Molecular In My Pocket™...

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	Diagnosis: Screening of indeterminate cytology thyroid nodules Prognosis Kinase inhibitor therapy selection/ response prediction	High specificity for papillary thyroid carcinoma (PTC) (*BRAF* V600E in ~45% PTC) Higher risk of recurrence in PTC, especially when it is with *TERT* promoter mutation Consideration of BRAF-targeted therapy in metastatic disease not amenable to RAI therapy	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	Diagnosis Prognosis Therapy selection/ response prediction In germline, risk of hereditary MTC	Medullary thyroid carcinoma (MTC) Somatic M918T mutation in sporadic MTC associated with aggressive clinical course and poor prognosis Consideration of targeted RET inhibitors or multi-kinase inhibitors in MTC patients with unresectable locally advanced or metastatic disease Inherited MTC (autosomal dominant): *MEN2A* (primarily in exons 10,11,13), *MEN2B* (exons 14 to 16) or familial MTC syndromes (exons 10, 11, 13 to 16)	NGS, pyrosequencing, Sanger sequencing, genotyping, or PCR-based assays with different sensitivity
***RET/PTC1* & *RET/PTC3* rearrangements**	*RET/PTC1* = fusion of *RET* with *CCDC6* *RET/PTC3* = fusion of *RET* with *NCOA4*	Diagnosis: Screening indeterminate cytology thyroid nodules Therapy selection/response prediction	Highly specific for PTC Consideration of targeted RET inhibitors or multi-kinase inhibitors	RT-PCR, NGS including RNA-Seq
***NTRK1, NTRK2, NTRK3* rearrangements**		Diagnosis: Screening indeterminate cytology thyroid nodules Therapy	Highly specific for PTC Consideration of targeted NTRK inhibitors in patients with advanced or aggressive disease.	RT-PCR, NGS including RNA-Seq
***PAX8/PPARG* rearrangement**		Diagnosis: Screening indeterminate cytology thyroid nodules	Primarily seen in follicular carcinomas, but may also been 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 disease.	FISH, RT-PCR, AMP

Biomarker	Specific Alterations/ Alternative terms	Indications	Result Interpretation Significance	Assay Techniques
Deficient DNA mismatch repair (dMMR):	Mutations in *MLH1, MLH3, MSH2, MSH5, MSH6*, and *PMS2*	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 2.2023 – May 18, 2023; NCCN.org. accessed 7/13/2023

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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