Comprehensive genomic profiling identifies novel NTRK fusions in neuroendocrine tumors

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ABSTRACT

CGP results from >60,000 cases were screened to identify NTRK fusion events from cases of neuroendocrine tumors. 2417 NET patients from diverse anatomic sites were identified. From this dataset, six cases harbored NTRK fusions which included intra- and inter-chromosomal translocations. A NTRK fusion frequency of approximately 0.3% was found across all subtypes of NETs. Three cases involved translocations of NTRK1 with unique fusion partners (GPATCH4, PIP5K1A, CCDC19). Co-occurring alterations occurred in five cases. NTRK alterations were identified in nearly the full spectrum of NETs, including from the small intestine, pancreas, lung, and others. With the late stage clinical development of NTRK TKIs (including entrectinib and larotrectinib), these findings may further inform targeted approaches to therapy in NET.

INTRODUCTION

We recently reported on a patient with the first identified NTRK fusion (ETV6:NTRK3) in a neuroendocrine tumor (NET) [1]. NTRK1, 2, and 3 encode the neurotrophic tropomyosin receptor kinase (TRK) family of receptor tyrosine kinases, TRKA, TRKB, and TRKC, respectively, and NTRK alterations are known to be oncogenic [2]. This patient was accrued to the STARTRK2 trial (NCT02568267) and experienced a dramatic and protracted response to entrectinib, an oral tyrosine kinase inhibitor (TKI) of the protein products of NTRK, ROS1, and ALK alterations. This response suggested that NTRK fusions may play an important role in NET pathogenesis made even more significant by the advent of NTRK targeting therapies. Next-generation sequencing (NGS) studies of NETs of the small intestine, pancreas, and lung had not previously revealed NTRK fusions in NETs [3–5]. We sought to interrogate a large NET database assayed with comprehensive genomic profiling (CGP) to document additional NTRK fusions in NET.

RESULTS

CGP was performed on specimens from 2417 NET patients from diverse anatomic sites in the course of clinical care. From this dataset, six cases harbored NTRK fusions which included intra- and inter-chromosomal translocations (see Table 1). Of these cases, five were females. The anatomic sites of origin included pancreas (n=2), uterus (1), lung (1), and unknown (2). Three cases involved translocations of NTRK1 with unique fusion partners (GPATCH4, PIP5K1A, CCDC19). Co-occurring alterations occurred in five cases. Of non-NTRK genes altered, TP53 was the most common occurring in 50% (3/6) cases. Additional co-occurring alterations included ARID1A, ATM, CDKN2A, EPHA3, MYC, NFE, PTEN, RB1, SLIT2, and SPTA1. Two cases had an alteration involving the MAPK pathway (KRAS G12D and KRAS Q61R); there were no other alterations involving RAF.
MEK, or ERK. The mean tumor mutational burden (TMB) was 3.81 mutations per DNA megabase (range 0.87-7.2 mut/Mb).

DISCUSSION

Our analysis identified six NET specimens with NTRK fusions out of a total of 2417 evaluated in the Foundation Medicine database. Including the index patient from the case report, we found a NTRK fusion frequency of approximately 0.3% across all subtypes of NETs. NTRK alterations were identified in nearly the full spectrum of NETs, including from the small intestine, pancreas, lung, and others. These gene fusions were diverse with NTRK1, 2, 3 fragments each attached to a unique fusion partner. Two patients had a NTRK1 fusion with co-occurring mutations in KRAS Q61R and KRAS G12D, respectively. Although it is unusual for KRAS mutations to co-occur with other driver tyrosine kinase alterations, this scenario has been reported with TKI efficacy despite the KRAS mutation [6]. In three patients, NTRK was 5’ to its fusion partner. Oncogenic NTRK fusions generally occupy the 3’ fusion position suggesting that the three 5’ NTRK fusions we report may not be functional. However, a variety of alternative oncogenic NTRK alterations have been reported, including point mutations, deletions, duplications, and other less well-described mechanisms [7–10]. In addition, the fusions we report satisfy Foundation Medicine’s reporting rules and therefore would have qualified for the STARTRK2 entrectinib registrational trial.

