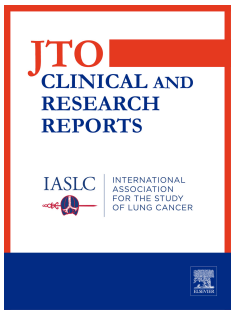

Primary Resistance to Larotrectinib in a Patient with Squamous Non-Small Cell Lung Cancer with Subclonal *NTRK1* Fusion: Case Report

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Title: Primary Resistance to Larotrectinib in a Patient with Squamous Non-Small Cell Lung Cancer with Subclonal *NTRK1* Fusion: Case Report

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Abstract

The neurotrophic receptor tyrosine kinase (*NTRK*) genes encode the tropomyosin receptor kinase (TRK) proteins. *NTRK* fusions lead to constitutively active, ligand-independent downstream signaling. *NTRK* fusions are implicated in up to 1% of all solid tumors and 0.2% of non-small cell lung cancer (NSCLC). Larotrectinib, a highly selective small molecule inhibitor of all three TRK proteins, has a response rate of 75% across a wide range of solid tumors. Mechanisms of primary resistance to larotrectinib are not well understood. We report a case of a 75-year-old male with minimal smoking history with *NTRK* fusion-positive metastatic squamous NSCLC with primary resistance to larotrectinib. We suggest subclonal *NTRK* fusion as a possible mechanism contributing to primary resistance to larotrectinib.

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Introduction

Neurotrophic receptor tyrosine kinase (*NTRK*) fusions drive ligand-independent signaling in a subset of non-small cell lung cancer (NSCLC). Larotrectinib, an FDA-approved inhibitor of the tyrosine kinases encoded by the *NTRK* genes (i.e., tropomyosin receptor kinases), induces significant and durable responses in NSCLCs harboring *NTRK* fusions. Intrinsic resistance to larotrectinib is rare. Here, we summarize the case of patient with metastatic NSCLC harboring an *NTRK1* fusion who experienced primary progression during first-line treatment with larotrectinib. This case provides potential insights into underlying factors contributing to an atypical treatment response to a highly efficacious therapy in a rare subset of NSCLC.

Case Presentation

A 75-year-old male with hypertension, hyperlipidemia, and a remote history of prostate cancer for which he had undergone prostatectomy presented with right-sided chest pain and dyspnea on exertion. The patient had minimal smoking history and had ceased smoking fifty years prior to presentation. On presentation, he was noted to have supraclavicular lymphadenopathy prompting referral for neck ultrasound which confirmed enlarged right supraclavicular nodes. Subsequent imaging demonstrated right supraclavicular lymphadenopathy (Figure 1A), a spiculated 4.3 cm right upper lobe mass (Figure 1B), extensive bilateral thoracic lymphadenopathy, right pleural thickening, bilateral solid pulmonary nodules, and a lytic sternal lesion. Brain MRI did not reveal intracranial metastases.

He underwent mediastinal nodal sampling via bronchoscopy with endobronchial ultrasound. Pathology from right paratracheal and subcarinal lymph node stations was consistent with non-small cell carcinoma (Figure 2A). By immunohistochemistry (IHC), the cells were diffusely positive for p40 and negative for TTF-1 and Napsin A, consistent with non-small cell carcinoma, favor squamous cell carcinoma. IHC also demonstrated loss of BRG1 nuclear expression in the vast majority of tumor cells (Figure 2B), while no membranous staining of PD-L1 was present. DNA- and RNA-based next-generation sequencing (NGS) identified a fusion juxtaposing exon 6 of the *F11R* gene to exon 10 of neurotrophic receptor tyrosine kinase 1 (*NTRK1)*, in addition to mutations in *KEAP1*

G364C, *STK11* splice alteration, *PIK3R1* N630T, and a deleterious *SMARCA4* splice alteration.

