Genomic Evolution and Personalized Therapy of an Infantile Fibrosarcoma Harboring an NTRK Oncogenic Fusion

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Introduction

Infantile fibrosarcomas are characterized by oncogenic fusions involving neurotrophic receptor tyrosine kinase genes (NTRK1, NTRK2, and NTRK3), which cause expression of oncoproteins with increased tropomyosin receptor kinase (TRK) activity. New TRK inhibitors such as larotrectinib are effective in the majority of patients with tumors expressing NTRK fusions including infantile fibrosarcoma. Secondary mutations within the ATP-binding pocket of the TRK kinase domain can lead to resistance to first-generation TRK inhibitors. Second-generation TRK inhibitors such as selitrectinib can overcome such resistance. Mechanisms of resistance to second-generation TRK inhibitors are not well understood, and possible therapeutic strategies are largely lacking in these cases.

Case Report

The 13-month-old patient presented to an outside hospital with progressing respiratory insufficiency requiring invasive mechanical ventilation after transfer to the Charité—Universitätsmedizin Berlin. Imaging displayed a large right-sided intrathoracic mass (Fig 1). Histopathological evaluation of the first two biopsies remained inconclusive. Two cycles of neoadjuvant polychemotherapy were administered (N4 according to the NB2016 Registry and vincristine, actinomycin D, cyclophosphamide [VAC] according to the Cooperative Soft Tissue Sarcoma Study Group [CWS]). Therapy response assessment according to the evaluation criteria for solid tumors, RECIST, demonstrated progressive disease (Fig 1). On the basis of a third biopsy (T1), molecular pathology analysis classified the tumor as an ETV6-NTRK3 fusion-positive infantile fibrosarcoma (Fig 2). The patient was enrolled in the phase I/Ii trial for the oral TRK inhibitor, larotrectinib, in pediatric patients with advanced solid or primary central nervous system tumors (LOXO-101, BAY2757556; 100 mg/m² twice a day; ClinicalTrials.gov identifier: NCT02637687). Tumor volume decreased > 98% (from 45 × 34 × 26 mm diameter = 480 mL to 23 × 22 × 25 mm in diameter = 6 mL) after 2 months of larotrectinib treatment (Fig 1; Data Supplement). Progressive disease was detected after total larotrectinib treatment duration of 4 months that required chemotherapeutic intervention with three VAC cycles because of recurrent respiratory symptoms (Fig 1). The tumor continued to grow under chemotherapy (Fig 1). At the molecular level (T2), single-nucleotide variant (SNV) analysis on the basis of whole-exome sequencing (WES) detected the NTRK3 p.G623R mutation in one of two analyzed tumor regions (Fig 3), which produces a protein incapable of binding larotrectinib. The patient was enrolled in the phase I/Ii trial designed to test safety, tolerability, and efficacy of the oral second-generation TRK inhibitor, selitrectinib (LOXO-195, BAY2731954; ClinicalTrials.gov identifier: NCT03215511). A partial response was achieved after 2.5 months, at a dose level of 43 mg/m² selitrectinib twice daily, but disease progressed after 3 months (Fig 1; Data Supplement). Selitrectinib was increased to 58 mg/m² twice daily, but the tumor continued to grow (Fig 1). A gross total tumor resection requiring bilobectomy was performed (T3 [time point 3]), and selitrectinib was resumed postoperatively. Disease progression 6 weeks postsurgery was treated with two cycles of the CWS iVAd regimen, to which the tumor partially responded (Fig 1). WES of T3 tumor tissue identified the xDFG motif p.G696A mutation in the NTRK3 gene (Fig 3). Therapy resistance to monotherapy with first- and second-generation TRK inhibitors prompted us to increase selitrectinib to a dose level of 87 mg/m² twice daily and combine it with the mitogen-activated protein kinase (MEK) 1/2 inhibitor, trametinib (0.032 mg/kg once daily) as oncogenic NTRK fusions are known to mediate elevated RAS/MAPK/ERK signaling cascade activity. The patient has remained...
free of disease progression on this two-drug combination for > 1 year. The safety profile of the two-drug combination selitrectinib/trametinib was favorable with no detectable evidence of organ toxicity.

