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2025 RESEARCH COLLECTION

A comprehensive collection of Afirma scientific abstracts presented in 2025



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Foreword



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Last year, we debuted the first edition of this collection to showcase all of the research throughout 2024 made possible by Afirma GSC and Afirma GRID (Genomic Resource for Intelligent Discovery),* a research-use-only resource for analysis of molecular characteristics that may ultimately help improve the understanding of how to manage patients with thyroid nodules and thyroid cancer. Research that presented compelling case studies, validated new molecular signatures, and furthered our understanding of thyroid disease.

2025 represented a significant leap forward with a greater number of abstracts made possible through Afirma GSC and Afirma GRID. These included several independent studies and multiple abstracts that received awards throughout the year. In addition, these abstracts weren't limited to being diagnostic or prognostic in nature; with studies pursuing potential novel therapeutic targets for advanced thyroid cancer as well.

It has been highly rewarding to collaborate with notable institutions not only across the United States, but around the world to make this research possible. It's been equally rewarding to see institutions generate novel independent research.

We hope that the studies in this collection provide a good demonstration of the spirit of discovery that we aspire to foster in researchers, and we hope that others are inspired to develop research of their own—research that innovates and contributes to a greater understanding of thyroid disease.

Thank you all for your contributions, and we hope you find the following collection illuminating.

*Ordering Afirma GSC is not a prerequisite to submitting a request for Afirma GRID.

Dr. Klopper joined Veracyte after serving as the Regional Service Chief for the Department of Endocrinology of Colorado Permanente Medical Group of Kaiser Permanente for four years. His post-graduate medical training included an internal medicine residency, post-doctoral research fellowship and endocrinology fellowship at The University of Colorado School of Medicine, where he joined the faculty in 2006 as an Assistant Professor of Medicine. He focused on thyroid cancer as a translational scientist looking at novel molecular targets for treating advanced thyroid cancer and was an investigator on an early clinical utility study of the original Afirma gene classifier for reducing unnecessary surgeries in thyroid nodules with indeterminate cytology. Dr. Klopper was promoted to Associate Professor of Endocrinology and Radiology prior to transitioning to Kaiser in 2015. Nationally, Dr. Klopper was the co-director of the Endocrine Society Introductory Hands-On Thyroid Ultrasound Workshop for five years and has served on the American Thyroid Association board of directors.

Analysis of Pleckstrin Homology Domain Containing S1 (*PLEKHS1*) Promoter Mutations in Pre-Operative Thyroid Nodule Samples

Analysis of Pleckstrin Homology Domain Containing S1 (*PLEKHS1*) Promoter Mutations in Pre-Operative Thyroid Nodule Samples

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KEY POINTS

- *PLEKHS1* C593T and G590A hotspot promoter mutations are associated with more aggressive thyroid tumor behavior in differentiated thyroid cancer independent of *TERT* promoter mutations.[1,2]
- To understand the potential diagnostic and prognostic utility of detecting *PLEKHS1* promoter mutations in indeterminate thyroid nodules, a cohort of 9,279 samples was tested for *PLEKHS1* hotspot promoter mutation status, Bethesda category, co-occurring mutations, and surgical outcomes (among a subset of 20 samples).
- This study found that in contrast to metastatic PTC, isolated *PLEKHS1* mutations found in indeterminate thyroid nodules do not correlate with a higher risk of malignancy (ROM) or more aggressive disease.

STUDY: IN SUMMARY

PLEKHS1 is a poorly characterized gene located on chromosome 10 that encodes the protein Pleckstrin homology domain containing S1. **Mutations at the C593T and G590 hotspots in the promoter region** (Figure 1) **are associated with more aggressive thyroid tumor behavior in differentiated thyroid cancer independent of *TERT* promoter mutations.**[1,2] Additional studies are needed, however, to fully understand the diagnostic and prognostic utility of detecting *PLEKHS1* promoter mutations among indeterminate thyroid nodules (ITN).

This study aimed to improve the understanding of this topic by **analyzing a large cohort of patient samples and reporting *PLEKHS1* promoter mutation prevalence, Bethesda category, co-occurring mutations, and surgical outcomes** (among a subset of 20 samples).

Out of a cohort of 9,279 patient samples collected between April 2023 and June 2024 with either Bethesda V/VI cytology or Bethesda III/IV cytology and an Afirma GSC Suspicious result, 60 were found to be positive for *PLEKHS1* C593T or G590A hotspot promoter mutations for a prevalence of 0.6%. Of these 60 samples, a subset of 20 samples with surgical histology were analyzed for pathology outcomes and co-occurring molecular alterations (Table 4). Out of this subset, 8 samples were ITN with an isolated *PLEKHS1* mutation, of which only 1 was malignant (a minimally invasive oncocytic carcinoma, found in row 8 of Table 4).

Ultimately, **this study found that in contrast to metastatic PTC, isolated *PLEKHS1* mutations found in indeterminate thyroid nodules do not correlate with a higher ROM or more aggressive disease,** though further studies are needed to fully understand the role that *PLEKHS1* mutations play in thyroid disease.

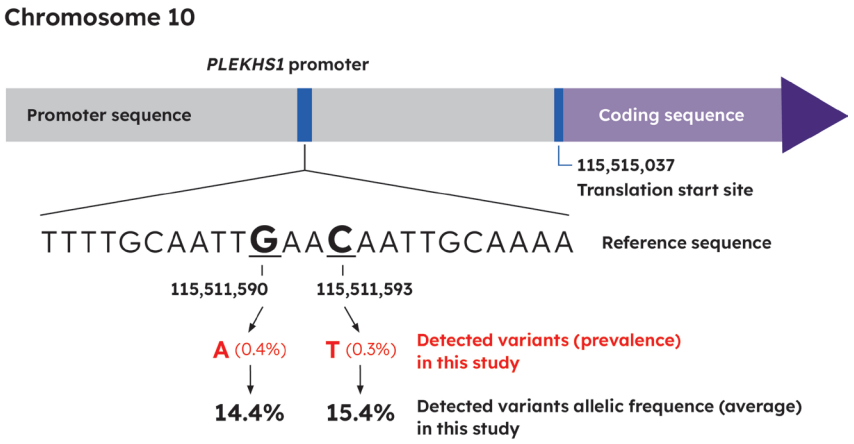
Access the poster layout

Schedule meeting with Medical Science Liaison

Introduction

- Pleckstrin homology domain containing S1 (*PLEKHS1)* (Figure 1) is a poorly characterized protein coding gene, whose C593T and G590A promoter mutations are associated with increased risk of lymph node and distant metastases,[1] RAI refractoriness,[2] and shorter survival[1] in differentiated thyroid cancer independent of *TERT* promoter (*TERT*p) mutations.

FIGURE 1. Genomic position and sequence of Pleckstrin Homology Domain Containing S1 (*PLEKHS1*) Promoter Mutations. Mutations coordinates are based on GRCh37/hg19.



- The diagnostic and prognostic significance of these mutations in thyroid nodules is unknown.
- Here we assess the potential impact of pre-operative detection of *PLEKHS1* promoter mutations in thyroid nodules.

Methods

- Targeted C593T and G590A *PLEKHS1* promoter mutations were assessed in 9,279 patient samples from April 2023 to June 2024 in indeterminate thyroid nodules (ITNs) with Bethesda (B) III/IV cytology (ITN) and Afirma GSC suspicious (GSC-S) results or with B V/VI cytology.
- A subset of consecutive cases positive for the targeted *PLEKHS1* promoter mutations with surgical histology (n=20) were analyzed for co-occurring molecular alterations and pathology outcomes.
  - Pathology outcomes were collected under WCG IRB #1384712.

Results

- *PLEKHS1* promoter mutations were assessed in 9,279 patient samples.
- The demographics of the 9,279 samples tested for the *PLEKHS1* C593T and G590A hotspot promoter mutations, along with the proportion with *TERT* promoter (*TERT*p) or *BRAF* p.V600E mutations are shown in Table 1.

Table 1. The median age, sex, proportion from each Bethesda category, and percent positive of *BRAF* p.V600E from 9,279 patient samples that are GSC-S or with BV/VI cytology and assessed for *PLEKHS1 C593T* and *G590A* promoter mutations.

Variable	Total (%)
Age (years) median IQR	54 [40-67]
Sex	
Male	2,448 (26.4%)
Female	6,829 (73.6%)
Bethesda group	
III	5,120 (55.2%)
IV	1,507 (16.2%)
V	1,108 (11.9%)
VI	1,177 (12.7%)
***BRAF*V600E+**	1,912 (20.6%)
***TERT*p+**	492 (5.3%)
***PLEKHS1*+**	60 (0.6%)

- *PLEKHS1* promoter mutations were positive in 60/9,279 (0.6%) of patient samples. The proportions of each hotspot mutation (C593T and G590A) assessed, and concomitant positive *TERT*p mutations and *BRAF* p.V600E mutations are shown in Table 2.

Table 2. *PLEKHS1* promoter mutations were positive in 60/9,279 (0.6%) of patient samples that were GSC-S or from BV/VI cytology. The proportions of each hotspot mutation (C593T and G590A) assessed, and concomitant positive *TERT*p mutations and *BRAF* p.V600E mutations are shown below.

Bethesda	Total	*BRAF*V600E+	*TERT*p+	*PLEKHS1* C593T+	*PLEKHS1* G590A+
III	25	2	0	11	14
IV	11	1	1	7	7
V	8	4	2	3	7
VI	16	11	1	7	10

- The proportion of *PLEKHS1* positive cases was highest in samples with Bethesda VI cytology (Table 3).

Table 3. The proportion of *PLEKHS1* positive cases from GSC-S or BV/VI samples was highest in samples with Bethesda VI cytology as shown below (*chi-square test p <0.01 compared to BIII).

Bethesda	Total	*PLEKHS1*+
III	5,293	25 (0.47%)
IV	1,507	11 (0.73%)
V	1,108	8 (0.72%)
VI	1,177	16 (1.36%)*

- Table 4 shows the case findings for 20 nodules with *PLEKHS1* positive mutations (15 from GSC-S ITN and 5 from BV/VI nodules). 40% (6/15) of ITN were malignant and met American Thyroid Association (ATA) criteria for low-risk cancer; 5/6 (83%) had co-alterations: *KRAS* p.G12D, *DICER1* p.E1705K, *NRAS* p.Q61R + *PIK3CA* p.E545K + *TERT* p.C228T, *NRAS* p.Q61R, and *FGFR2::VCL* fusion. 2/9 (22%) benign ITN had co-alterations (*PAX8::GLIS3* fusion and *NRAS* p.Q61R). Only 1/8 (12.5%) ITN with an isolated *PLEKHS1* mutation was malignant (minimally invasive oncocytic carcinoma). All 5 BV/VI nodules were malignant and met ATA criteria for high (2), intermediate (1), or low (2) risk cancer.

TABLE 4

Twenty case studies from *PLEKHS1* positive cases.

TT: Total Thyroidectomy
BLND: Bilateral Neck Dissection
CND: Central Neck Dissection
LND: Left Neck Dissection
FA: Follicular Adenoma
FVPTC: Follicular Variant of Papillary Thyroid Carcinoma
miFTC: Microcarcinoma Follicular Thyroid Carcinoma
miOTC: Minimally Invasive Oncocytic Thyroid Carcinoma
HN: Hyperplastic Nodule
IFVPTC: Invasive FVPTC

Nodule Location	Nodule size (cm)	Sex	Age	Bethesda	*PLEKHS1*_C593T	*PLEKHS1*_G590A	XA + *TERT* result	Surgery Type	(B)enign/ (M)alignant	Tumor Type	Tumor Size (cm)	Synoptic Data	ATA Risk Category
Middle Left	4.1	Female	58	III	–	Positive	–	TT	B	FA	4.3	–	–
Middle Right	2.8	Female	43	IV	Positive	–	*KRAS* p.G12D	R Lobectomy	M	FVPTC with Oncocytic Features	3.5	T2NxMx	Low
Isthmus	1.9	Male	66	III	Positive	–	–	L Lobectomy and Isthmusectomy	B	Adenomatoid Hyperplasia	1	–	–
Isthmus	2.1	Female	60	III	Positive	–	*DICER1* p.E1705K	Isthmusectomy	M	miFTC	2	T1bNxMx	Low
Lower Left	2.4	Male	59	III	Positive	–	–	L Lobectomy and Isthmusectomy	B	FA with Hürthle Change	2.1	–	–
Lower Left	1.2	Male	33	III	–	Positive	*PAX8::GLIS3*	Left Lobectomy	B	Hyalinizing Trabecular Tumor	0.9	–	–
Lower Left	2.6	Female	45	III	–	Positive	*NRAS* p.Q61R	TT	B	FH	2	–	–
Lower Right	4.2	Female	68	IV	Positive	–	–	Right Lobectomy	M	miOTC	4.3	T3aN0aMx	Low
Middle Right	1.8	Female	77	IV	Positive	Positive	*NRAS* p.Q61R, *PIK3CA* p.E545K, *TERT* p.C228T	TT	M	PTC	0.6	T1aN0aMx	Low
Left Side	0.69	Female	47	IV	–	Positive	*NRAS* p.Q61R	TT	M	PTC	0.2	T1aN0aMx	Low
Middle Right	2.9	Female	41	IV	Positive	Positive	*FGFR2::VCL*	TT	M	PTC (Warthin-like Subtype), Infiltrative	3	T2NxMx	Low
Left Side	3.2	Female	74	III	–	Positive	–	Left Lobectomy	B	FA	3.2	–	–
Middle Left	6.6	Female	69	III	–	Positive	–	Left Lobectomy	B	FA	4.8	–	–
Lower Right	1.2	Female	58	III	–	Positive	–	TT	B	FA	0.2	–	–
Left Side	4.7	Female	65	III	–	Positive	–	L Lobectomy and Isthmusectomy	B	HN	4.5	–	–
Middle Right	1.4	Female	60	VI	–	Positive	*BRAF* p.V600E and *DICER1* p.Q7R	TT + BLND	M	PTC	1.5	T1bN0aMx	Low
Middle Left	2.8	Female	65	VI	Positive	–	*BRAF* p.V600E	TT, CND, LND	M	PTC	4.7	T3bN1bMx	High
Upper Right	1	Female	55	V	–	Positive	*NTRK3::QSTM1*	R Lobectomy	M	IFVPTC	1.1	T1bNxMx	Low
Middle Left	2	Female	87	V	–	Positive	*BRAF* p.V600E	TT + CND	M	PTC	2.6	T2N1aMx	High
Right Side	3.95	Female	25	VI	Positive	–	*NRAS* p.Q61R	TT	M	PTC	4.8	T3aN0aMx	Intermediate

Discussion

- Molecular diagnostics can be harnessed to improve management of thyroid cancer and thyroid nodules.[3]
- Two studies showed *PLEKHS1* promoter mutations were associated with more aggressive DTC independent of *TERT*p mutations.
  - Xing et al Cancers 2020.[1]
    - Predict lymph node and distant metastases, and shorter overall and disease-free survival independent of *TERT*p mutations.
    - *PLEKHS1* over-expression enhanced AKT phosphorylation and invasiveness.
  - Jung et al Thyroid 2020.[2]
    - Associated with RAI refractoriness independent of *TERT* promoter mutation.

Conclusion

- *PLEKHS1* promoter mutations are rare in thyroid nodules undergoing molecular testing.
- In contrast with data in metastatic PTC, isolated *PLEKHS1* mutations detected in ITNs do not predict higher risk of cancer nor aggressive histology as compared with other Afirma GSC-S nodules.
- Further studies are needed to clarify the role of *PLEKHS1* promoter mutations in thyroid nodules and differentiated thyroid cancer to harness the full potential of molecular information for improving patient care.

References

1. Xing X, Mu N, Yuan X, Wang N, Juhlin CC, Stråått K, Larsson C, Xu D. *PLEKHS1* Over-Expression is Associated with Metastases and Poor Outcomes in Papillary Thyroid Carcinoma. *Cancers (Basel)*. 2020 Jul 31;12(8):2133. doi: 10.3390/cancers12082133. PMID: 32752127.
2. Jung CK, Jung SH, Jeon S, Jeong YM, Kim Y, Lee S, Bae JS, Chung YJ. Risk Stratification Using a Novel Genetic Classifier Including *PLEKHS1* Promoter Mutations for Differentiated Thyroid Cancer with Distant Metastasis. *Thyroid*. 2020 Nov;30(11):1589-1600. doi: 10.1089/thy.2019.0459. Epub 2020 May 19. PMID: 32326836.
3. Patel J, Klopper J, Cottrill EE. Molecular diagnostics in the evaluation of thyroid nodules: Current use and prospective opportunities. *Front Endocrinol (Lausanne)*. 2023 Feb 24;14:1101410. doi: 10.3389/fendo.2023.1101410. PMID: 36909304.

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

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

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KEY POINTS

- A 2021 study by Hu et al aimed to investigate the molecular findings across a large, real-world cohort of thyroid nodule fine needle aspiration (FNA) biopsies through the analysis of 50,644 consecutive BIII – BVI samples sent for Afirma GSC testing from August 2017 to September 2019.[1]
- The Afirma GSC database has since expanded to over 250,000 nodules with the added capability of *TERT* promoter mutation *(TERT*p*)* DNA analysis.[3]
- This study aimed to describe the updated genomic landscape of BIII-BVI thyroid nodules sent for Afirma GSC testing by assessing 252,510 consecutive thyroid tumors and reporting demographic, cytologic, and molecular findings.

STUDY: IN SUMMARY

Broad genomic analyses of thyroid nodules have historically been limited by small cohorts of less than 1,000 samples in size.[4-9] **In a 2021 study by Hu et al, the researchers reported the molecular findings** of thyroid nodule fine-needle aspiration (FNA) samples **using a large, real-world cohort of 50,644 consecutive (B)ethesda III - VI nodules assessed by Afirma GSC.**[1]

As the Afirma GSC database has since expanded to over 250,000 nodules with the added capability of *TERT*p DNA analysis, **the researchers in this study aimed to describe the updated genomic landscape of thyroid nodules sent for Afirma GSC testing in real world practice** with the benefit of a larger cohort and the addition of data on *TERT*p mutations.[3] This was done using 252,510 consecutive thyroid tumors with BIII-VI cytology undergoing Afirma GSC testing from 2018-2024; of which ~87,000 were suspicious and 8,627 samples received *TERT*p DNA analysis from 2023-2024.