No previous NGS analyses of NETs have identified NTRK gene fusions. We identified NTRK gene fusions in two pancreas NET patients despite the absence of these gene fusions among 102 pancreas NET patients screened with whole genome and RNA sequencing [4]. The Foundation Medicine analysis utilized targeted exome sequencing deploying intron baiting for all coding exons of NTRK1,2,3 with additional baits for introns 7-11 of NTRK1 and intron 12 of NTRK2. For a tumor fraction specimen of >20%, intron baiting was reported to have a sensitivity of 100% and a positive predictive value of >98% [11]. In contrast, whole genome and RNA sequencing have been preferred methods for detecting translocations that are large and have numerous upstream fusion partners, similar to NTRK translocations. The most likely explanations for the discrepancy of NTRK fusion detection among these reports is the low absolute frequency of NTRK fusions and the relatively small number of pancreatic NETs that were screened in the Scarpa, et al paper.

NTRK fusions have been detected at a low frequency in a variety of cancers, but appear to have a higher prevalence in rare cancers. Analysis of the RNA-seq data
set from The Cancer Genome Atlas detected NTRK fusions in only nine of 20 solid tumor types screened. In these nine tumor types the NTRK fusion prevalence ranged from 0.09% to 2.4% (Table 2) [12]. Our finding of 0.3% NTRK fusion rate in NETs indicates that these fusions are relatively common in NETs compared to many other malignancies.

This analysis has several limitations. We are missing information on stage, grade, Ki-67 status, and NET subtypes screened. Our report also lacks orthogonal validation to ensure the NTRK fusions we report are in-frame and functional. Foundation Medicine does not store tissue for this purpose so these studies were simply not possible. However, DNA based testing is well established for detecting clinically actionable NTRK fusions and is allowed for accrual to NTRK inhibitor trials.

Our report of a 0.3% prevalence rate is on par with genomic alterations that have impacted standard of care in other malignancies. Malignancies are increasingly defined by even ultra-rare genomic events. Late stage clinical development of entrectinib and larotrectinib, TKIs with high affinity binding for NTRK fusion protein products that have reported remarkable response and survival endpoints in various basket studies, makes the finding of NTRK fusions throughout the spectrum of NET subtypes clinically important [13, 14]. Although additional efforts can further clarify the prevalence of NTRK fusions in NET, determination of NTRK fusion status should be incorporated into the care of NET patients.

**MATERIALS AND METHODS**

The methods used for genomic profiling have been previously described [15, 16]. Formalin-fixed, paraffin-embedded slides or blocks from tumor samples were submitted to a Clinical Laboratory Improvement Amendments (CLIA)-certified, College of American Pathologists-accredited reference laboratory (Foundation Medicine, Cambridge, MA). Tumor samples submitted for profiling were reviewed by board-certified pathologists for tumor purity as well as the diagnosis made by the treating physicians. At least 50 ng of DNA per specimen was extracted. Next-generation sequencing was performed on hybridization-captured, adaptor ligation–based libraries to high, uniform coverage (> 500×) for all coding exons of 315 cancer-related genes and 28 genes commonly rearranged in cancer. Base substitutions, short insertions, deletions, copy number changes, gene fusions, and rearrangements were identified and reported for each patient sample. CGP results from >60,000 cases were reviewed from the Foundation Medicine database. NTRK fusion events from cases of neuroendocrine tumors were identified and reported as below.

### Table 2: Frequency of NTRK fusion products across multiple tumor types

| Tumor Type                                  | No. of Tumors harboring NTRK fusion product/Total No. Samples Tested | Percent (%) |
|---------------------------------------------|---------------------------------------------------------------------|-------------|
| Thyroid carcinoma                           | 12/498                                                              | 2.41        |
| Sarcoma                                     | 1/103                                                               | 0.97        |
| Colon adenocarcinoma                        | 2/286                                                               | 0.70        |
| Sarcoma                                    | 1/157                                                               | 0.64        |
| Head and neck squamous cell carcinoma       | 2/411                                                               | 0.49        |
| Brain low-grade glioma                      | 2/461                                                               | 0.43        |
| Skin cutaneous melanoma                     | 1/374                                                               | 0.27        |
| Lund adenocarcinoma                         | 1/513                                                               | 0.19        |
| Breast invasive carcinoma                   | 1/1072                                                              | 0.09        |

### Author contributions

Conception/Design: DS; Provision of study materials or patients: DS, SMA; Collection and/or assembly of data: DS, MSB, JH, DCP, GF, SMA; Data analysis and interpretation: DS, MSB, JH, DCP, GF, SMA; Manuscript writing: All authors; Final approval of manuscript: All authors.

### CONFLICTS OF INTEREST

DCP, GF, VAM, JSR, SMA are employees of and have equity interest in Foundation Medicine, Inc. DS has a patent for treating NTRK altered neuroendocrine tumors.

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