The challenges in the risk stratification of thyroid nodules and cancers: the role of molecular testing

BY MOHANNAD RAJAB AND RICK PAYNE

Around the world, molecular testing is becoming more widely used to personalise the management of thyroid nodules.

Thyroid nodules are relatively common. They are palpable in ~5% of the population, while high-resolution ultrasound (US) incidentally finds them in 19–68% of people. The likelihood of malignancy in these nodules is low, and distinguishing between benign and malignant nodules can pose a diagnostic challenge. Indeed, thyroidectomy (either partial or total) has served as the gold standard for confirming malignancy through histopathological examination. Unfortunately, up to 90% of patients undergoing such surgeries are found to have benign conditions, leading to unnecessary procedures. While thyroid surgery is generally well-tolerated, it carries notable risks and potential complications, and can also impose a significant financial burden [1].

Risk stratification challenges

US and fine needle aspiration (FNA) are considered the standard of care for assessing thyroid nodules. US is effective at identifying thyroid nodules with suspicious characteristics that necessitate further investigation through FNA. Cytological assessment is employed to offer risk stratification by accurately categorising 70–80% of thyroid nodules as benign or malignant. However, 20–30% of thyroid FNAs are labelled 'indeterminate for malignancy' (Figure 1). These nodules, also referred to as indeterminate thyroid nodules (ITNs), exhibit a broad spectrum of malignancy rates, from 5% to 75%. Besides cytological results, a mix of clinical and radiological findings is advocated by the American Thyroid Association (ATA) to determine the extent of surgery in low-risk thyroid cancers, which has been proven inaccurate by various studies. Despite these risk stratification techniques, the uncertainty regarding the malignancy risk and aggressiveness of thyroid nodules and malignancies continue to hinder the optimal treatment of these patients [1].

Molecular markers

The main theory of thyroid carcinogenesis involves the transformation of follicular cells into either differentiated or undifferentiated thyroid cancer, proceeding through a sequence of steps that include various genetic alterations. Driver alterations are usually responsible for initiating the cancer process, and secondary or promoter alterations drive progression from differentiated to undifferentiated cancer. These molecular changes are linked with unique gene expression and signaling pathways, resulting in diverse clinical features, histophenotypes and prognoses (Figure 2). Over recent decades, the molecular pathogenesis of many different types of thyroid cancer have been extensively studied. This concept has been effectively utilised by various commercial molecular tests to deliver valuable diagnostic, prognostic and therapeutic insights that influence the management and decision-making processes for patients with thyroid nodules and cancers [2].

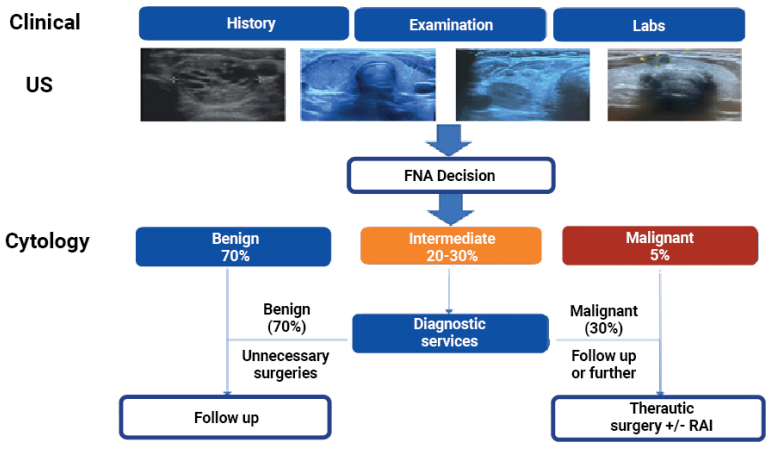


Figure 1: The decision-making scheme depending on the current standard of care method for the evaluation of thyroid nodules.