Written informed parental consent was received before inclusion in the respective clinical studies. The two-drug combination selitrectinib/trametinib was initiated as individual treatment attempt after written informed parental consent was obtained. Selitrectinib was purchased through the compassionate use program of Bayer (Leverkusen, Germany). Bayer had provided written permission to administer their investigational drug selitrectinib in combination with trametinib. Trametinib was administered as off-label use medication. Parental consent for the use of surplus biomaterial samples for research purposes is documented in the German Society of Pediatric Oncology and Hematology CWS registry. All clinical investigations were conducted in accordance with the Declaration of Helsinki. All clinical studies were approved by the appropriate institutional review boards. The investigators obtained written informed parental consent to publish this report. The details of molecular pathology analysis, tumor sequencing, and SNV and copy number variant analyses are supplied in the Data Supplement.14–25

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**FIG 1.** Treatment timeline and assessments. (A) Shown is the timeline of diagnosis and therapeutic interventions including drug therapies and surgery. Time points of whole exome sequencing studies and response evaluation by magnetic resonance imaging (MRI) and computed tomography (CT) scans in line with the response evaluation criteria in solid tumors (RECIST) are summarized below. (B) Shown are exemplarily selected images for each response evaluation time point. N4, chemotherapy regimen according to the NB2016 registry: doxorubicin, vincristine, cyclophosphamide; VAC, chemotherapy regimen according to the Cooperative Soft Tissue Sarcoma Study Group CWS of the German Society of Pediatric Oncology and Hematology (GPOH): vincristine, actinomycin-D, cyclophosphamide; I'VAd, chemotherapy regimen according to the Cooperative Soft Tissue Sarcoma Study Group CWS of the GPOH: ifosfamide, vincristine, doxorubicin; B, biopsy; R, resection; T, time point; n.a., not applicable; PD, progressive disease; PR, partial response; SD, stable disease; WES, whole exome sequencing.
Results

To characterize the genomic landscape of this infantile fibrosarcoma and define genetic alterations appearing under therapy, longitudinally collected tumor specimens were subjected to WES. SNV analysis of the T1 biopsy demonstrated two somatic mutations in cancer-related genes (PIK3R1 p.F46_Q457del, ARID1A p.W1686Csf*11; Fig 3). Spatial resolution was enabled at T2, in which only one tumor sample from the infantile fibrosarcoma (90x50x30mm in maximum diameter). The cut surface of the tumor was whitish and had some hemorrhagic (<10%) and some necrotic areas (<10%). FISH, fluorescent in situ hybridization; HE, hematoxylin and eosin; TRK, tropomyosin receptor kinase.

Discussion

We here report on a patient with an ETV6-NTRK3-driven infantile fibrosarcoma that developed resistance to first- and second-generation TRK inhibitors. The tumor rapidly responded to larotrectinib monotherapy but acquired resistance through an NTRK3 solvent-front mutation. Resistance was overcome with selitrectinib monotherapy, but an NTRK3 xDFG motif mutation again rendered the disease refractory. As exemplified by this patient, treatment with first- and second-generation TRK inhibitors can elicit rapid and strong responses in the treatment-naive and acquired resistant disease settings. The sequencing data reported provide no evidence to support a model of primary resistance. The reduction in tumor volume by >98% after 2 months of larotrectinib treatment is in line with the reported NTRK3 wild-type sequence at time point T1. Whether single-cell sequencing approaches of multiregion biopsies collected at initial diagnosis will unravel the sequential acquisition of two mutations that render the disease primarily resistant to monotherapy with TRK inhibitors remains to be investigated.

