ETV6-NTRK3 fusion and additional immunohistochemical evaluation (case 10). Although the morphologic spectrum of NTRK fusion tumors is expanding, these fusions were not detected in certain tumor subtypes. Collectively, we analyzed 261 common embryonal solid tumors (79 neuroblastomas, 29 medulloblastomas, 28 Wilms tumors, 13 atypical teratoid/rhabdoid tumors, and 11 hepatoblastomas), bone tumors (27 osteosarcomas and 24 Ewing sarcomas), and skeletal muscle tumors (50 rhabdomyosarcomas), and none had NTRK fusions. Thus, although it is difficult to exclude the possibility that NTRK fusions might occur in individual tumors of these subtypes, which accounted for about one third of all solid tumors in this cohort, they are likely to be rare.

Case 15 was a 6-month-old male infant with a left upper extremity mass. Histology findings suggested an intermediate- to high-grade mesenchymal neoplasm negative for an ETV6 rearrangement by fluorescence in situ hybridization (FISH). A novel fusion gene SPECC1L-NTRK3 (Fig. 3) and complicated CNAs were detected (Appendix Table A1). Given that surgical resection would have resulted in significant morbidity to the upper extremity musculature, the patient received neoadjuvant treatment with larotrectinib. The tumor shrank substantially, and a much more limited surgical resection was then performed after six cycles of treatment, which showed no residual tumor (Figs. 3B-C). The patient continued on larotrectinib postsurgery for six additional cycles. Magnetic resonance imaging 6 months after the surgery demonstrated no residual or recurrent mass. Prospectively, the rest of the patients who survived with the standard therapy may also benefit from TRK inhibitor therapy if their tumors progress or recur.

We assessed the clinical outcome of all NTRK fusion–positive patients for up to 46 months (median 21 months). All non-CNS, non-PTC solid tumors showed excellent outcome after surgical resection with or without postsurgery radiation or chemotherapy at a median follow-up of 32.5 months. The median follow-up time for patients with PTC was 21.5 months. Of the 6 ETV6-NTRK3–positive PTCs, none
have progressed to date and three achieved remission with or without receiving RAI therapy. Of the four NTRK1 PTCs, three of the four did not achieve remission with RAI. The median follow-up time for patients with CNS tumors was 21 months. The majority of NTRK-positive LGG demonstrated superb outcome with gross total resection without additional therapy. One patient with congenital glioblastoma and an ETV6-NTRK3 fusion died 3 months after birth, and the tumor specimen was obtained via autopsy. The prognostic significance of NTRK fusions in hematological malignancies is not clear. The patient with T-lymphoblastic lymphoma and a RBPMS-NTRK3 fusion achieved complete remission with standard chemotherapy and remains in remission at a 21-month follow-up. The patient with myeloid sarcoma and an NTRK3 fusion died during induction therapy. Evaluating other genomic alterations in NTRK fusion-positive tumors in this cohort, we found that SNVs are enriched in CNS tumors and hematological malignancies, whereas CNAs are mainly observed in non-CNS solid tumors (Fig. 1, Appendix Table A1). Although none of these co-occurring alterations are known drivers in these tumors, they may alter disease prognosis and/or play a role in the durability of and resistance to TRK inhibitors.

In summary, we studied the spectrum of NTRK-positive pediatric tumors in the largest cohort of unselected pediatric tumors to date and identified NTRK fusions in 2.22% of all tumors and 3.08% of solid tumors. Our review of 1,217 patients showed that NTRK fusions are more frequently seen in pediatric tumors than in adult tumors and involve a broader panel of fusion partners and a wider range of pediatric tumors than previously recognized. The identification of these NTRK fusions has facilitated precision cancer diagnosis and TRK inhibitor–targeted therapy. Our experience highlights the clinical utility of screening NTRK fusions for all pediatric tumors.

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**PRIOR PRESENTATION**

Portions of this data were presented at the 2019 Cancer Genomics Consortium Annual Meeting, Nashville, TN, August 12, 2019.