Molecular testing

Advances in thyroid tumour molecular characterisation have led to the creation of multiple commercial molecular tests that enhance cytology and US in improving risk stratification and decision-making for thyroid nodules and cancers. Molecular tests are classified as either rule-in or rule-out tests based on their ability to confirm or exclude malignancy. To rule out malignancy effectively, a test requires high sensitivity and a high negative predictive value (NPV). On the other hand, to confirm malignancy, high specificity and a high positive predictive value (PPV) are necessary. In a population with a cancer prevalence among ITNs of 20–40%, it is estimated that an NPV of 94% and a sensitivity of 90% are needed to rule out malignancy, while a specificity of 80% and a PPV of 60% are required to rule in malignancy [2]. Consequently, a rule-in test is more effective in scenarios with a higher prevalence of malignancy, aiding in refining surgical management, identifying more aggressive cancers, and predicting prognosis and outcomes. Conversely, a rule-out test is more beneficial when the prevalence of malignancy is low,

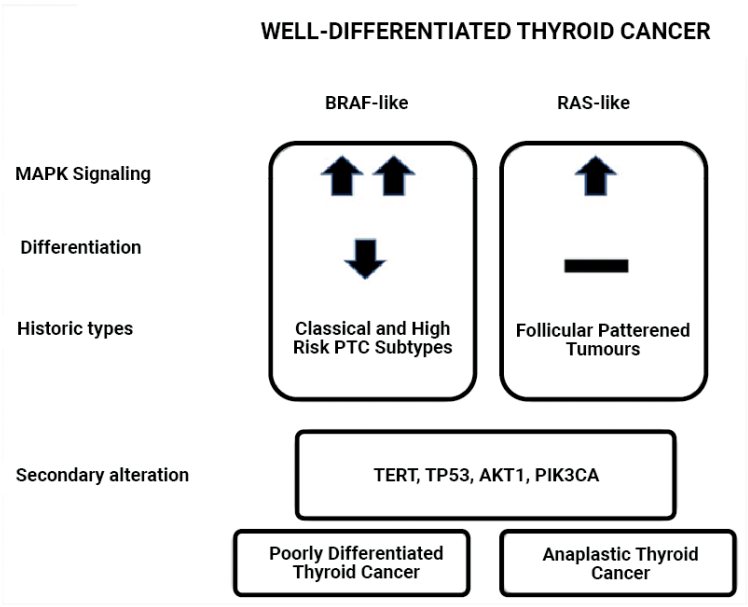
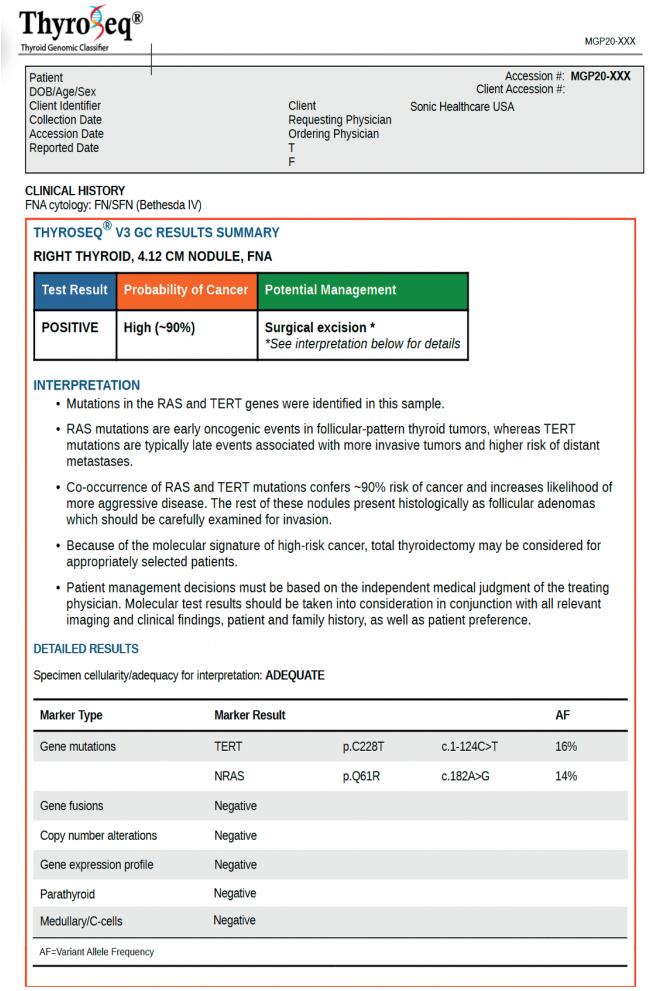