Based on findings from molecular testing, the patient was initiated on larotrectinib 100 mg twice a day as first line therapy for metastatic NSCLC. One month into treatment, he endorsed increased dyspnea, chest pain, unintentional weight loss, and enlarging right supraclavicular lymphadenopathy. Due to clinical concern for disease progression, expedited imaging was obtained which confirmed primary progression of disease involving the right supraclavicular region (Figure 1C), lungs (Figure 1D), pleura, and sternum, as well as increasing retrocrural lymphadenopathy. Given the unusual finding of intrinsic resistance to larotrectinib, additional studies—pan-TRK (tropomyosin receptor kinase) IHC and fluorescence in-situ hybridization (FISH)—were performed on the initial diagnostic biopsy tissue to confirm presence of the *NTRK* fusion (TRK IHC clone EPR17341, Abcam; TRK FISH probe RP11-1038N13- NTRK1 3' locus (chr1:156854507-156983651) and RP11-1047J23- NTRK1 5' locus (chr1:156569238-156781762)). TRK protein expression was observed in a minority of tumor cells (Figure 2C). Classic split ('break-apart') signal was present only in 11 of 50 evaluated cells (Figure 2D). Collectively, these findings suggested that the *NTRK1* fusion was a subclonal event. Broader NGS testing of the initial tumor biopsy utilizing a panel encompassing >500 genes did not identify additional putative mediators of primary resistance to larotrectinib. At disease progression, neither plasma genotyping nor repeat tissue biopsy were performed.

He was switched from larotrectinib to carboplatin and paclitaxel. After 2 cycles, imaging demonstrated response with decrease in right upper lobe mass, right pleural metastases, interlobular septal thickening, and retrocrural lymphadenopathy. However, after his third cycle of carboplatin and paclitaxel, imaging showed progression of disease involving the right supraclavicular node, pleura, sternum, and a new hepatic paracapsular nodule. He was transitioned to nivolumab.

Discussion

The *NTRK* genes encode the TRK proteins. *NTRK* fusions lead to constitutively active, ligand-independent signaling[1]. *NTRK* fusions are identified in up to 1% of all

solid tumors and 0.2% of NSCLC[1,2]. In the registrational studies that supported tumor-agnostic FDA approval, larotrectinib induced responses in 75% of patients, with responses observed in a variety of solid malignancies. In a recent update summarizing outcomes of 15 patients with *NTRK*-rearranged NSCLC who participated in these studies, objective responses were observed in 73% of patients with a median progression-free survival of 35.4 months[3]. Primary resistance to larotrectinib was rare. Indeed, in the phase 1 experience, only six patients (11%) had primary progression. Of these six patients, one patient was previously treated with TRK inhibitor and had a *NTRK3* G623R mutation, which interferes with larotrectinib binding[1]. For three of the remaining patients, central IHC testing of tumor tissue did not confirm the presence of *TRK* expression, raising the question of false positive *NTRK* fusion or a non-functional fusion that was not expressed at the protein level[1].

Given the rarity of *NTRK* fusions in NSCLC, the infrequent occurrence of intrinsic resistance to larotrectinib, and limited understanding of factors contributing to primary resistance to larotrectinib, we have summarized our patient's unusual clinical course. Our patient's case has several notable features, including subclonality of *NTRK* fusion, squamous histology, and co-alterations associated with aggressive tumor biology, including mutations in *SMARCA4, KEAP1*, and *STK11*. The issue of subclonality has been explored in the treatment of metastatic NSCLC with *EGFR* T790M mutations where clonality has been correlated with durability of response to osimertinib[4]. In our patient's case, it is possible that larotrectinib suppressed the minority *NTRK* fusion-positive population without exerting antiproliferative effect on the predominant population comprised of cells lacking the *NTRK* fusion. Of note, this situation is distinct from recent case reports in which acquired *NTRK* fusions emerged at resistance to RET and EGFR targeted therapies and were overcome by co-targeting the primary driver and the activated TRK protein in the subclonal resistant population[5,6].

