

# Durable Clinical Response to Entrectinib in *NTRK1*-Rearranged Non-Small Cell Lung Cancer

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**Introduction:** Chromosomal rearrangements involving neurotrophic tyrosine kinase 1 (*NTRK1*) occur in a subset of non-small cell lung cancers (NSCLCs) and other solid tumor malignancies, leading to expression of an oncogenic TrkA fusion protein. Entrectinib (RXDX-101) is an orally available tyrosine kinase inhibitor, including TrkA. We sought to determine the frequency of *NTRK1* rearrangements in NSCLC and to assess the clinical activity of entrectinib.

**Methods:** We screened 1378 cases of NSCLC using anchored multiplex polymerase chain reaction (AMP). A patient with an *NTRK1* gene rearrangement was enrolled onto a Phase 1 dose escalation study of entrectinib in adult patients with locally advanced or metastatic tumors (NCT02097810). We assessed safety and response to treatment.

**Results:** We identified *NTRK1* gene rearrangements at a frequency of 0.1% in this cohort. A patient with stage IV lung adenocarcinoma

with an *SQSTM1-NTRK1* fusion transcript expression was treated with entrectinib. Entrectinib was well tolerated, with no grade 3–4 adverse events. Within three weeks of starting on treatment, the patient reported resolution of prior dyspnea and pain. Restaging CT scans demonstrated a RECIST partial response (PR) and complete resolution of all brain metastases. This patient has continued on treatment for over 6 months with an ongoing PR.

**Conclusions:** Entrectinib demonstrated significant anti-tumor activity in a patient with NSCLC harboring an *SQSTM1-NTRK1* gene rearrangement, indicating that entrectinib may be an effective therapy for tumors with *NTRK* gene rearrangements, including those with central nervous system metastases.

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## INTRODUCTION

Chromosomal rearrangements resulting in expression of oncogenic receptor tyrosine kinase fusions occur in a subset of epithelial malignancies and can underlie sensitivity to tyrosine kinase inhibitors<sup>1</sup>. The tropomyosin-related kinase (Trk) proteins TrkA, TrkB, and TrkC are receptor tyrosine kinases encoded by *NTRK1*, *NTRK2*, and *NTRK3*, respectively, that normally function during neuronal development<sup>2</sup>. *NTRK1* gene rearrangements in NSCLC were first described among a population of NSCLC patients with adenocarcinoma histology and no detectable *EGFR* or *KRAS* mutation, or *ALK* or *ROS1* gene rearrangement<sup>3</sup>. Among this cohort, *NTRK1* rearrangements were detected at a frequency of 3% by fluorescence in situ hybridization (FISH). *NTRK1* gene rearrangements also occur at low frequencies in other solid tumor malignancies, including in colorectal carcinoma, intrahepatic cholangiocarcinoma, papillary thyroid cancer, spitzoid neoplasms, glioneuronal tumors, and sarcoma<sup>4,5</sup>. Gene rearrangements involving *NTRK2* and *NTRK3* have also been observed in a variety of solid tumor malignancies<sup>5</sup>. In all cases, the sequenced fusion gene product maintains the tyrosine kinase domain, supporting the hypothesis that kinase signaling through these fusion products promotes cell growth and survival.

Entrectinib is an orally available small molecule inhibitor of TrkA, TrkB, TrkC, ROS1 and ALK. In biochemical kinase assays, entrectinib inhibits TrkA with an IC<sub>50</sub> of 1.7 nM. Entrectinib inhibits cell proliferation, TrkA phosphorylation

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and downstream pathway activation in the KM-12 human colorectal cell line which expresses the *TPM3-NTRK1* fusion product<sup>6</sup>. In a KM-12 xenograft, entrectinib induces tumor regression and durable tumor stabilization<sup>6</sup>. Similar potency is observed in a model of patient-derived cells (PDC) from a colorectal cancer patient harboring *TPM3-NTRK1* fusion<sup>7</sup>. Entrectinib is currently in clinical development in trials for patients with locally advanced or metastatic solid tumor malignancies with molecular alterations (phase 1/2a, NCT 02097810) or gene rearrangements (phase 2, NCT 02568267) in *NTRK1*, *NTRK2*, *NTRK3*, *ROS1* or *ALK*.

