ThyroSeq®
Thyroid Genomic Classifier

Patient		Accession #:
DOB/Age/Sex		Client Accession #:
Client Identifier	Client	
Collection Date	Requesting Physician	
Accession Date	Ordering Physician	
Reported Date	T	
	F	

CLINICAL HISTORY
FNA cytology: FN/SFN (Bethesda IV)

THYROSEQ® V3 GC RESULTS SUMMARY

LEFT THYROID FNA

Test Result	Probability of Cancer or NIFTP	Potential Management
NEGATIVE	**Low (~3%)**	**Observation *** **See interpretation below for details*

INTERPRETATION

- The performed analysis was negative for all tested genetic alterations associated with thyroid cancer.
- Based on the results of studies at UPMC and prospective multicenter study, the probability of cancer in thyroid nodules with Bethesda III-IV cytology and negative ThyroSeq GC result is ~3%. This should be applicable to any clinical setting where the risk of malignancy in nodules with Bethesda III/IV cytology is within the expected range (<40%).
- According to the NCCN guidelines, if molecular testing, in conjunction with clinical and ultrasound features, predicts a probability of malignancy ~5% or less (comparable to benign FNA cytology), observation in lieu of surgical management may be considered.
- Patient management decisions must be based on the independent medical judgment of the treating physician. Molecular test results should be taken into consideration in conjunction with all relevant imaging and clinical findings, patient and family history, as well as patient preference.

DETAILED RESULTS

Specimen cellularity/adequacy for interpretation: **ADEQUATE**

Marker Type	Marker Result
Gene mutations	Negative
Gene fusions	Negative
Copy number alterations	Negative
Gene expression profile	Negative
Parathyroid	Negative
Medullary/C-cells	Negative

BACKGROUND

Diagnostic use of molecular markers in FNA samples with indeterminate cytology. Thyroid cancer is characterized by common occurrence of various genetic alterations (1). Based on the results of multicenter prospective double-blind study of ThyroSeq v3 (2,3), in the populations with pre-test cancer prevalence of 23-35%, the probability of cancer in thyroid nodules with Bethesda III (AUS/FLUS) and Bethesda IV (FN/SFN) cytology and negative ThyroSeq v3 test result is 2-3%. Based on our validation analysis, the probability of cancer in nodules with suspicious for malignant cells (Bethesda V) cytology and negative ThyroSeq test result is ~20% (data on file).

Patient management. Management of patients with thyroid nodules can be informed by the results of FNA cytology and ThyroSeq test. Importantly, in addition to the test results, clinical features of the nodule, patient history as well as patient preference must be considered when formulating the medical or surgical management approach.

ThyroSeq test result NEGATIVE:According to the National Comprehensive Cancer Network (NCCN) clinical practice guidelines (4), if molecular testing, in conjunction with clinical and ultrasound features, predicts a risk of cancer comparable to the risk of malignancy seen in a benign FNA cytology (approximately 5% or less), observation can be considered. Therefore, in those clinical situations where the pre-test probability of cancer in nodules with Bethesda III and IV cytology is <44%, negative ThyroSeq test results would confer the cancer probability of 5% or less (3), justifying observation in lieu of surgical management in appropriately selected cases. Because the probability of cancer in such nodules is comparable to benign FNA cytology, the management of patients may follow the recommendations for nodules with benign cytology, which, based on the 2015 American Thyroid Association (ATA) guidelines, should be determined based on ultrasound (US) pattern (5): For nodules that have high suspicion US pattern, repeat US and US-guided FNA within 12 months; for nodules with low to intermediate suspicion US pattern, repeat US at 12-24 months and if sonographic evidence of growth or development of new suspicious sonographic features, the FNA could be repeated or observation continued with repeat US, with repeat FNA in case of continued growth (Rec. #23 in ref. 5). In nodules with Bethesda V cytology and negative ThyroSeq result, the residual cancer risk of ~20% does not allow to avoid surgical management; thyroid lobectomy may be sufficient initial treatment for many of these patients as the majority of these nodules are expected to be benign.

ThyroSeq test result: CURRENTLY NEGATIVE:Test results are reported as currently negative when the sample is found positive for a low-risk (LR) gene mutation that alone is not sufficient for full cancer development (eg. PTEN, EIF1AX) and also found in a subpopulation of cells (6). Although at the time of sampling most of these nodules are benign, some of them may undergo clonal expansion and acquire additional mutations. In the absence of high suspicion US pattern or other clinical risk factors, many of these patients are likely to benefit from active surveillance with potential repeat of FNA and molecular testing in 1 year.

