# *NTRK, RET, BRAF,* and *ALK* Fusions in Thyroid Fine-Needle Aspirates (FNAs)


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## INTRODUCTION

Receptor tyrosine kinase fusions are being targeted for small molecule inhibitors to treat late-stage cancers, including advanced thyroid cancer (Figure 1). *NTRK* fusion inhibitors have received pan-cancer FDA approval. Clinical trials have included selective inhibitors of *ALK, BRAF, NTRK* and *RET*. The Afirma Genomic Sequencing Classifier and Xpression Atlas report 130 fusion pairs across Bethesda III-VI nodules, including these fusions. Here we report their prevalence in real-world clinical practice.

## METHODS

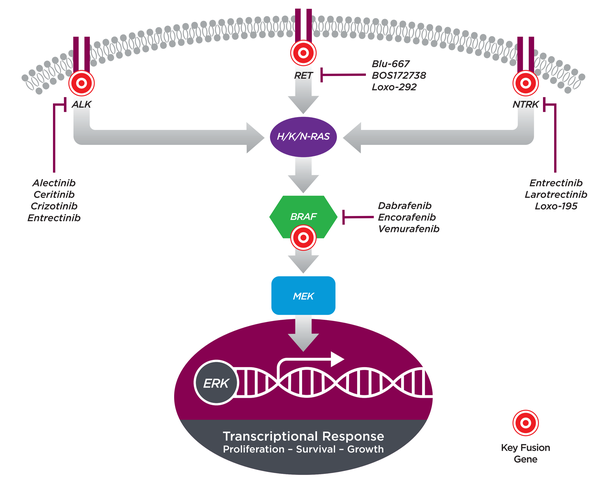
Anonymized data from 47,695 consecutive Bethesda III-VI nodule FNA samples submitted to Veracyte for molecular analysis were analyzed. Except for *RET/PTC*, gene pairs are listed alphabetically.

## RESULTS

Overall, 425 (0.89%) of all FNAs harbored an *ALK, BRAF, NTRK,* or *RET* fusion (Figure 2). *NTRK* fusions were the most common at 0.38% of all FNAs, followed by *RET* (0.32%), *BRAF* (0.13%), and *ALK* (0.06%) (Figure 2). The most common fusion observed for each was: *ETV6/NTRK3* (81% of *NTRK*), *RET/PTC1* (64.2% of *RET*), *BRAF/SND1* (47.5% of *BRAF*), and *ALK/STRN* (63.3% of *ALK*) (Figure 3). *RET* showed the highest diversity of fusions, with 8 unique fusion partners: *RET/PTC1, RET/PTC3* (29.8%), *ERC1/RET* (1.99%), *RET/RFP* (1.32%) with one observation of each of the remaining 4 partners (*RET/PTC2, RET/PTC7, AKAP13/RET, FKBP15/RET*).

*NTRK* fusions were observed in 0.28%, 0.57%, 1.88%, and 1.7% among Bethesda III, IV, V, and VI, respectively ($p < 0.001$). *RET* fusions were observed in 0.18%, 0.37%, 4.13%, and 2.43%, respectively ($p < 0.001$). *BRAF* and *ALK* fusions showed similar trends, although *ALK* fusions were not observed in Bethesda VI. (See Table 1)

FIGURE 1.
### The MAP Kinase Pathway.




In non-neoplastic cells, extracellular signals bind to and activate receptor tyrosine kinases. Those kinases in turn activate *RAS*, which activates *BRAF*, which activates *MEK*. *MEK* phosphorylates *ERK*, and phospho *ERK* translocates into the nucleus, activating a transcriptional response. Fusions have been observed in some of the key signaling proteins in this pathway, which uncouples the kinase domain from key regulatory domains within the protein; these genes are demarcated with a red circle. These fusion genes constitutively activate the signaling pathway in the absence of extracellular signals, which drives neoplastic growth. Because these are key drivers of tumor growth, these fusion kinases have been heavily targeted for selective therapeutic small molecules, with many in either clinical trials or approved by the FDA.

TABLE 1.
### The frequency of key kinase fusions across Bethesda categories from 47,695 consecutive FNAs.

| | Bethesda III (n = 36,476) | Bethesda IV (n = 8,630) | Bethesda V (n = 732) | Bethesda VI (n = 781) |
|---|---|---|---|---|
| ***NTRK1/NTRK3*** | 0.28% | 0.57% | 1.88% | 1.70% |
| ***RET*** | 0.18% | 0.37% | 4.13% | 2.43% |
| ***BRAF*** | 0.09% | 0.19% | 0.88% | 0.36% |
| ***ALK*** | 0.05% | 0.12% | 0.13% | 0.00% |

FIGURE 2.
### Sankey Diagram of *BRAF, RET, ALK, NTRK3,* and *NTRK1*.

