Patient			Accession #: **MGP21-**
DOB/Age/Sex		Client Accession #: / MRN:	
Client Identifier		Client / Ordering Laboratory	Sonic Healthcare USA ThyroSeq /CBLPATH, INC
Collection Date	02/23/2021		
Accession Date		Requesting Physician	
Reported Date		Ordering Physician	
		T	
		F	

CLINICAL HISTORY
Pathology diagnosis: Papillary thyroid carcinoma

THYROSEQ® V3 CRC RESULTS SUMMARY

RIGHT THYROID, FFPE TISSUE BLOCK

Test Result	Risk of Cancer Recurrence
POSITIVE	**High *** ***See interpretation below for details***

INTERPRETATION

- The specimen revealed BRAF V600E and TERT mutations, along with gene expression alterations.
- This molecular profile is characteristic of papillary carcinomas and related advanced, dedifferentiated cancers.
- Co-occurrence of these mutations is associated with a high risk for cancer recurrence and increased risk of locally invasive disease, lymph node and distant metastasis, and disease-related mortality.
- 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	33%
	BRAF	p.V600E	c.1799T>A	22%
Gene fusions	Negative			
Copy number alterations	Negative			
Gene expression profile	Positive			
Parathyroid	Negative			
Medullary/C-cells	Negative			

AF=Variant Allele Frequency

BACKGROUND

Thyroid cancer develops via progressive accumulation of genetic alterations, which serve not only as diagnostic but also as predictive markers. Prognostic value of specific molecular alterations detected in thyroid cancers have been well-established for several types of alterations (1-5). For example, detection of certain isolated alterations, such as RAS, PAX8/PPARG, or BRAF K601E is associated with the low risk for disease recurrence. Finding of other isolated alterations, such as BRAF V600E mutation, confer an intermediate risk of recurrence and unfavorable disease outcome. Finally, a number of other alterations such as TERT promoter mutations, denotes a high risk of aggressiveness in thyroid cancers, particularly when they represent a “late hit” found in combination with early driver mutations such as RAS or BRAF V600E mutations. Other late driver mutations and markers of aggressive thyroid cancer are TP53, AKT1, and PIK3CA mutations. These alterations can be used cancer risk stratification and to guide patient management.

ThyroSeq v.3 CRC test includes all main genetic alterations reported as predictive markers for thyroid cancer and allows to stratify thyroid cancer as low risk, intermediate risk, and high risk for recurrence. The analytical validation of the test was previously reported (7). The algorithm for cancer risk stratification was validated and reported in a case-control study of 55 patients with differentiated thyroid cancer and distant metastases matched with patients with differentiated thyroid cancer without distant metastases after >5 years of follow-up (6). The study found that ThyroSeq v.3 CRC low-risk signature is associated with 0.2-0.4% probability of distant metastasis, intermediate-risk signature with a 5-9% probability, and high-risk signature with a 19-34% probability of distant metastasis.

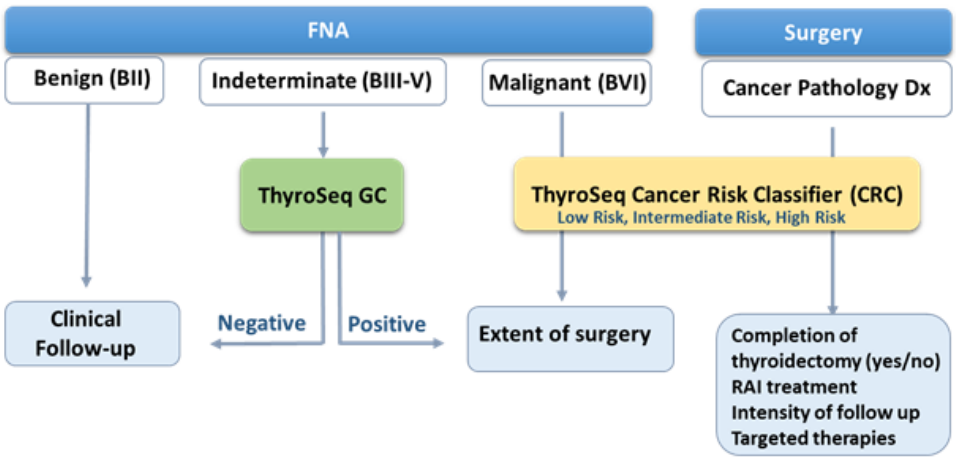
Based on the management guidelines from the American Thyroid Association (ATA) (7), thyroid cancer risk stratification is important for selecting the appropriate extent of surgery (lobectomy vs total thyroidectomy), radioactive iodine (RAI) treatment, and intensity of follow-up. Most thyroid cancers are indolent and these patients are at low risk for disease recurrence after cancer removal. These patients can be treated by lobectomy and are unlikely to benefit from RAI administration and TSH suppression (7-9). On the other hand, patients with high-risk cancers would benefit from up-front total thyroidectomy, which facilitates post-operative RAI administration and disease monitoring.

ThyroSeq CRC test application for thyroid cancer risk stratification may be considered for cytology samples with positive for malignancy (Bethesda VI) cytology and surgically removed cancer samples (6,8). In advanced forms of thyroid cancer, testing for mutations (eg. BRAF V600E) and gene fusions (eg. NTRK1,3) may additionally help with selection of targeted therapies (10-12).

References

1. Yip, L., et al. Ann Surg, 2015. 262(3): p. 519-25. 2. Song, Y.S., et al. Cancer, 2016. 122(9): p. 1370-9. 3. Labourier, E. et al. Diagn Cytopathol, 2021. 49(4): p. E175-e180. 4. Xing, M., et al. J Clin Oncol, 2015. 33(1): p. 42-50. 5. Yoo, S.K., et al. Nat Commun, 2019. 10(1): p. 2764. 6. Nikiforova, M , et al. Cancer, 2018; 7.Yip, L., et al. Cancer, 2021;127(11):1779-1787. 7. Haugen, B.R., et al. Thyroid, 2016. 26(1): p. 1-133. 8. Chin, P.D., et al. Endocr Pathol, 2020. 9. Hier, J., et al. J Otolaryngol Head Neck Surg, 2021. 50(1): p. 29. 10. Bible KC, et al. Nat Rev Clin Oncol. 2016;13:403-16; 11. Kummar S, et al. Target Oncol. 2018;13:545-556. 12. Bible, K.C., et al. Thyroid, 2021. 31(3): p. 337-386.

***Fig. 1.** Potential management of patients with thyroid cancer diagnosed on FNA (Bethesda VI) or surgically removed thyroid samples based on cancer risk prediction by ThyroSeq Cancer Risk Classifier (CRC).*



METHODOLOGY

Nucleic acids (DNA/mRNA) are isolated from thyroid FNA samples collected in the ThyroseqPreserve solution, from fixed cytology smears, or from FFPE 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 Cancer Risk Classifier (CRC) algorithm is used for data analysis. Test results are reported as Low Risk (low risk of cancer recurrence), Intermediate Risk (intermediate risk of cancer recurrence), or High Risk (high risk of cancer recurrence). Specimen adequacy, mutation type, gene expression and CNA profiles are reported in the Detailed Results section. 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
BRAF	NM_004333.4	chr7:140453136A>T

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 ThyroSeq / CBLPATH, INC.

Sample 1: 10 unstained and 1 stained slides received and labeled

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: