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

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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. 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. 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. 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. Gene rearrangements involving NTRK2 and NTRK3 have also been observed in a variety of solid tumor malignancies. 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 IC50 of 1.7 nM. Entrectinib inhibits cell proliferation, TrkA phosphorylation and downstream pathway activation in the KM-12 human colorectal cell line which expresses the TPM3-NTRK1 fusion product. In a KM-12 xenograft, entrectinib induces tumor regression and durable tumor stabilization. Similar potency is observed in a model of patient-derived cells (PDC) from a colorectal cancer patient harboring TPM3-NTRK1 fusion. 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. 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’ (RP11-1047123) 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) ≤ 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. 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 PBI dimerization domain of SQSTM1 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. 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/m2 PO daily. The drug was well tolerated, with possibly related adverse events of grade 1 dysguesia, 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.
Entrectinib in NTRK1-rearranged NSCLC

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

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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-γ (PLC-γ), and phosphatidylinositol 3-kinase (PI3K) pathways is thought to mediate cell differentiation and survival3. NTRK1 gene rearrangements, which result in expression of TrkA fusion proteins, represent one of the newest oncogenic drivers in NSCLC. Like gene rearrangements

**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.
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 MPRIP-NTRK1 or CD74-NTRK1 gene fusion results in phosphorylation of TrkA, anchorage independent growth, and tumor formation in nude mice1. Pharmacologic inhibition of TrkA signaling in cell lines expressing constitutively active TrkA causes decreases cell viability in vitro.1,4,7,12 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 rearrangement4, 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 previously3. 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 cases12. Furthermore, the AMP assay has demonstrated 100% sensitivity (95% confidence interval 99.3–100%) compared to FISH for detection of gene rearrangements involving ALK, ROS1 and RET7. The inconsistency between our results and those previously published3 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 rearrangements13. 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 rearrangements3. 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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REFERENCES