Although sustainable responses to TRK inhibition exist, the sequential acquisition of two NTRK3 mutations under therapy pressure reported here demonstrates that close disease monitoring is warranted. Cross-sectional imaging studies are the current gold standard for monitoring intrathoracic lesions. Although this patient was monitored on a monthly basis, stepwise acquisition of resistance to first- and second-generation TRK inhibitor therapy resulted in rapid progressive disease that required intensive care measures. Whether liquid biopsy–based diagnostics can better support resistance monitoring, therapy decisions, and response evaluation for tumors harboring oncogenic NTRK fusions remains an open question. Increasing evidence suggests...
FIG 3. Molecular profiling of multi-sample sequencing data. (A) Mutation data derived from WES. Shown are all samples with an average coverage of more than 40X. Mutations were filtered against the cosmic cancer gene census v9. Only variants with a variant allele frequency above 10%, no variant reads in the matched normal and a predicted functional impact of moderate or high are shown. Darker colors indicate mutations that have been called by MuTect2. Lighter shades represent variants that had supporting reads upon detailed inspection. The NTRK3 G→R mutation at time point T3 is caused by a G→C mutation on the nucleotide level, whereas the samples marked with an asterisk, show variant read evidence of a G→A mutation that also results in a G→R amino acid exchange. (B) Copy number profiles for samples T1 and T3. Genomic segments are colored based on their absolute copy number. Diploid regions are shown in white, losses in shades of blue, gains in red. Only events affecting segments of 5MB or larger are shown. (C) Time line representing the order of events leading from the ancestral cell to the last analyzed tumor sample. SNVs and fusion events are shown above the time line, copy number variants beneath it. The coloring of individual events is consistent with panels A) and B). B, biopsy; R, resection; T, time point.

that follow-up using liquid biopsies is feasible for patients with fusion-positive sarcomas.27,28

The primary mutational spectrum in this infantile fibrosarcoma was very low, with only two mutations detected in cancer-related genes. The PIK3R1 gene affected by an in-frame deletion encodes for the p85 regulatory subunit of phosphoinositide 3-kinases, which regulate signaling pathways important for cell proliferation, survival, adhesion, and motility.29 PI3K mutations have been linked to cancer,30 primary immunodeficiencies,31 and developmental disorders.32 The ARID1A gene affected by a frameshift deletion is part of the SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex regulating eukaryotic gene expression, SWI/SNF complex mutations occur in 20% of human cancers, and ARID1A has the highest mutation rate across all SWI/SNF complex components.33 ARID1A mutations were shown to be negatively associated with checkpoint immunotherapy responses and patient survival in different cancer entities.34

The only mutation occurring under therapy affected the CLTCL1 gene, a member of the clathrin heavy chain family required for mitotic progression and cytokinesis.35 CLTCL1 mutations have been reported in oral and lung squamous cell carcinoma,36,37 meningioma,38 and a rare case of thyroid follicular dendritic cell sarcoma.39 The newly occurring copy number alterations detected at T3 may be attributable to the CLTCL1 mutation–induced impairment of mitotic spindle stabilization. Altogether, genomic profiling in temporal and spatial resolution of this infantile fibrosarcoma identified a very low number of cancer-related, but undruggable, mutations. The borderline priority of all three overexpressed genes (FGFR1, YES1, and CTLA4) and the exhausted chemotherapeutic options prompted us to turn to downstream signaling cascades of the oncogenic NTRK3 p.G623R p.G696R fusion protein, which include PI3K, RAS/MAPK/ERK, and PLCG1/PLCG2.13 Combining selitrectinib with trametinib, to also inhibit MEK1/MEK2 activity, resulted in 1 year free of disease progression, thus providing insights into precision medicine strategies under conditions of acquired resistance to first- and second-generation TRK inhibition.

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AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO’s conflict of interest policy, please refer to www.asco.org/rcw or ascopubs.org/author-center.

Karsten Nysom
Honoraria: Y-mAbs Therapeutics
Consulting or Advisory Role: Bayer, EUSA Pharma, Y-mAbs Therapeutics Inc.
Travel, Accommodations, Expenses: Bayer
Anton G. Henssen
Research Funding: Bayer

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT
The authors thank Kathy Astrahantseff for critical reading of the manuscript.