

Successful Targeted Therapy of Refractory Pediatric *ETV6-NTRK3* Fusion-Positive Secretory Breast Carcinoma

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CASE REPORT

This 14-year-old girl from a small farming village in rural Bangladesh presented in 2010 at 8 years of age with a lump in the left breast. There was no significant past medical or family history of cancer. She underwent a lumpectomy with an initial diagnosis of fibroadenoma. One year later, she presented with a recurrent ipsilateral breast mass and underwent a second lumpectomy, and her diagnosis was revised to secretory breast carcinoma on the basis of repeat histopathology evaluation. She received two cycles of chemotherapy with fluorouracil, doxorubicin, and cyclophosphamide. Approximately 1 year later, her disease recurred locally, and she underwent a simple mastectomy with axillary lymph node dissection, followed by four cycles of carboplatin and docetaxel. In 2014, she had a chest wall recurrence and underwent a local resection followed by two cycles of vinorelbine and gemcitabine. One year later she developed a second left chest wall recurrence and was found to have bilateral lung metastases. The patient underwent resection of the chest wall mass and received two cycles of combination chemotherapy with ifosfamide, doxorubicin, dacarbazine, and mesna. Less than a year later, she presented with a recurrent fungating mass in the left chest wall and was treated with two cycles of carboplatin and paclitaxel, with no clinical benefit.

Having exhausted all available options, her treating oncologist presented her case at a virtual multidisciplinary tumor board organized by the Global Cancer Institute, a nonprofit organization focused on improving care of underserved patients with cancer. Given the histologic diagnosis of secretory breast carcinoma, the board recommended molecular testing for an *ETV6-NTRK3* fusion, the pathognomonic genomic alteration in this cancer type.¹ To accomplish this, a formalin-fixed paraffin-embedded (FFPE) tumor block was

located and analyzed at Memorial Sloan Kettering Cancer Center (MSKCC), where the presence of an in-frame *ETV6-NTRK3* fusion was confirmed by anchored multiplex RNA sequencing (Figs 1A and 1B) and separately by targeted hybridization capture-based DNA and ultimately whole-genome sequencing (WGS). Immunohistochemistry with a pan-tropomyosin receptor kinase (TRK) antibody confirmed overexpression in a perinuclear nuclear pattern, consistent with a fusion involving the nuclear transcription factor ETV6 (Fig 1C). Of note, both targeted capture-based sequencing and WGS also identified a *TERT* promoter mutation, a common alteration in solid tumors that leads to increased expression of telomerase² but to our knowledge has never been reported previously in secretory breast carcinoma.

