Leveraging RNA Sequencing for Pre-Operative Immunophenotyping of *BRAF*V600E+ Thyroid Nodules

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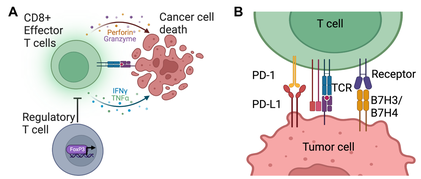
INTRODUCTION

- Beyond predicting malignancy risk in indeterminate thyroid nodules, molecular testing may provide pre-operative molecular prognostic information and identify opportunities for targeted therapy in aggressive thyroid cancers.
- Prior studies of *BRAF*V600E+ papillary thyroid cancer using surgical specimens found increased expression of checkpoint ligands and an unfavorable effector T cell anti-tumor response, including decreased effector CD8+ T cells and increased FoxP3+ T regulatory cells, compared to PTC without *BRAF*V600E mutation (Figure 1).
- The objective of this study was to evaluate immunophenotyping using the Afirma Genomic Sequencing Classifier (GSC), which leverages whole exome RNA sequencing of thyroid nodule fine needle aspirates and specific cancer gene mutation analyses.

FIGURE 1



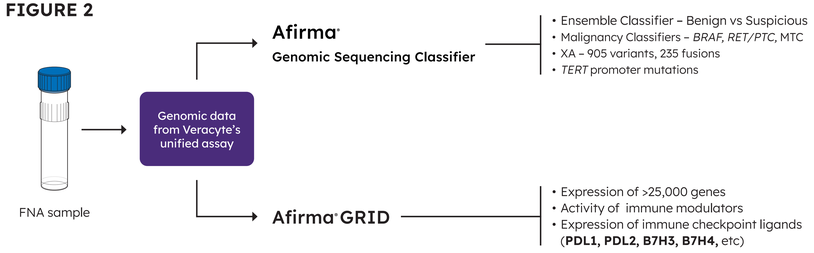
A. Balance of CD8+ effector and FoxP3+ T regulatory cells impacts effective anti-tumor immune response.

B. Tumor expression of checkpoint ligands impairs anti-tumor immune responses.

METHODS

- Retrospective analysis of 47,695 molecularly tested thyroid nodules by Afirma GSC as part of routine clinical care.
- Gene expression of tumor-associated checkpoint ligands (PDL1, PDL2, B7H3, and B7H4), as well as immune effector cell markers (CD8A, and FOXP3) normalized to protein tyrosine phosphatase receptor type C (PTPRC - encoding CD45), were compared between GSC-Benign (B) (96% NPV for malignancy), GSC-Suspicious (S) (47% PPV for malignancy), and likely malignant (Bethesda-V/VI cytology (SFM/M)) cohorts (figure 2).
- GSC-S and Bethesda SFM/M cohorts were stratified by *BRAF*V600E+ or wildtype (WT) status.

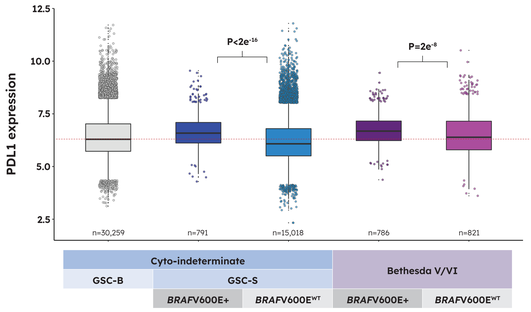
FIGURE 2



RESULTS

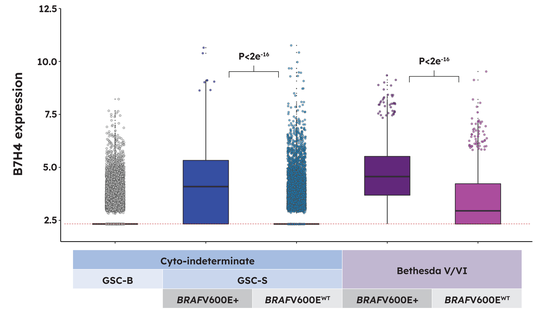
FIGURE 3: Expression of immune checkpoint ligands and T cell-related genes across thyroid nodules stratified by malignancy risk and *BRAF*V600E status

3a



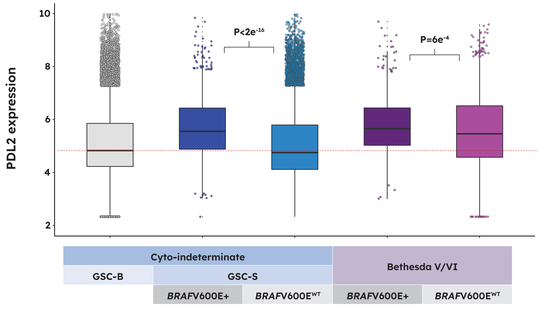
PDL1 expression is higher in samples with *BRAF*V600E+ variants compared to *BRAF*V600EWT in both GSC-S and Bethesda V/VI groups.

3d



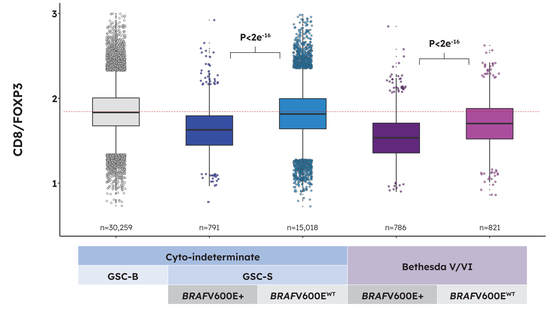
B7H4 expression is higher in samples with *BRAF*V600E+ variants compared to *BRAF*V600EWT in both GSC-S and Bethesda V/VI groups.

3b



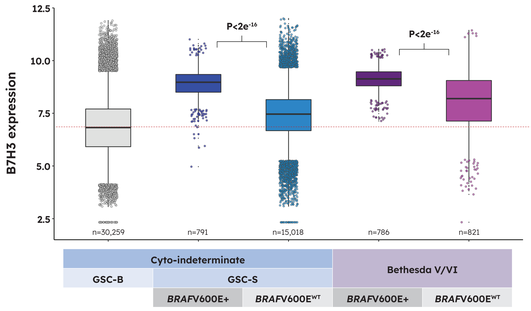
PDL2 expression is higher in samples with *BRAF*V600E+ variants compared to *BRAF*V600EWT in both GSC-S and Bethesda V/VI groups.

3e



Ratio of CD8/FOXP3 is lower in samples with *BRAF*V600E+ variants compared to *BRAF*V600EWT in both GSC-S and Bethesda V/VI groups suggesting a decreased effector CD8+ T cell to T regulatory cell ratio. Both CD8A and FOXP3 were normalized by PTPRC (encoding CD45).

3c



B7H3 expression is higher in samples with *BRAF*V600E+ variants compared to *BRAF*V600EWT in both GSC-S and Bethesda V/VI groups.

CONCLUSIONS

- RNA expression profiles provided by Afirma GSC molecular testing confirms the expected immunophenotype of BRAFV600E+ thyroid cancers in both the GSC-S and a cytologically-defined Bethesda-V/VI cohorts.
- mRNA expression based Afirma GSC evaluation may allow for pre-operative immunophenotyping of thyroid cancers, as an alternative to traditional immunohistochemistry techniques on surgical specimens.
- Future prospective studies are warranted to evaluate whether the Afirma GSC assay can predict thyroid cancer response to immune checkpoint inhibitor therapy.