## MATERIALS AND METHODS

### AMP-PCR to Identify *NTRK1* Gene Rearrangements in NSCLC

For detection of fusion transcripts involving *NTRK1* from clinical samples, we implemented AMP, as previously described<sup>8</sup>. The sequencing library targets known fusion exons in multiple oncogenes including *ALK*, *ROS1*, *RET* and *NTRK1*.

### Fluorescence In Situ Hybridization (FISH)

Cases with suspected *NTRK1* gene rearrangements were further confirmed using FISH. We used a break-apart FISH approach using BAC clones corresponding to the 5' (RP11-1047J23) and 3' (RP11-1038N13) sequences flanking the *NTRK1* gene labeled by nick translation in green and red, respectively. FFPE slides were de-paraffinized, treated with protease, and co-denatured with FISH probes using a Hybrite slide processor (Abbott Molecular), washed, counterstained, and cover-slipped FISH slides and analyzed using an Olympus BX61 fluorescence microscope equipped with red, green, and DAPI filters. Images were captured and analyzed using Cytovision software (Genetix Inc., San Jose, CA).

### Phase 1 Clinical Trial of Entrectinib

The Phase 1 dose escalation study of entrectinib in adult patients with locally advanced or metastatic tumors is ongoing (NCT02097810). Eligibility criteria include locally advanced or metastatic solid tumor malignancy with a *NTRK1*, *NTRK2*, *NTRK3*, *ROS1* or *ALK* molecular alteration, measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, and Eastern Cooperative Oncology Group (ECOG) performance status (PS)  $\leq 2$ . Patients with controlled asymptomatic central nervous system (CNS) involvement of disease are allowed in the absence of anticonvulsant therapy. Toxicities are graded using Common Terminology Criteria for Adverse Events (CTCAE) v4.0, and responses are measured using RECIST v1.1.

## RESULTS

Between July 1 2013 and September 15 2015, we performed AMP testing on 1378 NSCLC tumor specimens and identified two with *NTRK1* gene rearrangements (0.1%, 95% confidence interval 0.01%, 0.5%). One was a *TPM3-NTRK1* rearrangement previously described<sup>8</sup>. In a second case, we identified a fusion transcript containing sequence from *SQSTM1* (sequestosome 1) and *NTRK1*. Primers extending

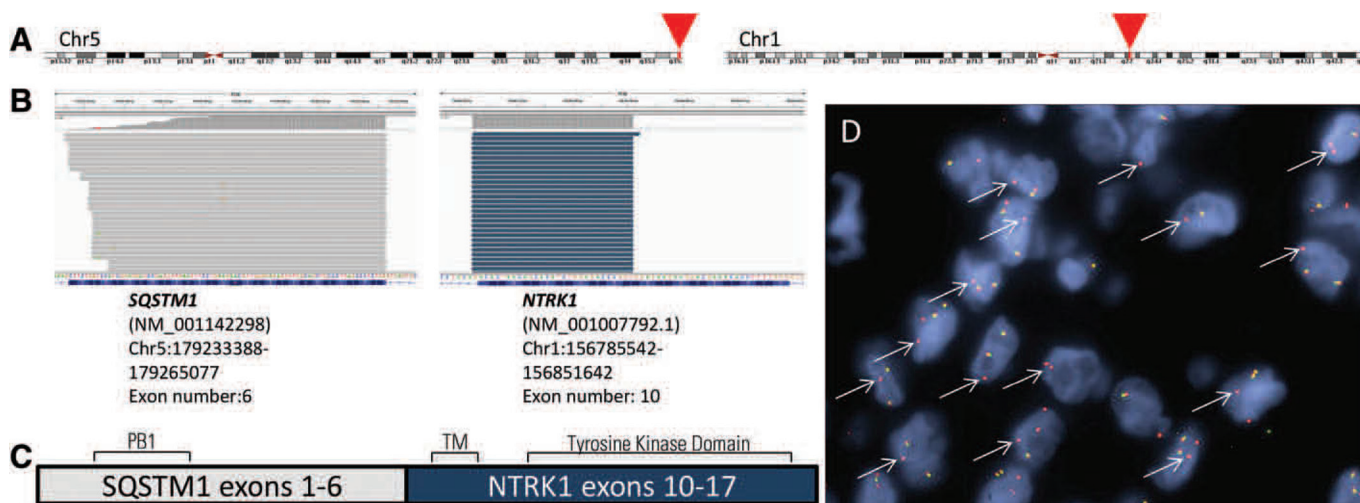
from exon 10 of *NTRK1* amplified contiguous sequence that mapped to exon 6 of *SQSTM1* (Figure 1A–B). The junction of the amplified fusion transcript lay at the exon boundaries, and resulted in an in-frame fusion. The predicted fusion gene product includes the PB1 dimerization domain of *SQSTM1*<sup>9</sup> with the tyrosine kinase domain of *TrkA* (Figure 1C). The *NTRK1* rearrangement was confirmed by FISH (Figure 1D). Of note, *SQSTM1* has previously been described as a fusion partner with *ALK* in NSCLC and in B-cell lymphoma<sup>10,11</sup>. Based on these results, we hypothesized that the fusion protein in this patient's tumor was expressed and functional.