The study reported:

Demographic/cytologic data
- The median patient age was 59.3 years, 76.5% of patients were female, and median nodule size was 2.2 cm (Table 1).
- The proportion of parathyroid and medullary thyroid carcinoma classifier positive lesions were < 1%, while > 78% of nodules referred for testing were Bethesda 3 (Table 1).
- 69% of indeterminate nodules (BIII/BIV) were classified as GSC-B.

Alteration data
- *BRAF*V600E was the most frequent variant, particularly in nodules with BV/VI cytology (Table 2.B).
- *NRAS* was the most prevalent RAS variant, most common in BIII/IV nodules (Table 2.B).
- *TERT*p mutations, alone or co-occurring with *BRAF*V600E were most common in BVI (Table 2.B).

Fusion data
- Fusions were identified in 6% of non-GSC-Benign nodules, most often in BV nodules (Table 2.B).
- Most prevalent fusions involved *RET* proto-oncogene, seen in Bethesda V/VI > III/IV (Table 2.B).
- *PPAR*γ fusions were primarily detected in Bethesda III/IV (Table 2.B).

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Schedule meeting with Medical Science Liaison

FULL STUDY

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).

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

- 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).

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

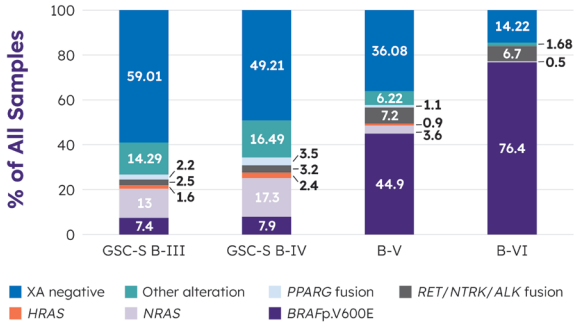
B. Most common expressed variants by Bethesda category

	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

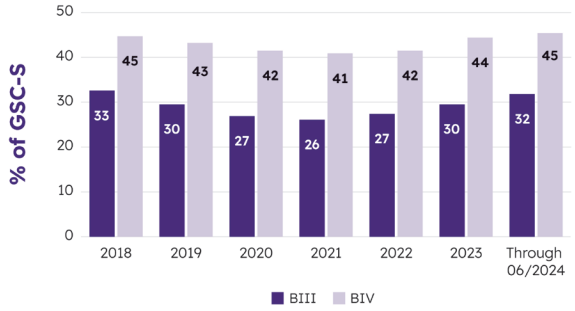
	Bethesda Category			
	III	**IV**	**V**	**VI**
TOTAL	**56,657**	**17,347**	**6,239**	**6,290**
RET	**399 (0.7%)**	**133 (0.8%)**	**202 (3.2%)**	**247 (3.9%)**
NTRK3	551 (1%)	227 (1.3%)	125 (2%)	74 (1.2%)
NTRK1	72 (0.1%)	22 (0.1%)	20 (0.3%)	25 (0.4%)
ALK	123 (0.2%)	57 (0.3%)	31 (0.5%)	7 (0.1%)
BRAF	**263 (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



- 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) ($p < 0.001$).

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.
  - *PPAR*γ fusions were primarily detected in Bethesda III/VI.
- Temporal shifts in GSC-S rates are of interest and warrant further investigation.

1. Hu MI, Waguespack SG, Dosiou C, Ladenson PW, Livhits MJ, Wirth LJ, Sadow PM, Krane JF, Stack BC, Zafereo ME, Ali SZ, Weitzman SP, Hao Y, Babiarz JE, Kennedy GC, Kloos RT. Afirma Genomic Sequencing Classifier and Xpression Atlas Molecular Findings in Consecutive Bethesda III-VI Thyroid Nodules. *J Clin Endocrinol Metab.* 2021 Jul 13;106(8):2198-2207. doi: 10.1210/clinem/dgab304. PMID: 34009369; PMCID: PMC8277199.
2. Iyer PC, Dadu R, Barque A, Zanelli C, Zheng X, Jiang H, Walsh PS, Hao Y, Huang J, Klopper JP, Kloos RT, Cabanillas M. Analytical Validation of a Telomerase Reverse Transcriptase (*TERT*) Promoter Mutation Assay. *J Clin Endocrinol Metab.* 2024 Aug 13;109(9):2269-2273. doi: 10.1210/clinem/dgae134. PMID: 38441247; PMCID: PMC11318993.
3. Angell TE, Wirth LJ, Cabanillas ME, Shindo ML, Cibas ES, Babiarz JE, Hao Y, Kim SY, Walsh PS, Huang J, Kloos RT, Kennedy GC, Waguespack SG. Analytical and Clinical Validation of Expressed Variants and Fusions From the Whole Transcriptome of Thyroid FNA Samples. *Front Endocrinol (Lausanne)*. 2019 Sep 11;10:612. doi: 10.3389/fendo.2019.00612. PMID: 31572297; PMCID: PMC6749016.
4. Xu B, Fuchs T, Dogan S, et al. Dissecting anaplastic thyroid carcinoma: a comprehensive clinical, histologic, immunophenotypic, and molecular study of 360 cases. *Thyroid*. 2020;30(10):1505-1517.
5. Ciampi R, Romei C, Ramone T, et al. Genetic landscape of somatic mutations in a large cohort of sporadic medullary thyroid carcinomas studied by next-generation targeted sequencing. *Iscience*. 2019;20:324-336.
6. Pozdeyev N, Gay LM, Sokol ES, et al. Genetic analysis of 779 advanced differentiated and anaplastic thyroid cancers. *Clin Cancer Res.* 2018;24(13):3059-3068.
7. Liang J, Cai W, Feng D, et al. Genetic landscape of papillary thyroid carcinoma in the Chinese population. *J Pathol.* 2018;244(2):215-226.
8. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. *Cell.* 2014;159(3):676-690.
9. Ye L, Zhou X, Huang F, et al. The genetic landscape of benign thyroid nodules revealed by whole exome and transcriptome sequencing. *Nat Commun.* 2017;8:15533.

The Genomic Landscape of Medullary Thyroid Carcinoma Identified by the Afirma RNA-Sequencing Classifier: Insights from a Large Real-World Cohort

The Genomic Landscape of Medullary Thyroid Carcinoma Identified by the Afirma RNA-Sequencing Classifier: Insights from a Large Real-World Cohort

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KEY POINTS

- Medullary thyroid carcinomas (MTC) are rare but more likely to cause death than more common types of thyroid cancer.[4,5]
- Cytopathological evaluation of thyroid nodule fine needle aspiration (FNA) biopsies may miss more than half of MTC.[6,7]
- This study characterized the landscape of MTC classifier positive samples across a large real-world cohort of Afirma GSC tested thyroid nodules.

STUDY: IN SUMMARY

Medullary thyroid carcinomas (MTC) are rare, but more likely to cause death than more common types of thyroid cancer.[4,5] Cytopathological evaluation of thyroid fine needle aspiration (FNA) biopsies misses more than half of MTC, and failure to identify these malignancies puts the patient at risk of receiving insufficient preoperative evaluation and initial thyroid surgery inconsistent with accepted guidelines.[4,6,7] Conversely, early MTC detection and treatment are associated with improved patient outcomes.[8]

Veracyte developed a microarray based MTC classifier to identify these malignancies, which was validated for BIII-BIV nodules.[9] **When Afirma migrated to an RNA-sequencing platform, a new RNA-sequencing-based MTC classifier was developed using machine learning which subsequently demonstrated 100% sensitivity in identifying MTC.**[10,11]

In this study, the researchers aimed to use this classifier to characterize the landscape of MTC across a large real-world cohort of FNA samples. Out of 252,510 FNA samples tested with Afirma GSC, 732 were classified as MTC. Using these MTC-positive samples, the study reported demographic (table 1), cytologic (table 1), molecular (table 2), and pathway expression data (figure 1 and 2).

Figure 1 shows that *RAS*-driven MTC demonstrated the highest ERK activity, followed closely by *RET*-driven MTC, both of which demonstrated significantly higher ERK signaling expression than non-*RET*-non-*RAS* samples. Ultimately, **this study described the genomic landscape of MTC, generating novel insights into a cohort of MTC positive samples that were not diagnosed on cytopathology.**

Access the poster layout

Schedule meeting with Medical Science Liaison

FULL STUDY

Introduction

- The Afirma RNA-sequencing Medullary Thyroid Carcinoma (MTC) classifier, a component of the Afirma Genomic Sequencing Classifier (GSC), was previously validated as a reliable tool for preoperative identification of MTC from fine-needle aspiration (FNA) specimens.[1,2]
- We aimed to characterize the genomic landscape of MTC identified using this classifier in a large real-world cohort of FNA samples.

Methods

- We retrospectively analyzed MTC positive samples by the Afirma MTC classifier in the Veracyte CLIA laboratory between January 2018 and June 2024.
- Genomic variants identified by the Afirma Xpression Atlas (XA) were characterized.[3]

Results

Demographic and cytologic characteristics

- Among 252,510 FNA samples tested, 732 (0.3%) were classified as MTC.
- Median patient age was 63.2 years (IQR, 52.1-72.1) and median nodule size was 2.1 cm (IQR, 1.6-3.0).
- On cytology, 71% (520/732) of nodules were Bethesda III or IV, 18% were Bethesda V and 11% were Bethesda VI (Table 1).

Table 1. Demographic data of MTC classifier positive thyroid nodules

	Total (n=732)
Median age (yrs) [IQR]	63.2 [52.1-72.1]
Median nodule size (cm) [IQR]	2.1 [1.6-3]
Sex	
Male	295 (40.3%)
Female	437 (59.7%)
Bethesda Category	
III	315 (43%)
IV	205 (28%)
V	132 (18%)
VI	80 (11%)

Molecular characteristics

- 73% of MTC-positive thyroid nodules had at least one pathogenic variant identified by XA, most commonly in *RET* (53%) and *RAS* (19%) with no significant difference across Bethesda categories (Table 2).
  - Most *RET* alterations were in codons 918 (19%) and 634 (10%), while *HRAS* was the most frequently altered *RAS* isoform (15%).

Table 2. Expressed molecular variants identified by XA in MTC+ thyroid nodules

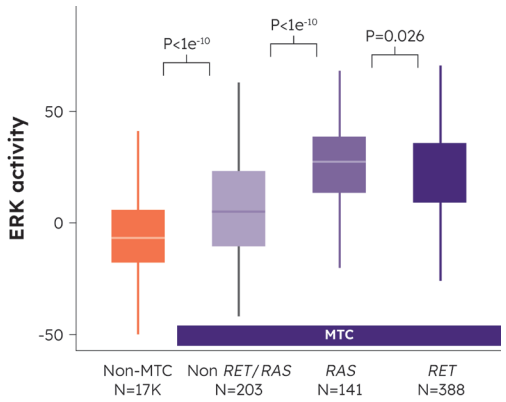
		Bethesda Category			
	All MTC	**III**	**IV**	**V**	**VI**
Total	732	315	205	132	80
Any variant	533 (73%)	231 (73%)	151 (74%)	94 (71%)	57 (71%)
RET	388 (53.01%)	172 (54.6%)	105 (51.22%)	71 (53.79%)	40 (50%)
M918	140 (19.13%)	53 (16.83%)	37 (18.05%)	30 (22.73%)	20 (25%)
C634	74 (10.11%)	38 (12.06%)	14 (6.83%)	14 (10.61%)	8 (10%)
C609	39 (5.33%)	23 (7.3%)	10 (4.88%)	4 (3.03%)	2 (2.5%)
C630	37 (5.05%)	17 (5.4%)	10 (4.88%)	7 (5.3%)	3 (3.75%)
C618	24 (3.28%)	9 (2.86%)	11 (5.37%)	2 (1.52%)	2 (2.5%)
C620	22 (3.01%)	8 (2.54%)	6 (2.93%)	5 (3.79%)	3 (3.75%)
V804	22 (3.01%)	7 (2.22%)	13 (6.34%)	2 (1.52%)	0 (0%)
A883	10 (1.37%)	4 (1.27%)	2 (0.98%)	3 (2.27%)	1 (1.25%)
C611	8 (1.09%)	2 (0.63%)	1 (0.49%)	4 (3.03%)	1 (1.25%)
L790	8 (1.09%)	6 (1.9%)	1 (0.49%)	0 (0%)	1 (1.25%)
HRAS	112 (15.3%)	51 (16.19%)	35 (17.07%)	17 (12.88%)	9 (11.25%)
Q61	84 (11.48%)	42 (13.33%)	24 (11.71%)	13 (9.85%)	5 (6.25%)
G13	27 (3.69%)	9 (2.86%)	11 (5.37%)	3 (2.27%)	4 (5%)
KRAS	29 (3.96%)	7 (2.22%)	11 (5.37%)	5 (3.79%)	6 (7.5%)
G13	21 (2.87%)	5 (1.59%)	8 (3.9%)	3 (2.27%)	5 (6.25%)
Q61	8 (1.09%)	2 (0.63%)	3 (1.46%)	2 (1.52%)	1 (1.25%)
NRAS	1 (0.14%)	1 (0.32%)	0 (0%)	0 (0%)	0 (0%)

- Mutually exclusive oncogenic fusions were identified in 8 non-*RET*/*RAS* SNV mutated samples [*EML4*::*ALK* (n=1), *MKRN1*::*BRAF* (n=6), and *SPECC1L*::*RET* (n=1)].
- Additional isolated altered variants identified in non-*RET*/*RAS* samples included *AKT1, DICER1, PIK3CA,* and *TP53*.

Pathway expression

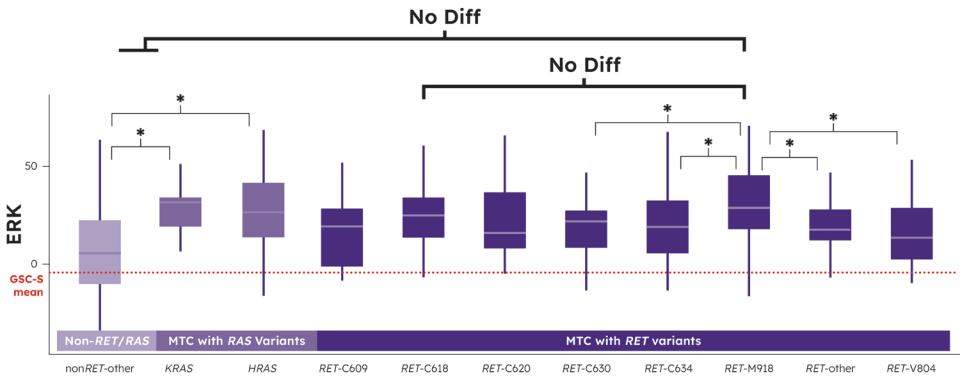
- ERK signaling activity was highest in *RAS*-mutated MTC-positive samples, followed by *RET*-mutated and non *RET/RAS* MTC, with all MTC-positive groups showing greater activity than non-MTC Afirma GSC-suspicious samples (Figure 1).

Figure 1. ERK signaling expression activity in MTC-positive and non-MTC GSC-S samples



- Across MTC-positive samples, *RET* M918T and *HRAS* variants were associated with the highest degree of ERK acritivty (Figure 2).

Figure 2. ERK signaling expression activity across MTC-positive samples



*p<0.01

Conclusions

- This large study reinforces the known genomic landscape of MTC.
- Additionally, our findings highlight that a subset of thyroid nodules sent for Afirma GSC testing are unrecognized MTCs, underscoring the value of molecular testing in improving preoperative diagnostic accuracy.
- Gene expression analysis showed that ERK activity was slightly lower in *RET*-driven than in *RAS*-driven MTC (p=0.02), but significantly higher in both groups compared with non-*RET*/non-*RAS* samples ($p<1^{-10}$).
- Further study is needed to molecularly define the nearly 30% of specimens that lacked detectable alterations.

References

1. Patel KN, Angell TE, Babiarz J, Barth NM, Blevins T, Duh QY, Ghossein RA, Harrell RM, Huang J, Kennedy GC, Kim SY, Kloos RT, LiVolsi VA, Randolph GW, Sadow PM, Shanik MH, Sosa JA, Traweek ST, Walsh PS, Whitney D, Yeh MW, Ladenson PW. Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules. *JAMA Surg*. 2018 Sep 1;153(9):817-824. doi: 10.1001/jamasurg.2018.1153. PMID: 29799911; PMCID: PMC6583881.
2. Iyer PC, Dadu R, Barque A, Zanelli C, Zheng X, Jiang H, Walsh PS, Hao Y, Huang J, Klopper JP, Kloos RT, Cabanillas M. Analytical Validation of a Telomerase Reverse Transcriptase (*TERT*) Promoter Mutation Assay. *J Clin Endocrinol Metab*. 2024 Aug 13;109(9):2269-2273. doi: 10.1210/clinem/dgae134. PMID: 38441247; PMCID: PMC11318993.
3. Angell TE, Wirth LJ, Cabanillas ME, Shindo ML, Cibas ES, Babiarz JE, Hao Y, Kim SY, Walsh PS, Huang J, Kloos RT, Kennedy GC, Waguespack SG. Analytical and Clinical Validation of Expressed Variants and Fusions From the Whole Transcriptome of Thyroid FNA Samples. *Front Endocrinol (Lausanne)*. 2019 Sep 11;10:612. doi:10.3389/fendo.2019.00612. PMID: 31572297; PMCID: PMC6749016.
4. Wells SA, Jr., Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid* 2015;25(6):567–610; doi:10.1089/thy.2014.0335.
5. Randle RW, Balentine CJ, Leverson GE, et al. Trends in the presentation, treatment, and survival of patients with medullary thyroid cancer over the past 30 years. *Surgery* 2017;161(1):137–146; doi:10.1016/j.surg.2016.04.053.
6. Workman AD, Soylu S, Kamani D, et al. Limitations of preoperative cytology for medullary thyroid cancer: Proposal for improved preoperative diagnosis for optimal initial medullary thyroid carcinoma specific surgery. *Head Neck* 2021;43(3):920–927; doi:10.1002/hed.26550.
7. Trimboli P, Treglia G, Guidobaldi L, et al. Detection rate of FNA cytology in medullary thyroid carcinoma: A meta-analysis. *Clin Endocrinol (Oxf)* 2015;82(2):280–285; doi:10.1111/cen.12563.
8. Lennon P, Deady S, White N, et al. Aggressive medullary thyroid cancer, an analysis of the Irish National Cancer Registry. *Ir J Med Sci* 2017;186(1):89–95; doi:10.1007/s11845-016-1455-1.
9. Kloos RT, Monroe RJ, Traweek ST, et al. A genomic alternative to identify medullary thyroid cancer preoperatively in thyroid nodules with indeterminate cytology. *Thyroid* 2016;26(6):785–793; doi:10.1089/thy.2016.0001.
10. Patel KN, Angell TE, Babiarz J, et al. Performance of a genomic sequencing classifier for the preoperative diagnosis of cytologically indeterminate thyroid nodules. *JAMA Surg* 2018;153(9):817–824; doi:10.1001/jamasurg.2018.1153.
11. Randolph GW, Sosa JA, Hao Y, et al. Preoperative Identification of Medullary Thyroid Carcinoma (MTC): Clinical Validation of the Afirma MTC RNA-Sequencing Classifier. *Thyroid*. 2022;32(9):1069-1076. doi:10.1089/thy.2022.0189.

