Durable Clinical Response to Crizotinib in *IRF2BP2-NTRK1* Non–small-cell Lung Cancer

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Clinical Practice Points

- Chromosomal rearrangements involving neurotrophic tropomyosin receptor kinases (NTRK1, 2, and 3) are detected in 0.1% to 1% of non–small-cell lung cancer.
- An in-frame gene rearrangement involving *NTRK1* and the 5' fusion partner encoded by Interferon Regulatory Factor 2 Binding Protein 2 (*IRF2BP2*) in a lung adenocarcinoma was reported. No other oncogenic alterations were identified, supporting that *IRF2BP2-NTRK1* gene fusion acts as a potent oncogenic driver.
- The patient achieved a durable clinical response to crizotinib (progression-free survival, 16 months) prior to disease progression in new metastatic sites.
- Mutational profiling of tumor biopsy samples found copy number increases of *HGF*, *IL7R*, *PAK3*, and *RICTOR* genes but not *NTRK1* resistance mutations post-crizotinib, likely because crizotinib represents a less potent inhibitor compared with pan-TRK inhibitors including larotrectinib and entrectinib, raising the possibility that tumors may still have been TRK-dependent and the patient could benefit from more potent TRK inhibitors.

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Introduction

Chromosomal rearrangements resulting in the expression of oncogenic receptor tyrosine kinase fusions occur in a subset of epithelial malignancies and can underlie the sensitivity to tyrosine kinase inhibitors.[1] The 3 neurotrophic tropomyosin receptor kinase (*NTRK*) genes *NTRK1*, *NTRK2*, and *NTRK3* encode tropomyosin receptor kinases (TRK) TRKA, TRKB, and TRKC, respectively, which play roles in the development of nervous system as receptors for nerve growth factors, are estimated to occur at a frequency of approximately 0.1% to 1% in non–small-cell lung cancer (NSCLC),[2,3] whereas the frequency was relatively higher (3.3%) in a lung adenocarcinoma population.[4] In particular, *NTRK1* fusions have been detected at a frequency of 12% in papillary thyroid cancers, with *TPM3 (tropomyosin 3)-NTRK1* being the most common gene rearrangement.[5,6] *NTRK1* gene fusions also occur at low prevalence in other solid tumor malignancies, including in colorectal carcinoma and sarcoma.[7] Fusion partners of *NTRK1* previously described include *MPRIP, CD74, SQSTM1,* and *MPIRP*.[2] In this study, we report an in-frame gene fusion involving *NTRK1* and the 5' fusion partner encoded by *Interferon Regulatory Factor 2 Binding Protein 2* (*IRF2BP2)* in a patients with lung adenocarcinoma. We reviewed the patient's clinical outcomes to multiple lines of chemotherapy and targeted therapies including crizotinib, and further investigated the mutation profiles of pre- and post-crizotinib *IRF2BP2-NTRK1* tumor biopsy samples by targeting a panel of 422 cancer-relevant genes using next generation sequencing.

Case Report

Chest x-rays showed that a 51-year-old female presented with partial consolidation in her left lower lung lobe during the regular physical examination in 2010. The cytology of pleural effusion was positive for adenocarcinoma cells likely of lung origin, but the

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primary remained occult. She remained untreated until 2012 when the computed tomography (CT) scans showed enlarged lymph nodes in her left supraclavicular (Figure 1A). The patient underwent lymph node biopsy and was confirmed with adenocarcinoma of lung origin (Figure 1B). The biopsy specimen tested negative for both *EGFR* and *ALK* mutations using the amplification-refractory mutation system at the Northern Jiangsu People's Hospital, Yangzhou, Jiangsu, China.

