

Preoperative mRNA Expression Signatures Associated with 2025 American Thyroid Association (ATA) Risk of Recurrence Categories

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INTRODUCTION

- Molecular testing refines the risk of malignancy in indeterminate thyroid nodules (ITN) with (B)ethesda III or IV cytology, yet its preoperative prognostic value remains unclear.
- The Afirma Genomic Sequencing Classifier (GSC) was validated in a study where indeterminate thyroid nodules were prospectively collected and there was blinded central histopathology review.
 - The Afirma GSC has a 96% negative predictive value for thyroid cancer with a benign result from ITN.
- The Afirma GSC utilizes whole-transcriptome-derived RNA sequencing that allows for the development of the Afirma Genomic Resource for Intelligent Discovery (GRID).

AIM

The aim of this study is to evaluate the ability of preoperative messenger RNA (mRNA) expression signatures derived from Afirma GRID to distinguish tumors with lower versus higher 2025 ATA risk of recurrence.

METHODS

- This was a retrospective, single-center study of thyroid cancer cases that underwent Afirma GSC testing as part of routine clinical care.
- 2025 ATA risk of recurrence labels were assigned and ATA low and low-intermediate (L-I) risk groups were combined and compared with intermediate-high (I-H) and high-risk groups.
- Expressed variants and fusions and molecular data from the Afirma GRID were compared between the groups.
- The low/L-I group was used as the reference and logistic regression was used to associate GRID signatures with the ATA risk classification.

RESULTS

- A total of 72 cases met inclusion criteria with the following ATA 2025 risk classification:
 - 26 low, 19 low-intermediate, 16 intermediate-high, and 11 high.
- Between the low/L-I and I-H/high groups, there was no significant difference in Bethesda category or common thyroid cancer associated variants and fusions (Table 1).
- Amongst GRID signatures, intermediate-high/high risk cancers correlated with increased expression of Apical Junction hallmark (OR 3.91, $p < 0.01$), and Invasion Signature Score (OR 3.35, $p = 0.03$), and decreased expression of Immune Content estimation (OR 0.34, $p = 0.04$) (Table 2).
 - Differences were most pronounced between the low-intermediate and intermediate-high risk groups (Figure 1 A-C).

CONCLUSIONS

- In this study, well described mutations, such as *BRAFp.V600E*, did not distinguish recurrence risk using ATA 2025 criteria, suggesting that detection of variants and fusions alone is insufficient for prognostic stratification.
- mRNA expression of Apical Junction, Invasion Signature Score, and Immune Content estimation were associated with higher-risk disease.
 - These findings identify transcriptional signatures as potential preoperative prognostic markers that may refine thyroid cancer risk assessment among tumors arising from the same Bethesda cytologic category and with the same expressed variants and fusions.
- Incorporation of these molecular markers may enhance individualized surgical and surveillance decision-making.

TABLE 1
Bethesda category and common variants and fusions by ATA risk groups

	Low/Interlow	High/Interhigh	pvalue*
Total	45	27	
Bethesda			
III/IV	35 (77.8%)	20 (74%)	0.77
V/VI	10 (22.2%)	7 (26%)	
<i>BRAFp.V600E</i>	10 (22.2%)	9 (33.3%)	0.41
<i>RAS</i> variants	9 (20%)	4 (14.8%)	0.75
<i>ALK/NTRK/RET</i> fusions	4 (9%)	3 (11.1%)	1

* Chi-square test

TABLE 2
Afirma GRID data point differences between ATA risk of recurrence groups

GRID scores	High/Interhigh vs Low/Interlow (ref)		Interhigh vs Interlow (ref)	
	Odd Ratio (OR)	pvalue	Odd Ratio (OR)	pvalue
Angiogenesis hallmark	1.56	0.36	1.11	0.88
Apical junction hallmark	3.91	0.009	12.13	0.002
Apical surface hallmark	1.27	0.62	1.11	0.88
Apoptosis hallmark	2.09	0.17	1.75	0.45
DNA repair hallmark	0.6	0.3	0.41	0.21
E2F targets hallmark	0.72	0.5	0.28	0.08
Epithelial mesenchymal transition hallmark	1.99	0.16	2.79	0.15
Estrogen response early hallmark	1.82	0.23	3.77	0.06
Estrogen response late hallmark	2.73	0.06	7.78	0.02
Hedgehog signaling pathway	2.4	0.08	3.6	0.08
Hypoxia hallmark	0.76	0.61	1.33	0.68
Inflammatory response hallmark	0.49	0.14	0.28	0.08
mTOR complex1 signaling hallmark	0.55	0.23	0.7	0.6
<i>TP53</i> pathway hallmark	2.06	0.14	1.85	0.37
PI3k akt mtor signaling hallmark	0.63	0.35	0.63	0.51
TGF beta signaling hallmark	1.95	0.18	3.6	0.08
Interferon gamma response hallmark	0.6	0.3	0.54	0.37
Activated CD4	0.56	0.26	0.6	0.48
Activated CD8	0.81	0.67	1.11	0.88
Immune content estimation	0.34	0.04	0.24	0.06
Immunomodulators	1.56	0.36	2.79	0.15
M2 to M1 macrophage ratio	1.23	0.67	1.38	0.64
PDL1 signaling	1.39	0.5	2.17	0.27
T cell exhaustion	1.07	0.9	0.83	0.78
T cell regulatory	2.35	0.12	6.61	0.04
Cancer associated fibroblast	1.16	0.76	2.44	0.21
T cell accumulation	0.61	0.33	0.27	0.06
NIS expression	1.3	0.61	0.98	0.98
Hürthle (Oncocytic) cell index score	1.22	0.83	NA	NA
Invasion signature score	3.35	0.03	5.09	0.07
Lymph node metastasis signature score	1.73	0.3	1.02	0.98

OR and pvalue calculated from logistic regression.

FIGURE 1
Expression of significant GRID data points by ATA risk of recurrence category