Development of a Prognostic Cell Cycle Progression Score in Preoperative Thyroid Tumor Samples to Predict Prognosis

Development of a Prognostic Cell Cycle Progression Score in Preoperative Thyroid Tumor Samples to Predict Prognosis

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KEY POINTS

- Alterations in cell cycle regulatory genes, which control the progression of cells through the cell cycle, may lead to abnormal cellular proliferation, tumor development, and malignancy.
- To investigate the prognostic potential of these genes, a novel cell cycle progression (CCP) activity z-score was developed using the expression of 47 genes implicated in cell cycle progression. The CCP score was used to stratify 2,205 Bethesda category V and VI fine needle aspiration samples sent for Afirma testing into 4 quartiles (Q1 representing the lowest 25% and Q4 representing the top 25%).
- This study found that relative to Q1, Q4 was enriched with *TERT* promoter mutations, disease progression, stage IV disease, and a shorter time to disease progression, suggesting that the CCP score may be predictive of tumor aggressiveness.

STUDY: IN SUMMARY

The process a cell must complete to grow and divide, a fundamental feature of life, is referred to as the 'cell cycle.' This cycle includes three main checkpoints (G1, G2, and M), where the cell is evaluated before proceeding to the next phase. Cell cycle regulatory genes produce proteins that either promote progression (such as cyclins and CDKs) or halt it (such as p53 and Rb), ensuring accurate and controlled cell division.

Though current molecular testing of thyroid nodules can confidently rule out cancer, the prognostic value of molecular markers to predict thyroid cancer aggressiveness is suboptimal. **Alterations in cell cycle regulatory genes may lead to cancer, which is why the researchers in this study chose to investigate their prognostic potential.**

First, **the researchers identified 47 genes from The Cancer Genome Atlas (Thyroid) involved in cell cycle progression** (listed along the right side axis of figure 1 and 2). Then, **the median activity of these 47 genes was measured and lower and higher values relative to the median expression created a novel CCP activity Z-score.** This score was analyzed in reference to clinicopathologic outcomes from the TCGA, *TERT* promoter mutation status, and common oncogenic alterations reported by the Afirma Xpression Atlas, as well as other molecular markers of tumor aggressiveness (such as MACIS score and stage IV disease, as seen at the top right of figure 1).

TCGA samples were then stratified into quartiles based on their CCP activity z-score, with Q4 representing the top 25% and Q1 representing the lowest 25% (Figure 1). After this was done, the researchers found that **relative to Q1, Q4 was enriched with *TERT* promoter mutations, disease progression, and stage IV disease, and was also associated with a shorter time to disease progression, suggesting the CCP score may prove a useful prognostic tool for tumor aggressiveness,** especially in tumors lacking distinct alterations such as *BRAF*V600E.

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Schedule meeting with Medical Science Liaison

Introduction

- The American Thyroid Association (ATA) thyroid nodule management guidelines follow a clinicopathological risk stratification system based on risk factors, which subdivides patients into high-risk, intermediate-risk and low-risk cohorts, providing prognostic and predictive information to facilitate clinical decisions.[1] However, many prognostic signatures lack reproducibility due to individual heterogeneity. Given the increasing use of lobectomy in thyroid cancer, as well as even less invasive approaches (e.g. active surveillance) there is a need for more comprehensive pre-operative classifying tools.
- While *BRAF*V600E and *RAS* mutations have an established role in diagnosis of thyroid cancer, their use for prognostication in thyroid cancer is more limited due to tumor heterogeneity and presence of other factors. Secondary mutations have been shown to confer worse prognosis in thyroid cancer, and especially mutations in *TERT* promoter and genes in the *PI3K/AKT/mTOR* pathway.[2,3]
- Alterations in cell cycle regulatory genes may lead to abnormal cellular proliferation, tumor development, and malignancy. Disruptions in the cell cycle can contribute to the development and progression of the disease.
- By leveraging the whole transcriptome derived Afirma Genomic Sequencing Classifier (GSC) thyroid nodule molecular testing platform,[4] a mRNA expression-based signature on genes implicated in cell cycle progression was developed.
- The objective of this study was to characterize cell cycle proliferation genes in thyroid nodules/cancer and assess the molecular and clinical associations of their expression.

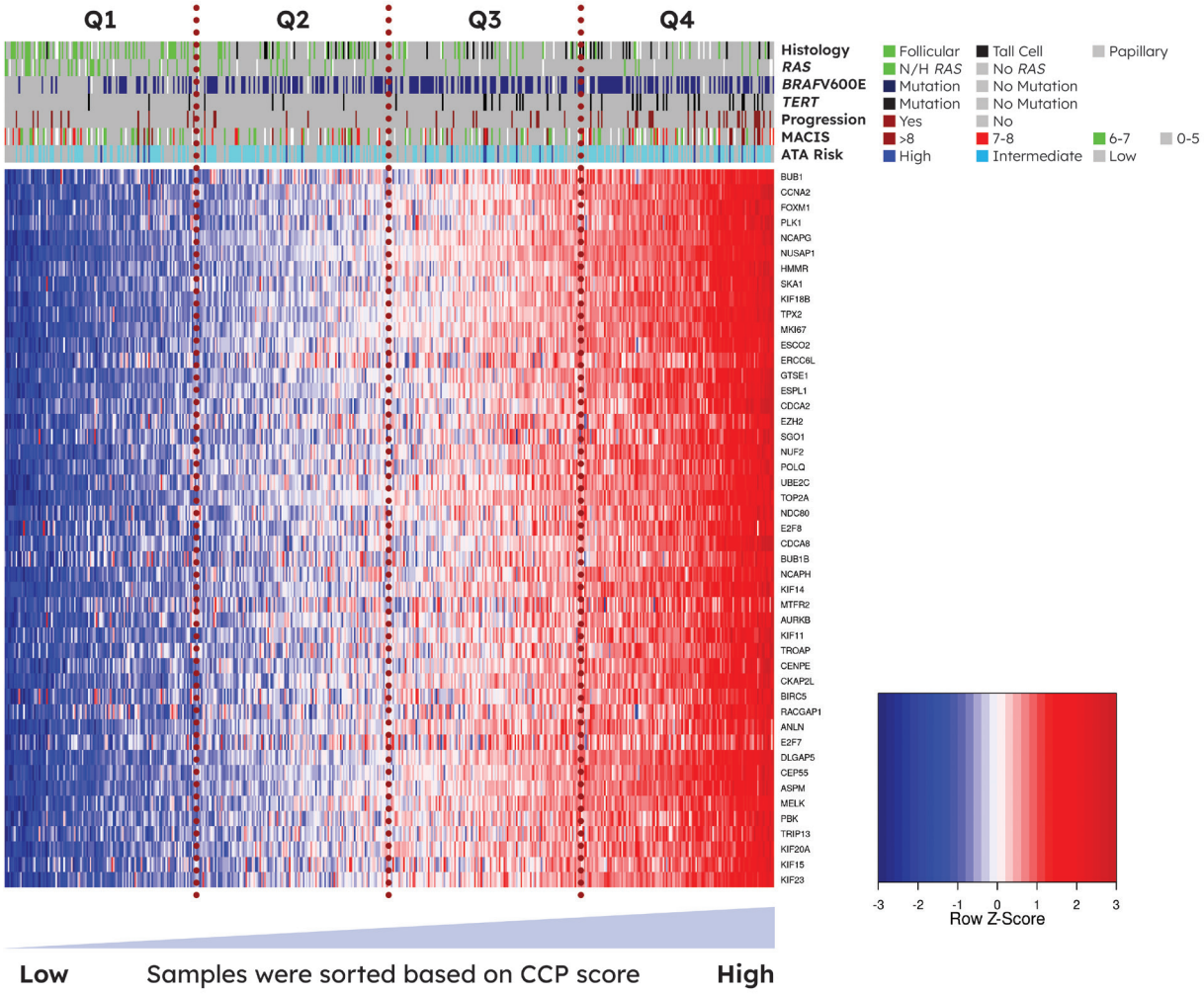
Methods

- A set of 47 genes implicated in cell cycle progression (CCP) (i.e. *TOP2A, MKI67*) was identified from The Cancer Genome Atlas (TCGA) Thyroid.
- A CCP activity z-score was derived from the expression of the 47 genes and subsequently associated with genomic alterations and outcome data in TCGA.
- 2,205 fine needle aspiration (FNA) samples with (B)ethesda V/VI cytology sent for Afirma testing were extracted from the Afirma thyroid nodule database.
  - The CCP score was analyzed in reference to *TERT* promoter mutation status and common oncogenic alterations reported by the Afirma Xpression Atlas (XA – the variant and fusion panel), as well as other molecular markers of tumor aggressiveness.
  - Fisher's exact test was used to assess statistical differences.

Results

- TCGA samples were stratified based on the CCP score and then grouped into 4 quartiles (Q4: top 25%, Q3: 50-75%, Q2: 25-50%, Q1: low 25%) (Figure 1).

Figure 1. Quartiles of CCP scores in TCGA showing differential gene expression and associated histology, mutation, TCGA disease progression, MACIS, and high risk category



- Comparing Q4 to Q1, Q4 was enriched with *TERT* promoter mutations (14.4% vs 2.4%, p<0.001), disease progression (20% vs 8%, p=0.001), MACIS (Metastases, Age, Completeness of resection, Invasion, and Size) score > 8 (9.6% vs 3.2%, p=0.06), and stage IV disease (13.6% vs 5.6%, p=0.05). The Q4 group is associated with a shorter time to disease progression (HR: 2.56, 95% CI [1.23-5.3], p=0.01) relative to the Q1 group (Table 1).

Table 1. Relative proportion of mutations, risk of progression, histology, cancer risk, MACIS score, and disease stage by CCP quartile in TCGA data

	Q1 (n=125)	Q2 (n=124)	Q3 (n=124)	Q4 (n=125)	p-value (Q4 vs Q1)
*BRAF*p.V600E	31 (24.8%)	61 (49.2%)	72 (58.1%)	70 (56%)	**p<0.001**
N/H *RAS*	27 (21.6%)	13 (10.5%)	6 (4.8%)	7 (5.6%)	**p<0.001**
TERT	3 (2.4%)	4 (3.2%)	10 (8.1%)	18 (14.4%)	**p<0.001**
Progression	10 (8%)	6 (4.8%)	10 (8.1%)	25 (20%)	**p=0.001**
Histology					**p<0.001**
Tall cell	1 (0.8%)	11 (8.9%)	9 (7.3%)	13 (10.4%)	
PTC	67 (53.6%)	82 (66.1%)	85 (68.5%)	87 (69.6%)	
FTC	48 (38.4%)	23 (18.5%)	17 (13.7%)	11 (8.8%)	
Risk					**p<0.001**
High	4 (3.2%)	5 (4%)	8 (6.5%)	7 (5.6%)	
Intermediate	51 (40.8%)	69 (55.6%)	61 (49.2%)	75 (60%)	
Low	59 (47.2%)	42 (33.9%)	42 (33.9%)	28 (22.4%)	
MACIS					
MACIS>8	4 (3.2%)	4 (3.2%)	6 (4.8%)	12 (9.6%)	
MACIS7-8	11 (8.8%)	11 (8.9%)	4 (3.2%)	12 (9.6%)	
MACIS6-7	21 (16.8%)	13 (10.5%)	15 (12.1%)	14 (11.2%)	
MACIS<6	75 (60%)	85 (68.5%)	86 (69.4%)	68 (54.4%)	
Disease Stage					**p=0.05**
I	60 (48%)	65 (52%)	74 (60%)	67 (54%)	
II	18 (14.4%)	16 (13%)	12 (10%)	3 (2.5%)	
III	30 (24%)	23 (18.5%)	21 (17%)	27 (21.6%)	
IV	7 (5.6%)	13 (11%)	7 (6%)	17 (13.6%)	

- In Afirma B V/VI samples, Q4 CCP score was more enriched with *TERT* promoter mutations (11.2% vs 4.9%, p<0.001) compared to Q1, but less enriched with *BRAF*V600E (40% vs 69%, p<0.001), and *RAS* family variants (1.8% vs 6.4%, p<0.001) (Figure 2).

Figure 2. CCP quartiles by genomic alterations in Afirma Bethesda V/VI samples

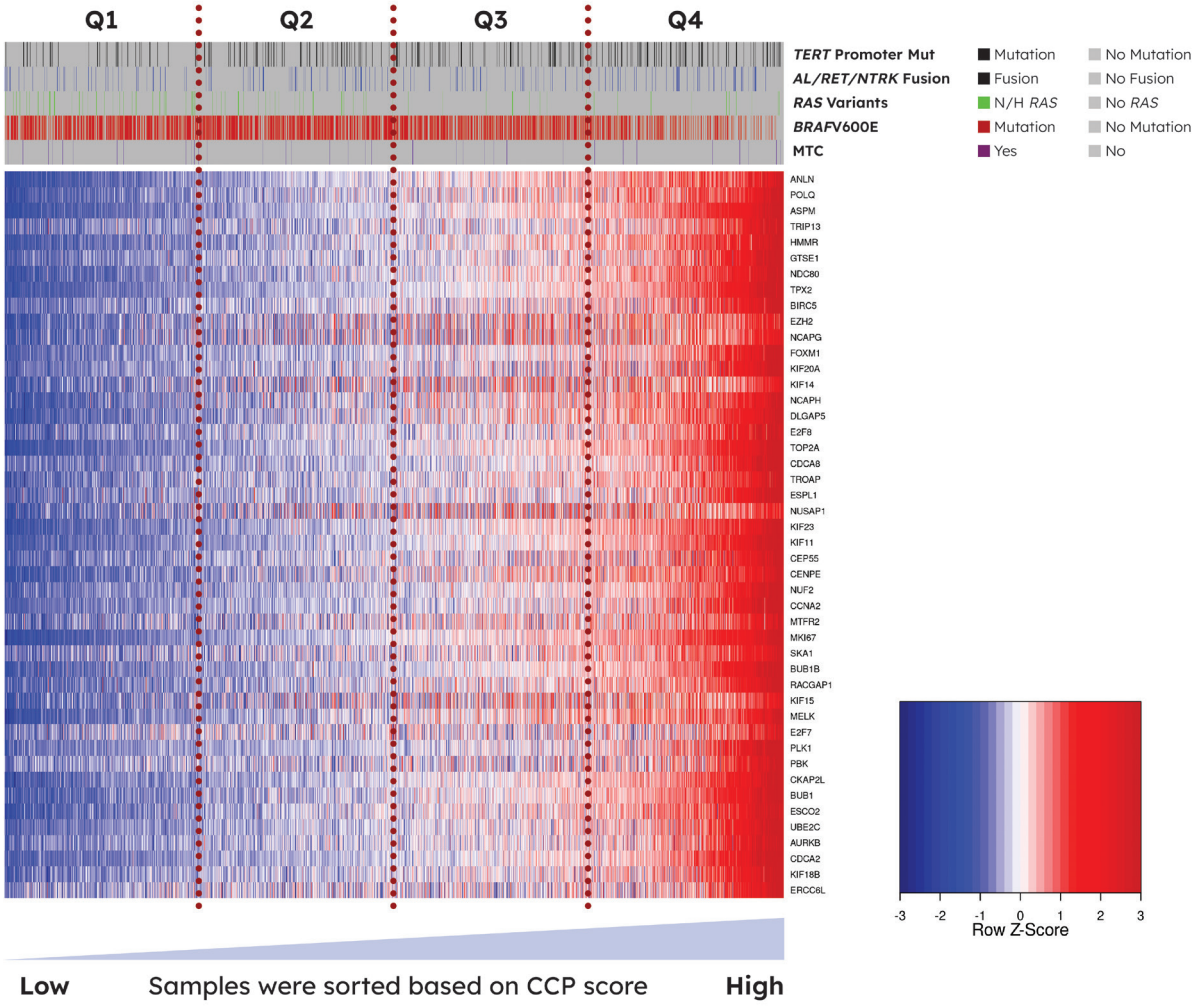


Table 2. Relative proportion of mutations, sex, nodules size, and age by CCP quartile

	Q1	Q2	Q3	Q4	p-value (Q4 vs Q1)
Total	**551**	**552**	**550**	**552**	
Key Genomic Alterations					
TERT+	27 (4.9%)	56 (10.1%)	61 (11.1%)	62 (11.2%)	**p<0.001**
*BRAF*V600E+	382 (69.3%)	393 (71.2%)	350 (63.6%)	223 (40.4%)	**p<0.001**
PTEN/*PIK3* variants	0 (0%)	0 (0%)	9 (1.6%)	5 (0.9%)	
RAS	35 (6.4%)	21 (3.8%)	8 (1.5%)	10 (1.8%)	**p<0.001**
RET/ALK/NTRK fusions	33 (6%)	37 (6.7%)	29 (5.3%)	40 (7.2%)	
Sex					
Male	156 (28.3%)	163 (29.5%)	133 (24.2%)	155 (28.1%)	
Female	395 (71.7%)	389 (70.5%)	416 (75.6%)	397 (71.9%)	
Nodule Size (cm)					
<1	27 (4.9%)	23 (4.2%)	29 (5.3%)	41 (7.4%)	
1-1.99	288 (52.3%)	257 (46.6%)	255 (46.4%)	277 (50.2%)	
2-2.99	130 (23.6%)	153 (27.7%)	138 (25.1%)	121 (21.9%)	
3-3.99	59 (10.7%)	60 (10.9%)	64 (11.6%)	52 (9.4%)	
≥4	42 (7.6%)	43 (7.8%)	54 (9.8%)	54 (9.8%)	
Age (years)					
<21	13 (2.4%)	20 (3.6%)	23 (4.2%)	19 (3.4%)	
21-39	135 (24.5%)	167 (30.3%)	165 (30%)	156 (28.3%)	
40-59	219 (39.7%)	196 (35.5%)	195 (35.5%)	217 (39.3%)	
60-74	145 (26.3%)	135 (24.5%)	120 (21.8%)	119 (21.6%)	
≥75	39 (7.1%)	34 (6.2%)	47 (8.5%)	41 (7.4%)	

Conclusions

- The CCP score was associated with *TERT* promoter mutations and more advanced disease state in the TCGA cohort, and similarly with *TERT* promoter mutations in Afirma B V/VI samples.
- In the pre-operative setting, the CCP score may serve as proxy of tumor aggressiveness, especially in thyroid tumors lacking distinct oncogenic alterations, such as *BRAF*V600E, and with variable histologic phenotype.
- Further studies integrating histopathology and recurrence information are necessary to better characterize the applicability of CCP score in the pre-operative evaluation of thyroid tumors.