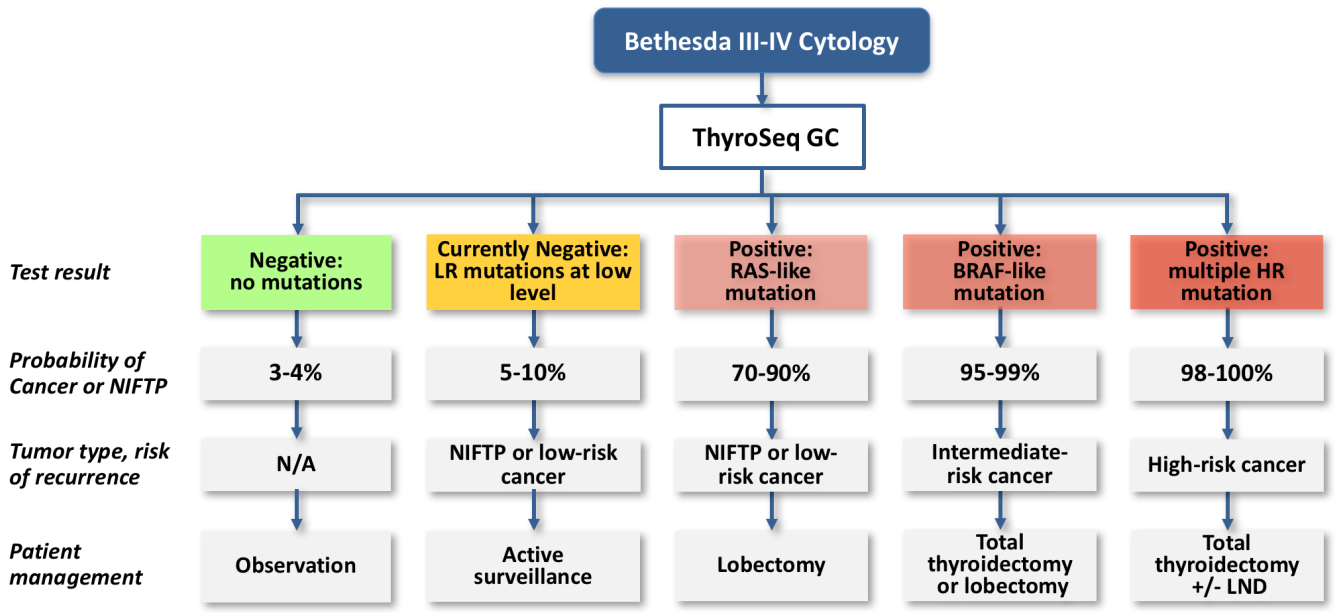
ThyroSeq test result POSITIVE:For these nodules, the type and level of mutation(s) provide further refinement of the probability of cancer and allow estimating cancer aggressiveness/risk. According to the ATA risk stratification system for thyroid cancer, the risk of structural disease recurrence can be low (~1-5%), intermediate (~10-20%), and high (30-55%) (5).

ThyroSeq test positive for an isolated RAS or RAS-like mutation (e.g. BRAF K601E, PPARG fusions) predicts a high probability (~80%) of either low-risk cancer (2,3) or a pre-cancerous tumor, NIFTP (7). Many of these nodules may be managed by therapeutic lobectomy, which is currently recommended by the ATA guidelines for low-risk papillary and follicular carcinomas (Rec. #35 in ref. 5) and NIFTP (8).

ThyroSeq test positive for an isolated BRAF V600E or BRAF V600E-like mutation (e.g. RET/PTC, BRAF fusions) confers a very high (>95%) probability of cancer that typically is at intermediate risk for recurrence (9). According to the ATA guidelines (5), BRAF-mutated unifocal intrathyroidal carcinoma is low risk for disease recurrence and therefore may be treated with thyroid lobectomy alone, whereas 1-4 cm intrathyroidal BRAF-positive PTC is an intermediate-risk tumor, where total thyroidectomy or lobectomy should be considered based on clinical and US findings.

ThyroSeq test positive for multiple high-risk (HR) mutations (e.g. BRAF V600E and TERT) is virtually diagnostic of cancer and predicts an elevated risk of disease recurrence by the ATA guidelines (5) and of tumor-related mortality by several studies (10-12). Most of these patients would likely benefit from total thyroidectomy, with possible consideration for regional lymph node dissection if one of the mutations is BRAF V600E (13).