The patient then received 4 cycles of cisplatin (40 mg, days 1-3) and pemetrexed (800 mg, day 1) and 2 cycles of pemetrexed monotherapy. Stable disease was achieved according to Response Evaluation Criteria In Solid Tumors (RECIST v1.1)[8] until the restaging CT scans detected enlarged lymph nodes in the patient's left clavicle in April 2015 (Figure 1C). The patient then received gefitinib orally for 1 month, followed by 2 cycles of nedaplatin (40 mg, days 1-3) and pemetrexed (800 mg, day 1). However, the patient showed rapid progressive disease without significant clinical improvement. The patient underwent repeated lymph node biopsy in the left supraclavicular in December 2015, and was confirmed with lung adenocarcinoma (Figure 1D). The patient's formalin-fixed paraffin-embedded tumor samples were subject to comprehensive mutational profiling by targeting a panel of 422 cancer-related genes using capture-based targeted next generation sequencing at Nanjing Geneseeq Technology, Jiangsu, China. An in-frame gene rearrangement involving *IRF2BP2* exon 1 (transcript id: NM_182972) and *NTRK1* exon 8-16 (transcript id: NM_002529) was identified at a mutant allele frequency of 6% (Figure 2A). Given that both *NTRK* and *IRF2BP2* are located in chromosome 1q, *IRF2BP2 E1-NTRK1 E8* likely resulted from an inversion of the chromosome arm 1q (Figure 2B). No concurrent oncogenic alterations were detected. Wong et al reported that crizotinib showed clinical benefits in a patient with *LMNA-NTRK1* fusion-mediated fibrosarcoma with nearly complete response.[9] Although crizotinib is a less potent inhibitor of TRKs compared with larotrectinib (formerly ARRY-470) or entrectinib (formerly RXDX-101),[4,10-12] the patient immediately started on crizotinib (250 mg/twice a day) orally in January 2016 owing to the unavailability of these pan-TRK inhibitors. The patient achieved stable disease for approximately 16 months on crizotinib according to RECIST v1.1 (Figure 3A, B, E), until the restaging CT scans detected new metastatic sites including the chest (pleural nodule, Figure 3C) and the brain (Figure 3D) in June 2017. However, the lymph node site in the left supraclavicular remained with stable disease according to RECIST (Figure 3E). Tumor biopsy sample of the pleural nodule was subject to genetic testing against the same 422-gene panel by targeted next generation sequencing at Nanjing Geneseeq Technology. *IRF2BP2-NTRK1* fusion was detected at a mutant allele frequency of 11.5%. In addition, copy number increases of *HGF*, *IL7R*, *PAK3*, and *RICTOR* were observed when disease progressed on crizotinib. Genetic testing result was unavailable for tumor metastases of the patient's brain. Crizotinib was soon discontinued. The patient received 4 cycles of avitin and albumin paclitaxel starting in November 2017.

Discussion

TRK signaling is normally involved in neuronal development, synaptic function, and plasticity. Wild-type TRKA, TRKB, and TRKC function through ligand-dependent dimerization and eventually cause the activation of downstream signaling via the mitogen-activated protein kinase, phospholipase C-γ, and phosphatidylinositol 3-kinase pathways in mediating cell differentiation and survival. *NTRK* gene fusions, which result in the abnormal expression of TRK fusion proteins, represent approximately 0.1% to 1% of the

Figure 1 Computed Tomography Scans Showing Enlarged Lymph Nodes in the Patient's Left Supraclavicular (A) and Left Clavicle (C) and Histopathologic Appearance Showing Adenocarcinoma Cells Likely of Lung Origin in Those Sites (B, D). Red Arrowheads: Enlarged Lymph Nodes