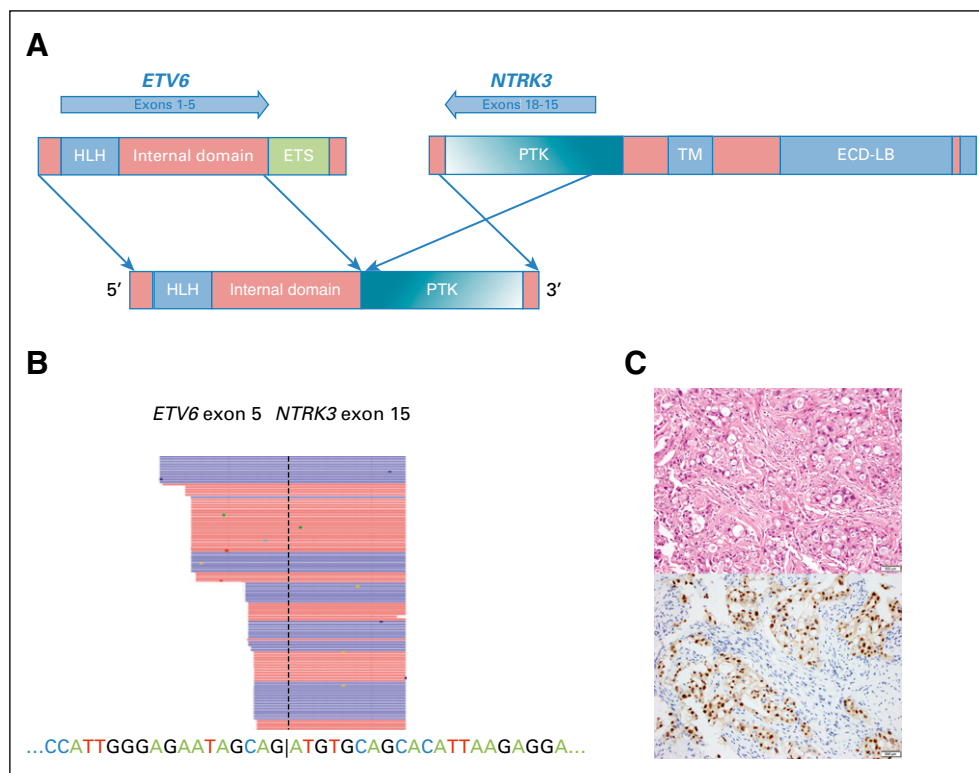
After confirmation of an expressed *ETV6-NTRK3* fusion, which is known to result in constitutive TrkC kinase activity,¹ treatment with a Trk inhibitor was recommended by MSKCC pediatric and medical oncology teams. Ultimately, with approval from the US Food and Drug Administration (FDA) and Loxo Oncology (Stamford, CT), a single patient use protocol for larotrectinib (LOXO-101) was written and opened for this patient at MSKCC.

METHODS

Anchored Multiplex RNA Sequencing

The tumor was analyzed using the MSK-Solid Fusion assay, a targeted RNA-based panel that uses the Archer Anchored Multiplex polymerase chain reaction technology³ and next-generation sequencing to detect gene fusions. Unidirectional gene-specific primers were designed to target specific exons in 35 genes known to be involved in oncogenic fusions in solid tumors. RNA was extracted from tumor FFPE material, followed by cDNA synthesis and library preparation. Anchored

Fig 1. Pathologic, molecular, and immunohistochemical analysis of secretory breast carcinoma. (A) Schematic illustration of the protein structure and transcript sequence of the *ETV6* (NM_001987)-*NTRK3* (NM_002530) in-frame fusion product. Exons 1 to 5 of *ETV6* are fused to exons 15 to 18 of *NTRK3*, which include the kinase domain. (B) Representation of cDNA sequencing reads supporting the *ETV6*-*NTRK3* fusion transcript. Red and blue rectangles represent bidirectional sequencing chimeric reads, where the exact location of the fusion breakpoint is indicated with a dotted vertical line and with an I symbol in the fusion transcript sequence. (C) Histopathologic findings typical for secretory breast carcinoma, including tubuloalveolar growth pattern, cytoplasmic vacuolization with eosinophilic secretions, and areas of hyalinization (hematoxylin and eosin). Immunohistochemical staining for pan-Trk expression revealed diffuse, strong positive staining in a nuclear pattern (Abcam, mAb EPR17341, 1:250). ECD-LB, extracellular ligand binding domain; ETS, E26 transformation-specific binding domain; HLH, helix-loop-helix domain; PTK, protein tyrosine kinase domain; TM, transmembrane domain.



Multiplex polymerase chain reaction amplicons were then sequenced on an Illumina MiSeq and the data analyzed using the Archer software (V4.0.10).

Targeted Hybridization Capture DNA Sequencing

The Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets assay was conducted as previously described.^{4,5} In brief, DNA was extracted from an FFPE biopsy of the patient's chest wall metastasis and from her blood leukocytes. Barcoded libraries from tumor and normal samples were captured, sequenced, and subjected to a custom pipeline to identify somatic mutations. Sequencing consisted of deep sequencing of all exons and selected introns of a custom 410 cancer-associated gene panel. The median depth of coverage across all exons was 972×.