The patient is a male who was 45 years old when he was diagnosed with stage IV lung adenocarcinoma in 2013. He had a 30 pack year smoking history, and he developed progressive disease despite four prior lines of therapy, including carboplatin and pemetrexed, pembrolizumab, docetaxel, and vinorelbine. At the time of enrollment, the patient had an ECOG performance score of 2 with baseline chest wall pain, dyspnea at rest, and an oxygen requirement of 3L/min by nasal cannula. He had a palpable left chest wall mass measuring approximately 5 cm in diameter with associated palpable 1 cm satellite nodules extending from the mass into the left axilla. Staging head CT demonstrated 15–20 brain metastases that were asymptomatic and new compared to the most recent prior brain MRI from 18 months earlier.

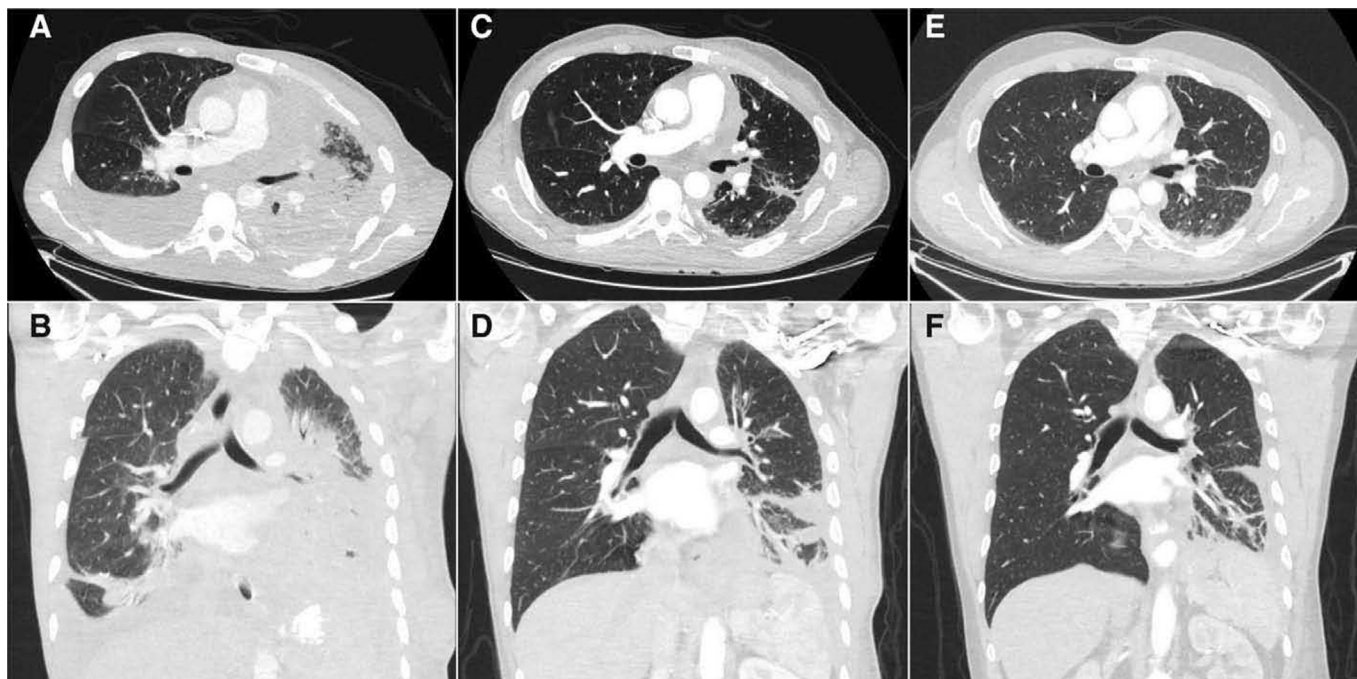
He enrolled in a phase 1 trial with entrectinib at 400 mg/m<sup>2</sup> PO daily. The drug was well tolerated, with possibly related adverse events of grade 1 dysphagia, grade 1 paresthesias, and grade 2 fatigue that all subsequently resolved. Within three weeks of starting treatment, the patient reported resolution of pain and dyspnea, and no longer required supplemental oxygen.

