MON-640

Retrospective Analysis of mRNA Expression Based Signatures of Thyroid Tumor Invasion and Metastases

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INTRODUCTION

- The American Thyroid Association thyroid cancer initial risk stratification system is driven primarily by the extent of vascular and extrathyroidal extension, as well as the presence of lymph node metastases.[1]
- Minimizing surgical intervention for thyroid tumors from indeterminate thyroid nodules (ITN - Bethesda III and IV cytology) is often preferred to mitigate surgical complications and lessen the need for thyroid hormone supplementation post-operatively if clinical outcomes are not worsened.
- By leveraging the whole transcriptome derived Afirma Genomic Sequencing Classifier (GSC) thyroid nodule molecular testing platform, mRNA expression-based signatures were developed to predict thyroid tumors with a low risk (>95% NPV) of clinically significant invasion and metastases, as part of the Afirma Genomics Resource for Intelligent Discovery (GRID).[2]
- The objective of this study was to analyze the performance of these signatures on a retrospective cohort of patients with ITN who had Afirma GSC-suspicious findings and then underwent thyroid surgery.

METHODS

- Local pathology reports blinded to the tumor behavior signature result were scored for the extent of thyroid tumor invasion or metastases.
- Tumors with extensive vascular invasion (INV) or extrathyroidal extension are labeled as high risk (and otherwise are low risk). Tumors with lymph node metastases (LNM) that either have documented >2mm tumor deposits, >40% of central nodes involved, or any lateral lymph node metastases are labeled as high risk (and otherwise are low risk) (Table 1).
- Predictive signatures were developed utilizing literature-derived signatures as well as differentially expressed genes when comparing samples with or without clinically significant invasion/metastases as the basic building blocks. Machine learning algorithms were employed to develop the final candidate signatures (Figure 1).
- The Afirma GRID invasion and metastases signatures were applied to correlate with the pathology scoring.

RESULTS

- There were 144 (70%) females and 59 (30%) males with a median age of 54 [IQR 40-65] (Table 2).
- Cytology included 152 (75%) Bethesda III and 51 (25%) Bethesda IV nodules.
- 109 of 203 (53%) samples were malignant (including NIFTP) at final histology (Table 2).
- Two samples had high risk LNM, and four samples had high risk INV based on local histopathology.
- The INV signature ruled out 57% of the samples with 99% NPV and 57% specificity.
  - There was one false negative INV signature in a sample that had extra-thyroidal extension (ETE) into the overlying strap muscle on final histopathology from a left lobectomy (Figure 2). The patient had a completion right lobectomy that was benign. No lymph nodes were removed at either procedure. The patient received 50 mCi of RAI and has had an excellent response to therapy.
  - There was no statistical difference in the percentage of females (57%) and males (54%) ruled out (chi-squared test p=0.19) (Figure and Table 3).
- The LNM signature ruled out 55% of the samples with 100% NPV and 55% specificity.
  - There was no statistical difference in the percentage of females (58%) and males (46%) ruled out (chi-squared test p=0.10) (Figures and Table 3).

TABLE 1.
Tumor behavior labeling criteria

Invasion	Score
Extra-thyroidal	High Risk
Extensive vascular (≥4 vessels)	High Risk
Minimal vascular (<4 vessels)	Low Risk
None	Low Risk

Lymph Node Metastases	Score
Lateral neck nodes	High Risk
Central neck nodes ≥2mm tumor deposit or ≥40% of LN involved	High Risk
Central neck nodes <2mm tumor deposit and <40% of LN involved	Low Risk
None (including Nx)	Low Risk

FIGURE 1.
A lab developed test machine learning pipeline to develop novel tumor behavior signatures: >200 different variations of models were tested.

TABLE 2.
Clinico-genomics characteristics of the research cohort; Total (n) = 203

Age (median IQR)	**54 [40-65]**
Gender	
Male	59 (29%)
Female	144 (71%)
Bethesda Cytology Category	
III	152 (75%)
IV	51 (25%)
Invasion scoring	
Low risk: no invasion	170 (83.7%)
Low risk: min invasion (<4 vessels)	29 (14.3%)
High risk: Extensive vascular invasion (≥4)	3 (1.4%)
High risk: Extensive extra-thyroidal	1 (0.5%)
Lymph node metastasis scoring	
Low risk: no nodes	199 (98%)
Low risk: central neck <2 mm or <40% LN involvement	2 (1%)
High risk: central neck ≥2 mm or ≥40% LN involvement	2 (1%)
High risk: Lateral neck nodes	0

Histology	
PTC	26 (12.8%)
FTC	10 (4.9%)
HCC	13 (6.4%)
MTC	2 (1%)
FvPTC	19 (9.4%)
NIFTP	37 (18.2%)
FA	54 (26.6%)
FH	16 (7.9%)
HA	19 (9.4%)

FIGURE 2.
A bee swarm plot of invasion and LN metastasis models. Samples below the threshold line are predicted to have a low risk of invasion or lymph node metastases.

