Abstract #1227

DETECTING EXPRESSED VARIANTS AND FUSIONS IN RNA-SEQ DATA FROM THYROID FNAS

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Objective: The Afirma RNASeq platform provides genomic content that is utilized by the Genomic Sequencing Classifier (GSC) to classify cytologically indeterminant FNAs as molecularly benign or suspicious. In addition to mRNA expression, the platform captures genomic changes such as fusions and nucleotide variants. The GSC already reports RET/PTC fusions and the BRAF V600E variant. Here we markedly expand the panel of reported genomic changes and compare their detection from the RNASeq platform against both DNA and RNA independent reference methods.

Methods: A panel of 761 nucleotide variants from 346 genes and 130 unique fusion pairs from 184 genes was derived from the literature, including The Cancer Genome Atlas. 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.

Results: We examined the nucleotide variants with both DNA AmpliSeq and RNASeq in 501 FNAs. 175 of 501 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), while in GSC naïve, Bethesda V/VI nodules, BRAF V600E (n=79), NRAS Q61R (n=7), and SPOP P94R (n=3) variants were most common. Comparing the two methods, we observed 74% Positive Percent Agreement (PPA), >99% Negative Percent Agreement (NPA), and 98.5% confirmation rate. The limit of detection was 5%.

We also examined gene fusions with the RNA reference method and RNASeq in 695 consecutive clinical FNAs. 61 of 695 FNAs contained a 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. Comparing the two methods, we observed 82% PPA, >99% NPA, and >99% confirmation rate. The limit of detection was 10%.

Discussion: Agreement between the RNASeq platform and comparative methods for gene fusions and variants is high.

Conclusion: Prior studies have suggested overall modest sensitivity and specificity for cancer with this gene panel alone. However, when used among nodules destined for surgery, the genomic information obtained from the nodules' actively transcribed genes may inform diagnosis, prognosis, and cell-signali g pathway activation that may alter patient management.