Interestingly, our patient's tumor harbored a concurrent *SMARCA4* mutation with BRG1 deficiency. *SMARCA4* mutations are seen in 10% of NSCLC and are most commonly associated with smoking history[7]. Mutations in *SMARCA4*, as well as the *KEAP1* mutation noted in our patient's tumor tissue, have been linked to resistance to targeted therapy in preclinical models and retrospective clinical series[7-9]. Specifically,

preclinical studies suggest that KEAP1 loss alters cell metabolism and promotes survival of oncogene-driven cancer cells treated with therapies that suppress receptor tyrosine kinase and mitogen activated pathway kinase signaling[10]. However, as these studies did not include *NTRK*-rearranged NSCLC, the impact of these mutations on sensitivity to TRK-directed therapies remains to be established. Finally, our patient's tumor was of squamous histology; squamous differentiation has been implicated in primary and acquired resistance to various targeted therapies[11-13].

Conclusion

In summary, we describe several tumor features that may contribute to primary resistance to larotrectinib, including clonality of the *NTRK* fusion. We suggest considering prioritizing genotype-agnostic therapeutic strategies over TRK inhibitors as initial therapy for *NTRK*-rearranged NSCLCs with these adverse molecular and histologic features.

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Figure Legends

Figure 1. (A) Baseline CT neck demonstrates right supraclavicular lymphadenopathy and (B) a right upper lobe mass (C) CT with increased size of right supraclavicular lymphadenopathy after initiation of larotrectinib (D) CT with increased size of right upper lobe mass and nodular interlobular septal thickening after initiation of larotrectinib

Figure 2. (A) A H&E section showed non-small cell carcinoma in solid clusters. Immunohistochemistry for lung lineage markers confirmed a squamous differentiation. (B) The vast majority of tumor cells demonstrated loss of BRG1 nuclear expression by Immunohistochemistry. (C) Pan-TRK protein expression was observed in a minority of tumor cells by immunohistochemistry. (D) FISH using a break-apart probe to the NTRK1 locus revealed rearrangement of the NTRK1 locus visualized as split green 5' and red 3' NTRK1 signals (arrows) as well as isolated 5' signals (circled). The tumor cells exhibited an average of 3 copies of the NTRK1 locus with the predominant abnormal FISH pattern being isolated 5' only signals in 30% of cells with an additional 22% of cells with split 5' and 3' signals.

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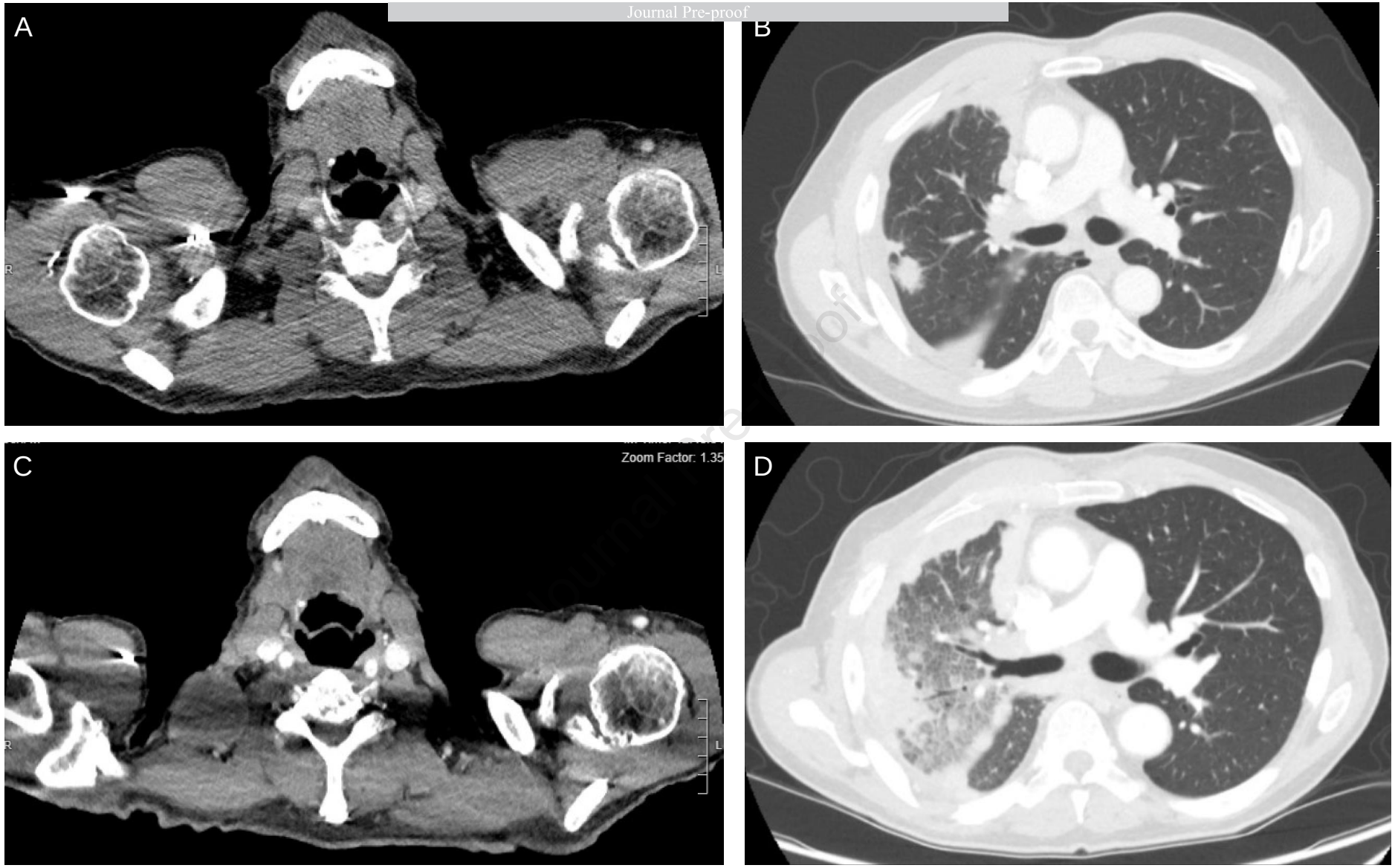

