Pan Solid Tumor Identification of NTRK Fusions Utilizing RNA Sequencing Identifies Diverse Fusion Partners

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ABSTRACT

Introduction:
NTRK gene fusions were the first genomic alteration with an FDA approved pan-solid tumor targeted therapy. While rare, identification of NTRK fusions is critical for optimal patient care but can be challenging to detect due to the variety of fusion partners and large intronic regions.
Methods:
We analyzed data from 6,730 FFPE solid tumor samples that had comprehensive genomic profiling (CGP), including non-small cell lung carcinoma (NSCLC, n=2410), breast carcinoma (BC, n=459), skin basal cell carcinoma (BCC, n=2), angiosarcoma (AS, n=10), melanoma (n=135), colorectal carcinoma (CRC, n=815), pancreatic carcinoma (PC, n= 233), and ovarian carcinoma (OC, n=102). CGP included RNA sequencing for gene fusions and DNA sequencing for detection of genomic alterations (GAs).
Results:
We identified *NTRK* fusions (*NTRK1* = 5, *NTRK2* = 3, and *NTRK3* = 8) by RNA sequencing in 16 patients (age 26-84 years, mean=60 years, 50% male, 50 % female) in NSCLC (n=8, 0.33%), BC (n=1, 0.22%), BCC (n=1, 50%), CRC (n=2, 0.25%), Melanoma (n=1, 0.74%), AS (n=1, 10%), PC (n=1, 0.43%) and, OC (n=1, 0.98%). Fusion breakpoints were present in *NTRK1* introns 1 and 11, *NTRK2* introns 2, 3, 9, and 15, and *NTRK3* introns 3, 5, 14, and 19, spanning 0.32 Mb. 10 novel fusions were identified: *HMCN1-NTRK1, ASTN2-NTRK2, MSANTD3-NTRK2, PRKACA-NTRK2, ERBB2-NTRK3, FAM174B-NTRK3, PIAS1-NTRK3, SIN3A-NTRK3*, and *TCF-NTRK3*. Previously described fusions (*LMNA-NTRK1, PEAR-NTRK1, RABGAP1L-NTRK1, TP53-NTRK1, KANK1-NTRK3, AGBL1-NTRK3*, and *SASH-NTRK3*) were also identified. This clinical cohort did not contain NTRK-associated cancers (inflammatory myofibroblastic tumors, secretory breast cancers, or high-grade pediatric gliomas). Despite lacking these tumor types with frequent *NTRK* fusions, the rate of *NTRK* fusions across all solid tumors in this cohort was 0.24%.
In NSCLC with *NTRK* fusions, *TP53* was the most common recurrent GA (n=7/8). Half of NSCLC cases had co-occurring driver alterations, with *KRAS* (G12C, G13D), *EGFR* (S752_I759del), *BRAF* (G649A) identified. Outside of NSCLC, the only co-occurring driver GAs were an *ERBB2* amplification (BC) and an *ITSN-ALK* fusion (PC). One CRC sample was MSI-high and 47% of samples had a tumor mutational burden > 10 mutations/Mb (TMB-high). 50% of the NSCLC were TMB-high.
Conclusions:
NTRK1, NTRK2, and *NTRK3* fusions are clinically relevant driver alterations across solid tumor types. These fusions are difficult to detect, as the breakpoints occur across large intronic regions and they have many partner genes, with 10 novel fusion partners identified in this study. These data emphasize how important CGP with RNA sequencing is to identify all *NTRK* fusions for optimal patient treatment.

Background

- Neurotrophic tropomyosin receptor kinase (*NTRK*) gene fusions are oncogenic drivers in a variety of pediatric and adult solid tumors but are found at a low frequency (<1%).[1]
- >60 known fusion partners have been identified for *NTRK*1/2/3 across multiple tumor types. The pathogenicity of an in-frame fusions results in constitutive activation of the TRK receptor.[2]
- Routine assessment for *NTRK* fusions and treatment with TRK inhibitors have been recommended in 25 different tumor types.[3]
- *NTRK* gene fusions were the first genomic alteration with an FDA approved pan-solid tumor targeted therapy. While rare, identification of *NTRK* fusions is critical for optimal patient care.[4]
- In this study, we aim to describe the landscape of *NTRK*1/2/3 fusions detected across solid tumors by RNA sequencing and characterize the co-alterations detected in patients with *NTRK* fusion positive cancers.

METHODS

- We analyzed data from 6,730 FFPE solid tumor samples that had comprehensive genomic profiling (CGP) during the course of routine clinical care, including samples of: non-small cell lung carcinoma (NSCLC, n=2410), breast carcinoma (n=459), skin basal cell carcinoma (n=2), angiosarcoma (n=10), melanoma (n=135), colorectal carcinoma (CRC, n=815), pancreatic carcinoma (n= 233), and ovarian carcinoma (n=102).
- OmniSeq® INSIGHT was utilized for CGP in this study and is a next generation sequencing-based (NGS) assay for the detection of genomic variants, signatures, and immune gene expression in FFPE tumor tissue. DNA sequencing with hybrid capture is used to detect small variants in the full exonic coding region of 523 genes (single and multi-nucleotide substitutions, insertions, and deletions), copy number alterations in 59 genes (gains and losses), as well as analysis of microsatellite instability (MSI) and tumor mutational burden (TMB) genomic signatures. RNA is sequenced with hybrid capture is used to detect fusions and splice variants in 55 genes, in addition to mRNA expression in 64 immune genes.

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2. Kummar S, Lassen UN: TRK Inhibition: **A New Tumor-Agnostic Treatment Strategy**. Target Oncol 2018, 13(5):545-556.
3. Klink AJ, Kavati A, Gassama AT, Kozlek T, Gajra A, Antoine R: **Timing of NTRK Gene Fusion Testing and Treatment Modifications Following TRK Fusion Status Among US Oncologists Treating TRK Fusion Cancer**. Target Oncol 2022, 17(3):321-328.
4. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A, Bruno DS, Chang JY, Chirieac LR, D'Amico TA *et al*: **Non-Small Cell Lung Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology**. J Natl Compr Canc Netw 2022, 20(5):497-530.

Results

Patient profiled by Comprehensive Genomic Profiling

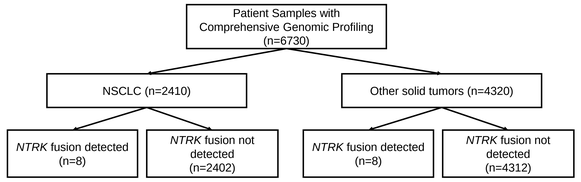


Figure 1: Identification of patient cohort

	Non-Small Cell Lung Carcinoma (n=2,410)	Breast Carcinoma (n=459)	Colorectal Carcinoma (n=815)	Basal Cell Carcinoma (n=2)	Ovarian Carcinoma (n=102)	Pancreatic Carcinoma (n=233)	Angiosarcoma (n=10)	Melanoma (n=135)
Fusion Prevalence (%) *NTRK* 1, 2, &3	8 (0.33%)	1 (0.22%)	2 (0.25%)	1 (50%)	1 (0.98%)	1 (0.43%)	1 (10%)	1 (0.74%)
Median Age - (Years)	58 (26-83)	43	61 (56-66)	63.5 (43-84)	51	75	74	74
Gender, n (%)								
Male	4 (50%)	-	1 (50%)	1 (50%)	-	1 (100%)	-	1 (100%)
Female	4 (50%)	1 (100%)	1 (50%)	-	1 (100%)	-	1 (100%)	-
Stage, N (%)								
III	-	-	-	-	-	-	-	-
IV	5 (62.5%)	-	1 (50%)	-	-	1 (100%)	-	-
Unknown	3(37.5%)	1 (100%)	-	2 (100%)	1 (100%)	-	1 (100%)	1 (100%)

Table 1: Patient Demographics

Frequency of *NTRK* alterations by tumor type

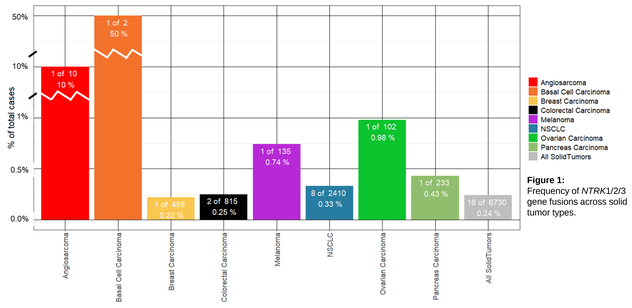


Figure 1: Frequency of *NTRK*1/2/3 gene fusions across solid tumor types.