WGS

WGS was performed using Illumina X10 paired end sequencing standard methods under the auspices of the Cancer Alliance Study in collaboration with the New York Genome Center. Tumor was sequenced to approximately 80× with a matched normal sample at 60×. Single-base substitutions were called using CaVEMan (Cancer Variants through Expectation Maximization; <http://cancerit.github.io/CaVEMan/>), as described previously.⁶

Copy number and cellularity information for CaVEMan were predicted with the Battenberg algorithm⁶ using 1,000 Genomes⁷ loci within the next-generation sequencing data. Small somatic insertions and indels were identified using a modified version of Pindel (<https://github.com/cancerit/cgpPindel>).⁸ Structural rearrangements were detected by an in-house algorithm, BRASS (Breakpoints via Assembly; <https://github.com/cancerit/BRASS>). To improve specificity, a number of custom postprocessing filters were applied. Mutational signature analysis of the substitutions was performed using the R package DeconstructSigs.⁹ Small insertion/deletions were interrogated for the presence of either short tandem repeat or microhomology at the breakpoints, as described previously.¹⁰ Data relating to this patient will be deposited in the European Genome-phenome Archive.¹¹

Trk Immunohistochemistry

Expression of TrkC, the protein product of *NTRK3*, was detected via immunohistochemistry with anti-pan-Trk Abcam monoclonal antibody EPR17341 in a 1:250 dilution. A positive control (testicular seminiferous epithelium)¹² and a negative control (benign hepatocytes) were stained simultaneously. Positive staining of tumor cells was defined by tumor cells showing immunohistochemical

expression of Trk with at least weak (1+) intensity in a membranous, cytoplasmic, and/or nuclear pattern.

Larotrectinib Treatment

The treatment dose of the oral pan-Trk inhibitor larotrectinib was 100 mg twice per day. This corresponds to the recommended phase II adult dose in an ongoing trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02576431) identifier: NCT02576431).

RESULTS

On arrival to MSKCC, the patient reported significant pain at the recurrent chest wall tumor site. Physical examination revealed a 10.4×8.5 cm fungating chest mass with multiple satellite lesions scattered over her chest wall ([Fig 2](#)). Baseline computerized tomography (CT) scan also revealed numerous pulmonary metastases as well as bone metastases involving the sternum and vertebrae ([Fig 3A](#)). After obtaining written informed consent, treatment with larotrectinib was initiated. She noted marked improvement of tumor-related pain within 3 days of starting therapy. Significant and rapid reduction in size of the left chest mass was achieved within 1 week of starting therapy, with near-complete resolution after 2 months of therapy ([Figs 2 and 3B](#)). Furthermore, CT imaging of her chest revealed

near-complete resolution of the pulmonary metastases ([Fig 3B](#)). Her response is ongoing for approximately 4 months. The patient tolerated larotrectinib well, with the only drug-related toxicities being two discrete episodes of dizziness (grade 1 and 2) associated with postural change. She was provided with a 6-month supply of larotrectinib and has since returned home for continued treatment by her oncologists in Gopalganj, Bangladesh, with plans to return to MSKCC twice a year for follow-up.

To further investigate the genomic landscape of this patient's secretory breast carcinoma, a fresh pretreatment tumor biopsy was obtained and paired tumor-normal WGS performed. A total of 697 somatic alterations consisting of 530 single-nucleotide substitutions, 157 deletions (indels), and 10 rearrangements were identified, although only two of these resulted in alteration of a coding region, and of these, only the *ETV6-NTRK3* fusion was clonal. Counting both synonymous and nonsynonymous mutations, the overall mutation burden was remarkably low (1.8×10^{-4} subs/Mb), even when compared with other pediatric tumor types known to harbor low mutation rates.¹³

Analysis of the clonal structure of the genome revealed that the *ETV6-NTRK3* and *TERT* promoter mutations were both clonal (present in

Fig 2. Clinical response in chest wall mass. Photographic images of the bulky left chest mass before initiation of larotrectinib treatment (baseline) followed by photographs on days +6, +20, and +54 of therapy.