Figure 1

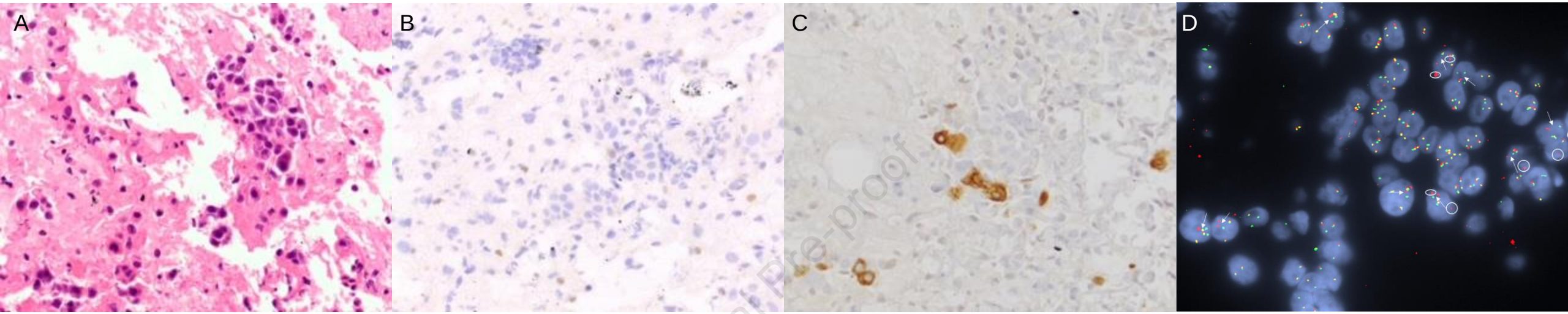

Figure 2

Primary Resistance to Larotrectinib Arising from Baseline Subclonal NTRK1 Fusion in Non-Small Cell Lung Cancer

MCB: Writing - original draft and review and editing. **JST:** Writing - review and editing. **MMK:** Writing - review and editing, resources. **LLR:** Writing - review and editing, resources. **IDJ:** Conceptualization, writing - original draft and review and editing.