Restaging CT scans at 26 days demonstrated RECIST partial response of -47% (Figure 2 A–D). There was resolution of the prior right-sided pleural effusion, marked interval re-expansion of the left upper and lower lobes, and partial resolution of the previous diffuse consolidative opacity in the left lung. There was decreased ground glass and septal thickening of the tumor in other areas of the left upper lobe, thought to represent improvement of lymphangitic spread of disease. There was decreased pleural thickening on the left. In the mediastinum, there was significant interval regression of previous bulky bilateral lymphadenopathy. In the abdomen, there was significant improvement of previous para-aortic lymphadenopathy. There was increased sclerosis of previously visualized bone metastases, consistent with treatment response. The left-sided chest wall mass was smaller and flatter on exam, and the satellite nodules were no longer palpable. The radiographic response was confirmed and ongoing in subsequent scans. At day 155, restaging scans demonstrated further tumor reduction, -77% compared to baseline (Figure 2 E, F). There was ongoing improvement of the left lower lobe consolidation and ongoing decreased size of mediastinal lymph nodes. The previous left chest wall mass was no longer palpable or visible on scans. He had no new sites of disease involvement.

The patient also had a complete response of all brain metastases on entrectinib. Fifteen to 20 baseline brain metastases had been identified, the largest of which were in the left occipital region, the right thalamus, and the left cerebellum. These measured up to 1.7 cm in diameter (Figure 3A–C). At day 26, a head CT with contrast demonstrated near resolution



**FIGURE 1.** *SQSTM1-NTRK1* fusion transcripts detected by AMP in a NSCLC. (A) Contiguous reads from amplified transcripts mapped to the locations of *SQSTM1* on chromosome 5 and *NTRK1* on chromosome 1 (red arrowheads). (B) Visualization of subset of sequence read pileup showing fusion reads. The y axis represents read coverage. The x axis represents reference bases and their respective codons below. Shown in gray are read portions corresponding to the randomly ligated universal adapter end with staggered distribution of reads and differing start positions. Shown in blue are read portions corresponding to the anchored end targeted with GSP1 and GSP2. The fusion is in-frame with respect to both transcripts. (C) Schematic drawing of gene fusion involving *SQSTM1* and *NTRK1*. TM, transmembrane domain. Not drawn to scale. (D) FISH confirmation *SQSTM1-NTRK1* rearrangement showed individual 3' (red only) probe signals that represent a region downstream of the *NTRK1* gene along with normal paired green and red signals that represent non-rearranged alleles. White arrows highlight representative red only signals.