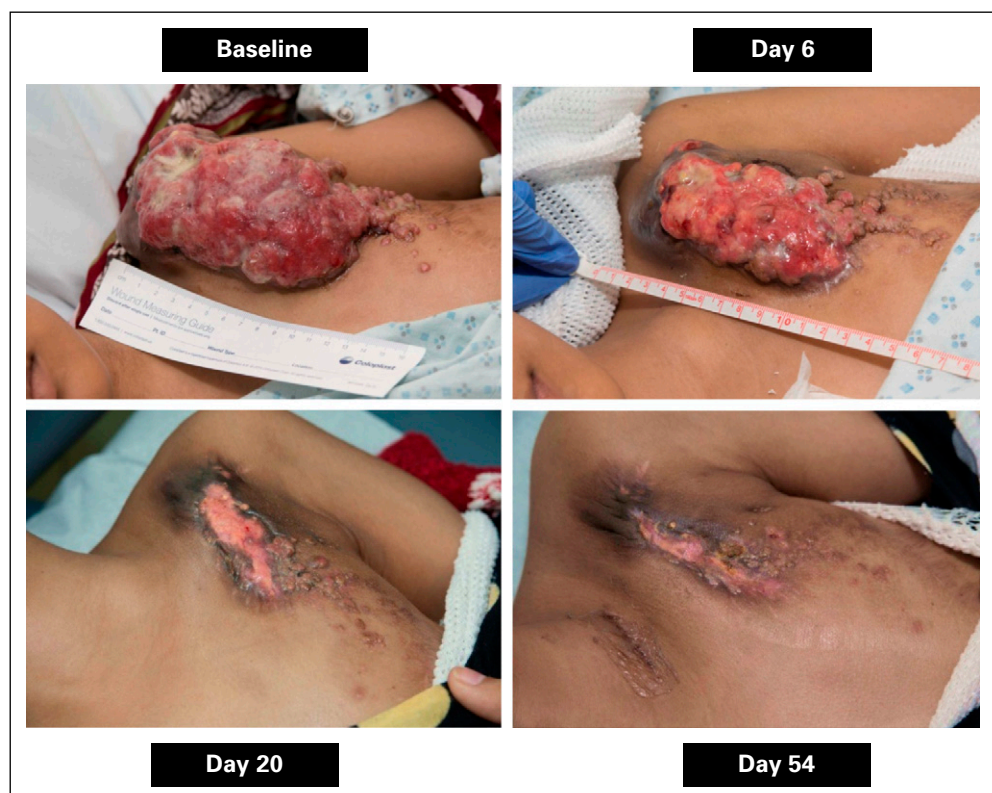
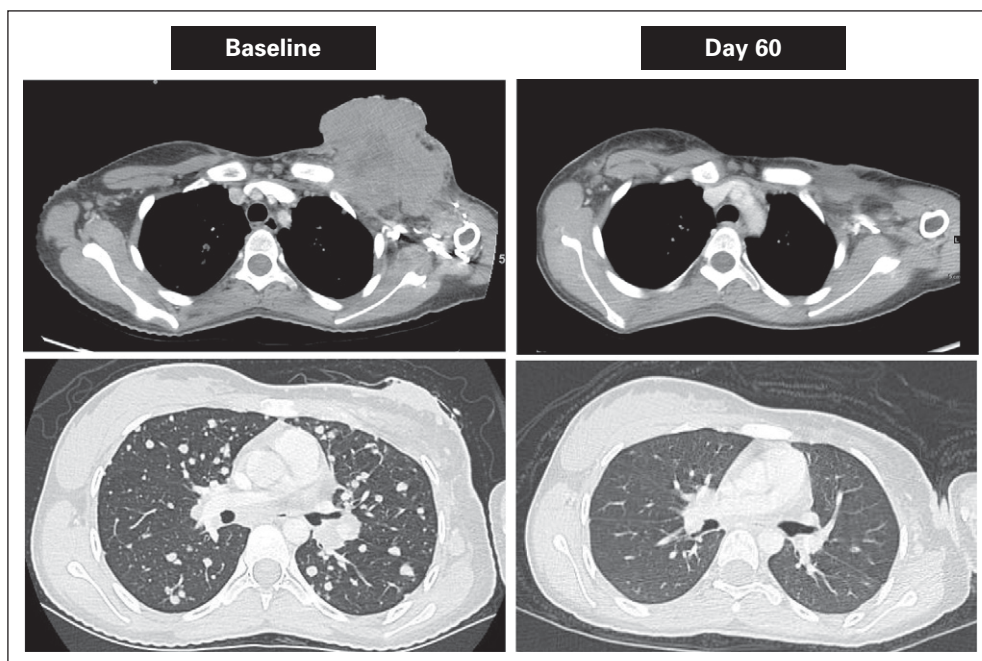