Figure 2: The progression of some thyroid tumours with BRAF-like and RAS-like molecular alterations.

helping to prevent diagnostic surgeries for benign thyroid nodules. Currently, multiple MTs can detect specific thyroid-related molecular alterations, further contributing to more personalised decision-making and management of thyroid nodules and cancers. Generally, genotyping-based tests for thyroid FNAs do not yield a simple 'negative' or 'positive' result. Rather, these tests present a spectrum of cancer likelihood (including insights on tumour type and prognosis) based on the type, number and allelic frequency of identified molecular alterations (Figure 3). The clearly defined relationships between a tumour's molecular profile and its histophenotype and prognosis allow for the classification of tumours into low-, intermediate-, and high-molecular risk groups (MRG). These classifications can then serve as a supplement to clinical, ultrasound and cytological data to enable more precise management decisions [2].

The role of molecular testing

Molecular testing initially aimed to exclude malignancies in ITNs and reduce unnecessary diagnostic surgeries. Validation studies of all major commercial molecular tests achieved satisfactory outcomes with a benign call rate (BCR) between 41% and 61% in contexts where the prevalence of thyroid cancer among ITNs is 20–40% [1]. External validation



ThyroSeq®
Thyroid Genomic Classifier

MGP20-XXX

Patient		Accession #:	MGP20-XXX
DOB/Age/Sex		Client Accession #:	
Client Identifier			
Collection Date	Client	Sonic Healthcare USA	
Accession Date	Requesting Physician		
Reported Date	Ordering Physician		
	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 *** **See interpretation below for details*

INTERPRETATION

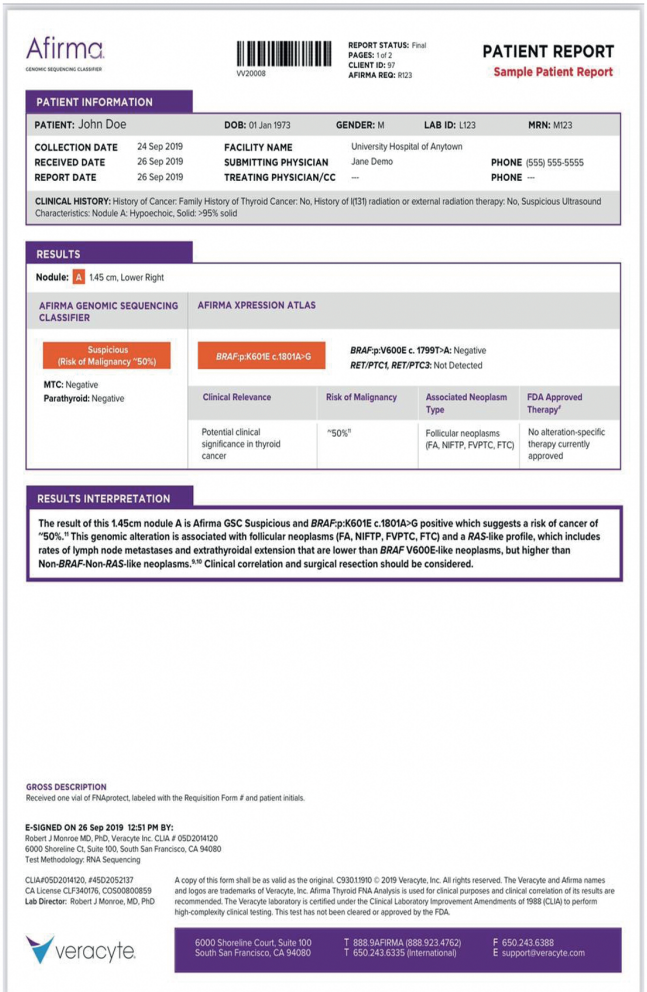
- Mutations in the RAS and TERT genes were identified in this sample.
- RAS mutations are early oncogenic events in follicular-pattern thyroid tumors, whereas TERT mutations are typically late events associated with more invasive tumors and higher risk of distant metastases.
- Co-occurrence of RAS and 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