NTRK gene structure and rearrangement locations

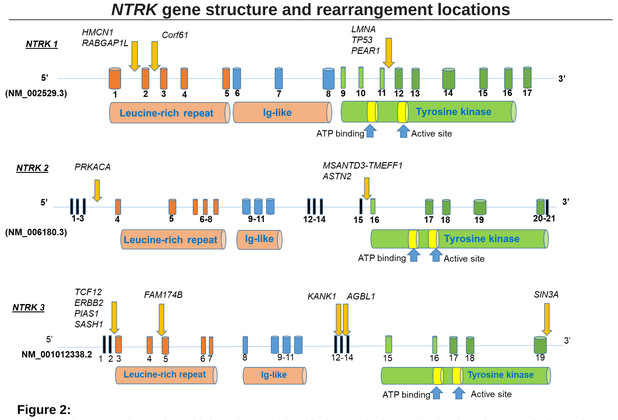


Figure 2:
*NTRK*1/2/3 gene schematics with locations of identified gene fusions. Fusion locations are indicated by a yellow arrow. In all cases, *NTRK1/2/3* was the 3' gene in the gene fusion with an intact tyrosine kinase domain.

RESULTS

	Length (kbp)	Fusion Partner Gene
NTRK1 (1q23.1)		
Intron 1	26.2	HMCN1, RABGAP1L
Intron 2	22.1	C1orf61
Intron 11	0.5	LMNA, TP53, PEAR1
NTRK 2 (9q21.33)		
Intron 3	0.5	PRKACA
Intron 15	6.2	MSANTD3-TMEFF1, ASTN2
NTRK 3 (15q25.3)		
Intron 2	71.6	TCF12, ERBB2, PIAS1, SASH1
Intron 4	36.0	FAM174B
Intron 12	93.2	KANK1
Intron 13	92.1	AGBL1
3' downstream		SIN3A

Table 2: Location of gene fusions, with corresponding intron sizes. Previously known fusions are colored green, novel fusions are colored red.

- Breakpoints for gene fusions occurred across multiple introns, including in *NTRK1* introns 1 and 11, *NTRK2* introns 3, and 15, and *NTRK3* introns 3, 5, 14, and 19, spanning a total of 0.32 Mb of genomic sequence.
- 10 novel fusions were identified and are colored red in the table.
- 7 previously known fusions were identified and are colored green.
- One case had two concurrent gene fusions, *HMCN1-NTRK1 and C1orf61-NTRK1.*

NTRK gene fusions identified

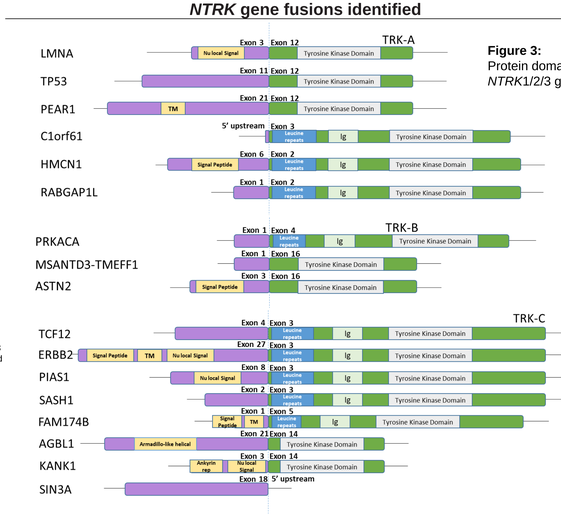
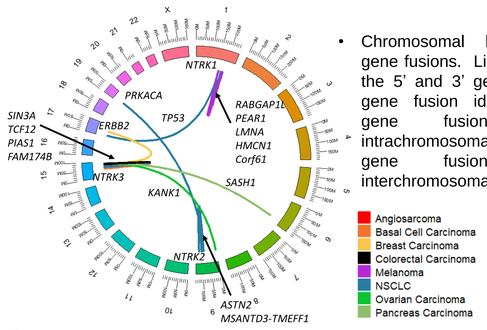


Figure 3:
Protein domains of *NTRK*1/2/3 gene fusions.