Fig 3. Radiographic response of chest wall lesion and lung metastases. Chest computed tomography scan demonstrates a large exophytic soft tissue mass arising from the left anterior chest wall and numerous nodules throughout both lungs (baseline). Repeat imaging (day +60) demonstrates a marked decrease in the size of the left anterior chest wall mass and near-complete resolution of lung nodules.



100% of the tumor cells). In addition, WGS also identified two reciprocal inversion events leading to a 9,500-bp deletion containing the first exon of *CDKN2A*, followed later in this tumor's evolution by a subclonal copy-neutral loss of heterozygosity as a result of loss of the wild-type allele and duplication of the mutated allele (Fig 4). Although the allele with focal deletion of *CDKN2A* seemed to be clonal, loss of the other allele was not. Therefore, biallelic inactivation of *CDKN2A* was a subclonal event in this tumor. Germline analysis for evaluation of cancer predisposition gene mutations was not performed. We will discuss this option with the family on their follow-up visit to MSKCC.

DISCUSSION

NTRK1, *NTRK2*, and *NTRK3* encode for the TrkA, TrkB, and TrkC receptor tyrosine kinases. Oncogenic gene fusions involving the *NTRK* family of genes have been identified across numerous different tumor types and seem to result in constitutive activation of Trk kinase activity.¹⁴ Larotrectinib is an orally administered highly selective inhibitor, with nanomolar activity against all three Trk protein isoforms.¹⁵ Substantial tumor regressions with larotrectinib have been reported in a child with congenital fibrosarcoma bearing the *ETV6-NTRK3* fusion, as well as an adult with a soft tissue sarcoma harboring an *LMNA-NTRK1* fusion.^{16,17} Preliminary activity has also been described in multiple other *NTRK* fusion-positive

cancer types with larotrectinib.¹⁸ In July 2016, the FDA granted breakthrough designation to larotrectinib for the treatment of unresectable or metastatic solid tumors bearing *NTRK* fusions.¹⁹

Although targeted therapy of its extramammary counterpart has been reported,²⁰ this is the first report of a patient with secretory carcinoma of the breast treated with a Trk inhibitor. Secretory breast carcinoma is an extremely rare tumor type, accounting for < 0.02% of breast cancer cases.²¹ It is classically an indolent tumor, with primary management usually consisting of surgical resection. Although initially believed to occur primarily in children, these tumors have subsequently been identified in both pediatric and adult-age patients.²² Histologically, secretory breast carcinomas are characterized by their low-grade appearance, granular or vacuolated cytoplasm, and abundant intracellular and extracellular secretory material.¹⁹ Furthermore, these tumors are generally triple negative for expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2.²² Although axillary involvement is seen in a significant proportion of patients, widely metastatic disease is uncommon.²¹ As a result, the role of radiation and chemotherapy is not established for patients with locally advanced or metastatic disease.