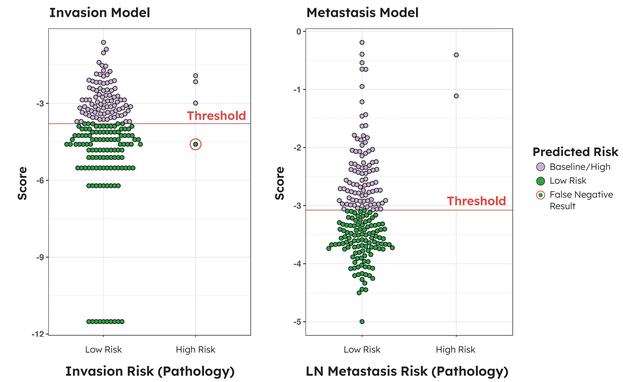
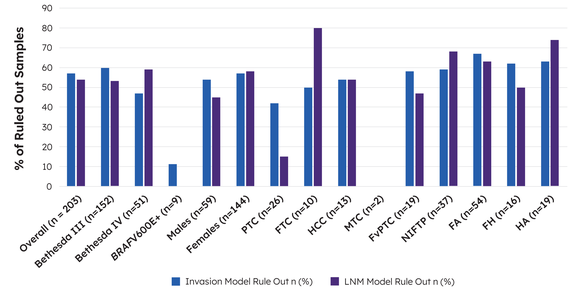


TABLE AND FIGURE 3.
Number and percentage of samples ruled out by the models across different subgroups



	Invasion Model Rule Out n(%)	LNM Model Rule Out n (%)
Overall (n=203)	115 (57%)	111 (54.7%)
Bethesda III (n=152)	91 (60%)	81 (53.3%)
Bethesda IV (n=51)	24 (47%)	30 (59%)
*BRAF*V600E+ (n=9)	1 (11%)	0
Males (n=59)	32 (54.2%)	27 (45.7%)
Females (n=144)	83 (57.6%)	84 (58.3%)
PTC (n=26)	11 (42.3%)	4 (15.4%)
FTC (n=10)	5 (50%)	8 (80%)
HCC (n=13)	7 (54%)	7 (54%)
MTC (n=2)	0	0
FvPTC (n=19)	11 (58%)	9 (47.4%)
NIFTP (n=37)	22 (59.5%)	25 (67.6%)
FA (n=54)	36 (66.7%)	34 (63%)
FH (n=16)	10 (62.5%)	8 (50%)
HA (n=19)	12 (63%)	14 (73.7%)

CONCLUSIONS

- These data confirm the ability of the Afirma GRID INV and LNM signatures to effectively rule out clinically significant higher-risk tumor features in >50% of the studied cohort with >95% NPV.
  - The low prevalence of aggressive histology from nodules with indeterminate cytology makes development of a rule in signature for aggressive disease very challenging.
- Prospective studies should be performed to confirm the utility of Afirma GRID tumor behavior signatures to optimize and individualize surgical decision-making, decreasing the burden of unnecessary extent of thyroid surgery while maintaining outstanding clinical outcomes.
- Future research opportunities include developing tumor behavior signatures for lesions with Bethesda V/VI cytology and/or a *BRAF*V600E mutation.

References

1. Haugen et al. Thyroid. 2016 Jan;26(1):1-133.
2. Golding et al. ENDO 2023. JES. Volume 7, Issue Supplement_1, October–November 2023.

Abbreviations Key

PTC: Papillary thyroid cancer
FTC: Follicular thyroid cancer
HCC: Hürthle cell (oncocytic) cancer
MTC: Medullary thyroid cancer
FvPTC: Follicular variant of papillary thyroid cancer
NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear features
FA: Follicular adenoma
FH: Follicular hyperplasia
HA: Hürthle cell (oncocytic) adenoma