The Genomic Landscape of 250,000+ Thyroid Nodules Undergoing Exome-Enriched RNA Sequencing

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INTRODUCTION

- The Afirma Genomic Sequencing Classifier (GSC) is used to risk stratify and reclassify cytologically indeterminate thyroid nodules to molecularly benign or suspicious.
- The Afirma Xpression Atlas (XA) panel detects 905 variants and 235 fusion pairs from 593 predefined genes using exome-enriched RNA-sequencing.[1]
- In 2021, genomic analysis of 50,000+ consecutive thyroid nodules with Bethesda III-VI (BIII-VI) cytology assessed by Afirma GSC was published, showing that almost one-half of BIII/IV Afirma GSC suspicious and most BV/VI nodules had at least one genomic alteration identified, which may optimize personalized treatment decisions.[2]

STUDY RATIONALE AND AIMS

- The Afirma molecular database now includes over 250,000 samples.
- Additionally, since 2023, Afirma GSC includes optional *TERT* promoter (*TERT*p) DNA analysis,[3] that can be requested by the ordering provider.
- We aimed to describe the updated genomic landscape of BIII-BVI thyroid nodules sent for Afirma GSC testing in real world practice, including data on *TERT*p mutations, when available.

METHODS

- Cytologic and molecular differences were assessed in 252,510 consecutive thyroid tumors with BIII-VI cytology undergoing Afirma GSC testing from 2018-2024.
- *TERT*p was assessed in 8,627 samples from 2023-2024.

RESULTS

- Median patient age was 59.3 years. 76.5% of patients were female, and median nodule size was 2.2 cm. The proportion of parathyroid (PTA) and medullary thyroid carcinoma (MTC) classifier positive lesions were < 1%, while > 78% of nodules referred for testing were Bethesda 3 (Table 1).
- 140,565 (71%) BIII and 23,101 (57%) BIV nodules were classified as GSC-(B)enign.
  - Overall, 69% of indeterminate nodules (BIII/BIV) were classified as GSC-B.
- The proportion of XA variants increased with higher Bethesda category classifications (Table 2). Higher risk variants and fusions were enriched in nodules with BVI cytology, while *RAS* mutations were predominant in BIII/BIV nodules (Table 2, Figure 1).
- There was a significant decrease in GSC-S rates among BIII/IV nodules from 2018-2021 and reversal of that trend from 2021-2024 (Figure 2) ($p < 0.001$).

TABLE 1.
Demographic data of tested nodules

	Total (n=252,510)
Median age (yrs) [IQR]	59.3 [46.4-69.4]
Median nodule size (cm) [IQR]	2.2 [1.6-3.1]
Sex	
Male	57,061 (22.6%)
Female	193,085 (76.5%)
PTA	1,496 (0.6%)
MTC	833 (0.3%)
Bethesda category among non-PTA/MTC	
III	197,222 (78.1%)
IV	40,448 (16.0%)
V	6,239 (2.5%)
VI	6,290 (2.5%)

PTA: Parathyroid Classifier **MTC:** Medullary Thyroid Carcinoma Classifier

TABLE 2.
A. Proportion of molecular alterations by Bethesda category

	Bethesda Category			
	III	**IV**	**V**	**VI**
Total*	**56,657**	**17,347**	**6,239**	**6,290**
Any XA variant	20,363 (35.9%)	7,571 (43.6%)	3,429 (55.0%)	4,961 (78.9%)
Any XA fusion	2,938 (5.2%)	1,264 (7.3%)	581 (9.3%)	462 (7.4%)

* with GSC-S or Bethesda V or VI

B. Most common expressed variants by Bethesda category

	Bethesda Category			
	III	**IV**	**V**	**VI**
Total	**56,657**	**17,347**	**6,239**	**6,290**
***BRAF*p.V600E+**	**4,220 (7.4%)**	**1,378 (7.9%)**	**2,799 (44.9%)**	**4,806 (76.4%)**
*BRAF*p.K601E+	577 (1%)	235 (1.4%)	13 (0.2%)	2 (0%)
NRAS	**7,376 (13%)**	**3,002 (17.3%)**	**224 (3.6%)**	**31 (0.5%)**
HRAS	**4,640 (8.2%)**	**1,781 (10.3%)**	**116 (1.9%)**	**11 (0.2%)**
KRAS	**892 (1.6%)**	**422 (2.4%)**	**59 (0.9%)**	**19 (0.3%)**
EIF1AX	280 (0.5%)	127 (0.7%)	19 (0.3%)	5 (0.1%)
DICER1	920 (1.6%)	312 (1.8%)	42 (0.7%)	40 (0.6%)
TSHR	786 (1.4%)	113 (0.7%)	80 (1.3%)	27 (0.4%)
SPOP	468 (0.8%)	148 (0.9%)	48 (0.8%)	10 (0.2%)
TP53	87 (0.2%)	47 (0.3%)	9 (0.1%)	22 (0.3%)
PIK3CA	48 (0.1%)	49 (0.3%)	22 (0.4%)	40 (0.6%)
JAK2	151 (0.3%)	27 (0.2%)	3 (0%)	6 (0.1%)
OBSCN	95 (0.2%)	24 (0.1%)	17 (0.3%)	17 (0.3%)
FAT1	74 (0.1%)	21 (0.1%)	10 (0.2%)	10 (0.2%)
TG	49 (0.1%)	13 (0.1%)	1 (0%)	0 (0%)
AKT1	22 (0%)	10 (0.1%)	4 (0.1%)	6 (0.1%)
EZH1	29 (0.1%)	4 (0%)	9 (0.1%)	0 (0%)
GNAS	32 (0.1%)	3 (0%)	1 (0%)	1 (0%)
Total with *TERT* profile	4,952	1,464	1,060	1,151
***TERT*p**	**140 (2.8%)**	**125 (8.5%)**	**83 (7.8%)**	**124 (10.8%)**
C228	115 (2.3%)	96 (6.5%)	71 (6.7%)	107 (9.3%)
C250T	27 (0.5%)	29 (2%)	14 (1.3%)	19 (1.6%)
TERT+BRAF	**35 (0.7%)**	**19 (1.3%)**	**50 (4.7%)**	**110 (9.6%)**
TERT+NRAS	27 (0.5%)	33 (2.3%)	7 (0.7%)	1 (0.1%)