Secretory breast carcinomas are characterized by the *ETV6-NTRK3* fusion. The broad genomic evaluation performed in this case, along with

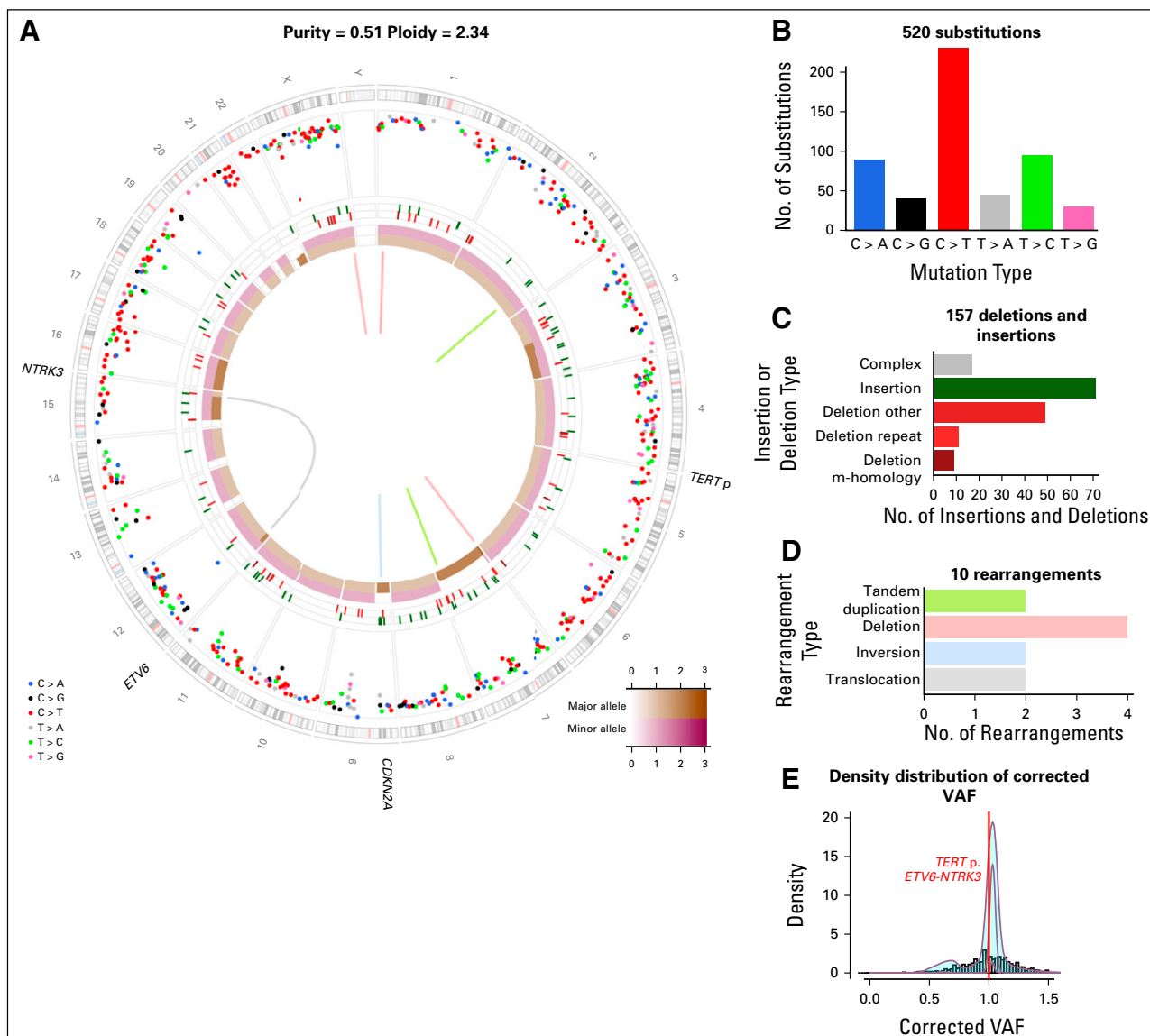


Fig 4. Circos plot summarizing the whole-genome sequencing analysis. The two innermost tracks depict the integer copy number changes for the major (brown) and minor allele (dark pink). The outermost track shows the intermutation distance for substitutions, each plotted according to the type of nucleotide change. The middle track shows the genomic positions of the small insertion (green) and deletion (red) along the genome. Rearrangements are plotted as arcs inside

