

ONCOLOGY: Molecular Biomarkers of Thyroid Cancer

Sample Type to Test: Fine needle aspirates; Cytology smears; formalin-fixed, paraffin-embedded tissue (FFPE); peripheral blood/buccal swabs for germline testing

Biomarker	Specific Alterations/ Alternative terms	Indications	Result Interpretation Significance	Assay Techniques
BRAF	Variants in codons 600, 601, especially V600E	<p>Diagnosis: Screening of indeterminate cytology thyroid nodules</p> <p>Prognosis</p> <p>Kinase inhibitor therapy selection/ response prediction</p>	<p>High specificity for papillary thyroid carcinoma (PTC) (<i>BRAF</i> V600E in ~45% PTC)</p> <p>Higher risk of recurrence in PTC, especially when it is with <i>TERT</i> promoter mutation</p> <p>Consideration of BRAF-targeted therapy in metastatic disease not amenable to RAI therapy</p>	NGS, pyrosequencing, Sanger sequencing, or PCR-based genotyping assays with different sensitivity
RAS: HRAS, NRAS, and KRAS	Variants in codons 61, 12, 13	Diagnosis: Screening of indeterminate cytology thyroid nodules	Frequently seen in follicular adenomas, follicular carcinomas, NIFTP, and invasive follicular variant of PTC	NGS, pyrosequencing, Sanger sequencing, genotyping, or PCR-based assays with different sensitivity
RET	M918T; A883F; variants in C634, C609, C611, C618, C620, C630; G533C; D631Y; K666E; E768D; L790F; V804L; V804M; S891A; R912P	<p>Diagnosis</p> <p>Prognosis</p> <p>Therapy selection/ response prediction</p> <p>In germline, risk of hereditary MTC</p>	<p>Medullary thyroid carcinoma (MTC)</p> <p>Somatic M918T mutation in sporadic MTC associated with aggressive clinical course and poor prognosis</p> <p>Consideration of targeted RET inhibitors or multi-kinase inhibitors in MTC patients with unresectable locally advanced or metastatic disease</p> <p>Inherited MTC (autosomal dominant): <i>MEN2A</i> (primarily in exons 10,11,13), <i>MEN2B</i> (exons 14 to 16) or familial MTC syndromes (exons 10, 11, 13 to 16)</p>	NGS, pyrosequencing, Sanger sequencing, genotyping, or PCR-based assays with different sensitivity
RET/PTC1 & RET/PTC3 rearrangements	<i>RET/PTC1</i> = fusion of <i>RET</i> with <i>CCDC6</i> <i>RET/PTC3</i> = fusion of <i>RET</i> with <i>NCOA4</i>	<p>Diagnosis: Screening indeterminate cytology thyroid nodules</p> <p>Therapy selection/response prediction</p>	<p>Highly specific for PTC</p> <p>Consideration of targeted RET inhibitors or multi-kinase inhibitors</p>	RT-PCR, NGS including RNA-Seq
NTRK1, NTRK2, NTRK3 rearrangements		<p>Diagnosis: Screening indeterminate cytology thyroid nodules</p> <p>Therapy</p>	<p>Highly specific for PTC</p> <p>Consideration of targeted NTRK inhibitors in patients with advanced or aggressive disease.</p>	RT-PCR, NGS including RNA-Seq
PAX8/PPARG rearrangement		Diagnosis: Screening indeterminate cytology thyroid nodules	Primarily seen in follicular carcinomas, but may also be seen at lower frequencies in follicular adenomas and the follicular variant of PTC	RT-PCR, NGS including RNA-Seq
ALK rearrangement		For advanced, progressive, or threatening disease to identify actionable mutations	Consideration of targeted therapy in patients with advanced or aggressive diseases.	FISH, RT-PCR, AMP

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Deficient DNA mismatch repair (dMMR):	Mutations in <i>MLH1</i> , <i>MLH3</i> , <i>MSH2</i> , <i>MSH5</i> , <i>MSH6</i> , and <i>PMS2</i>	For advanced, progressive, or threatening disease to identify actionable mutations	Consideration of immune checkpoint inhibitors therapy in patients with advanced or aggressive disease	NGS, Sanger sequencing
Microsatellite instability (MSI)	MSI-H	For advanced, progressive, or threatening disease to identify actionable mutations	Consideration of immune checkpoint inhibitors in patients with advanced or aggressive disease	PCR
Tumor mutational burden (TMB)	TMB-H (high): ≥ 10 mutations/megabase [mut/Mb]	For advanced, progressive, or threatening disease to identify actionable mutations	Poorer prognostic indicator Consideration of immune checkpoint inhibitors in patients with advanced or aggressive disease	NGS

Abbreviations: RAI - radioactive iodine; NIFTP - noninvasive follicular thyroid neoplasm with papillary-like nuclear features; PTC - papillary thyroid carcinoma; MTC - medullary thyroid carcinoma; NGS – next generation sequencing; RT-PCR – Reverse Transcription PCR, AMP - anchored multiplex PCR

Where to Test: Testing should be performed in laboratories that are certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity (molecular pathology) testing.

References:

National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology. Thyroid Carcinoma. Version 1.2025 – March 27, 2025; NCCN.org. accessed 7/15/2025

Haugen BR, *et al.* 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*. 2016 Jan;26(1):1-133.

Wells SA Jr, *et al.* American Thyroid Association Guidelines Task Force on Medullary Thyroid Carcinoma. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid*. 2015 Jun;25(6):567-610.

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