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THYROID FNA ANALYSIS

SAT-1227

Detecting Expressed Variants and Fusions in RNASeq Data from Thyroid FNAs

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Detecting Expressed Variants and Fusions in RNASeq Data from Thyroid FNAs

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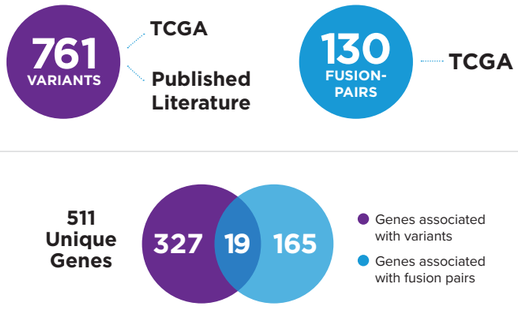
INTRODUCTION

- Veracyte's Afirma RNASeq platform provides genomic content utilized by the Genomic Sequencing Classifier (GSC) to classify cytologically indeterminate fine needle aspirations (FNAs) as molecularly benign or suspicious. In addition to mRNA expression, the platform captures genomic changes, such as fusions and nucleotide variants, some of which imply cancer. The GSC already reports *RET/PTC* gene fusions and the *BRAF* V600E variant.
- The objective of this study was to expand the panel of reported genomic changes and compare their detection by the RNASeq platform against DNA and RNA independent reference methods.

METHODS

A panel of 761 nucleotide variants from 346 genes and 130 unique fusion pairs from 184 genes (Xpression Atlas) was derived from the literature, including The Cancer Genome Atlas[1] (Figure 1). A cohort of FNA samples from GSC Suspicious Bethesda III/IV nodules, and GSC Naïve Bethesda V/VI nodules were further examined for nucleotide variants and fusions. These included the Afirma GSC validation cohort and de-identified samples from the Veracyte clinical laboratory. A custom DNA AmpliSeq variant panel and separate RNA AmpliSeq fusion panel plus TaqMan fusion assays were developed as reference methods. Molecular testing was performed while blinded to all other information.

FIGURE 1.
Xpression Atlas Panel Derivation



The Xpression Atlas was derived from published literature and The Cancer Genome Atlas (TCGA)[1]

RESULTS

- Nucleotide variants were examined with both DNA AmpliSeq and RNASeq in 501 FNAs. 175 of 501 (34.9%) FNAs contained a variant by the reference method. In GSC Suspicious Bethesda III/IV FNAs, the most common variants were *NRAS* Q61R (n=60), *HRAS* Q61R (n=30), *NRAS* Q61K (n=19), *KRAS* Q61R (n=7), *HRAS* Q61K (n=7), and *HRAS* G13R (n=5). In GSC Naïve Bethesda V/VI nodules, the most common variants were *NRAS* Q61R (n=7) and *SPOP* P94R (n=3) (Table 1).
  - Comparing the two methods, a 74% Positive Percent Agreement (PPA), >99% Negative Percent Agreement (NPA), and 98.5% confirmation rate were observed. The limit of detection was 5% (Table 1).
- Gene fusions were also examined with the RNA reference method and RNASeq in 695 consecutive clinical FNAs. 61 of 695 (8.8%) FNAs contained a gene fusion by the reference method. *PAX8/PPARG* (n=16), *ETV6/NTRK3* (n=13), *ALK/STRN* (n=6), and *RET/PTC1* (n=6) fusions were observed most frequently (Table 2).
  - Comparing the two methods, we observed 82% PPA, >99% NPA, and >99% confirmation rate. The limit of detection was 10% (Table 2).