Fig. 1.*Potential management of patients with Bethesda III-IV cytology. (LR – low-risk; HR – high-risk; NIFTP – non-invasive follicular thyroid neoplasm with papillary-like nuclear features; LND – lymph node dissection)*



Other applications.In addition to the primary application for indeterminate FNA cytology, ThyroSeq may have clinical utility in a subset of FNA samples with benign (Bethesda II) or malignant (Bethesda VI) cytology. Up to 30% of nodules with benign cytology but suspicious clinical features have detectable mutations, and most of those are found malignant after surgery (14,15). One study showed that testing for BRAF and RAS mutations decreased the false-negative rate of cytology from 4.8% to 0.4%, concluding that molecular testing could be helpful, but only in the presence of clinical suspicion for malignancy (15). In cytology samples with positive for malignancy (Bethesda VI) cytology and in surgically removed cancer samples, ThyroSeq testing may contribute to thyroid cancer risk stratification and optimizing patient management, including decision-making related to the use of radioiodine (16-18). In advanced thyroid cancer, mutational status may impact selection of targeted therapies (19).

References

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METHODOLOGY

Nucleic acids (DNA/mRNA) are isolated from thyroid FNA samples collected in the ThyroseqPreserve solution or from fixed samples. If required, manual microdissection is performed from unstained slides under the microscope with H&E guidance. The NGS analysis is applied to detect SNVs/indels, gene fusions (GF), gene expression alterations (GEA), and copy number alterations (CNAs) in targeted regions of 112 thyroid-cancer related genes (AGGF1, AGK, AKAP13, AKAP9, AKT1, ALK, APC, BANP, BCL2L11, BRAF, C7orf10, CALCA, CCDC149, CCDC30, CCDC6, CCNY, CHEK2, CHGA, CITED1, CREB3L2, CTNNB1, DICER1, EIF1AX, EML4, EP300, ERBB4, ERC1, ETV6, EZH1, EZR, FAM114A2, FAM193A, FARSB, FGFR2, FKBP15, GFPT1, GLIS3, GNAS, GOLGA5, GORASP2, GTF2IRD1, HOOK3, HRAS, IDH1, IDH2, IGF2BP3, IRF2BP2, KIAA1217, KIAA1598, KIF5B, KLK1, KRAS, KR T20, KRT7, KTN1, LOC389473, LTK, MACF1, MEN1, MET, MKRN1, NCOA4, NF2, NRAS, NTRK1, NTRK3, OFD1, PAX8, PCM1, PGK1, PICALM, PIK3CA, POR, PPARG, PRKAR1A, PTEN, PTH, RAF1, RBPMS, RET, RNF213, ROS1, SLC26A11, SLC5A5, SND1, SPECC1L, SQSTM1, SS18, SSBP2, STK11, STRN, SYN2, TBL1XR1, TERT, TFG, THADA, TP53, TPM3, TPR, TRA2A, TRIM24, TRIM27, TRIM33, TRIM61, TSC2, TSHR, UACA, VCL, VHL, WARS, ZBTB8A, ZC3HAV1). The Torrent Suite v5.2.2, Variant Explorer v2 and Genomic Classifier (GC) algorithm is used for data analysis. Test results are reported as Negative (low probability of malignancy) or Positive (high probability of malignancy). Specimen adequacy, mutation type, gene expression and CNA profiles are reported in the Detailed Results section. In FNA samples, GC sensitivity is 93% and specificity is 81% (2). The GC limits of detection (LOD) is 6-12% of thyroid cells (3). Analytical sensitivity (PPA) and analytical specificity (PPV) for SNVs/indels is >99%/99% at 3-5% AF (6-10% of tumor cells), for GF is >99%/99% at >1-3% of tumor cells, for GEA is >99%/99% at 10% of tumor cells, and for CNA is 92%/100% with LOD 20-25% of tumor cells in FNA samples and 40-70% of tumor cells in FFPE samples.The assay minimal required sequencing depth is 500x. Genetic regions that did not meet minimal sequencing coverage requirements are specified in the report as failed.

LOW COVERAGE HOTSPOTS OBSERVED IN THE FOLLOWING GENES

NONE

GROSS DESCRIPTION

1 FNA vial(s) labelled with patient name and identifiers received from CBLPATH, INC.

DISCLAIMER

ThyroSeq is a diagnostic test that was developed and its performance characteristics determined by the UPMC Molecular and Genomic Pathology laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research only. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. ThyroSeq test does not sequence genes in their entirety and mutations outside of mutation hotspots, some insertions and deletions, some novel gene fusions, and genomic alterations below sensitivity cut offs may not be detected. This test does not provide information on germline or somatic status of detected mutations. Certain sample characteristics may result in reduced sensitivity, including sample heterogeneity, low sample quality, and other causes. The information in this report must be used in conjunction with all relevant clinical information and does not intend to substitute clinical judgement. Decisions on patient care must be based on the independent clinical judgement of the treating physician. A treating physician's decision should not be based solely on this or any other single tests or the information in this report.

Electronically signed out by:

Arivarasan Karunamurthy, MD

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