The Genomic Landscape of Preoperative FNAs positive for the Afirma GSC Medullary Thyroid Cancer Classifier

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BACKGROUND

The Afirma Genomic Sequencing Classifier (GSC) uses RNA sequencing to assess FNA specimens from cytologically indeterminate thyroid nodules, which are also tested for specific molecular aberrations associated with thyroid cancer via a suite of highly accurate malignancy classifiers (figure 1). This suite can also be applied independently to Bethesda V/VI nodules. The Afirma Xpression Atlas (XA) (figures 1 and 2) is an RNA sequencing-based add-on test that reports expressed nucleotide variants and fusions across 511 cancer-associated genes (figure 3), when added to Afirma GSC and/or malignancy classifiers.

Medullary thyroid cancer (MTC) is a rare subtype of thyroid cancer that can appear in every Bethesda category. Previously, the Afirma MTC classifier demonstrated 100% sensitivity and specificity among 211 FNA samples that included 21 MTCs.[1] Herein, we report the prevalence and genomic landscape of MTC classifier positive (MTC+) FNA samples in the Veracyte Clinical Laboratory.

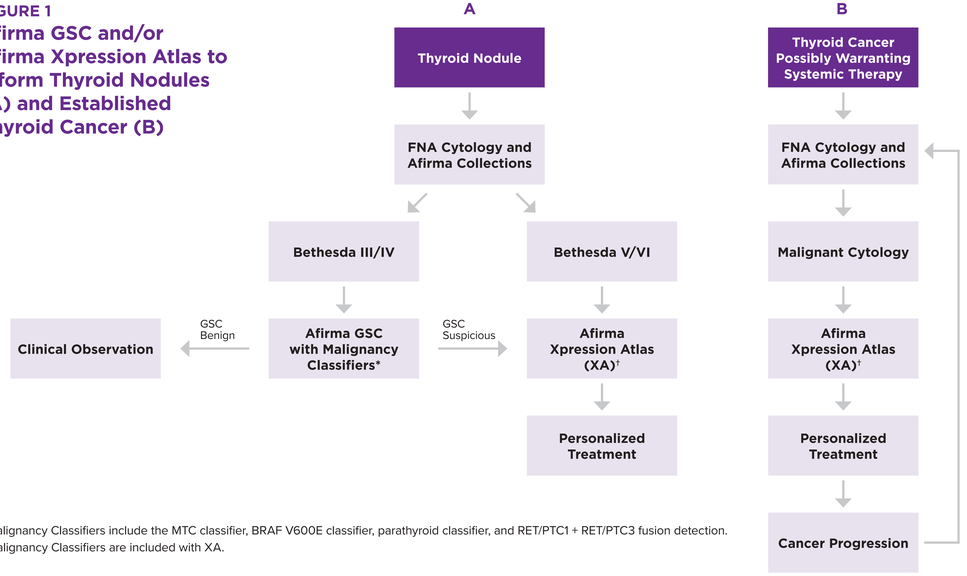
METHODS

All Afirma GSC and malignancy classifier tests run in the Veracyte Clinical Laboratory between July 2017 and November 2018 were deidentified and examined for MTC+ cases. Afirma XA was run on all MTC+ cases and all variants and fusions were tabulated.

RESULTS

Examination of 22,130 FNAs revealed 77 MTC+ cases. Among consecutive Afirma GSC tests, 28 were Bethesda III (0.16% out of 17,245) and 27 were Bethesda IV (0.65% out of 4,182). Provider-ordered Afirma testing was done on an additional 14 and 8 MTC cases from Bethesda V and VI nodules, respectively. Examining all MTC+ samples revealed that 55.8% harbored a *RET* variant (+/- others), 9.1% contained a *KRAS* variant (+/- others), 6.5% had an *HRAS* variant, 2.6% possessed fusions, and 26.0% included no variant/fusion (figure 4).

FIGURE 1
Afirma GSC and/or Afirma Xpression Atlas to Inform Thyroid Nodules (A) and Established Thyroid Cancer (B)



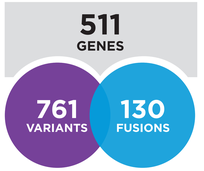
* Malignancy Classifiers include the MTC classifier, BRAF V600E classifier, parathyroid classifier, and RET/PTC1 + RET/PTC3 fusion detection.
† Malignancy Classifiers are included with XA.

FIGURE 2
Use of RNA Sequencing in Afirma Xpression Atlas to Detect Expressed DNA Variants

Proposed mechanism: modifier turns off gene expression so the mutated gene is not active in the cell and the variant is not found in the mRNA

No modifier to turn off gene expression so variant appears in the mRNA

FIGURE 3
Afirma Xpression Atlas



VARIANT EXAMPLES

BRAF
DICER1
EIF1AX
H/K/N-RAS
RET
TG
TP53
ZFHX3

FUSION GENE EXAMPLES

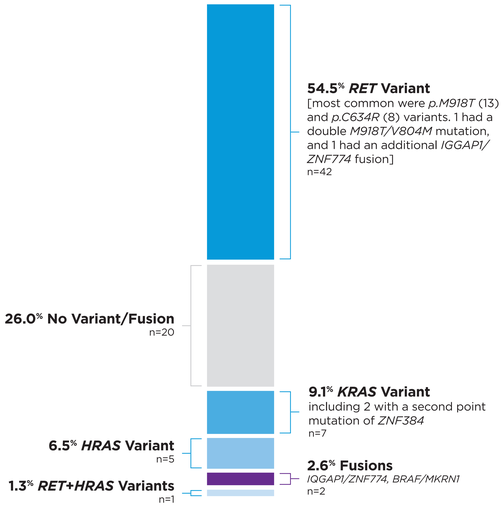
ALK
BRAF
NTRK
PAX8
RET

TARGETED THERAPY EXAMPLES

ALK	*HRAS*	*PAX8/PPAR-γ*
BRAF	*MET*	*RET*
EGFR	*NTRK*	*ROS1*

Using RNA sequencing, Afirma Xpression Atlas reports expressed genomic variants and fusions among 511 genes. Therapies targeting some of these alterations are available or under investigation.

FIGURE 4
Variants and Fusions Identified in 77 MTC+ Samples Using the Afirma Xpression Atlas



CONCLUSIONS

In indeterminate FNA samples, the Afirma GSC can help to clarify the risk of MTC. In our cohort of Bethesda III-VI MTC+ FNAs, the Afirma XA identified a variant or fusion in 74.0%. Limitations of this study include the lack of knowledge regarding germline *RET* status (which should be checked in all patients with an identified *RET* variant or confirmed MTC) and final pathology on MTC+ samples. Future studies may investigate how the preoperative identification of a known MTC driver mutation in a biopsy sample can inform the pre-operative evaluation, the surgical plan, and the potential role of targeted therapy (figures 1 and 3).

Reference

1. Randolph G, et al. Clinical Validation of the Afirma Genomic Sequencing Classifier for Medullary Thyroid Cancer (oral abstract 29). Thyroid 2017;27: A-105.