References

1. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*. 2016;26(1):1-133. doi:10.1089/thy.2015.0020.
2. Liu R, Xing M. *TERT* Promoter Mutations in Thyroid Cancer. *Endocr Relat Cancer*. 2016;23(3):R143-R155. doi:10.1530/ERC-16-0034.
3. Zhang J, Baran J, Cros A, et al. A Pan-Cancer Proteogenomic Atlas of *PI3K/AKT/mTOR* Pathway Alterations. *Cancer Cell*. 2017;31(6):820-832.e3. doi:10.1016/j.ccell.2017.05.002.
4. Alexander EK, Kennedy GC, Baloch ZW, et al. Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology. *N Engl J Med*. 2012;367(8):705-715. doi:10.1056/NEJMoa1203208.

Retinoic Acid Receptor (RAR) and Retinoid X Receptor (RXR) Expression in Preoperative Thyroid Tumor Samples

Retinoic Acid Receptor (RAR) and Retinoid X Receptor (RXR) Expression in Preoperative Thyroid Tumor Samples

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KEY POINTS

- Retinoids influence cell growth and differentiation through binding to retinoid receptors, of which there are 6 major subtypes (RARα, -β, -γ, and RXRα, -β, -γ).[4-5,7-8]
- This study investigated the relationship between retinoid receptors and thyroid tumors by measuring the mRNA expression of these 6 isoforms by Bethesda category, Afirma GSC result, and *BRAF*V600e status.
- The RARγ and RXRγ isoforms demonstrated similar high relative expression by cytology, Afirma GSC result, and higher risk mutation status, and may prove to be good therapeutic targets in advanced thyroid cancer.

STUDY: IN SUMMARY

Retinoids are a group of chemical compounds related to vitamin A that play a role in a variety of biological processes ranging from embryonic development to immune response. **Retinoid therapy has demonstrated success in the treatment of certain cancers (e.g., Acute Promyelocytic Leukemia) and its potential as a therapeutic in other solid cancers is of research interest.**[9,10,11]

Retinoids influence cell growth and differentiation by binding to proteins known as retinoid receptors, of which there are two families: **RAR (Retinoic Acid Receptor)** and **RXR (Retinoid X Receptor).**[4,5,7,8] Each family has 3 subtypes (α, β, and γ) for a total of 6 isoforms: **RARα, RARβ, RARγ, RXRα, RXRβ,** and **RXRγ**.

RXRγ is generally not detectable in healthy thyroid tissue. It can be found in cancerous thyroid tumors, but the quantity can vary significantly from tumor to tumor. **The level of RXRγ predicts how well thyroid cancer cells will respond to retinoid treatment in cell lines.**[2]

The researchers in this study decided to investigate these retinoid isoforms to better understand their relationship with thyroid tumors. They did this by investigating the mRNA expression of these 6 RAR and RXR isoforms by Bethesda category, Afirma GSC result, and *BRAF*V600e status. Another cohort with *TERT* promoter mutational status was analyzed by variant and fusion alterations and genomic markers of tumor aggressiveness.

Results were provided by isoform. Notably, **RARγ and RXRγ demonstrated similar relatively high expression by cytology, Afirma GSC result, and higher risk mutation status, and may prove to be good therapeutic targets in advanced thyroid cancer.**

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Schedule meeting with Medical Science Liaison

Introduction

- Thyroid cancer will account for ~2% of all new carcinoma diagnoses in the US in 2025.[3]
- Approximately 40,000 new cases of thyroid cancer are diagnosed in the US each year, and ~3000 of these patients die each year.
- An estimated 5-10% of patients have advanced thyroid cancer that is unresponsive to surgical and radioiodine therapy.[1]
- Retinoids influence cell growth and differentiation through retinoid receptors, retinoic acid (RAR) and retinoid X receptor (RXR).[4-5, 7-8]
  - Six major subtypes of receptor have been identified, which are encoded by separate genes (RARα, -β, -γ, and RXRα, -β, -γ).
- The RXRγ isoform is undetectable in normal thyroid and variably expressed in malignant tumors, and this receptor predicts response to retinoid treatment in cell lines.[2]
  - A phase II trial with the retinoid bexarotene was stopped prematurely due to toxicity and low efficacy.[6]

Goal of the study

Hypothesis

Retinoid receptors will be differentially expressed in thyroid tumors based on (B)ethesda cytology category, Afirma GSC result, and mutational status.

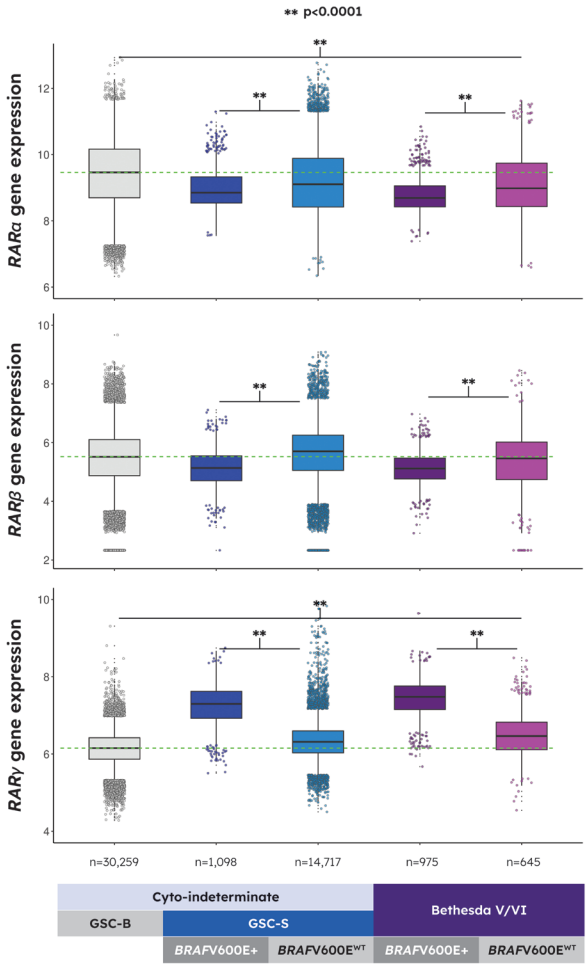
Methods

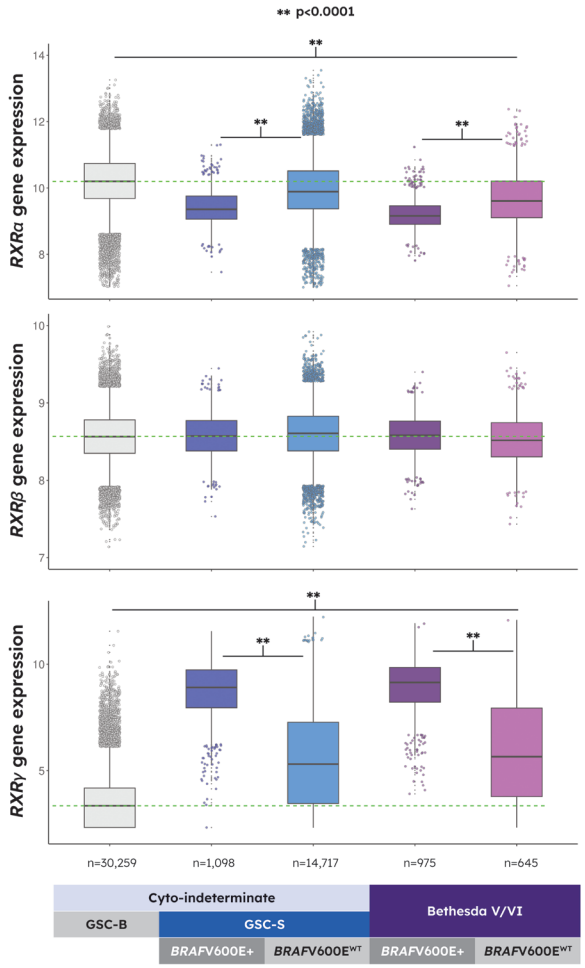
- mRNA expression of the six retinoic acid (RAR) and retinoid X receptor (RXR) isoforms was analyzed across thyroid nodules sent for Afirma testing.
- Differential expression was explored across a cohort of nodules with BIII/IV cytology and Afirma benign (GSC-B) or suspicious (GSC-S) categories, BV/VI nodules, and relative to *BRAF*V600E status.
- Another cohort with *TERT* promoter mutational status was analyzed along with expressed variant and fusion alterations as well as other genomic markers of tumor aggressiveness.

Results

- RARα
  - Lower expression in BV/VI vs GSC-B
- RARβ
  - Lower expression in *BRAF*V600E+ tumors
- RARγ
  - Higher mRNA expression in GSC-S and BV/VI
  - Highest expression in *BRAF*V600E+ tumors

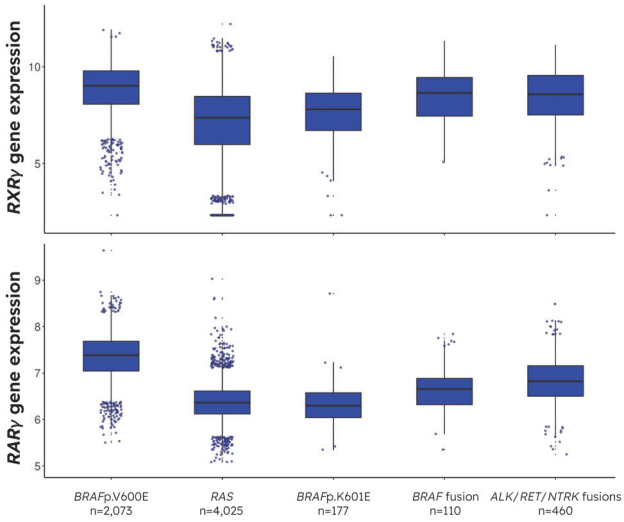
- RXRα
  - Lower expression in GSC-S and Bethesda V/VI samples
  - Lower expression in samples with *BRAF*V600E mutation
- RXRβ
  - No differences in expression
- RXRγ
  - Higher expression in GSC-S and BV/VI versus GSC-B
  - Highest expression in *BRAF*V600E+ tumors





RARγ and RXRγ vs. other alterations in GSC-S & BV/VI

Expression of RARγ and RXRγ is higher in samples with *BRAF*p.V600E compared to other alterations (p< 0.001 for all)



Conclusion

- RARγ and RXRγ appear to be coordinately expressed based on cytology, Afirma GSC and mutation status.
- RARγ and RXRγ expression may be driven by MAPK signaling.
- RARγ and RXRγ may be good therapeutic targets in advanced thyroid cancer.
- Future studies should evaluate the underlying mechanism of how *BRAF*V600E and MAPK signaling may affect RARγ and RXRγ expression.

References

1. Haugen BR. Management of the patient with progressive radioiodine non-responsive disease. *Semin Surg Oncol*. 1999;16(1):34-41. doi:10.1002/(sici)1098-2388(199901/02)16:1<34::aid-ssu7>3.0.co;2-2.
2. Haugen BR, Larson LL, Pugazhenthi U, et al. Retinoic acid and retinoid X receptors are differentially expressed in thyroid cancer and thyroid carcinoma cell lines and predict response to treatment with retinoids [published correction appears in *J Clin Endocrinol Metab*. 2008 Nov;93(11):4553]. *J Clin Endocrinol Metab*. 2004;89(1):272-280. doi:10.1210/jc.2003-030770.
3. Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. Cancer statistics, 2025. *CA Cancer J Clin*. 2025 Jan-Feb;75(1):10-45. doi: 10.3322/caac.21871. Epub 2025 Jan 16. PMID: 39817679.
4. Grünwald F, Pakos E, Bender H, et al. Redifferentiation therapy with retinoic acid in follicular thyroid cancer. *J Nucl Med*. 1998;39(9):1555-1558.
5. Grüning T, Tiepolt C, Zöphel K, Bredow J, Kropp J, Franke WG. Retinoic acid for redifferentiation of thyroid cancer--does it hold its promise?. *Eur J Endocrinol*. 2003;148(4):395-402. doi:10.1530/eje.0.1480395.
6. Klopper J, Kane M, Jimeno A, et al. A Phase II Trial of Bexarotene for Advanced Differentiated Thyroid Cancer. *Thyroid*. 2015;25(5):563-564. doi:10.1089/thy.2014.0399.
7. Simon D, Koehrle J, Reiners C, et al. Redifferentiation therapy with retinoids: therapeutic option for advanced follicular and papillary thyroid carcinoma. *World J Surg*. 1998;22(6):569-574. doi:10.1007/s002689900436.
8. Simon D, Körber C, Krausch M, et al. Clinical impact of retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot study. *Eur J Nucl Med Mol Imaging*. 2002;29(6):775-782. doi:10.1007/s00259-001-0737-6.
9. Huang ME, Ye YC, Chen SR, et al. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood*. 1988;72(2):567-572. *Blood*. 2016;128(26):3017. doi:10.1182/blood-2016-11-750182.
10. Castaigne S, Chomienne C, Daniel MT, et al. All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. *Blood*. 1990;76(9):1704-1709.
11. Tallman MS. Therapy of acute promyelocytic leukemia: all-trans retinoic acid and beyond. *Leukemia*. 1998;12 Suppl 1:S37-S40.

Vitamin D Signaling Expression Markers in Thyroid Tumors

Vitamin D Signaling Expression Markers in Thyroid Tumors

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KEY POINTS

- Studies have demonstrated associations between 25(OH)D deficiency and higher thyroid cancer risk, as well as vitamin D receptor *(VDR)* polymorphisms and thyroid cancer, but the relationship between vitamin D activity and tumor aggressiveness and disease progression remains unclear.[1,2,3]
- The researchers in this study aimed to examine the relationship between vitamin D activity and thyroid cancer by characterizing *VDR*, VD regulatory enzymes, and VD pathway target gene expression in thyroid nodules sent for Afirma testing.
- The study found that GSC Suspicious BIII/IV nodules and BV/VI nodules exhibited lower vitamin D signaling than GSC Benign nodules, and *BRAF*-like alterations demonstrated lower vitamin D signaling than *RAS*-like alterations. A novel VD activity score was inversely correlated with ERK signaling, returned a lower score among *BRAF*V600E+ nodules than *BRAF*V600^{WT} in both GSC-S and malignant nodules, and may serve as a novel prognostic marker.

STUDY: IN SUMMARY

Research has found that patients who are deficient in **25(OH)D** (25-hydroxyvitamin D, the predominant circulating metabolite of vitamin D), tend to be at a higher risk of developing thyroid cancer.[1,2] 25(OH)D is the precursor to the active form of vitamin D, called **calcitriol,** which binds to a receptor protein called **vitamin D receptor *(VDR)*** to mediate most of the biological functions of vitamin D.[6] Researchers have also identified a connection between polymorphisms of these *VDR* genes and thyroid cancer.[3]

It's uncertain, however, if 25(OH)D deficiency contributes to more aggressive thyroid tumor behavior. **While connections between vitamin D activity and thyroid cancer have been discovered, the exact relationship between Vitamin D Activity and tumor aggressiveness and disease progression isn't clear.** The researchers in this study aimed to examine this relationship by **characterizing *VDR*, VD regulatory enzymes, and VD pathway target gene expression in thyroid nodules sent for Afirma testing.**

This was done by analyzing the Afirma database to analyze the expression of *VDR* (Figure 1), VD regulatory enzymes (*CYP27A1, CYP27B1,* and *CYP24A1,* Figure 2) and four vitamin D target genes (achieved through a novel Vitamin D Activity Score that provides the average expression level of *CAMP, CD14, ORM1,* and *CLMN,* Figure 3). The expression of each was characterized by Afirma GSC result, Bethesda category, and *BRAF* mutational status. The VD activity score was further characterized by expressed variants and fusions (Figure 4).

The study found that GSC Suspicious BIII/IV nodules and BV/VI nodules exhibited lower vitamin D signaling than GSC Benign nodules, and *BRAF*-like alterations demonstrated lower vitamin D signaling than *RAS*-like alterations. The novel VD activity score was inversely correlated with ERK signaling, returned a lower score among *BRAF*V600E+ nodules than *BRAF*V600^{WT} in both GSC-S and malignant nodules, and may serve as a novel tumor behavior marker.

