

Patient	Accession #: MGP20-XXX
DOB/Age/Sex	Client Accession #:
Client Identifier	Client: Sonic Healthcare USA
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

RIGHT THYROID, 4.12 CM NODULE, FNA

Test Result	Probability of Cancer	Potential Management
POSITIVE	High (~90%)	Surgical excision * <i>*See interpretation below for details</i>

INTERPRETATION

- Mutations in the RAS and TERT genes were identified in this sample.
- RAS mutations are early oncogenic events in follicular-pattern thyroid tumors, whereas TERT mutations are typically late events associated with more invasive tumors and higher risk of distant metastases.
- Co-occurrence of RAS and TERT mutations confers ~90% risk of cancer and increases likelihood of more aggressive disease. The rest of these nodules present histologically as follicular adenomas which should be carefully examined for invasion.
- Because of the molecular signature of high-risk cancer, total thyroidectomy may be considered for appropriately selected patients.
- 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	AF
Gene mutations	TERT	p.C228T c.1-124C>T 16%
	NRAS	p.Q61R c.182A>G 14%
Gene fusions	Negative	
Copy number alterations	Negative	
Gene expression profile	Negative	
Parathyroid	Negative	
Medullary/C-cells	Negative	

AF=Variant Allele Frequency

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), 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 ~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 molecular tests like ThyroSeq (3,4). Importantly, in addition to the test results, imaging and other clinical features of the nodule, patient history as well as patient preference must be considered.

ThyroSeq test result NEGATIVE: According to the National Comprehensive Cancer Network (NCCN) clinical practice guidelines (5), if molecular testing, in conjunction with clinical and ultrasound features, predicts a risk of malignancy comparable to the risk of malignancy seen with a benign FNA cytology (~5% or less), active surveillance 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 (2), 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 (Recommendation #23 in ref. 6). 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.

ThyroSeq test result CURRENTLY NEGATIVE: Test results are reported as currently negative when the sample is found positive for a low-risk and/or low-level gene mutation, DNA copy number alterations (CNA) or gene expression alterations (GEA) that alone is not sufficient for full cancer development (7). 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 suspicious US features or other clinical risk factors, many of these patients are likely to benefit from active surveillance with repeat of clinical exam and potentially 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 allows 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%) (6).

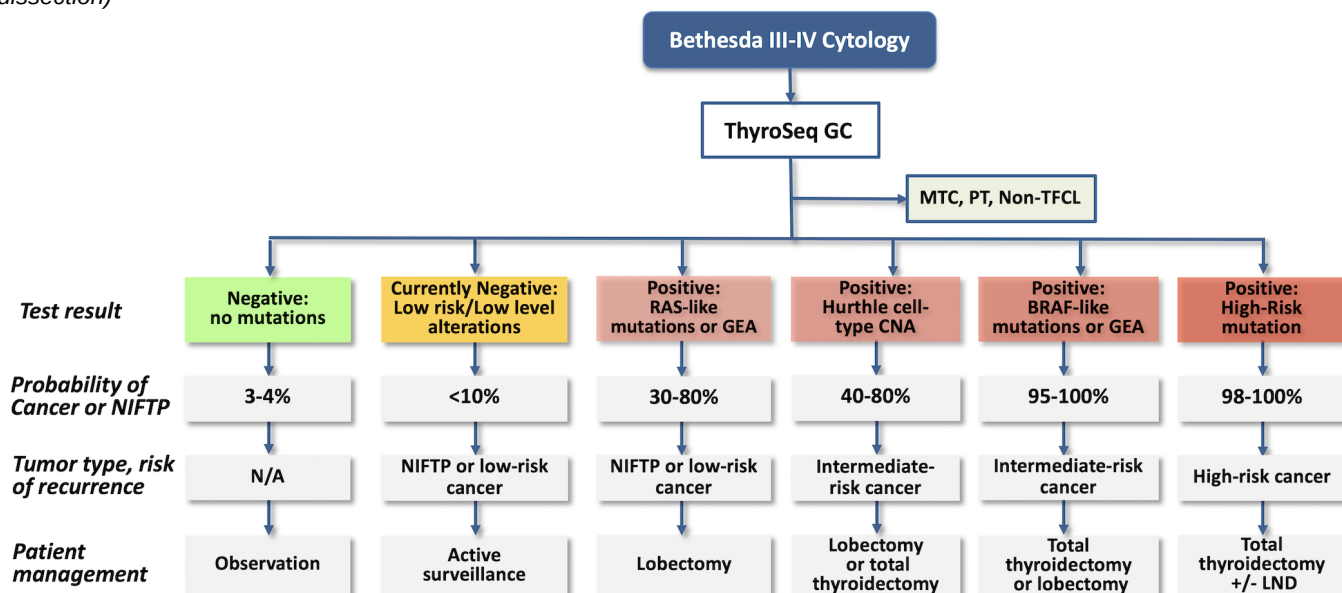
ThyroSeq test positive for an isolated RAS mutation or RAS-like alteration (e.g. BRAF K601E mutation, THADA fusion, RAS-like GEA) indicates that the nodule is a tumor (not hyperplasia) and predicts, depending on specific alteration, a 30-80% probability of either a low-risk cancer (2) or a pre-cancerous 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 (Recommendation #35 in ref. 6) and NIFTP (8).

ThyroSeq test positive for isolated Hurthle cell-type copy number alterations (CNA) confers, in different nodule size groups, a 40-80% probability of Hurthle cell carcinoma, whereas the rest of these nodules are benign Hurthle cell adenomas (2,9,10).

ThyroSeq test positive for an isolated BRAF V600E or BRAF V600E-like alteration (e.g. RET/PTC, BRAF fusions, BRAF V600E-like GEA) confers a very high (>95%) probability of cancer (2). According to the ATA guidelines (6), BRAF-mutated unifocal intrathyroidal carcinoma <1 cm in size has low risk for recurrence and therefore may be treated with thyroid lobectomy alone, whereas 1-4 cm 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) confers a very high probability of cancer and predicts an increased risk of disease recurrence by the ATA guidelines (6) and of tumor-related mortality (11-12). 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 patient with Bethesda III-IV cytology nodules. (MTC - medullary thyroid carcinoma; PT - parathyroid; Non-TFCL - non-follicular thyroid cell lesion; GEA - gene expression alterations; CNA - copy number alterations; 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). In one study, 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 forms of thyroid cancer, testing for mutations (eg. BRAF V600E) and gene fusions (eg. NTRK1,3) using ThyroSeq may impact selection of targeted therapies (5,19,20).

References

1. Agrawal N, et al. *Cell*. 2014;159:676-90;
2. Steward D, et al. *Thyroid*. 2017;27:A-168;
3. Durante C, et al. *JAMA*. 2018;319:914-924;
4. Mayson SE, et al. *Endocrinol Metab Clin North Am*. 2019;48:85-97;
5. 2019 National Comprehensive Cancer Network (NCCN) clinical practice guidelines for thyroid carcinoma. <https://www.nccn.org/>
6. Haugen BR, et al. *Thyroid*. 2016;26:1-133;
7. Nikiforova MN, et al. *Cancer*. 2018;124:1682-1690;
8. Haugen BR, et al. *Thyroid*. 2017;27:481-483;
9. Ganly I, et al. *Cancer Cell*. 2018;34:256-270.e5;
10. Gopal RK, et al. *Cancer Cell*. 2018;34:242-255.e5;
11. Xing M, et al. *J Clin Oncol*. 2014;32:2718-26;
12. Song YS, et al. *Cancer*. 2016;122:1370-9;
13. Howell GM, et al. *Ann Surg Oncol*. 2013;20:47-52;
14. Nikiforov YE, et al. *J Clin Endocrinol Metab*. 2009;94:2092-8;
15. Proietti A, et al. *Cancer Cytopathol*. 2014;122:751-9;
16. Tufano RP, et al. *Medicine (Baltimore)*. 2012;91:274-86;
17. Xing M, et al. *Lancet*. 2013;381:1058-69;
18. Xing M, et al. *J Clin Oncol*. 2009;27:2977-82;
19. Bible KC, et al. *Nat Rev Clin Oncol*. 2016;13:403-16;
20. Kummar S, et al. *Target Oncol*. 2018;13:545-556;

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, KRT20, 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.

Additional details of DNA sequence variants

Gene	Transcript	Genomic Position
TERT	NM_198253.2	chr5:1295228G>A
NRAS	NM_002524.4	chr1:115256529T>C

LOW COVERAGE HOTSPOTS OBSERVED IN THE FOLLOWING GENES

NONE

GROSS DESCRIPTION

1 part(s) labeled with patient name and identifiers received from Sonic Healthcare USA.

Sample 1: One (1) FNA vial received and labeled with patient name and identifiers

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:

Peter Lucas, MD, PhD
03/05/2020 15:51