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**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

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### TABLE A1. Co-Occurring Molecular Findings of NTRK Fusion–Positive Tumors

| Pt ID | Fusion Gene | 3’ partner* | 5’ partner* | SNVs/Indels* | CNAs |
|-------|-------------|-------------|-------------|--------------|------|
| 1     | KANK1-NTRK2 | KANK1 (NM_015158.3) exon 3 | NTRK2 (NM_006180.4) exon 12 | Not identified | Anaplastic regions: loss of partial chr 9p including CDKN2A/B; gain of partial chr 9p including JAK2, CD274, and PAX5; and gain of partial 9q including GNAQ, NTRK2, ABL1, and TSC1. |
| 2     | C2orf44-NTRK2 | C2orf44 (NM_025203.2) exon 4 | NTRK2 (NM_006180.4) exon 16 | Not identified | Not identified |
| 3     | QKI-NTRK2 | QKI (NM_006775.2) exon 6 | NTRK2 (NM_006180.3) exon 16 | SETD2 (NM_014159.6), c.5287_5290del (p.Q1764Pfs*3) (VAF = 0.11) | Not identified |
| 4     | KCTD16-NTRK2 | KCTD16 (NM_020768.3) exon 3 | NTRK2 (NM_006180.4) exon 16 | CHEK2 (NM_007194.3), c.470T>C, (p.I157T) (VAF = 0.44) | Not identified |
| 5     | TRIM24-NTRK2 | TRIM24 (NM_003852.3) exon 12 | NTRK2 (NM_006180.4) exon 16 | Not identified | Not identified |
| 6     | SPECC1L-NTRK2 | SPECC1L (NM_015330.4) exon 6 | NTRK2 (NM_006180.4) exon 12 | ATM (NM_000051.3), c.2921+1G>A, (p.?) (VAF = 0.51) | Loss of chr 22 and gain of partial chr 6p including IRF4, TPMT, HIST1H3B, and HIST1H1C. |
| 7     | ETV6-NTRK3 | ETV6 (NM_001987.4) exon 4 | NTRK3 (NM_002530.3) exon 14 | CDKN1B (NM_004064.4), c.25G>A (p.G9R) (VAF = 0.44) | Not identified |
| 8     | RBPMS-NTRK3 | RBPMS (NM_006867.3) exon 5 | NTRK3 (NM_002530.3) exon 14 | NOTCH1 (NM_017617.3), c.4745_4746insAGACTTCCC (p.P1582E1583insDFP) (VAF = 0.14) | cnLOH of partial chr 9p. |
| 9     | TPM3-NTRK1 | TPM3 (NM_152,263.3) exon 8 | NTRK1 (NM_002529.3) exon 12 | GATA2 (NM_001145661.1), c.1085G>A, (p.R362Q) (VAF = 0.51) | Not identified |
| 10    | ETV6-NTRK3 | ETV6 (NM_001987.4) exon 5 | NTRK3 (NM_002530.3) exon 15 | FANCC (NM_000136.2), c.355_360delinsA (p.S119Nfs*8) (VAF = 0.47) | Gain of chr 3; loss of partial chr 4 including FGFR3; gain of chr 7; loss of partial chr 9p including CDKN2A/B; gain of partial chr 12p including KDM5A and CCND2; gain of partial chr 15q including MAP2K1 and NTRK3; and loss of chr 22 |
| 11    | TFG-NTRK3 | TFG (NM_006070.5) exon 6 | NTRK3 (NM_002530.3) exon 14 | Not identified | Loss of partial chr 3q including GATA2, EPHB1, and FOXL2; loss of partial chr 5p including SDHA, TERT, IL7R, and RICTOR; gain/loss of partial chr 7q; loss of partial chr 9p; loss of partial chr 10p; LOH of partial chr 11q; and loss of partial chr 15q including IDH2 and BLM |
