# Identification of Tropomyosin Kinase Receptor (TRK) Point Mutations in Cancer



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## Introduction

- Trk A, B and C, (encoded by NTRK1, NTRK2, and NTRK3 genes, respectively) along with their neurotrophin ligands, regulate growth, differentiation and survival of neurons.
- Chromosomal rearrangements resulting in kinase fusions have been described across the NTRK gene family, and may contribute to tumorigenesis in diverse clinical settings.
- LOXO-101, the only molecule to selectively inhibit the Trk kinases, has recently entered clinical trials.
- Characterizing the full set of NTRK alterations capable of driving tumorigenesis will enable the rational selection of patients likely to benefit from Trk inhibition.
- In an effort to identify non-fusion driver mutations in NTRK, we compiled a database of NTRK point mutations from data provided by Foundation Medicine (FMI) and Compendia BioSciences (Compendia).
- By filtering and rank-ordering the database we generated a short list of high priority putative activating mutations for experimental validation.

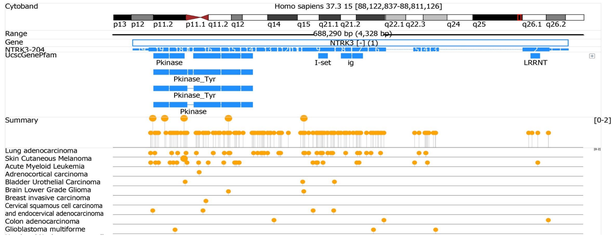
## Methods

- **Starting Database**: 1823 mutations in NTRK1, NTRK2 & NTRK3 were compiled from data provided by Foundation Medicine (FMI) and Compendia Biosciences on 42,155 tumor samples.
- **Filtering**: Mutations that are synonymous, lead to loss of a canonical stop codon, cause truncation of the kinase domain, or are mapped to an alternative transcript that does not contain a full kinase domain were filtered due to questionable functional relevancy.
- **Clustering**: For each codon with one or more mutations present, all mutations within the given, preceding & subsequent codons were clustered together. Duplicate clusters were then removed.
- **Scoring & Ranking**: Mutation clusters were ranked for follow-up by combining several component scores into a single score via multiplication:
- The hotspot score captures the prevalence of the mutation cluster:
  - size of this cluster / size of largest cluster
- The domain score captures the functional relevance of the protein region harboring the cluster to oncogenesis:
  - kinase domain = 1, Ig-like domains = 0.9, leucine rich repeats = 0.3, none = 0.05
- The co-alteration score captures the likelihood that the mutation cluster contains drivers based on the presence of co-occurring mutations in other oncogenes.
- The exac score captures the rarity of the mutations in a large collection of germline samples, as a measure of relevance to oncogenesis.
- The conservation score captures how conserved the region of the protein is across placental mammals, as a measure of functional relevance.
- **Expression Filtering**: An additional filter requiring that at least one mutation in a given cluster be associated with expression of the relevant NTRK gene above background was applied after clustering.
- **Structure Modeling**: Specific residues were mapped onto the appropriate crystal structures and a judgment made on activation potential based on structure and knowledge of kinase regulation.

## Results

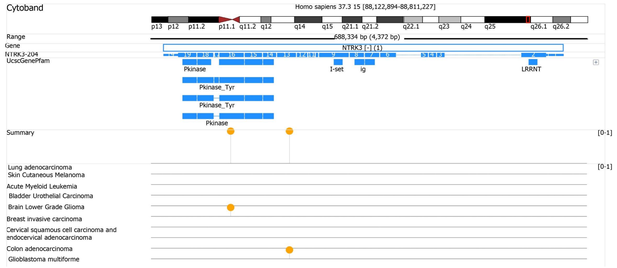
### Classic Hotspots Were Not Observed

- Although hundreds of mutations were found in NTRK1, NTRK2 and NTRK3, they tend to be:
  - Spread uniformly across the primary structure.
  - More common in cancers that are associated with carcinogens known to generate point mutations (tobacco & UV exposure).
- As an example, see figure for NTRK3 below, in which mutations are indicated as gold balls. The top row compiles all mutations, which are then broken out by histologies in the subsequent rows below.
- This pattern suggests that most NTRK point mutations are passengers.