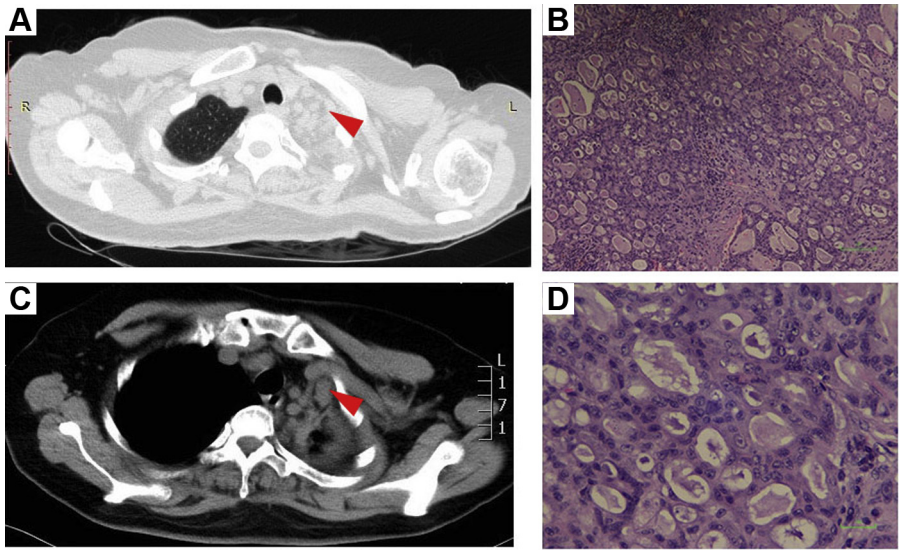
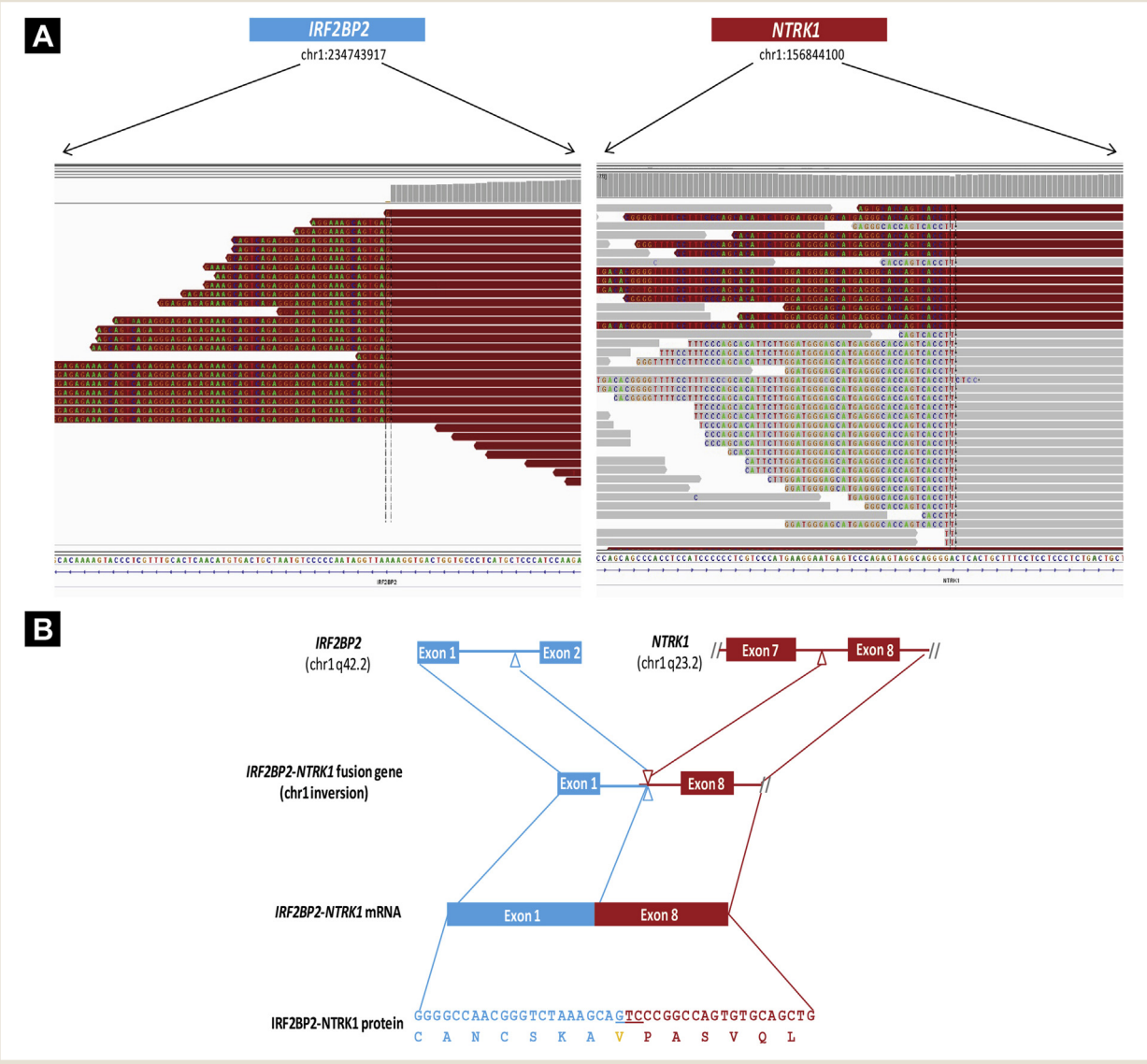