| 12    | RBPMS-NTRK3 | RBPMS (NM_006867.3) exon 5 | NTRK3 (NM_002530.3) exon 14 | Not identified | Low-level mosaic triploidy of chr 1, 2, 3, 7, 10, 12, 13, and 18; four copies of chr 6 and 15; loss of partial chr 16p (2-3 copies); loss of chr 19 (2-3 copies); and cnLOH of partial Xp (two copies). |
| 13    | ETV6-NTRK3 | ETV6 (NM_001987.4) exon 5 | NTRK3 (NM_002530.3) exon 15 | Not identified | Gain of chr 17 |
| Pt ID | Fusion Gene | Pt Fusion Gene | Partner Gene | Partner Gene | SNVs/Indels | CNAs |
|------|-------------|----------------|--------------|--------------|-------------|------|
| 14   | ETV6-NTRK3  | ETV6 (NM_001987.4) exon 5 | NTRK3 (NM_002530.3) exon 14 | Not identified | Not identified |
| 15   | SPECII-NTRK3 | SPECII (NM_015330.4) exon 9 | NTRK3 (NM_002530.3) exon 14 | Not identified | cnLOH of partial chr 3p including SETD2, RHOA, BAP1, and PBRM1; loss of partial chr 9p including ABL1 and TSC1; loss of chr 11p and 14q; complex CNV for partial chr 15q including loss of exons 1-11 of NTRK3 and gain of exons 12-18 of NTRK3; complex CNV of partial chr 17q including loss of BRCA1 and gain of SPOP, RNF43, PPM1D, BRIP1, CD79B, PRKAR1A, and RPTOR; complex CNV of partial chr 22q including gain of CRKL, MAPK1, and SMARCB1; and loss of EP300. |
| 16   | PRDX1-NTRK1 | PRDX1 (NM_002574.3) exon 5 | NTRK1 (NM_002529.3) exon 14 | MUTYH (NM_012222.2), c.1178G>A (p.G393D) (VAF = 0.46) | Loss of partial chr 1p, chr 9, and partial chr 15q. |
| 17   | STRN3-NTRK3 | STRN3 (NM_014574.3) exon 3 | NTRK3 (NM_002530.3) exon 14 | Not identified | Not identified |
| 18   | ETV6-NTRK3  | ETV6 (NM_001987.4) exon 5 | NTRK3 (NM_002530.3) exon 14 | Not identified | Loss of partial chr 15q including IDH2, BLM, and IGF1R. |
| 19   | ETV6-NTRK3  | ETV6 (NM_001987.4) exon 4 | NTRK3 (NM_002530.3) exon 14 | TET2 (NM_001127208.2), c.5152G>T (p.V1718L) (VAF = 0.48) | Not identified |
| 20   | IRF2BP2-NTRK1 | IRF2BP2 (NM_182,972.2) exon 2 | NTRK1 (NM_002529.3) exon 10 | Not identified | Not identified |
| 21   | ETV6-NTRK3  | ETV6 (NM_001987.4) exon 4 | NTRK3 NM_002530.3 | Not identified | Not identified |
| 22   | ETV6-NTRK3  | ETV6 (NM_001987.4) exon 4 | NTRK3 (NM_002530.3) exon 14 | Not identified | Not identified |
| 23   | ETV6-NTRK3  | ETV6 (NM_001987.4) exon 5 | NTRK3 (NM_002530.3) | N/A (solid part canceled because of poor sample quality) | N/A (solid part canceled because of poor sample quality) |
| 24   | SQSTM1-NTRK1 | SQSTM1 (NM_003900.4) exon 4 | NTRK1 (NM_002529.3) exon 12 | Not identified | Not identified |
| 25   | TPR-NTRK1   | TPR (NM_003292.2) exon 21 | NTRK1 (NM_002529.3) exon 12 | Not identified | Not identified |
| 26   | TPR-NTRK1   | TPR (NM_003292.2) exon 9 | NTRK1 (NM_002529.3) exon 12 | Not identified | Not identified |
| 27   | ETV6-NTRK3  | ETV6 (NM_001987.4) exon 4 | NTRK3 (NM_002530.3) exon 14 | Not identified | Not identified |

Abbreviations: Chr, chromosome; VAF, variant allele frequency. *Based on GRCh37.