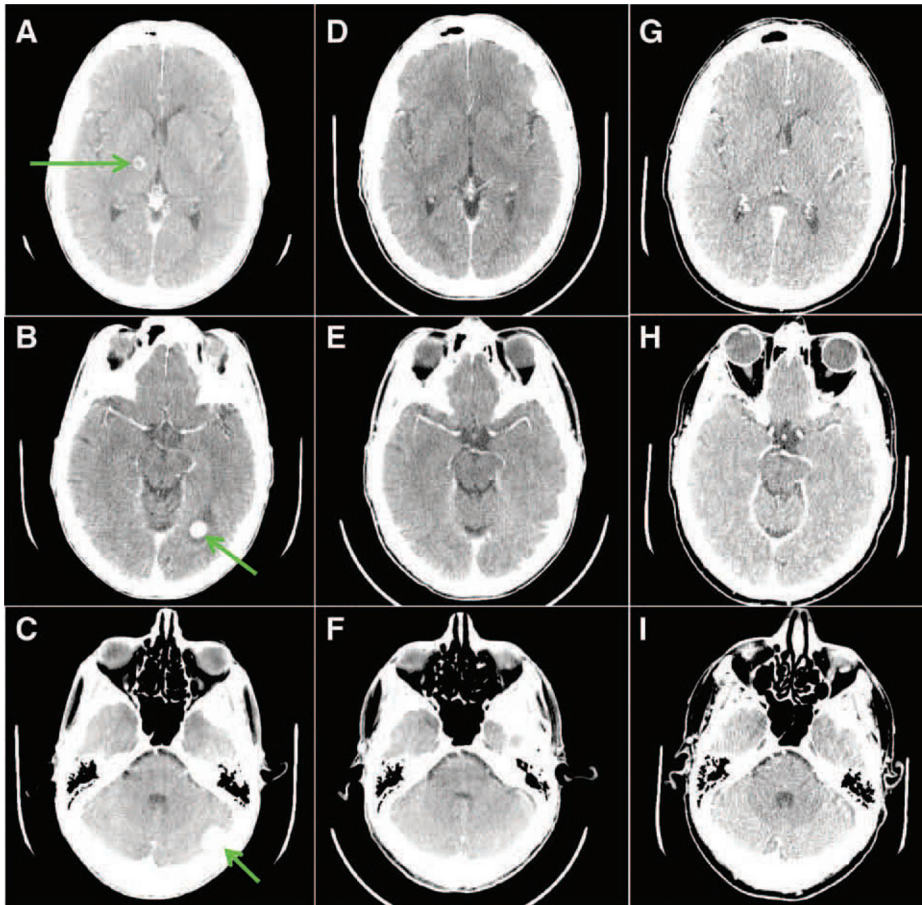


**FIGURE 2.** Partial response to entrectinib. Horizontal (A) and coronal (B) images of the chest at day -7 (baseline scan), day 26 (C, D), and day 155 (E, F) on entrectinib.

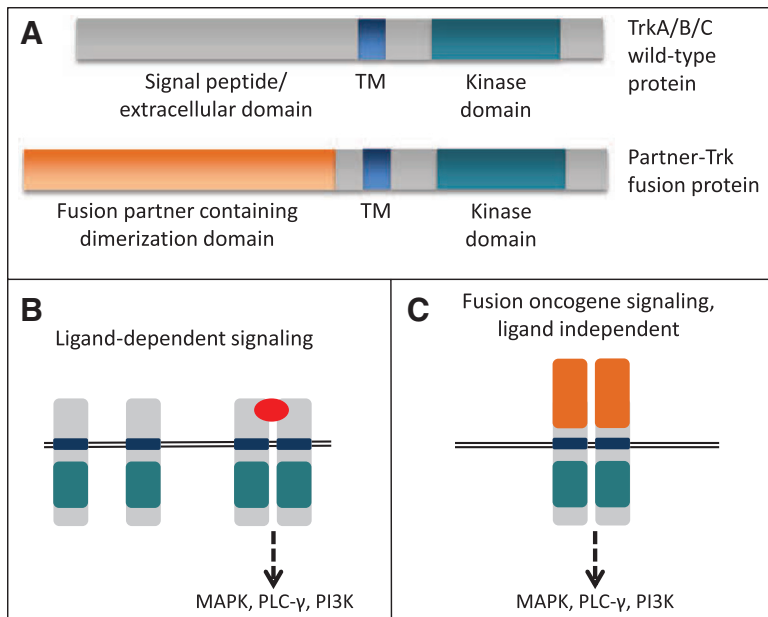
of these metastases (Figure 3 D–F), and by day 155 the patient continued to have a complete response of all brain metastases (Figure 3 G–I). To date, the patient has continued on entrectinib for over 6 months with ongoing partial response and current duration of response 4.1 months.

### DISCUSSION

Trk signaling is normally involved in neuronal development, synaptic function and plasticity. Wild-type TrkA, TrkB and TrkC, function through ligand-dependent dimerization, leading to phosphorylation of tyrosine residues within



**FIGURE 3.** Complete response of brain metastases to entrectinib. (A-C) Baseline head CT scan at day -7 demonstrating metastases (green arrows) in the right thalamus (A), left occipital lobe (B) and left cerebellum (C). (D-I) Restaging head CT scans at day 26 (D-F) and day 155 (G-I) on entrectinib.