the robust clinical response to a single-agent TRK inhibitor, support previous data demonstrating *ETV6-NTRK3* as a driver lesion.²³ The 14-year-old reported here, despite being heavily pretreated for 6 years and having a high burden of disease, achieved an almost immediate and complete response to larotrectinib that remains ongoing with minimal toxicity. Significant activity and durable responses have also been established in adult patients with NTRK-rearranged tumors. Recent results from a phase I study reported objective responses in all seven patients enrolled whose tumors harbored NTRK fusions. Furthermore, most patients had ongoing responses > 1 year in duration, with the longest response approaching 2 years.²⁴

Whole-genome sequencing analysis reveals a simple mutation and clonal structure, predominantly defined by the fusion gene, mutation in the *TERT* locus, and loss of *CDKN2A*. The rarity of secondary mutations in translocation-associated pediatric tumors has been previously described²⁵⁻²⁷ and implies potentially significant susceptibility of the fusion to targeted agents. All genomic studies in this report were performed on recurrent tumor specimens. The material from the primary tumor was not available. However, because it was the only clonal event in a coding region identified by WGS, along with its known status as the defining alteration in this subtype of breast cancer and prior functional data confirming its activating and transforming nature,^{1,24} we can infer *ETV6-NTRK3*

the *circos*. (B) Mutation signature analysis of the substitutions using deconstructSigs. Only the first 10 signatures are shown. (C) Summary of the insertion and deletion data. (D) Summary of rearrangement data. (E) Distribution of corrected variant allele frequency (VAF) identifying the *ETV6-NTRK3* fusion and *TERT* promoter mutation as clonal events.

fusion as the primary mitogenic driver in this patient's tumor from the initial presentation onward.

Along with being the first detailed genomic evaluation of secretory breast carcinoma to our knowledge, and establishing the extraordinary response of this subtype of breast cancer to larotrectinib, this report also describes a unique and dynamic collaboration between multiple international academic centers, global health initiatives, and the pharmaceutical industry. The Global Cancer Institute began organizing virtual multidisciplinary tumor boards connecting oncology experts based in the United States and Western Europe with physicians from low- and middle-income countries in 2012, with the goal of improving care of patients with cancer in underserved populations worldwide.²⁸ Although these tumor boards have typically focused on improving

standard practice in the developing world, here we demonstrate how these boards can also lead to the implementation of investigational therapy with dramatic benefit. Through collaboration of three large US academic centers, including MSKCC, Johns Hopkins, and Massachusetts General Hospital, and the participation of Loxo Oncology, the FDA, and the Professor Dr Obayedullah Ferdousi Foundation Cancer Hospital and Research Institute in Bangladesh, not only were we able to provide profound clinical benefit to a patient who had exhausted all available conventional therapies but also, through her extraordinary response, we were able to directly demonstrate that *ETV6-NTRK3* is an oncogenic driver in secretory breast cancer.

DOI: <https://doi.org/10.1200/PO.17.00034>

Published online on ascopubs.org/journal/po on August 15, 2017.

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Financial support: Andrew L. Kung

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Manuscript writing: All authors

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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. 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/rwc or po.ascopubs.org/site/ifc.

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Research Funding: Syros Pharmaceuticals (Inst)

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No relationship to disclose

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No relationship to disclose

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We thank Muhammed Yunus for his significant assistance in facilitating the patient's travel to the United States for treatment. We also thank Penelope Sinanian for her logistical support in the treatment of this patient. We thank Joseph Olechnowicz for editorial assistance.

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Support

Supported by National Institutes of Health Grants No. P30 CA008748 and P30CA006973. The Cancer Alliance Study is supported by an unrestricted grant from IBM to the New York Genome Center. Travel for the patient and her mother was supported by Loxo Oncology. Housing was provided by Ronald MacDonald House. All clinical care was covered by philanthropic support from Memorial Sloan Kettering Cancer Center.

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