Figure 2 Identification of IRF2BP2-NTRK1 Rearrangement in a Lung Adenocarcinoma Patient. A, Next Generation Sequencing Reads in the 2 IRF2BP2 and NTRK1 Fusion Breakpoints in Chromosome 1 Were Visualized by Integrated Genomics Viewer (IGV). The Exact Breakpoints Are Shown in the Figure. B, A Scheme Showing the In-frame IRF2BP2-NTRK1 Rearrangement at Chromosome, mRNA, and Protein Level

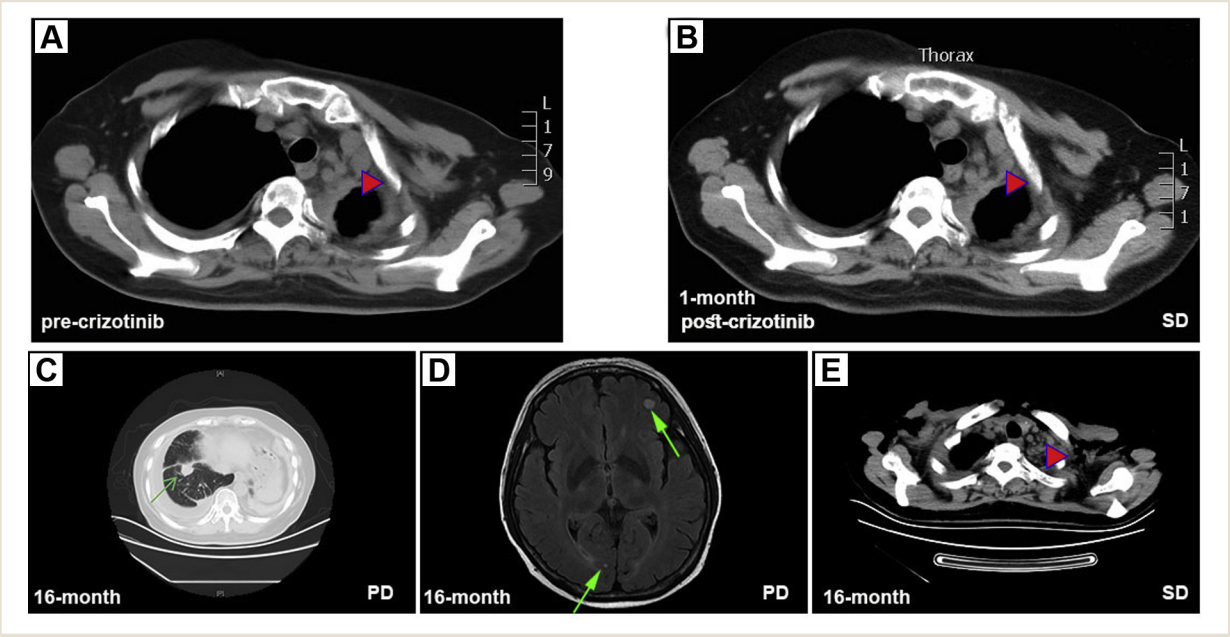


Abbreviations: IRF2BP2 = Interferon Regulatory Factor 2 Binding Protein 2; NTRK1 = (neurotrophic tropomyosin receptor kinase 1.