TABLE 1.
761 Nucleotide Variant Panel Results

Sub-cohort	# Samples (n=501)	Most Common Variants	RNA Access Only	AmpliSeq DNA Only	Both	PPA†	NPA†	Confirmation†
GSC Suspicious Bethesda III/IV nodule FNAs	290	• *NRAS* Q61R (n=60; 20.7%) • *HRAS* Q61R (n=30; 10.3%) • *NRAS* Q61K (n=19; 6.6%) • *BRAF* V600E (n=8; 2.8%) • *KRAS* Q61R (n=7, 2.4%) • *HRAS* Q61K (n=7; 2.4%) • *HRAS* G13R (n=5; 1.7%)	2	39	113	74.3% [67-81]	100% [100-100]	98.3% [94-100]
GSC Naïve Bethesda V/VI nodule FNAs	211	• *BRAF* V600E (n=79; 37.4%) • *NRAS* Q61R (n=7; 3.3%) • *SPOP* P94R (n=3; 1.4%)	0	8	21	72.4% [53-87]	100% [100-100]	100% [84-100]
Both	501		2	47	134	74% [67-80]	100% [100-100]	98.5% [95-100]

†Calculated without *BRAF* V600E classifier

TABLE 2.
130 Gene Fusion Panel Results

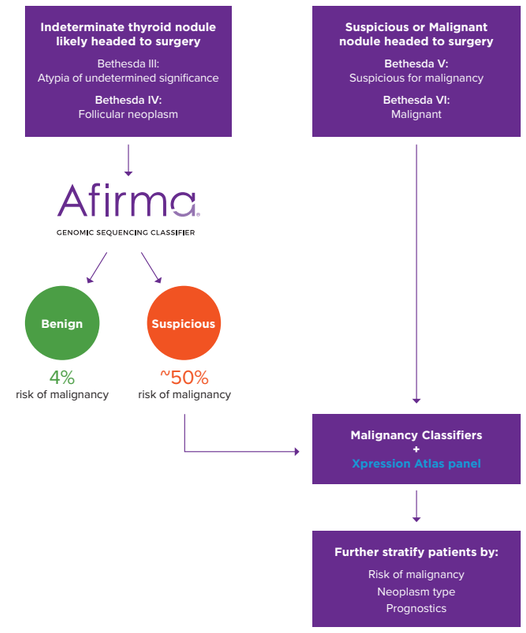
Sub-cohort	# Samples (n=695)	Most Common Fusions	RNA Access Only	AmpliSeq RNA/ TaqMan only	Both	PPA*	NPA*	Confirmation*
GSC Suspicious Bethesda III/IV nodule FNAs	634	• *PAX8/PPARG* (n=16; 2.5%) • *ETV6/NTRK3* (n=13; 2.0%) • *ALK/STRN* (n=5; 0.8%) • *RET/PTC1* (n=4; 0.6%) • *RET/PTC3* (n=3; 0.5%)	0	10	46	82.1% [70-91]	100% [100-100]	100% [92-100]
GSC Naïve Bethesda V/VI nodule FNAs	61	• *RET/PTC1* (n=2; 3.3%) • *ALK/STRN* (n=1; 1.6%) • *BRAF/SND1* (n=1; 1.6%) • *BRAF/MACF1* (n=1; 1.6%)	0	1	4	80% [28-99]	100% [100-100]	100% [40-100]
Both	695		0	11	50	82% [70-91]	100% [100-100]	100% [93-100]

*Based on comparison to RNA AmpliSeq and TaqMan fusion assays targeting 117 fusions.

CONCLUSIONS

- Agreement between the Afirma RNASeq platform and independent reference methods for nucleotide variants and gene fusions is high. Positive confirmation rates were >98.5% for both.
- Prior studies suggest overall modest sensitivity and specificity for cancer with this gene panel alone.[2,3] However, when used for thyroid nodules destined for surgery, genomic data obtained from the nodules' actively transcribed genes may inform risk of malignancy, diagnosis/ neoplasm type, prognosis, and cell-signaling pathway activation that may alter management of patients (Figure 2). Incorporating the RNASeq platform into the diagnostic flow has the potential to enhance and increase confidence in patient risk stratification.

FIGURE 2.
How the Xpression Atlas Panel Can Fit into Clinical Practice



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