Chromosomal location of gene fusions



- Chromosomal location of gene fusions. Lines connect the 5' and 3' gene in each gene fusion identified. 11 gene fusions were intrachromosomal and 5 gene fusions were interchromosomal.

Figure 4
Chromosomal location of gene fusions.

RESULTS

Co-mutation plots

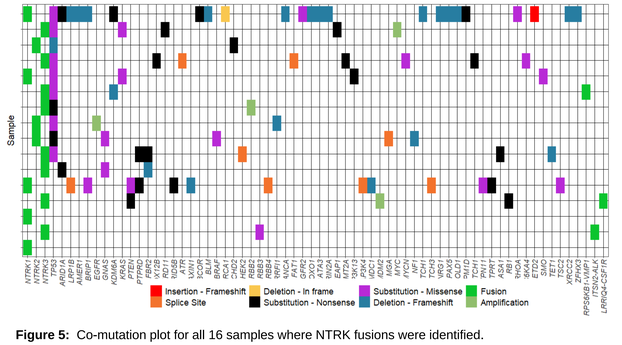


Figure 5: Co-mutation plot for all 16 samples where NTRK fusions were identified.

Co-mutation plots for recurring genomic alterations

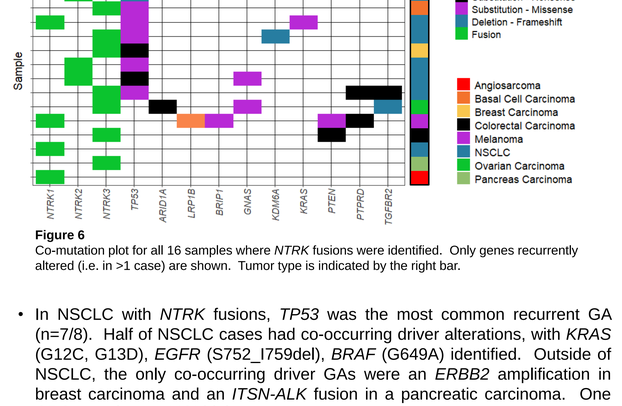


Figure 6
Co-mutation plot for all 16 samples where *NTRK* fusions were identified. Only genes recurrently altered (i.e. in >1 case) are shown. Tumor type is indicated by the right bar.

- In NSCLC with *NTRK* fusions, *TP53* was the most common recurrent GA (n=7/8). Half of NSCLC cases had co-occurring driver alterations, with *KRAS* (G12C, G13D), *EGFR* (S752_I759del), *BRAF* (G649A) identified. Outside of NSCLC, the only co-occurring driver GAs were an *ERBB2* amplification in breast carcinoma and an *ITSN-ALK* fusion in a pancreatic carcinoma. One CRC sample was MSI-high.

CONCLUSIONS

- *NTRK1*, *NTRK2*, and *NTRK3* fusions are clinically relevant driver alterations across solid tumor types.
- These fusions are difficult to detect, as the breakpoints occur across large intronic regions and they have many partner genes.
- *NTRK* fusions occur both within and between chromosomes.
- Of the 17 *NTRK* kinase fusions detected across 16 samples (*NTRK1*=6, *NTRK2*=3, *NTRK3*=8) across multiple tumor types, 10 had novel fusion partners.
- Co-occurring drivers were identified in a significant fraction of patients, with 50% of NSCLC cases having other drivers.
- These data emphasize the value of CGP using a well-designed RNA sequencing assay to identify all *NTRK* fusions for optimal patient care.