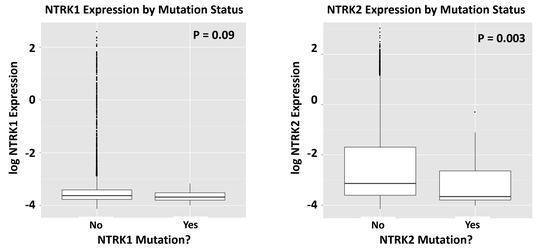

### Expressed Point Mutations Are Rare

- Due to the passenger pattern observed and the relatively rare expression of the NTRK genes in tumors, we decided to filter our analysis to the subset of mutation clusters with evidence of expression from at least one tumor sample (RPKM > 1).
- As an example, for NTRK3 (see below) the number of clusters was reduced from 732 to 7.



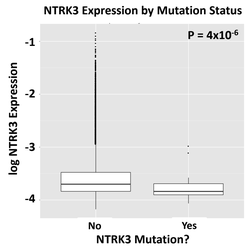

### Point Mutations Are Found Preferentially in Tumors With Lower NTRK Expression

- The reduction of mutation clusters after including an expression requirement led us to examine whether NTRK mutations are, in fact, enriched in tumors where NTRK is expressed at low levels or not expressed at all.
- The results shown for NTRK1, NTRK2, & NTRK3 mutations from TCGA in the box plots below indicate that NTRK point mutations are found preferentially in tumors with lower NTRK expression.
- This pattern is consistent with non-expressed genes lacking (1) selective pressure and (2) transcription-coupled repair. This further suggests that NTRK point mutations are, on the whole, passengers.







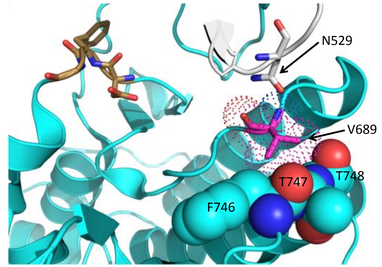



### Some Expressed Point Mutations Appear Oncogenic

| gene | mutated samples | mutations | domain | hotspot score | domain score | co-alteration score | exac score | conservation score | combined score |
|---|---|---|---|---|---|---|---|---|---|
| NTRK2 | 5 | A314E, A314G, A314V, L315F | Ig-like C2-type 2 | 0.25 | 0.90 | 1.00 | 0.97 | 1.00 | 0.22 |
| NTRK2 | 2 | V689M | Protein kinase | 0.10 | 1.00 | 1.00 | 1.00 | 1.00 | 0.10 |
| NTRK2 | 5 | M240I, N241D, E242K | Ig-like C2-type 1 | 0.25 | 0.90 | 0.40 | 1.00 | 1.00 | 0.09 |
| NTRK2 | 1 | I264M | Ig-like C2-type 1 | 0.05 | 0.90 | 1.00 | 1.00 | 1.00 | 0.04 |
| NTRK2 | 4 | A440S, A440T, A440V | N/A | 0.20 | 0.05 | 1.00 | 0.98 | 1.00 | 0.01 |
| NTRK2 | 10 | T426I, G427S, R428Q | N/A | 0.50 | 0.05 | 0.50 | 0.87 | 0.82 | 0.01 |
| NTRK2 | 3 | G401A, G401E, G401R | N/A | 0.15 | 0.05 | 1.00 | 1.00 | 0.98 | 0.01 |

- These are the 7 mutation clusters with evidence of expression in at least one tumor.
- The tumors harboring these mutations represent a diverse range of histologies including lung adenocarcinoma, colon adenocarcinoma and melanoma.
- The scores represent the oncogenic potential of these mutations, as described in the methods section.

### Model of TrkB Valine 689




TrkB Model based on PDB entry 4ASZ

Backbone atoms of Val689 in TrkB are modeled to interact with Asn529 in the juxta-membrane domain, potentially stabilizing the auto-inhibited conformation. When Val689 is mutated to Met, the larger sidechain is predicted to clash with residues 746-748 in the C-terminal domain resulting in a conformational change that may de-stabilize the auto-inhibited structure.

## Summary

- NTRK expression is rare in tumors of most histologies and point mutations are often found in tumors that have no detectable NTRK expression. It will therefore be important when considering NTRK point mutations as a predictive marker to ensure the mutated NTRK allele is actually expressed.
- While rare, we did identify a few mutation clusters associated with NTRK expression that are potentially activating and need to be further characterized with cell biology experimental approaches.