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Introduction

- 25(OH)D deficiency has been associated with higher thyroid cancer risk; however, it is unclear if it mechanistically affects biologic behavior and aggressiveness.[1,2]
- Studies have demonstrated an association between vitamin D receptor (*VDR*) polymorphisms and thyroid cancer.[3]
- *BRAF*V600E is the most common mutation seen in PTC.[4]
- *CYP27B1* encodes 1α-hydroxylase which converts 25(OH)D to $1,25(OH)_2D$.
- *CYP24A1* encodes for 25-hydroxyvitamin D3-24-hydroxylase which converts 25(OH)D and $1,25(OH)_2D$ to inactive metabolites.
- Increased expression of *VDR* (CYP24A1) is seen in PTC as well as tumors that have *BRAF*V600E mutations.[5]
- It is unclear how Vitamin D Activity relates to tumor aggressiveness and disease progression.

Study AIM

The aim of this study was to characterize *VDR*, VD regulatory enzymes, and VD pathway target gene expression in thyroid nodules sent for Afirma Genomic Sequencing Classifier (GSC) molecular testing.

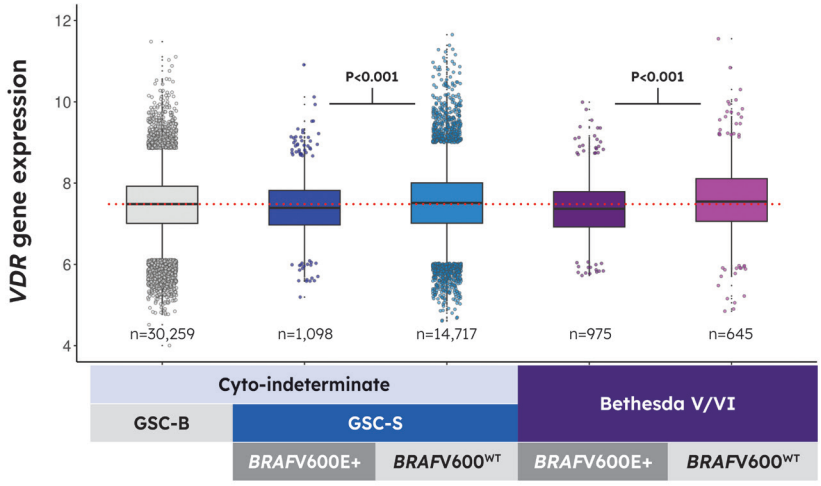
Methods

- The Afirma thyroid nodule molecular database (DB) was analyzed to:
  - Characterize vitamin D expression and genes involved in its synthesis in Afirma GSC-(B)enign and GSC-(S)uspicious (B)ethesda III/IV nodules and BV/VI malignant nodules.
  - Further characterize expression according to *BRAF*V600E+ vs *BRAF*wt and other molecular variants.
- The mRNA expression levels of four vitamin D target genes were evaluated.
  - *CAMP, CD14, ORM1, CLMN*
- A Vitamin D Activity Score was developed as a measure of VD signaling and correlated with Afirma result, expressed variant and fusion status, and ERK signaling.
  - This score is the average expression level of the 4 vitamin D target genes.

Results

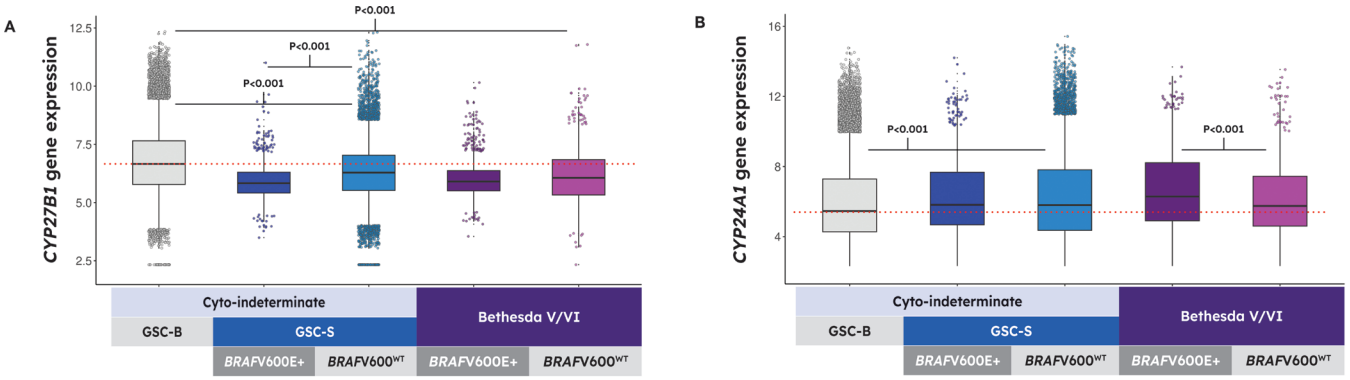
- 47,695 thyroid nodules from the Afirma DB were analyzed.
  - 30,259 BIII/IV (GSC-B)
  - 15,815 BIII/IV (GSC-S)
  - 1,621 BV/VI
- *VDR* expression is lower in the presence of *BRAF*V600E relative to *BRAF*wt (figure 1).

Figure 1. *VDR* expression by Afirma GSC, Bethesda category, and *BRAF* mutational status



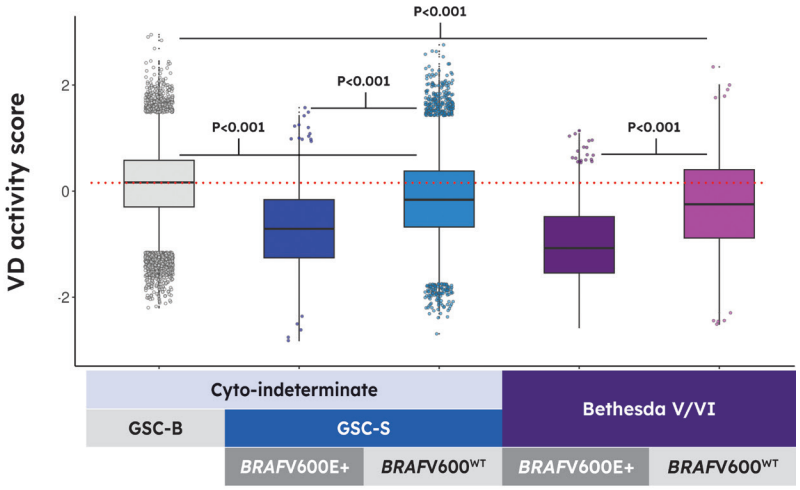
- *CYP27A1* (sterol 27-hydroxylase – conversion of VD to 25(OH)D) expression was not significantly different from GSC-B levels in any other category.
- *CYP27B1* (1α-hydroxylase – generation of active $1,25(OH)_2D$) expression was lower in GSC-S and BV/VI nodules compared to GSC-B nodules and even lower in *BRAF*V600E mutant GSC-S and BV/VI nodules compared to those with *BRAF*wt (figure 2a).
- *CYP24A1* (24-hydroxylase – catabolism of $1,25(OH)_2D$) expression was higher in GSC-S compared to GSC-B, and in BV/VI nodules, and higher still in *BRAF*V600E compared to *BRAF*wt subgroups (figure 2b).

Figure 2. *CYP27B1* (1α-hydroxylase – generation of active 1,25 VD) (A) and *CYP24A1* (24-hydroxylase – catabolism of 1,25 VD) (B) expression by Afirma GSC, Bethesda category, and *BRAF* mutational status



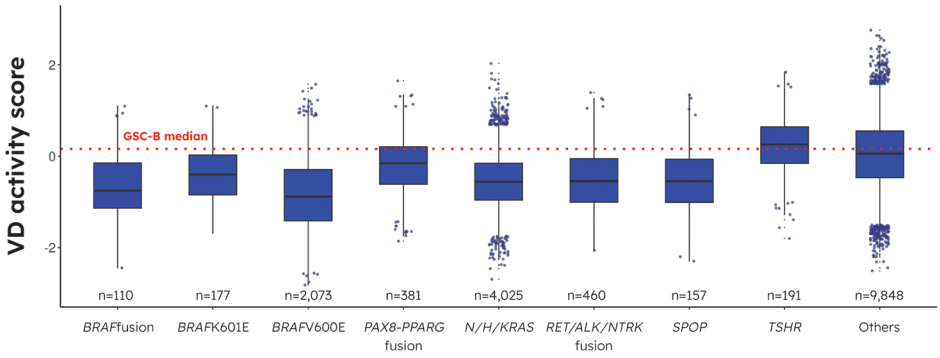
- The four VD signaling target genes (*CAMP, CD14, ORM1, CLMN*) showed expression levels that were lower in GSC-S and BV/VI nodules compared to GSC-B nodules and even lower in *BRAF*V600E mutant GSC-S and BV/VI nodules compared to those with *BRAF*wt.
- The novel VD activity score followed the same pattern, with the lowest levels in samples with *BRAF*V600E (figure 3).

Figure 3. VD activity score by Afirma GSC, Bethesda category, and *BRAF* mutational status



- The VD activity score in nodules with *RAS* like mutations (*N/H/KRAS* and *PAX8::PPARγ*) was lower than the median level with GSC-B results and higher than *BRAF*V600E (figure 4).

Figure 4. VD activity score in GSC-S and BV/VI nodules by expressed variants and fusions



- The VD activity score was strongly positively correlated with *VDR* expression (r=0.45) and strongly negatively correlated with ERK activity (r= -0.48).

Conclusion

- Bethesda III/IV-GSC-S and Bethesda V/VI malignant nodules have reduced VD signaling compared with GSC-benign nodules.
- *BRAF*-like alterations have lower VD signaling than *RAS*-like alterations.
- Novel VD Activity score was lowest in *BRAF*V600E+ GSC-S and malignant nodules when compared to those negative for *BRAF*V600E.
  - VD Activity score is inversely correlated with ERK signaling.
  - VD Activity score may be a novel marker predicting tumor behavior, especially in nodules that do not express canonical mutations.

References

1. Zhao, J., Wang, H., Zhang, Z., Zhou, X., Yao, J., Zhang, R., Liao, L., & Dong, J. (2019). Vitamin D deficiency as a risk factor for thyroid cancer: A meta-analysis of case-control studies. *Nutrition* (Burbank, Los Angeles County, Calif.), 57, 5–11. https://doi.org/10.1016/j.nut.2018.04.015.
2. Hu, Y., Xue, C., Ren, S., Dong, L., Gao, J., & Li, X. (2024). Association between vitamin D status and thyroid cancer: a meta-analysis. *Frontiers in nutrition*, 11, 1423305. https://doi.org/10.3389/fnut.2024.1423305.
3. Maciejewski, A., & Lacka, K. (2022). Vitamin D-Related Genes and Thyroid Cancer-A Systematic Review. *International journal of molecular sciences*, 23(21), 13661. https://doi.org/10.3390/ijms232113661.
4. Wei, X., Wang, X., Xiong, J., Li, C., Liao, Y., Zhu, Y., & Mao, J. (2022). Risk and Prognostic Factors for *BRAF*^{V600E}Mutations in Papillary Thyroid Carcinoma. *BioMed research international*, 2022, 9959649. https://doi.org/10.1155/2022/9959649.
5. Zou, M., BinHumaid, F. S., Alzahrani, A. S., Baitei, E. Y., Al-Mohanna, F. A., Meyer, B. F., & Shi, Y. (2014). Increased CYP24A1 expression is associated with *BRAF*(V600E) mutation and advanced stages in papillary thyroid carcinoma. *Clinical endocrinology*, 81(1), 109–116. https://doi.org/10.1111/cen.12396.
6. Bikle DD. Vitamin D: Production, Metabolism, and Mechanism of Action. [Updated 2025 Jun 15]. In: Feingold KR, Ahmed SF, Anawalt B, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK278935/.

The Genomic Landscape of Thyroid Nodules in Patients ≥75 Years Old

The Genomic Landscape of Thyroid Nodules in Patients ≥75 Years Old

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KEY POINTS

- Malignant nodules in patients 75 or older tend to behave more aggressively than those in younger patients, but data on the subject is limited.[1]
- This study evaluated the molecular and transcriptomic profiles of thyroid nodules in patients 75 or older versus younger patients.
- Molecularly tested nodules from patients ≥75 years old demonstrated generally favorable variant/fusion profiles and an overall higher benign call rate, suggesting an increased confidence in more conservative management in older patients.

STUDY: IN SUMMARY

Thyroid nodules are more common in older adults, and while most of these nodules are benign, those that are malignant tend to behave more aggressively.[1] Data on the subject is limited, however. The researchers in this study aimed to help address this limitation by **reporting on the molecular and transcriptomic profiles of thyroid nodules in patients ≥75 years in age versus those in younger patients.**

This study evaluated 250,200 nodules from the Afirma GSC database from August 2017 – June 2024, of which 8,627 samples received *TERT* promoter mutation analysis. Additionally, 17,000 GSC (S)uspicious or (B)ethesda category V/VI nodules were assessed using 3 signatures of tumor aggressiveness (an ERK signaling score, EMT signaling score, and a *BRAF-RAS* (BRS) score), and expression of the Human Molecular Signatures Database (MSigDB) hallmarks of cancer pathways.

The study found that patients ≥75 years of age had:

- a higher rate of BIII cytology than patients 20-54 years of age
- a lower rate of BV/VI cytology compared to patients ≤74 years of age
- a higher rate of Afirma GSC-Benign molecular results than younger age groups
- significantly lower ERK and EMT activity expression among advancing age groups while there is no change in the BRS score (Figure 1).
- greater expression in UV response, *KRAS* signaling, *MTORC1* signaling, and immune signaling markers, among others than younger patients
- lower expression of hedgehog, TGFβ and EMT signaling markers than younger patients

Molecularly tested nodules from patients ≥75 years old demonstrated generally favorable variant/fusion profiles and an overall higher benign call rate, suggesting an increased confidence in more conservative management in older patients may decrease unnecessary surgeries in this population.

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Schedule meeting with Medical Science Liaison

FULL STUDY

Introduction

- Thyroid nodules are increasingly common in older adults; most are benign.[1]
- In patients ≥75 years, malignant nodules often behave more aggressively, making accurate risk stratification essential.
- The Afirma Genomic Sequencing Classifier (GSC) is an exome-enriched RNA-seq assay validated in adults ≥21 years that refines malignancy risk in Bethesda III/IV nodules, potentially avoiding unnecessary surgery.[2]
- Limited data exist on the genomic and transcriptomic landscape of thyroid nodules in patients ≥75 years.
- The goal of this study was to characterize the molecular and transcriptomic profiles of thyroid nodules in patients ≥75 years compared with younger cohorts.

Methods

- The Afirma GSC database was queried from August 2017 – June 2024.
- Nodules that had adequate genomic material from follicular cells were identified to analyze expressed variants and fusions among Afirma GSC-(S)uspicious and nodules with Bethesda V/VI cytology.
  - Nodules from patients <20 yrs were excluded.
  - *TERT* promoter mutations were analyzed from March 2023 – June 2024.
- A subset of samples with available transcriptomic were analyzed for ERK and EMT signaling expression, the *BRAF-RAS* (BRS) score, and expression of the Human Molecular Signatures Database (MSigDB) hallmarks of cancer pathways.
- Patients were stratified into the following age cohorts:
  - 20-54 yrs
  - 55-74 yrs
  - ≥75 yrs

Results

- 250,200 were included in the analysis. Expressed variants and fusions are reported amongst nodules that were Afirma GSC-(S)uspicious or that had Bethesda V/VI cytology.
  - 8,627 samples with *TERT* promoter mutation testing were analyzed.
  - A subset of 17,000 GSC-S or B V/VI samples with available transcriptomic were analyzed for ERK and EMT signaling expression, the *BRAF-RAS* (BRS) score, and expression of the Human Molecular Signatures Database (MSigDB) hallmarks of cancer pathways.

Table 1. Proportion of Bethesda cytology categories by age group

- Patients ≥75 yrs had a higher rate of BIII cytology compared to those 20-54 yrs.
- Patients ≥75 yrs had lower rates of BV/VI cytology compared to those ≤74 yrs.

	Age groups		
	20-54	**55-74**	**≥75**
Total	**99,671**	**116,788**	**32,141**
B-III	75,632 (76%)	94,472 (80.9%)	26,044 (81%)*
B-IV	16,847 (16.9%)	18,155 (15.5%)	5,136 (16%)
B-V	3,416 (3.4%)	2,182 (1.9%)	535 (1.7%)**
B-VI	3,779 (3.8%)	1,978 (1.7%)	426 (1.3%)**

*p <0.01 compared to 20-54 yrs; **p <0.01 compared to ≤74 yrs

- Patients ≥75 years had larger median nodule size (2.4 cm [1.7-3.3]) compared to 55-74 years (2.2 cm [1.6-3.1]) and <54 years (2.2 cm [1.5-3.3]) (p <0.01).

Table 2. Afirma GSC-B call rates by age group (a) and sex (b)

(a)

	Age groups		
	20-54	**55-74**	**≥75**
Total	**92,479**	**112,627**	**31,180**
GSC-B	56,015 (60.6%)	83,390 (74%)	23,648 (76%)*

*p <0.01 in ≥75 years compared to younger ages.

(b)

	Age groups (Male)		
	20-54	**55-74**	**≥75**
Total	**15,398**	**28,988**	**9,094**
GSC-B	8,832 (57.4%)	19,584 (67.5%)	6,237 (68.6%)

	Age groups (Female**)		
	20-54	**55-74**	**≥75**
Total	**77,058**	**83,613**	**22,084**
GSC-B	47,169 (61.2%)	63,789 (76.3%)	17,409 (79%)

**p <0.01 in females compared to males.

- Patients ≥75 years had a higher Afirma GSC-(B)enign rate compared to younger age groups (Table 2a).
  - Females had a higher rate of Afirma GSC-B than males in all age groups (Table 2b).

Molecular alterations were detected at different rates across age groups in indeterminate nodules (BIII/IV) that are Afirma GSC-S (Table 3a) and BV/VI nodules (Table 3b).

