

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 | |
| 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 |
| BRAFp.V600E+ | 4,220 (7.4%) | 1,378 (7.9%) | 2,799 (44.9%) | 4,806 (76.4%) |
| BRAFp.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 |
| TERTp | 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

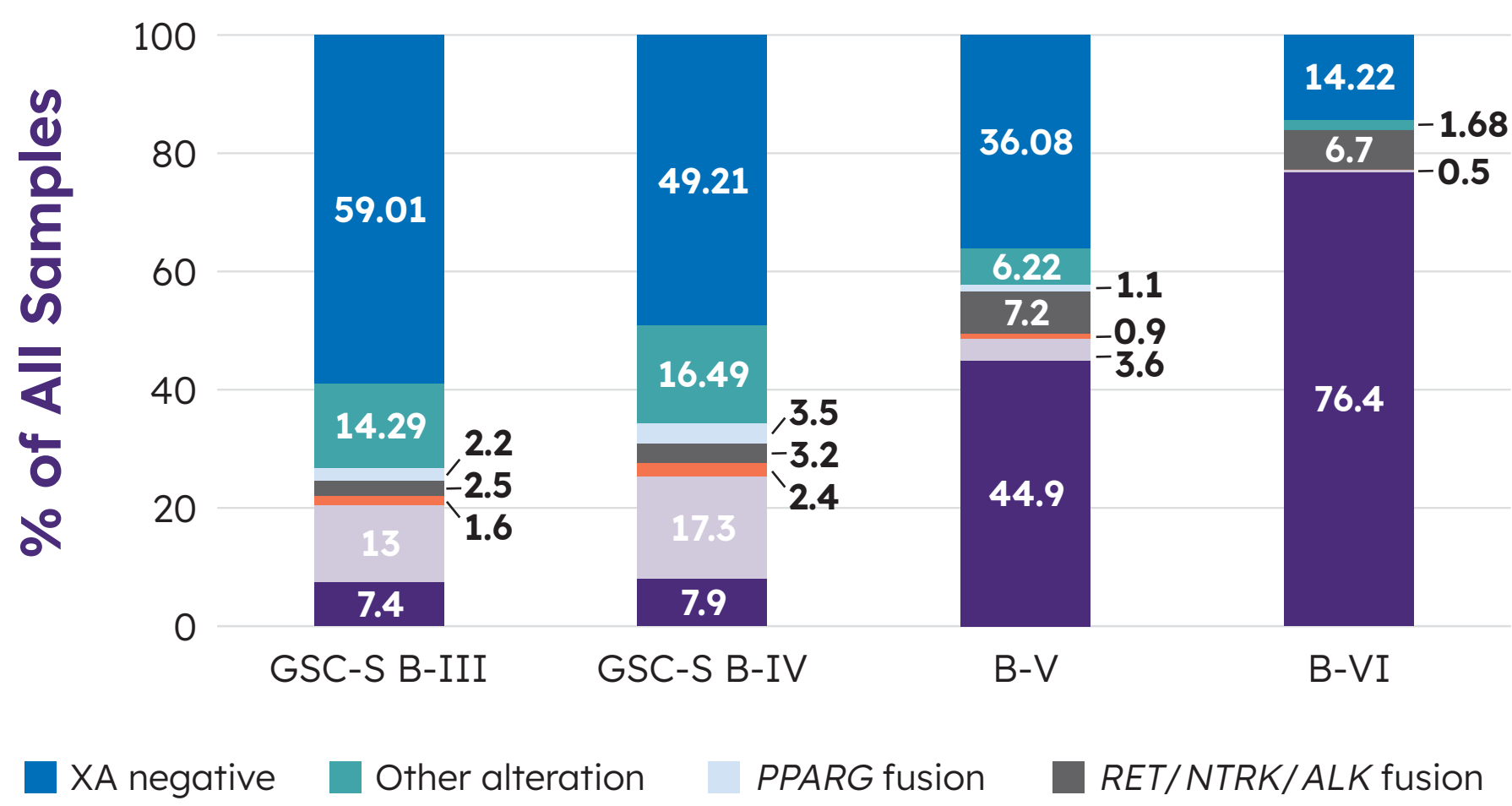
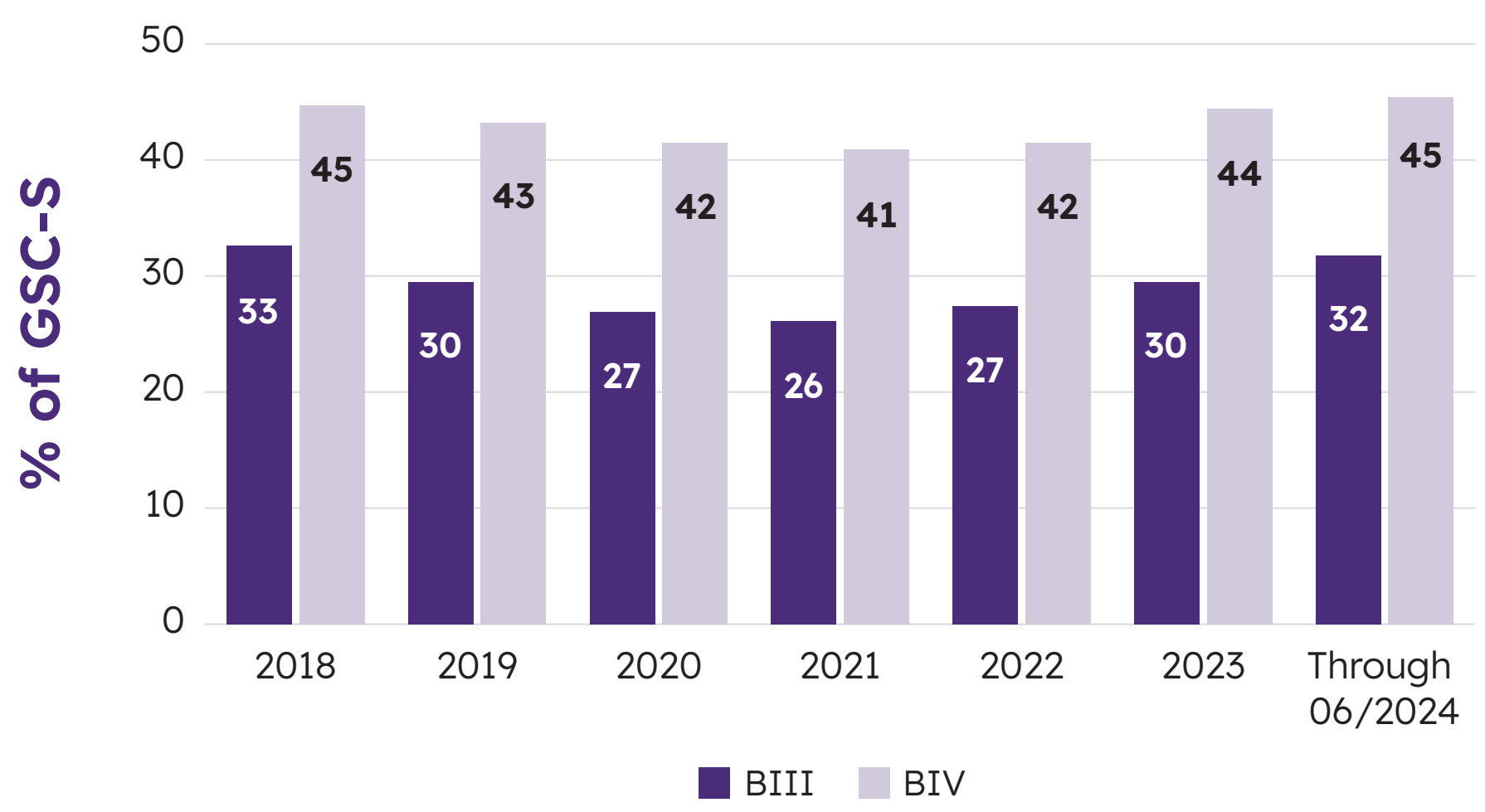


FIGURE 2.
Proportion of GSC-S results from Afirma tested BIII/IV nodules between 2018 and June 2024



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.

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