population with NSCLC.[2] Herein, we report a patient with lung adenocarcinoma with *NTRK1* gene abnormally fused to *IRF2BP2*, resulting in the constitutive activation of TRKA kinase, the IRF2BP2-NTRK1 fusion oncoprotein, which is likely to be the driver mutation because no concurrent oncogenic alteration was detected.

Aside from *IRF2BP2, NTRK1* fusion partners that have previously been described include *SQSTM1, TRP, TPM3, MPIR,* and *LMNA*.[2] However, therapeutic options for patients with *NTRK* fusions were limited prior to the discovery of pan-TRK inhibitors, including larotrectinib and entrectinib. It has previously shown that crizotinib, a tyrosine kinase inhibitor of ALK and ROS1, achieved durable response in a patient diagnosed with *LMNA-NTRK1* fusion-mediated congenital infantile fibrosarcoma with a progression-free survival of more than 8 months.[9] However, another case with *MPRIP-NTRK1* rearrangement lung adenocarcinoma remained as stable disease according to RECIST for only 3 months on crizotinib.[4] In this case study, the patient achieved a RECIST stable disease on crizotinib for approximately 16 months until disease progression in new metastatic sites including the chest (pleural nodule) and the brain. These findings are consistent with previous findings that crizotinib represents a less potent inhibitor when compared with larotrectinib or entrectinib, which potently inhibit TRKs at low nanomolar concentrations

Figure 3 Computed Tomography Scans Showing Enlarged Lymph Nodes in the Patient's Left Supraclavicular Pre-crizotinib (A) and Stable Disease 1 Month Post-crizotinib (B), New Metastases in the Chest (C) and the Brain (D) 16 Months Post-crizotinib, Whereas Disease Remained Stable in the Patient's Left Supraclavicular (E). Red Arrowheads: Enlarged Lymph Nodes. Green Arrows: New Metastases In The Chest And The Brain



in vitro.[4,11] Further clinical trials in large cohorts showed that larotrectinib had marked and durable antitumor activity in both adult and pediatric *TRK*—fusion-positive patients with cancer with locally advanced or metastatic solid tumors, regardless of the age of the patient or of the tumor type,[13] although resistance mechanism inevitably develops.[14] Meanwhile, it has recently been reported at ESMO 2018 that entrectinib, a small molecule that inhibits *TRKs*, *ROS1*, and *ALK* rearrangements, induced clinically meaningful and durable responses in *NTRK*—fusion-positive solid tumors, type agnostic, with and without disease spreading to the central nervous system.[15] Such agents may soon grant clinicians and patients with *NTRK*-rearranged fusions more potent therapeutic options during treatment selection.

Lastly, in contrast to the emergence of resistance mutations in *NTRK1* genes including G595R, F589L, and G667S/C following the treatment of larotrectinib or entrectinib,[13,16] copy number increases of *HGF*, *IL7R*, *PAK3*, and *RICTOR* genes were observed in the patient's post-crizotinib sample likely because crizotinib has much lower potency against TRK. For example, the 2-fold increase of hepatocyte growth factor that encodes the ligand of c-MET may represent a bypass resistance mechanism against crizotinib, in line with a prior study by Ko et al reporting that overexpressing hepatocyte growth factor-induced excessive autocrine signaling thus activating MET both in vitro and in vivo, leading to EGFR-TKI resistance.[17] This finding raised the possibility that tumors may still be TRK-dependent, and the patient could benefit from larotrectinib or entrectinib.

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Disclosure

Q.O., X.W., and Y.W.S. are the shareholders or employees of Geneseeq Technology Inc. Canada. The remaining authors have no conflicts of interest to declare.

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