Assessment of *BRAF* Fusions in 177,227 Thyroid Nodules by Exome-Enriched RNA-Seq Testing

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INTRODUCTION

- The *BRAF*p.V600E alteration is a canonical driver of Papillary Thyroid Carcinoma (PTC) oncogenesis via its activation of the MAPK pathway.
- Fusions comprise a rare subset of *BRAF* alterations in cancer.
- In melanomas harboring *BRAF* fusions, encouraging responses to MEK inhibitors suggest that *BRAF* fusions may be a therapeutic target.
- Given their potential actionability and limited information in thyroid cancer, we examined the landscape of *BRAF* fusions in thyroid tumors in the largest thyroid nodule molecular database.

METHODS

- Exome-enriched RNA-sequencing data were analyzed from consecutive thyroid nodule FNA specimens that underwent Afirma Genomic Sequencing Classifier (GSC) and Xpression Atlas (XA) testing in the Veracyte CLIA-certified laboratory.
- *BRAF* fusions were identified from XA data, which reports 87 *BRAF* fusions.[1,2]
- Expression signatures were compared between thyroid nodules with *BRAF* fusions, *BRAF*p.V600E (class 1), or p.K601E (class 2).
- We also assessed *BRAF* fusions among thyroid carcinomas in AACR GENIE public data.

RESULTS

- In 177,227 thyroid nodule FNA samples, *BRAF* fusions were detected in 0.2% (n=382), ranging from 0.1% of Bethesda III nodules (n=189/139,245), 0.33% of Bethesda IV nodules (n=99/30013) to 1.2% of Bethesda V/VI nodules (n=47/3,886 and n=47/3,984, respectively) (Table 1). Of all nodules that had indeterminate cytology and were molecularly GSC-suspicious, 0.54% had *BRAF*-fusions detected.
- A total of 75 different partner genes were identified, most frequently *SND1* (25%), *AGK* (19%), *MKRN1* (10%), *WARS* (3%), *EXOC4* (2%), *TRIM24* (2%), and *ZC3HAV1* (2%) – all of which resided on chromosome 7q3 (except for *WARS* on chromosome 14) (Table 2).
  - Only 1/382 nodules with *BRAF* fusion had a concurrent *BRAF*p.V600E alteration and no concurrent *RAS* mutations were identified.
- Of nodules with Bethesda III or IV cytology, the Afirma GSC ensemble classifier categorized 99% of thyroid nodules with *BRAF* fusions as GSC-suspicious (n=378/382).
  - While not reported clinically, molecular alterations can be identified in Afirma GSC-benign thyroid nodules.
- In expression signature analyses, *BRAF*-fusions nodules displayed benign vs. malignancy classifier, *BRAF-RAS* (BRS), ERK, and follicular-mesenchymal transition (FMT) scores that were between those of nodules with *BRAF*p.V600E (n=6,600) or *BRAF*p.K601E (n=645).
  - Expression scores depended on fusion partners, with *AGK* and *MKRN1* fusions more like *BRAF*p.V600E nodules and *WARS* fusions more like *BRAF*p.K601E nodules.
- Analysis of sequencing data from 2,303 thyroid cancers in AACR GENIE identified 46 (2.0%) with *BRAF* fusions, including 3.1% of FTCs, 2.7% of PTCs, 1.6% of ATCs, and 0.9% of MTCs.

TABLE 1

Frequency of *BRAF* fusions by thyroid nodule cytology category

	BRAF fusion negative	*BRAF* fusion positive
Bethesda III	139,056	189 (0.13%)
Bethesda IV	30,013	99 (0.33%)
Bethesda V	3,839	47 (1.21%)
Bethesda VI	3,937	47 (1.18%)