Table 3. Proportion of expressed variants and fusions in Afirma GSC-S (a) and BV/VI nodules (b)

(a)

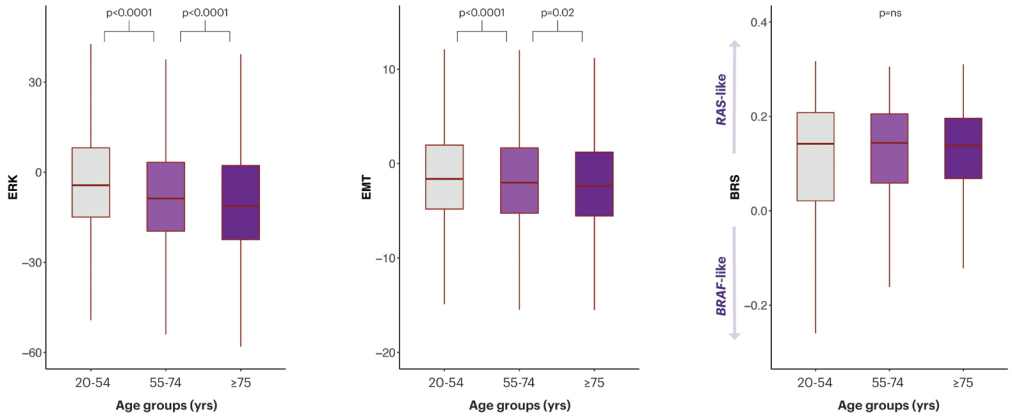
	Age groups	20-54	55-74	≥75	P values	
	Afirma GSC-S	36,449	29,235	7,530	20-54 vs ≥75	≥75 vs 55-74
VARIANTS	**Total variant alterations**	**14,861 (40.77%)**	**10,206 (34.91%)**	**2,515 (33.4%)**	<0.0001	0.01
	***BRAF*p.K601E**	462 (1.27%)	285 (0.97%)	55 (0.73%)	0.0001	ns
	***BRAF*p.V600E**	3,172 (8.7%)	1,969 (6.74%)	384 (5.1%)	<0.0001	<0.0001
	HRAS	3,257 (8.94%)	2,513 (8.6%)	607 (8.06%)	0.01	ns
	KRAS	637 (1.75%)	537 (1.84%)	129 (1.71%)	ns	ns
	NRAS	5,790 (15.89%)	3,578 (12.24%)	895 (11.89%)	<0.0001	ns
	DICER1	798 (2.19%)	293 (1%)	60 (0.8%)	<0.0001	ns
	EIF1AX	106 (0.29%)	204 (0.7%)	97 (1.29%)	<0.0001	<0.0001
	SPOP	193 (0.53%)	335 (1.14%)	88 (1.17%)	<0.0001	ns
	JAK2	22 (0.06%)	90 (0.3%)	66 (0.87%)	<0.0001	<0.0001
	PIK3CA	27 (0.07%)	50 (0.17%)	20 (0.26%)	<0.0001	ns
	TP53	47 (0.13%)	60 (0.2%)	27 (0.36%)	<0.0001	0.02
	TSHR	399 (1.09%)	377 (1.3%)	102 (1.35%)	ns	ns
FUSIONS	**Total fusion alterations**	**2,549 (6.99%)**	**1,282 (4.39%)**	**266 (3.53%)**	<0.0001	0.001
	RET	356 (0.98%)	127 (0.43%)	29 (0.39%)	<0.0001	ns
	NTRK3	541 (1.48%)	165 (0.56%)	31 (0.41%)	<0.0001	ns
	NTRK1	56 (0.15%)	29 (0.1%)	8 (0.11%)	ns	ns
	ALK	114 (0.31%)	50 (0.17%)	9 (0.12%)	0.005	ns
TERT	**Total**	**3,066**	**2,538**	**750**		
	TERT+	46 (1.5%)	138 (5.4%)	81 (10.8%)	<0.0001	<0.0001
	***TERT+BRAF*V600E**	5 (0.2%)	32 (1.3%)	17 (2.3%)	<0.0001	0.06

(b)

	Age groups	20-54	55-74	≥75	P values	
	BV/VI nodules	7,195	4,160	961	20-54 vs ≥75	≥75 vs 55-74
VARIANTS	**Total variant alterations**	**4,934 (68.58%)**	**2,736 (65.77%)**	**599 (62.33%)**	0.0001	0.05
	***BRAF*p.K601E**	4 (0.06%)	9 (0.22%)	2 (0.21%)	ns	nd
	***BRAF*p.V600E**	4,561 (63.39%)	2,426 (58.32%)	509 (52.97%)	<0.0001	0.002
	HRAS	68 (0.95%)	47 (1.13%)	12 (1.25%)	ns	ns
	KRAS	39 (0.54%)	26 (0.63%)	12 (1.25%)	0.01	ns
	NRAS	131 (1.82%)	87 (2.09%)	31 (3.23%)	0.005	0.05
	DICER1	53 (0.74%)	24 (0.58%)	4 (0.42%)	ns	ns
	EIF1AX	7 (0.1%)	9 (0.22%)	8 (0.83%)	<0.0001	0.007
	SPOP	21 (0.29%)	27 (0.65%)	10 (1%)	0.001	ns
	JAK2	3 (0.04%)	4 (0.1%)	2 (0.2%)	ns	ns
	PIK3CA	19 (0.26%)	28 (0.67%)	13 (1.35%)	<0.0001	ns
	TP53	10 (0.14%)	13 (0.31%)	6 (0.6%)	0.005	ns
	TSHR	39 (0.54%)	58 (1.39%)	8 (0.83%)	ns	ns
FUSIONS	**Total fusion alterations**	**716 (9.95%)**	**241 (5.79%)**	**39 (4.06%)**	<0.0001	0.04
	RET	328 (4.56%)	84 (2.02%)	8 (0.83%)	<0.0001	0.02
	NTRK3	151 (2.1%)	33 (0.79%)	3 (0.31%)	<0.0001	ns
	NTRK1	35 (0.49%)	7 (0.17%)	1 (0.1%)	ns	ns
	ALK	29 (0.4%)	7 (0.17%)	1 (0.1%)	ns	ns
TERT	**Total**	**1,275**	**723**	**175**		
	TERT+	45 (3.6%)	107 (14.8%)	55 (31.4%)	<0.0001	<0.0001
	***TERT+BRAF*V600E**	38 (3%)	81 (11.2%)	41 (23.4%)	<0.0001	<0.0001

ERK and EMT activity expression is significantly lower with advancing age groups while there is no change in the BRS score (Figure 1).

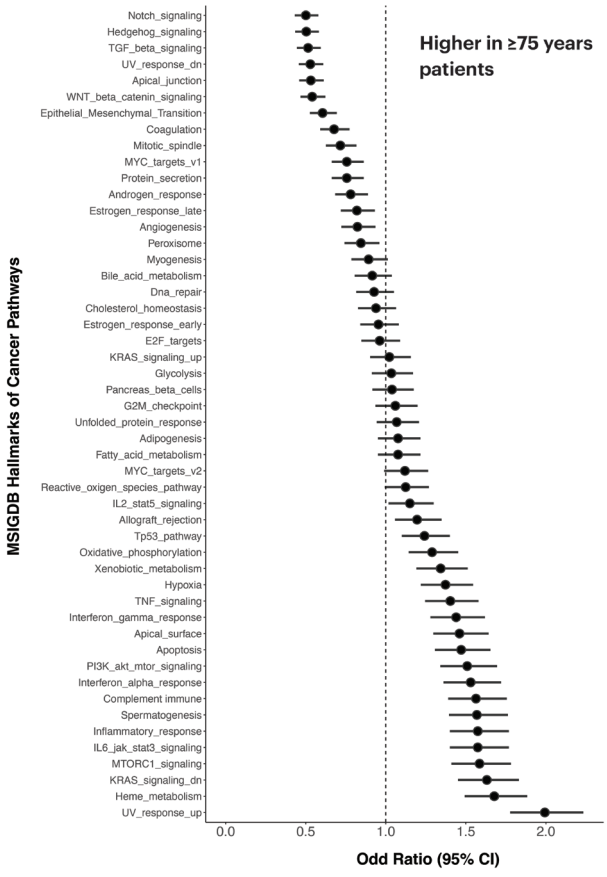
Figure 1. The activity expression of ERK and EMT and the BRS score by age groups



Hallmarks where the confidence intervals does not cross the reference line are statistically significant (p <0.05)

The expression of MSigDB hallmarks of cancer pathways are enriched in markers of UV response, *KRAS* signaling, *MTORC1* signaling and immune signaling, amongst others, in those ≥75 years vs younger patients. Hedgehog, TGFβ, and EMT signaling are lower (Figure 2).

Figure 2. MSigDB Hallmarks of Cancer Pathways in patients ≥75 years vs younger patients



Conclusion

- Molecularly tested nodules from patients ≥75 years have an overall higher benign call rate and generally favorable variant/fusion profiles.
- In BV/VI nodules, there are higher levels of alterations suggestive of aggressive thyroid cancer (e.g. *TERT*p).
- Differential gene expression analysis suggests nodules in patients ≥75 years have increased inflammatory changes, while younger patients demonstrate more neoplastic pathways.
- Future studies should be done with histopathology detail and longer-term oncologic outcomes to see if improved prognostic features from the above-described expression analysis can personalize treatment for thyroid cancer in older patients.
- These data suggest one can increase confidence in more conservative management in older patients and may decrease the need for unnecessary surgery in this population.

References

1. Wang Z, Vyas CM, Van Benschoten O, Nehs MA, Moore FD Jr, Marqusee E, Krane JF, Kim MI, Heller HT, Gawande AA, Frates MC, Doubilet PM, Doherty GM, Cho NL, Cibas ES, Benson CB, Barletta JA, Zavacki AM, Larsen PR, Alexander EK, Angell TE. Quantitative Analysis of the Benefits and Risk of Thyroid Nodule Evaluation in Patients ≥70 Years Old. *Thyroid*. 2018 Apr;28(4):465-471. doi: 10.1089/thy.2017.0655. Epub 2018 Apr 2. PMID: 29608439.
2. Patel KN, Angell TE, Babiarz J, Barth NM, Blevins T, Duh QY, Ghossein RA, Harrell RM, Huang J, Kennedy GC, Kim SY, Kloos RT, LiVolsi VA, Randolph GW, Sadow PM, Shanik MH, Sosa JA, Traweek ST, Walsh PS, Whitney D, Yeh MW, Ladenson PW. Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules. *JAMA Surg*. 2018 Sep 1;153(9):817-824. doi: 10.1001/jamasurg.2018.1153. PMID: 29799911; PMCID: PMC6583881.

Preoperative Thyroid Nodule mRNA Expression Signatures to Predict Postoperative Thyroid Histopathology

Preoperative Thyroid Nodule mRNA Expression Signatures to Predict Postoperative Thyroid Histopathology

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KEY POINTS

- The primary goal of molecular testing in indeterminate cytology is to improve diagnostic accuracy. In Bethesda category V and VI nodules, molecular testing is primarily useful to provide prognostic information.
- This study aimed to assess the ability of 31 molecular signatures from the Afirma Genomic Resource for Intelligent Discovery (GRID) to preoperatively differentiate between American Thyroid Association (ATA) low risk cancers and intermediate or high-risk thyroid cancers.
- The immune activation, cell cycle progression, and hypoxia hallmark signatures demonstrated significantly different mRNA expression between ATA low and intermediate risk thyroid cancers.

STUDY: IN SUMMARY

The Afirma Genomic Resource for Intelligent Discovery (GRID) is a research use only tool that contains a variety of signatures and data points that may provide molecular data to improve upon prognostic information in a research setting.

This study aimed to assess the ability of 31 of these GRID signatures to preoperatively differentiate between American Thyroid Association (ATA) low risk cancers and intermediate or high-risk thyroid cancers. This was done using a retrospective cohort of 163 adult patients who had Afirma GSC testing and final histopathology reports associated, 119 of which had either an Afirma GSC-(S)uspicious result or BV or VI cytology. Surgical pathology was stratified by ATA risk of recurrence criteria, then correlated with GRID signatures and expressed mutations.

Ultimately, this study found that while classically described canonical mutations like *BRAF*V600E didn't differentiate between low and intermediate risk thyroid cancers (compare the "low" and "intermediate" ATA risk columns for *BRAF*p.V600E in table 1), **a number of the signatures demonstrated significantly different mRNA expression between ATA low and intermediate risk categorization, including the immune activation** (particularly the "Activated CD4" and "T cell accumulation" signatures in table 2), **cell cycle progression** (the "*E2F* targets hallmark" signature in table 2), **and hypoxia hallmark signatures**.

Access the poster layout

Schedule meeting with Medical Science Liaison

FULL STUDY

Introduction

- The primary role for thyroid nodule molecular testing is to risk stratify nodules with indeterminate cytology (ITN – those with (B)ethesda III/IV cytology).
  - The primary objective is to find molecularly benign nodules and thus avoid unnecessary diagnostic surgery.
- Molecular testing may also provide prognostic information in ITN when suspicious for thyroid cancer.
  - For BV/VI thyroid nodules, the main goal of molecular testing is to obtain prognostic information.
- The Afirma Genomic Sequencing Classifier (GSC) is an exome-enriched RNA sequencing test that utilizes genomic classifiers to risk stratify ITN and reports on expressed variants and fusions in Afirma GSC-(S)uspicious nodules and in nodules with BV/VI cytology.
  - Expressed variants and fusions are reported as part of the Afirma Xpression Atlas (XA).
- The Afirma Genomic Resource for Intelligent Discovery (GRID) is a research use only tool that contains mRNA based molecular signatures and data points beyond expressed variants and fusions that may provide molecular data for research to improve upon the current prognostic information associated with clinically reported data.
- This study aimed to evaluate Afirma GRID for molecular signatures to preoperatively differentiate American Thyroid Association (ATA) low risk from intermediate/high risk thyroid cancers.

Methods

- A retrospective cohort study of adult patients presenting to a community otolaryngology practice for evaluation of thyroid nodules between 2017-2025 was conducted.
- A total of 163 patients who had Afirma GSC testing (ordered at the discretion of the clinical provider) had final histopathology reports associated.
  - 119 had Afirma GSC-S result or had BV/VI cytology.
- Surgical pathology stratified by ATA risk of recurrence criteria was correlated with expressed mutations and Afirma GRID signatures.
- Of the 119 cases, 75 samples had risk assigned (cases that were histologically benign or NIFTP did not have ATA risk assigned).
- There were 49 ATA low risk cancers, 25 intermediate risk, and 1 high risk.
  - The 1 high risk case was included with the intermediate risk category for data analysis.
- The cohort with ATA risk assigned was evaluated for Afirma XA variant and fusion results.
- Of those patients with ATA risk assigned, 60 had Afirma GRID data available for analysis (Table 2).
- The expression level of GRID signatures relative to the median level of all GSC-S lesions, or those with BV/VI cytology, in the database was used to calculate odds ratios (OR) correlated with ATA low vs intermediate risk thyroid cancer using logistic regression.
  - A higher OR is consistent with ATA intermediate risk cancer.

Results

- Of nodules with BV/VI cytology, 17 were intermediate risk and 23 were low risk.
- **XA results had no association with ATA risk.**
- One lesion with *TERT*p + *BRAF*p.K601E mutations was in an ATA low risk cancer.

Table 1. Expressed variants associated with ATA risk categories

		ATA risk		
		Low	Intermediate	High
	Total	**49**	**25**	**1**
Variants	***BRAF*p.V600E**	14 (28%)	7 (28%)	1
	HRAS	3 (6.1%)	2 (8%)	0
	NRAS	5 (10.2%)	1 (4%)	0
	EIF1AX	0	1 (4%)	0
	TSHR	1 (2%)	1 (4%)	0
	***BRAF*p.K601E+*TERT*p.C228T**	1 (2%)	0	0
Fusions	***AGK*::*BRAF***	1 (2%)	0	0
	CCDC6*::*RET	0	2 (8%)	0
	ETV6*::*NTRK3	2 (4.1%)	0	0
	PAX8*::*PPARG	3 (6.1%)	0	0
	CREB3L2*::*PPARG	1 (2%)	0	0

Table 2. The data is presented as a continuous OR or relative to being above or below the 50th percentile of the signature

A. OR of the evaluated samples relative to the median GSC-S percentile score of GRID signatures

	Continuous		Category (>50% vs ≤50%)	
Signature	**OR**	**p value**	**OR**	**p value**
Angiogenesis hallmark	1.9	0.54	0.9	0.86
Apical junction hallmark	2.3	0.41	1.9	0.31
Apical surface hallmark	0.4	0.39	0.7	0.58
Apoptosis hallmark	2.8	0.34	1.4	0.58
DNA repair hallmark	1	0.96	1.1	0.86
***E2F* targets hallmark**	**6.8**	**0.05**	2.7	0.09
Epithelial mesenchymal transition hallmark	1.1	0.91	0.8	0.71
Estrogen response early hallmark	0.3	0.32	0.7	0.54
Estrogen response late hallmark	0.4	0.35	0.5	0.24
Hedgehog signaling pathway	1.2	0.88	0.9	0.82
Hypoxia hallmark	**0.1**	**0.01**	**0.2**	**0.02**
Inflammatory response hallmark	2.1	0.55	1	0.99
mTOR complex1 signaling hallmark	0.7	0.76	1	0.94
TP53 pathway hallmark	0.3	0.15	**0.3**	**0.05**
Pi3k akt *mtor* signaling hallmark	1.2	0.86	1.6	0.43
TGF beta signaling hallmark	0.9	0.89	1.2	0.71
Interferon gamma response hallmark	4.7	0.25	1.8	0.33
Activated CD4	**17.8**	**0.02**	1.6	0.43
Activated CD8	4.8	0.15	1.6	0.43
Immune content estimation	3.5	0.3	2.5	0.12
Immunomodulators	0.5	0.52	0.6	0.42
M2 to M1 macrophage ratio	0.3	0.39	0.4	0.13
PDL1 signaling	0.6	0.64	1	0.94
T cell exhaustion	0.4	0.42	0.9	0.87
T cell regulatory	0.4	0.37	0.6	0.3
Cancer associated fibroblast	0.6	0.68	0.7	0.55
T cell accumulation	**13.9**	**0.03**	**3.3**	**0.04**
NIS expression	0.8	0.79	0.8	0.65
Hürthle Oncocytic cell index score	0.8	0.89	2.1	0.39
Invasion signature score	0.8	0.82	0.8	0.69
Lymphnode metastasis signature score	6.3	0.12	7.4	0.06

B. OR of the evaluated samples relative to the median BV/VI percentile score of GRID signatures

	Continuous		Category (>50% vs ≤50%)	
Signature	**OR**	**p value**	**OR**	**p value**
Angiogenesis hallmark	3.2	0.25	1.7	0.32
Apical junction hallmark	1.7	0.65	0.7	0.63
Apical surface hallmark	0.6	0.6	1.1	0.84
Apoptosis hallmark	2.5	0.33	1.4	0.53
DNA repair hallmark	0.8	0.84	0.9	0.9
E2F targets hallmark	6.2	0.06	2.7	0.09
Epithelial mesenchymal transition hallmark	2.7	0.34	1.3	0.68
Estrogen response early hallmark	0.3	0.29	0.8	0.67
Estrogen response late hallmark	0.5	0.57	0.8	0.67
Hedgehog signaling pathway	1.3	0.77	1.2	0.78
Hypoxia hallmark	**0**	**0.01**	**0.2**	**0.01**
Inflammatory response hallmark	2.6	0.41	2	0.23
mTOR complex1 signaling hallmark	0.7	0.75	0.6	0.43
TP53 pathway hallmark	0.1	0.08	0	0.99
Pi3k akt *mtor* signaling hallmark	0.6	0.61	0.6	0.43
TGF beta signaling hallmark	0.8	0.86	1.4	0.56
Interferon gamma response hallmark	4.9	0.2	**4.1**	**0.02**
Activated CD4	**22.6**	**0.02**	**6.2**	**0.01**
Activated CD8	4.3	0.21	2.1	0.21
Immune content estimation	3.4	0.31	2	0.26
Immunomodulators	0.5	0.51	0.7	0.56
M2 to M1 macrophage ratio	0.3	0.32	0.6	0.32
PDL1 signaling	1	0.97	2.5	0.2
T cell exhaustion	0.3	0.18	0.4	0.11
T cell regulatory	0.4	0.35	0.6	0.32
Cancer associated fibroblast	1.4	0.77	1	0.98
T cell accumulation	7.1	0.09	1.9	0.38
NIS expression	0.8	0.78	0.8	0.68
Hürthle Oncocytic cell index score	0.7	0.8	1.3	0.73
Invasion signature score	1	0.99	1.3	0.67
Lymphnode metastasis signature score	2.9	0.26	1.6	0.43

ATA intermediate risk thyroid cancers appear to have higher immune activity and lower hypoxia hallmark signature compared to low risk thyroid cancers.