On the left are partner genes, and on the right are the key kinase genes. The thickness of vertical bar denotes the number of each partner observed. The thickness of the grey connection reveals the proportion each partner makes. Two partners have multiple connections (*ERC1* to *BRAF* and *RET*; *TFG* to *NTRK1* and *ALK*).

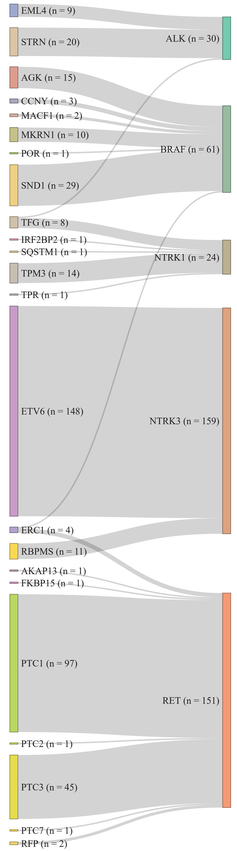
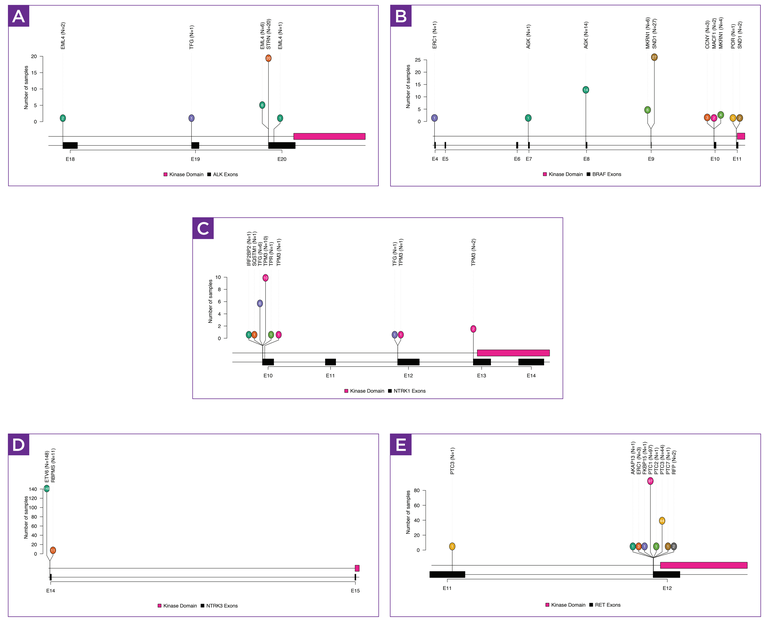



FIGURE 3.
### Key kinase fusions, and the location and frequency of their fusion partners.




Each key kinase gene is shown with the kinase domain highlighted in pink. The exon structure of the gene is shown below. Each fusion is shown at it's location, and the height of the bar indicates the number of fusions observed at that position. The gene name of the partner is shown at the top of each bar.

(A) *ALK* fusions. The most frequently observed fusion partner was *STRN*.

(B) *BRAF* fusions. The most frequently observed fusion partner was *SND1*.

(C) *NTRK1* fusions. The most frequently observed fusion partner was *TPM3*.

(D) *NTRK3* fusions. The most frequently observed fusion partner was *ETV6*.

(E) *RET* fusions. The most frequently observed fusion partner was *PTC1* (*CCDC6*).

Other *RET* gene partners are *PTC2* (*PRKAR1A*), *PTC3* (*NCOA4*), *PTC7* (*TRIM33*), *RFP* (*TRIM27*).

## CONCLUSIONS

Whole-transcriptome RNA-seq demonstrated differences in the prevalence of *ALK, BRAF, NTRK,* and *RET* fusions across Bethesda categories with Bethesda V being the highest, suggesting that prevalence of malignancy may not be the only predictor of fusion occurrence. Future studies need to determine if detection of precision medicine candidates at pre-operative FNA can optimize initial treatment, predict response to treatment, and prioritize selective targeted therapy should systemic treatment be needed.