C. Most common expressed fusions by Bethesda category

	Bethesda Category			
	III	**IV**	**V**	**VI**
TOTAL	**56,657**	**17,347**	**6,239**	**6,290**
RET	**399 (0.7%)**	**133 (0.8%)**	**202 (3.2%)**	**247 (3.9%)**
NTRK3	551 (1%)	227 (1.3%)	125 (2%)	74 (1.2%)
NTRK1	72 (0.1%)	22 (0.1%)	20 (0.3%)	25 (0.4%)
ALK	123 (0.2%)	57 (0.3%)	31 (0.5%)	7 (0.1%)
BRAF	**263 (0.5%)**	**119 (0.7%)**	**71 (1.1%)**	**70 (1.1%)**
Any of above	1406 (2.5%)	558 (3.2%)	449 (7.2%)	423 (6.7%)
MET	27 (0%)	5 (0%)	7 (0.1%)	1 (0%)
VCL	50 (0.1%)	27 (0.2%)	6 (0.1%)	1 (0%)
TTC18	63 (0.1%)	24 (0.1%)	8 (0.1%)	12 (0.2%)
PPARγ	**1238 (2.2%)**	**614 (3.5%)**	**68 (1.1%)**	**10 (0.2%)**

FIGURE 1.
Proportion of expressed molecular variants and fusions in GSC-S BIII/IV nodules and BV/VI nodules

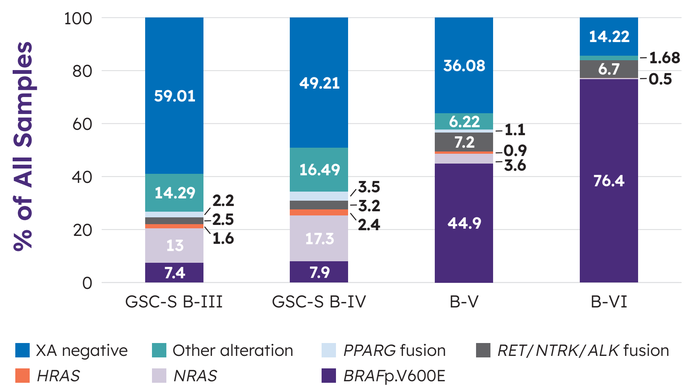
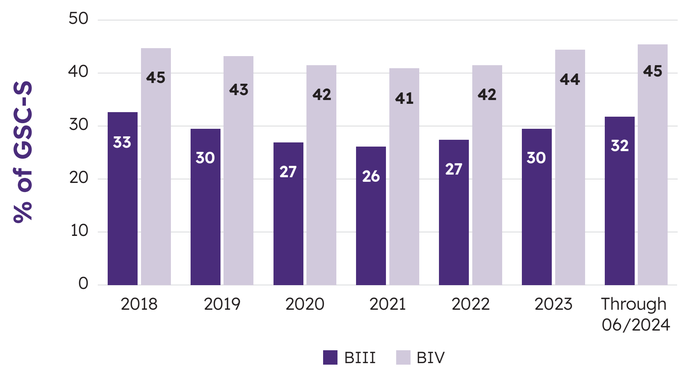


FIGURE 2.
Proportion of GSC-S results from Afirma tested BIII/IV nodules between 2018 and June 2024



DISCUSSION

- This large study describes the molecular landscape of ~87,000 samples from suspicious thyroid nodules assessed by the Afirma Genomic Sequencing Classifier.
- Our findings highlight the molecular alterations seen across Bethesda categories in suspicious thyroid nodules:
  - *BRAF*V600E is the most frequent variant, particularly in BV/VI.
  - *NRAS* is the most prevalent *RAS* variant, most common in BIII/IV nodules.
  - *TERT*p mutations, alone or co-occurring with *BRAF*V600E were most common in BVI.
  - Fusions were identified in 6% of non-GSC-Benign nodules, most often in BV.
  - Most prevalent fusions involved *RET* proto-oncogene, seen in Bethesda V/VI > III/IV.
  - *PPARγ* fusions were primarily detected in Bethesda III/VI.
- Temporal shifts in GSC-S rates are of interest and warrant further investigation.

References

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