Conclusion

- *BRAF*p.V600E and other classically described canonical mutations did not differentiate low from intermediate risk thyroid cancers in this cohort.
- The mRNA expression of immune activation, cell cycle progression, and hypoxia hallmark signatures were significantly different between thyroid cancers with ATA low and intermediate risk categorization.
- If validated for clinical use, GRID signatures may represent novel preoperative prognostic markers for molecularly suspicious ITN or nodules with BV/VI cytology.

The Expression of Cell-Surface Targets as Potential Prognostic and Therapeutic Markers in Thyroid Tumors

The Expression of Cell-Surface Targets as Potential Prognostic and Therapeutic Markers in Thyroid Tumors

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KEY POINTS

- Cell surface targets (useful diagnostic, prognostic, or therapeutic protein targets found on the surface of a cell) have had a large impact on cancer treatment, but interest in expanding this research to solid tumor types, such as thyroid cancer, has been limited by a lack of suitable targets.[10-13]
- The researchers in this study aimed to help address this by measuring the expression level of 78 cell surface targets across Bethesda III – VI thyroid nodules sent for Afirma GSC testing.
- TROP2 and NECTIN4 were expressed more in GSC-S nodules than GSC-B nodules and are targets for FDA approved antibody-drug conjugates in triple negative breast cancer and urothelial carcinoma, respectively.

STUDY: IN SUMMARY

Last year, Veracyte presented a poster regarding the expression of prostate-specific membrane antigen (PSMA) in indeterminate and malignant thyroid nodules.[2] PSMA is a protein overexpressed on the surface of malignant prostate cells and other solid tumor cells, making it a useful diagnostic target.[3-9]

PSMA is an example of a **cell surface target (CST) – a useful diagnostic, prognostic, or therapeutic target found on the surface of a cell.** In fact, *FOLH1* (the gene that encodes PSMA) is one of the cell surface targets investigated in this abstract.

CSTs have revolutionized treatment of blood cancers and there's interest in expanding this research to other solid tumors, **but research has been limited by a lack of suitable targets.**[10-13] **The researchers in this study aimed to help address this limitation by identifying potential prognostic and therapeutic targets for aggressive thyroid cancer.**

They did so by measuring the expression level of 78 CSTs across (B)ethesda III-VI thyroid nodules sent for Afirma GSC testing:

- First among 30,259 molecularly benign BIII/IV nodules to establish a baseline (Figure 1)
- Then among 15,815 molecularly suspicious BIII/IV nodules, relative to the molecularly benign nodules (Figure 2)
- Then among 1,621 BV/VI nodules, relative to the molecularly suspicious nodules (Figure 3)
  - Within these samples with *TERT*p DNA analysis ordered, the association of CST expression by *BRAF*V600E and *TERT* mutational status was also evaluated (Table 1)

CSTs with FDA approved drugs to target them were denoted in red. A cohort of 463 nodules tested with Afirma GSC and with surgery and final histopathology available to associate CST expression with histopathology outcomes — loco-regional lymph node metastases in particular (Figure 3). Logistic regression was used to calculate odd ratios (OR) to associate expression (top 25% vs others) with adverse features.

TROP2 (encoded by the gene *TACSTD2*) and NECTIN4 (encoded by *PVRL4*) stood out in particular; they were expressed more in GSC Suspicious nodules than GSC Benign nodules **and are targets for FDA approved antibody-drug conjugates in triple negative breast cancer and urothelial carcinoma,** respectively. **These, along with other expressed CSTs may represent novel therapeutic targets for thyroid carcinomas** – especially those lacking a targetable mutation or with a resistance to currently approved targeted therapies.

Access the poster layout

Schedule meeting with Medical Science Liaison

Introduction

Cell surface targets (CSTs) are increasingly important in oncology, with the evolution of cancer immunotherapies. Recognising cell surface targets that are specific to malignant cells has had significant impact in haematological malignancies and has expanded more recently to solid malignancies. One limiting factor in the application of these novel therapies is the lack of suitable targets, in part because these have not all been identified. While there is a well-developed pathway of surgery and radioactive iodine for differentiated thyroid cancer, the challenge of radioiodine refractory disease persists. Characterizing CSTs in thyroid cancer (TC) is currently understudied. We aimed to evaluate the baseline expression of CSTs in molecularly benign thyroid nodules, to compare this to thyroid cancer, and to identify targets that are associated with potentially aggressive forms of TC. These would have potential to be used in prognostication and as a therapeutic target.

Methods

- The Afirma Genomic Sequencing Classifier (GSC) utilizes exome-enriched next generation RNA sequencing (RNAseq) as the platform to develop classifiers and report on expressed variants and fusions for thyroid nodule clinical management.[1]
- The expression level of 78 CSTs was characterized across thyroid nodules with Bethesda III-VI cytology sent for Afirma Genomic Sequencing Classifier (GSC) molecular testing. The testing was requested at the discretion of the ordering clinician.
- CSTs that have FDA approved drugs to target them are denoted in red.
- We evaluated the "baseline" expression of CSTs in the Bethesda III/IV-GSC-(B)enign nodules (n=30,259).
- We then evaluated their expression of these CSTs in Bethesda III/IV GSC-(S)uspicious (n=15,815) nodules relative to the expression in GSC-B nodules.
- Finally, we evaluated the expression of these CSTs in Bethesda V/VI (n=1,621) nodules relative to the expression in GSC-S nodules.
- Within Bethesda V/VI samples with *TERT* promoter (*TERT*p) mutation profiling ordered (n=2,240), we evaluated the association of CST expression with *BRAF*p.V600E+ (n=1,348), *TERT*p+ (n=206) and *TERT*p+*BRAF*p.V600E+ (n=160).
- To associate the CST expression with histopathology outcomes, we evaluated a cohort of Afirma GSC tested nodules that had surgery and final histopathology available (n=463).
  - The primary outcome of interest was loco-regional lymph node metastases.
- Logistic regression was used to calculate odd ratios (OR) to associate expression (top 25% vs others) with adverse features.
- Pathology reports were collected under WCG IRB DHF 005-077.

Results

- *FN1, CD46, ADAM9, EPCAM, ERBB2, ALCAM* are highly expressed in these nodules.
- Most FDA approved targets have low expression (*TACSTD2:* TROP2, *PVRL4:* NECTIN4, *DLL3, FOLH1:* PSMA, *SSTR2*)

Figure 1. The baseline expression of CSTs in Afirma GSC-B samples.

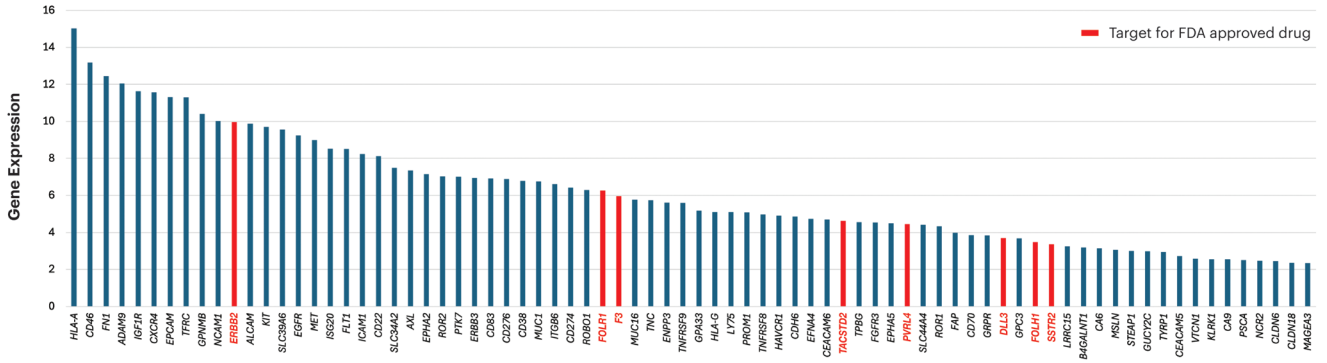
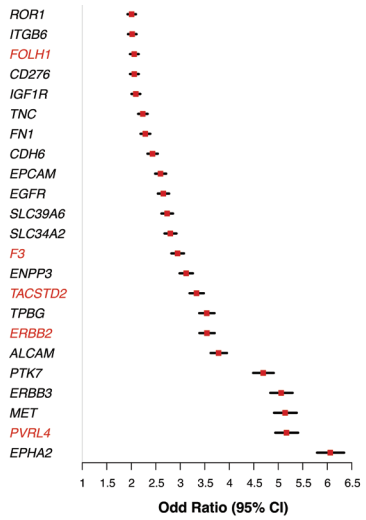


Figure 2

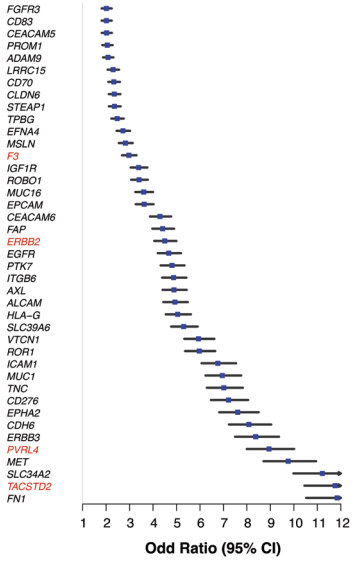
A. The odds ratio of CSTs associated with Afirma GSC-S samples relative to the GSC-B cohort (OR>2, p<0.001 for all).



CSTs that have FDA approved drugs to target them are denoted in red.

23 genes were strongly associated with GSC-S compared to GSC-B.

B. The odds ratio of CSTs associated with thyroid nodules with BV/VI cytology relative to the GSC-S cohort (OR>2, p<0.001 for all) .



CSTs that have FDA approved drugs to target them are denoted in red.

41 genes had OR>2, with *FN1* having the highest OR.

- *TACSTD2* (TROP2), a tumor associated calcium signal transducer 2, that is a target for antibody-drug conjugates that has FDA approval (Sacituzumab govitecan) in triple negative breast cancer.
- *PVRL4* (NECTIN4), has an antibody-drug conjugate (ADC) that has been FDA approved for urothelial carcinoma (Enfortumab vedotin).

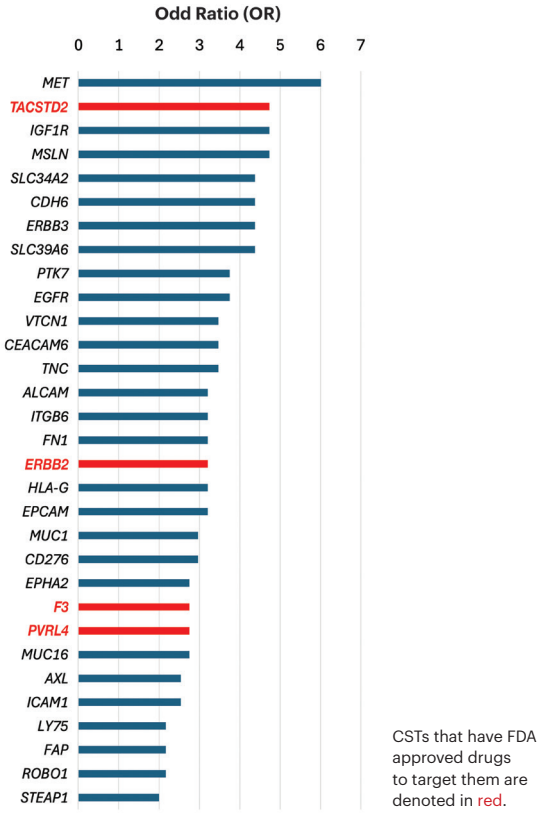
Table 1. The odds ratio of CSTs associated with thyroid nodules with BV/VI cytology with *TERT*p testing ordered.

Gene	*BRAF*p.V600E+	*TERT*p+	*TERT*p+*BRAF*p.V600E+
SLC34A2	**46.2**	**3.1**	**5.4**
TACSTD2	**39.7**	1.4	**2.1**
ERBB3	**22.8**	**2.3**	**3.7**
MET	**20.1**	**2.7**	**4.5**
PVRL4	**16.7**	**2.5**	**3.9**
FN1	**15.1**	**3.9**	**5.9**
IGF1R	**10.8**	0.8	1
EPHA2	**8.2**	1.2	1.6
MUC1	**7.8**	**3.3**	**4.9**
HLA-G	**6.7**	0.9	1.1
VTCN1	**6.1**	1.2	1.7
ERBB2	**5.2**	1.1	1.4
ALCAM	**4.3**	1.4	1.7
ITGB6	**4.2**	1.9	**2.4**
MSLN	**4.2**	1	1.2
FGFR3	**3.8**	1	1.3
EGFR	**3.7**	1.1	1.4
ROR1	**3.5**	1.6	1.8
CDH6	**3.3**	1	1.2
FAP	**3.2**	**2.8**	**2.7**
TNC	**3**	**2.6**	**2.6**
LRRC15	1.7	**2.5**	**2.5**
TPBG	1.2	**2.3**	**2.1**
SLC39A6	**2.8**	**2.1**	**2.6**
FOLR1	2	1.7	**2.3**
FOLH1	1.2	1.9	1.7

CSTs that have FDA approved drugs to target them are denoted in red.

- Samples that are *BRAF*p.V600E +.
  - *TACSTD2, PVRL4, ERBB2* are CSTs with FDA approved targeted drugs and high OR in this cohort.
- Samples that are *TERT*p+ or *TERT*p +*BRAF*p.V600E+.
  - *FN1* has the highest association with these adverse molecular findings.

Figure 3. When evaluating the association of CSTs with lymph node metastases, *MET*, TROP2, *SLC34A2* and *FN1* were significantly associated with lateral disease, >2mm central node deposits, or a high lymph node ratio (≥40%) (OR>2, p<0.001).



Conclusion

- TROP2 and NECTIN4 are targets for FDA approved antibody-drug conjugates in triple negative breast cancer and urothelial carcinoma, respectively. These were more highly expressed in Afirma GSC-S nodules compared to GSC-B.
- These and other expressed CSTs may represent novel prognostic markers in lieu of, or associated with, classic canonical driver mutations in aggressive thyroid carcinoma.
- Additionally, these CSTs may represent novel molecular targets for new therapies in thyroid carcinomas that do not express a targetable mutation, or which develop resistance to currently approved targeted therapies.
- As further immunotherapies arise, expanded knowledge of the cancer cell surface proteome is critical for applying new therapies in TC.