TABLE 2

***BRAF* fusion partner genes with proportion > 1%**

BRAF fusion partners	Proportion with reference to all samples with *BRAF* fusions (n=382)
SND1	96 (25.1%)
AGK	71 (18.6%)
MKRN1	38 (9.95%)
WARS	10 (2.6%)
EXOC4	9 (2.3%)
TRIM24	9 (2.3%)
ZC3HAV1	9 (2.3%)
SORBS2	8 (2.1%)
GBP1	7 (1.8%)
MACF1	7 (1.8%)
POR	6 (1.6%)
CCNY	5 (1.3%)
TNS1	5 (1.3%)
TNS3	4 (1%)
NRF1	4 (1%)
AGAP1	4 (1%)
AGAP3	4 (1%)
CDK5RAP2	4 (1%)
PDE10A	4 (1%)
ERC1	4 (1%)

TABLE 3

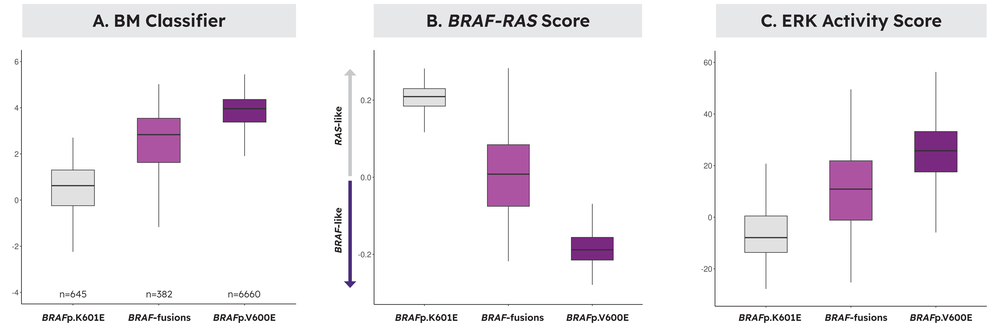
***BRAF* fusion partners in AACR GENIE (public resource) across different thyroid cancer histology**

These fusion partners were seen at least 1% of the time in the Afirma database.

	AACR GENIE			
BRAF fusion partners	PTC (n=1,626)	FTC (n=168)	APC (n=308)	PDTC (n=358)
SND1	4	—	—	—
AGK	1	—	—	—
MKRN1	4	—	—	—
WARS	—	—	—	—
EXOC4	1	—	—	—
TRIM24	1	—	—	—
ZC3HAV1	—	—	—	—
SORBS2	—	—	—	—
GBP1	—	—	—	—
MACF1	—	—	—	—
POR	1	—	—	—
CCNY	—	—	—	—
TNS1	—	—	—	—
TNS3	—	—	—	—
NRF1	—	—	—	1
AGAP1	—	—	—	—
AGAP3	—	—	—	—
CDK5RAP2	5	—	—	—
PDE10A	—	—	—	—
ERC1	—	—	1	—

FIGURE 1

The relative levels of:
A. BM (benign-malignancy) classifier
B. BRS (*BRAF*-RAS score)
C. ERK score
D. Follicular mesenchymal transition (FMT) score
E. Thyroid differentiation score (TDS) amongst thyroid tumors with *BRAF*-fusions, *BRAF*p.K601E, or *BRAF*p.V600E alterations.
All pair-wise comparisons are statistically significant (p<0.001).



CONCLUSION

- The detection of *BRAF* fusions and its many partners was enabled by the Afirma XA exome-enriched RNA-Seq panel.
- Molecular analysis of genomic signatures of aggressiveness (i.e. BM score, BRS score, TDS, ERK, and FMT showed *BRAF* fusions had expression levels between tumors with more aggressive *BRAF*p.V600E alterations and tumors with less aggressive *BRAF*p.K601E alterations.
  - Expression signatures varied by the *BRAF* fusion partner gene.
- The exome-enriched RNAseq Afirma GSC platform additionally allows for the discovery of rare and targetable alterations such as *RET, ALK,* and *FGFR2* fusions. More data is required to fully understand the clinical significance of thyroid tumors harboring these rare fusions.
- Our findings suggest that for otherwise *BRAF/RAS/NTRK/RET/ALK*-wildtype thyroid cancers, *BRAF* fusion testing could help identify patients who may benefit from *BRAF*/MAPK pathway inhibition.
- Future investigation of the full spectrum of *BRAF* fusion partners is needed.

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