**FIGURE 4.** Trk signaling. (A) Schematic diagrams showing wild-type TrkA, TrkB or TrkC protein, top, and showing an oncogenic fusion involving a partner gene that contains a dimerization domain and the kinase domain of TrkA, TrkB or TrkC, bottom. Of note, the fusion shown here includes the transmembrane (TM) domain, though Trk fusion proteins that lack the TM domain have also been described. (B) In the absence of ligand, left, Trk proteins do not dimerize or activate downstream signaling pathways. In the presence of ligand (red circle), Trk proteins dimerize, leading to downstream pathway activation. Double line represents the cell membrane. (C) Fusion oncogenes dimerize in a ligand-independent manner, leading to constitutive activation and downstream signaling. Proteins and their domains are not drawn to scale.

the cytoplasmic domain, recruitment of scaffold proteins, and activation of downstream signaling (Figure 4A, B). Signaling through the mitogen-activated protein kinase (MAPK), phospholipase C- $\gamma$  (PLC- $\gamma$ ), and phosphatidylinositol 3-kinase

(PI3K) pathways is thought to mediate cell differentiation and survival<sup>2</sup>. *NTRK1* gene rearrangements, which result in expression of TrkA fusion proteins, represent one of the newest oncogenic drivers in NSCLC. Like gene rearrangements

involving other receptor tyrosine kinases such as *ALK* or *ROS1*, *NTRK* gene fusions are thought to function through ligand independent dimerization and downstream pathway activation (Figure 4C). In cell lines that lack oncogenic potential at baseline, forced expression of either the *MPRIIP-NTRK1* or *CD74-NTRK1* gene fusion results in phosphorylation of TrkA, anchorage independent growth, and tumor formation in nude mice<sup>3</sup>. Pharmacologic inhibition of TrkA signaling in cell lines expressing constitutively active TrkA causes decreases cell viability *in vitro*<sup>3,4,7,12</sup>. It has therefore been hypothesized that *NTRK1* gene rearrangements in NSCLC drive tumor progression and may confer sensitivity to TrkA inhibition.

In a patient with *NTRK1*-rearranged NSCLC, treatment with entrectinib led to rapid and clinically significant improvement of disease with minimal side effects. Notably, all of the patient's CNS metastases, which were discovered on screening and were not treated with radiation, completely resolved on entrectinib, indicating potent CNS penetration and activity of the drug. This response indicates that, like *ALK* and *ROS1* rearrangements in NSCLC, TrkA fusions in NSCLC drive tumor growth and survival and are targetable. A recent case report describes a similar significant clinical response to Loxo-101, a small molecule pan-Trk inhibitor, in a patient with metastatic sarcoma harboring an *LMNA-NTRK1* gene rearrangement<sup>4</sup>, further supporting the hypothesis that *NTRK* gene rearrangements can act as potent oncogenic drivers in multiple tumor histologies. We conclude that entrectinib may be an effective anti-tumor therapy for patients with *NTRK1* gene rearrangements, including patients with metastatic CNS disease.

*NTRK1* gene rearrangements in NSCLC are rare. Our identification of *NTRK1* gene rearrangements in 0.1% of NSCLCs in this cohort is lower than the frequency of up to 3%, as described previously<sup>3</sup>. Consistent with our findings, however, is the observation that no *NTRK1* fusion transcripts were detected by RT-PCR among a cohort of 268 Japanese NSCLC surgical resection cases<sup>12</sup>. Furthermore, the AMP assay has demonstrated 100% sensitivity (95% confidence limit 99.3–100%) compared to FISH for detection of gene rearrangements involving *ALK*, *ROS1* and *RET*<sup>8</sup>. The inconsistency between our results and those previously published<sup>3</sup> may be partially explained by the fact that Vaishnavi and colleagues focused on a cohort of patients that had previously screened negative for other gene alterations by standard clinical testing, whereas our cohort was largely previously unscreened. Furthermore, FISH may detect chromosomal rearrangements that do not result in expression of a fusion transcript, or in which a fusion transcript is expressed at low levels, whereas AMP detects the fusion RNA transcript. Finally, we note that our cohort includes a mix of metastatic and early-stage NSCLCs. It is possible that *NTRK1* rearrangements may be more common in metastatic disease, as may be the case for *ALK* rearrangements<sup>13</sup>. Given the

low frequency of *NTRK1* gene rearrangements, screening using FISH may not be practical due to limitations of tissue availability or cost. Incorporation of *NTRK1* rearrangement testing into a multiplexed NGS based assay, as we have done using AMP, allows for simultaneous screening for *NTRK1* gene rearrangements among other more common gene rearrangements<sup>8</sup>. The profound and durable clinical response of a patient with *NTRK1*-rearranged NSCLC to entrectinib argues strongly for screening patients with both NSCLC and other solid tumor malignancies for *NTRK* gene rearrangements.

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