References

1. Walsh PS, Hao Y, Ding J, Qu J, Wilde J, Jiang R, Kloos RT, Huang J, Kennedy GC. Maximizing Small Biopsy Patient Samples: Unified RNA-Seq Platform Assessment of over 120,000 Patient Biopsies. *J Pers Med.* 2022 Dec 22;13(1):24. doi:10.3390/jpm13010024. PMID: 36675685.
2. Sadiq et al, Presented at Endocrine Society 2024.
3. Horoszewicz JS, Kawinski E, Murphy GP. Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. *Anticancer Res.* 1987;7(5B):927-935.
4. Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C. Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res.* 1997;3(1):81-85.
5. Troyer JK, Beckett ML, Wright GL Jr. Detection and characterization of the prostate-specific membrane antigen (PSMA) in tissue extracts and body fluids. *Int J Cancer.* 1995;62(5):552-558. doi:10.1002/ijc.2910620511.
6. Kawakami M, Nakayama J. Enhanced expression of prostate-specific membrane antigen gene in prostate cancer as revealed by in situ hybridization. *Cancer Res.* 1997;57(12):2321-2324.
7. Pandit-Taskar N, O'Donoghue JA, Divgi CR, et al. Indium 111-labeled J591 anti-PSMA antibody for vascular targeted imaging in progressive solid tumors. *EJNMMI Res.* 2015;5:28. Published 2015 Apr 29. doi:10.1186/s13550-015-0104-4.
8. Nomura N, Pastorino S, Jiang P, et al. Prostate specific membrane antigen (PSMA) expression in primary gliomas and breast cancer brain metastases. *Cancer Cell Int.* 2014;14(1):26. Published 2014 Mar 20. doi:10.1186/1475-2867-14-26.
9. Denmeade SR, Mhaka AM, Rosen DM, et al. Engineering a prostate-specific membrane antigen-activated tumor endothelial cell prodrug for cancer therapy. *Sci Transl Med.* 2012;4(140):140ra86. doi:10.1126/scitranslmed.3003886.
10. https://ashpublications.org/thehematologist/article/doi/10.1182/hem.V22.1.2025214/535048/Technology-SpotlightSurface-Target-Discovery-for.
11. Guan, Z., Zhang, Z., Wang, K. et al. Targeting myeloid cells for hematological malignancies: the present and future. *Biomark Res* 13, 59 (2025). https://doi.org/10.1186/s40364-025-00775-1.
12. Zhu, J., Zhou, J., Tang, Y. et al. Advancements and challenges in CAR-T cell therapy for solid tumors: A comprehensive review of antigen targets, strategies, and future directions. *Cancer Cell Int* 25, 313 (2025). https://doi.org/10.1186/s12935-025-03938-0.
13. Ranoa DRE, Sharma P, Schane CP, et al. Single CAR-T cell treatment controls disseminated ovarian cancer in a syngeneic mouse model. *J Immunother Cancer.* 2023;11(5):e006509. doi:10.1136/jitc-2022-006509.

Single-cell RNA Sequencing Analysis of Thyroid Nodule Fine Needle Biopsy Aspiration

Single-Cell RNA Sequencing Analysis of Thyroid Nodule Fine Needle Biopsy Aspiration

Caitlin Bell, MD,[1] Maria Albuja-Cruz, MD,[2] Mohammed Alshalalfa, PhD,[3] Brian Cervenka, MD,[4] Kristin Gleitsman, PhD,[3] Joshua P. Klopper, MD,[3] Chris Raeburn, MD,[2] Andrew Uzilov, PhD,[3] Bryan Haugen, MD,[1] Nikita Pozdeyev, MD[1,2]

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KEY POINTS

- Single-cell RNA sequencing (scRNAseq) could be used to identify subpopulations of cells in the tumor microenvironment and may provide unique genomic insights.
- The researchers aimed to assess the feasibility of scRNAseq on thyroid nodule FNA samples using 18 subjects referred for thyroid nodule FNA biopsy or undergoing thyroidectomy.
- The researchers were able to differentiate between benign and malignant samples via clustering of follicular cells, found that more aggressive cancers exhibit unique clusters with less differentiated cells, and found that most cells from a thyroid FNA are likely immune cells.

STUDY: IN SUMMARY

Bulk RNA and DNA sequencing has proven to be highly sensitive for molecular testing of indeterminate thyroid nodules. An alternate method of sequencing exists, however, known as **single-cell RNA sequencing** (or "scRNAseq") that **could be used to identify subpopulations of cells within the tumor microenvironment.**

This is because scRNAseq provides single cell data, here in the form of 2-dimensional plots, that can help to identify subtypes of cells. In these plots, each dot represents a single cell. These dots are clustered, with cells most similar to each other clustered close together. Figure 1 provides an example, with clusters 6 and 10 at the top of the plot representing follicular cells. **Little data exists on using this form of sequencing for thyroid nodules, especially from preoperative thyroid fine needle aspirate (FNA) samples, which is why the researchers decided to investigate further in this study.**

This was done using 18 subjects referred for thyroid nodule FNA biopsies or undergoing thyroidectomy, 16 of which completed scRNAseq, and 10 of which had high quality data (follicular cells present). Of these 10 high quality samples, 5 were benign, 3 were ATA low risk of recurrence (ROR) papillary thyroid cancer (PTC), and 2 were ATA high/intermediate ROR PTC.

The researchers were able to differentiate benign and malignant samples by follicular cell clustering and found that more aggressive cancers exhibited unique clusters featuring less differentiated cells (Figures 2 and 3), demonstrating the feasibility of scRNAseq for thyroid nodule FNA samples. They also found that most cells from an FNA are immune cells, likely from peripheral blood (see clusters 0-2,4, and 7-9 in figure 1).

Access the poster layout

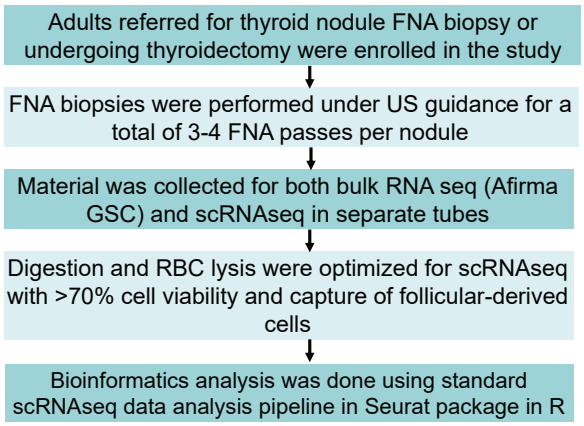
Schedule meeting with Medical Science Liaison

FULL STUDY

Objective

- Molecular testing with bulk RNA and DNA sequencing is used for thyroid nodule fine needle aspiration (FNA) biopsies with indeterminate cytology. While the sensitivity of these tests is excellent, specificity and prognostic information could be improved.
- Single cell RNA sequencing (scRNAseq) is a novel approach used to identify cell subpopulations in tumor microenvironments and has the potential to identify diagnostic and prognostic molecular data in cell clusters not identified in bulk sequencing.
- There is scarce data on the diagnostic potential for scRNAseq for thyroid cancer, especially from preoperative samples.
- **We sought to determine the feasibility of using scRNA seq on thyroid nodule FNA samples.**

Methods



Results

Enrollment Data

- Total of 18 subjects were enrolled → 16 completed scRNAseq → 10 had high quality data (presence of follicular cells)
- Average age was 54.8 years
- 6 women and 4 men
- 4 biopsies were performed in clinic; 6 in OR (prior to neck incision)

ScRNAseq Data

Cytology or surgical pathology diagnosis	N	Avg # total cells	Avg # follicular cells
Benign	5	4054	84
PTC (ATA low risk of recurrence (ROR))	3	3176	109
PTC (ATA high/intermediate ROR)	2	7809	1039

Figure 1. Example of scRNAseq from FNA sample.

Majority of clusters (0,1,2,4,7,8,9) are immune cells, likely from peripheral blood. Cluster 6 and 10 are follicular cells.

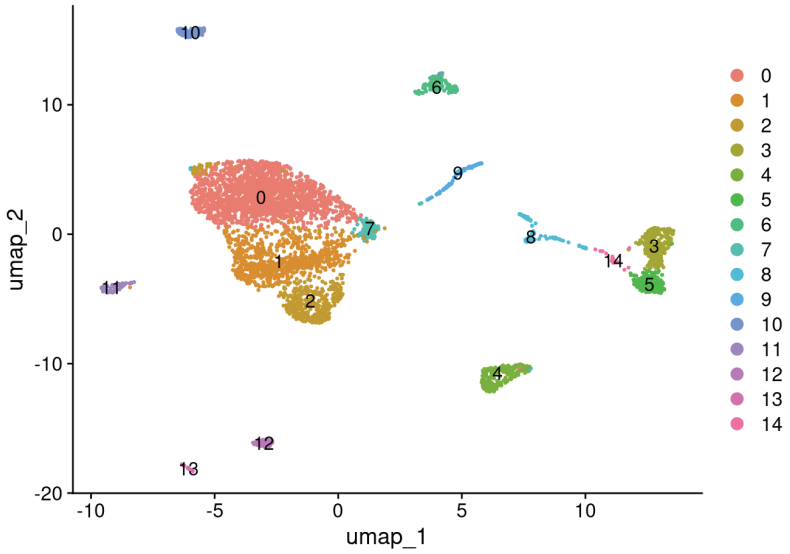


Figure 2. Reclustering follicular cells with high quality samples (n=7).

Two distinct clusters of follicular cells are identified; de-differentiation is seen in the smaller cluster.

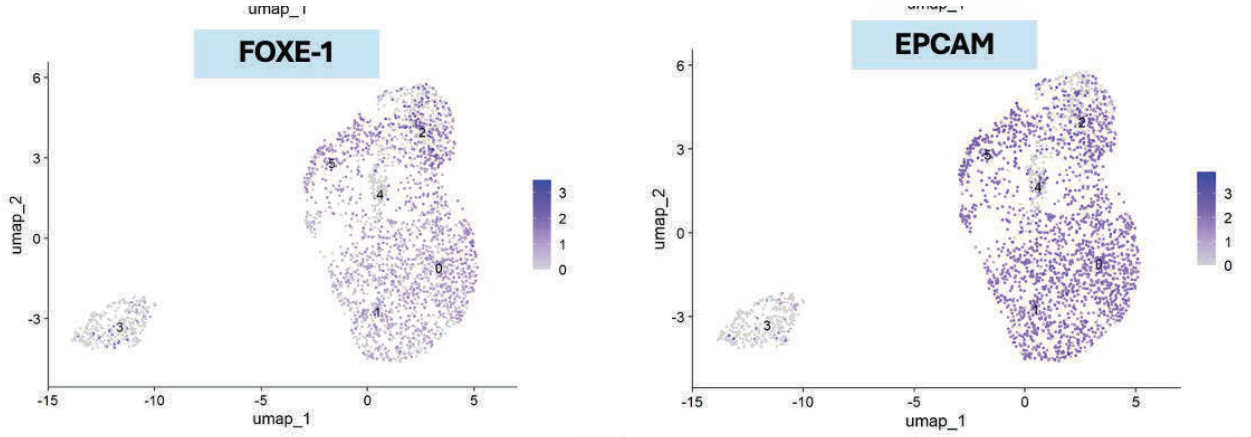
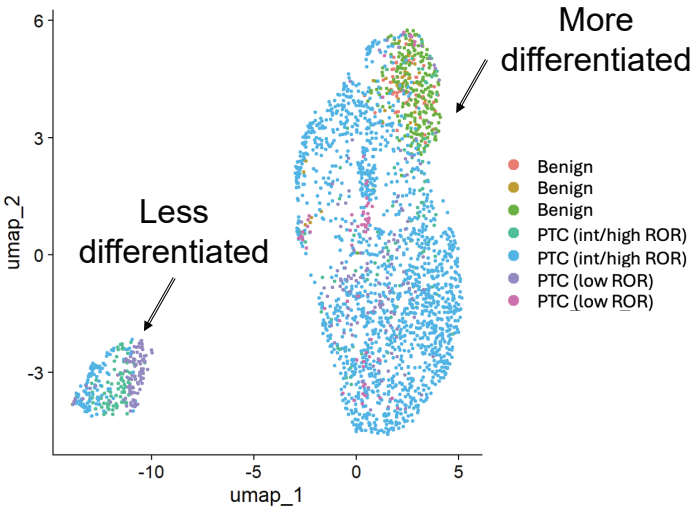


Figure 3. Identifying follicular cell clusters.
The less differentiated cluster contains cells from both intermediate/high risk for PTC ROR (blue and teal) and one low risk tumor (purple).



Conclusions

- We developed a protocol that demonstrates the feasibility of scRNAseq on thyroid nodule FNA samples.
- Our scRNAseq analysis showed that most cells from FNA are immune cells, likely from peripheral blood. Importantly, we were able to differentiate benign and malignant samples by follicular cell clustering.
- Furthermore, our scRNAseq analysis showed that more aggressive cancers seem to demonstrate unique clusters with less differentiated cells, which could enhance FNA molecular prognosis.
- Future studies will evaluate the concordance of scRNAseq from different cell populations with the Afirma GSC bulk RNAseq results.

Independent Abstracts and Conclusion

The following are independent studies that utilized data from Afirma GSC and Afirma GRID, presented at annual meetings throughout 2025.

Development of a Molecular Expression Profile-based Model to Predict High-Risk Histopathologic Features of Differentiated Thyroid Cancer

Justin Bauzon MD, Allan Siperstein MD, Michael Lui MD, Rafael Perez-Soto MD, MSc, Jee-Hye Choi MD, Joyce Shin MD, Gilman Plitt MD, PhD, Judy Jin MD, Gustavo Romero-Velez MD | Cleveland Clinic

- In a cohort of 445 patients that had Afirma GSC testing and operative resection, the investigators used significant variables from 30 distinct molecular signatures to develop a logistic regression model to preoperatively classify high-risk histopathologic features.
- The final model exhibited 93.3% accuracy, a sensitivity of 99.5%, and a negative predictive value of 83.3%.

Different Features of Aggressive Cancer Behavior Exhibit Clear Variations in mRNA Expression Patterns: An Analysis of Molecular Signatures in Differentiated Thyroid Cancer

Justin Bauzon MD, Michael Lui MD, Jee-Hye Choi MD, Rafael Perez-Soto MD, MSc, Joyce Shin MD, Gilman Plitt MD, PhD, Judy Jin MD, Gustavo Romero-Velez MD, Allan Siperstein MD | Cleveland Clinic

- Researchers wanted to understand whether hallmark features of differentiated thyroid cancer (DTC), such as angioinvasion or extrathyroidal extension, show distinct molecular expression patterns that could explain differences in tumor behavior.
- This was explored through 445 patients, of which 116 tumors exhibited at least one of five aggressive hallmark DTC features (tumor size >4cm, angioinvasion, extrathyroidal extension, lymph node metastases, or high-risk histologic variants) that were compared against 30 Afirma GRID cancer-related signatures.
- Comparison of the signatures among the 5 hallmark pathology features returned the following proportions with either significantly upregulated or downregulated expression levels: high-risk variants: 22/30 (73%); nodal metastases: 20/30 (67%); angioinvasion 18/30 (60%); tumor size >4cm: 8/30 (27%); extrathyroidal extension: 6/30 (20%), with the E2F signature overexpressed in all hallmark features except tumor size and the mTOR Complex 1 signaling overexpressed with angioinvasion, but underexpressed with metastatic nodes.

mRNA Expression Signatures Enhance Risk Stratification in Indeterminate Thyroid Nodules

Rachel Stemme BS, Joy Done MD, MHS, Anatoliy Rudin MD, FACS, Aarti Mathur MD, PhD, FACS, Lilah Morris-Wiseman MD, FACS | Johns Hopkins

- In 2025, the validation of two Afirma GRID (Genomic Resource for Intelligent Discovery) classifiers developed to rule out the risk of clinically significant thyroid tumor invasion (INV) and regional lymph node metastases (LNM) was published in the journal *Frontiers in Endocrinology*.
- To assess the ability of these classifiers to successfully classify indeterminate thyroid nodules and reduce unnecessary surgeries, the researchers in this study reviewed 154 Bethesda III or IV nodules from 150 patients that received Afirma Suspicious molecular results between 2020 and 2024 (all of whom had Afirma GRID testing and subsequent surgery), then risk-stratified based on final pathological results according the 2015 American Thyroid Association guidelines.
- The two GRID classifiers in this study return either low risk results (INV-LOW, LNM-LOW) or baseline/high risk results (INV-HIGH, LNM-HIGH); nodules with both INV-LOW and LNM-LOW results had an NPV of 85.7%.

The Impact of Nodule Size on Performance of Molecular Testing for Cytologically Indeterminate Thyroid Nodules

Athanasios Bikas MD, PhD,[1] Benjamin Altshuler BA,[1] Ellen Marqusee MD,[1] Sara Ahmadi MD,[1] Emma Su,[2] Gerard Doherty MD,[1] Theodora Pappa MD, PhD[2]

1. Brigham and Women's Hospital. 2. Dana-Farber Cancer Institute.

- Limited data exists on the performance of molecular testing for nodules larger than 4cm, so the researchers in this study investigated the impact of thyroid nodule size on the performance of molecular testing in indeterminate thyroid nodules.
- 823 cytologically indeterminate thyroid nodules that received Afirma GSC testing between 2017 and 2023 were divided into 3 groups based on size (<3 cm, 3–4 cm, and ≥4 cm) to compare molecular results and surgical outcomes.
- Significant molecular or histological differences were not observed between groups, suggesting that the performance of the Afirma GSC in nodules >4 cm is not different than in smaller nodules.

Evaluating Risk of Malignancy in Thyroid Nodules Using Genomics Resource for Intelligent Discovery (GRID) Molecular Profiling

Josie Yalovitser BA, Peter Callas PhD, Scott Anderson MD, Allison Ciolino MD, William Brundage MD, Damon Silverman MD, Mirabelle Sajisevi MD | University of Vermont

- The researchers in this study sought to evaluate the ability of 30 Afirma GRID (Genomic Resource for Intelligent Discovery) signatures in preoperatively distinguishing between benign and malignant pathology among nodules with Afirma Suspicious molecular results.
- This was done using a single institution retrospective analysis of 94 nodules from 2020-2025 that received an Afirma Suspicious result and either underwent lobectomy or total thyroidectomy, with pathology reports analyzed for final histopathologic diagnoses and classified as either American Thyroid Association "low-risk" or "intermediate/high-risk."
- Several signatures exhibited significantly different mRNA expression between benign nodules and NIFTP/malignant nodules, including the TGF-β signaling hallmark, mTOR signaling hallmark, Hürthle cell index, and PDL-1 signaling score.

CONCLUSION

Last year, thanks to collaborations with notable investigators and institutions from around the world, Veracyte was able to present research that demonstrated the ability of Afirma GRID (Genomic Resource for Intelligent Discovery) to glean new insights about thyroid nodules and thyroid cancer preoperatively.

This year, we're proud to not only present a greater number of abstracts, but to see institutions from around the world present their own research independently—research that pushes boundaries and advances the science around thyroid cancer. We maintain that whole-transcriptome-derived analysis will help drive cancer research and unlock findings that may ultimately be used in clinical care, and we're excited to see what the next year will bring.

If you're interested in conducting research of your own or would like to learn more about Afirma GSC, scan the QR code below to schedule a meeting with one of Veracyte's Medical Science Liaisons.

Schedule meeting with Medical Science Liaison

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