Afirma
GENOMIC SEQUENCING CLASSIFIER

VV20008

REPORT STATUS: Final
PAGES: 1 of 2
CLIENT ID: 97
AFIRMA REQ: R123

PATIENT REPORT
Sample Patient Report

PATIENT INFORMATION

PATIENT: John Doe		DOB: 01 Jan 1973		GENDER: M	LAB ID: L123	MRN: M123	
COLLECTION DATE	24 Sep 2019	FACILITY NAME	University Hospital of Anytown				
RECEIVED DATE	26 Sep 2019	SUBMITTING PHYSICIAN	Jane Demo			PHONE	(555) 555-5555
REPORT DATE	26 Sep 2019	TREATING PHYSICIAN/CC	---			PHONE	---

CLINICAL HISTORY: History of Cancer: Family History of Thyroid Cancer: No, History of I(131) radiation or external radiation therapy: No, Suspicious Ultrasound Characteristics: Nodule A: Hypoechoic, Solid: >95% solid

RESULTS

Nodule: A 1.45 cm, Lower Right

AFIRMA GENOMIC SEQUENCING CLASSIFIER

Suspicious (Risk of Malignancy ~50%)

MTC: Negative
Parathyroid: Negative

AFIRMA XPRESSION ATLAS

BRAF:p.K601E c.1801A>G

BRAF:p.V600E c. 1799T>A: Negative
RET/PTC1, RET/PTC3: Not Detected

Clinical Relevance	Risk of Malignancy	Associated Neoplasm Type	FDA Approved Therapy[2]
Potential clinical significance in thyroid cancer	~50%[1]	Follicular neoplasms (FA, NIFTP, FVPTC, FTC)	No alteration-specific therapy currently approved

RESULTS INTERPRETATION

The result of this 1.45cm nodule A is Afirma GSC Suspicious and *BRAF*:p.K601E c.1801A>G positive which suggests a risk of cancer of ~50%.[1] This genomic alteration is associated with follicular neoplasms (FA, NIFTP, FVPTC, FTC) and a *RAS*-like profile, which includes rates of lymph node metastases and extrathyroidal extension that are lower than *BRAF* V600E-like neoplasms, but higher than Non-*BRAF*-Non-*RAS*-like neoplasms.[8,10] Clinical correlation and surgical resection should be considered.

GROSS DESCRIPTION
Received one vial of FNAprotect, labeled with the Requisition Form # and patient initials.

E-SIGNED ON 26 Sep 2019 12:51 PM BY:
Robert J Monroe MD, PhD, Veracyte Inc. CLIA # 05D2014120
6000 Shoreline Ct, Suite 100, South San Francisco, CA 94080
Test Methodology: RNA Sequencing

CLIA#05D2014120, #45D2052137
CA License CLF340176, CO500800859
Lab Director: Robert J Monroe, MD, PhD

veracyte

6000 Shoreline Court, Suite 100
South San Francisco, CA 94080

T 888.9AFIRMA (888.923.4762)
T 650.243.6335 (International)

F 650.243.6388
E support@veracyte.com

Figure 3: Example of reports from different commercial molecular tests.

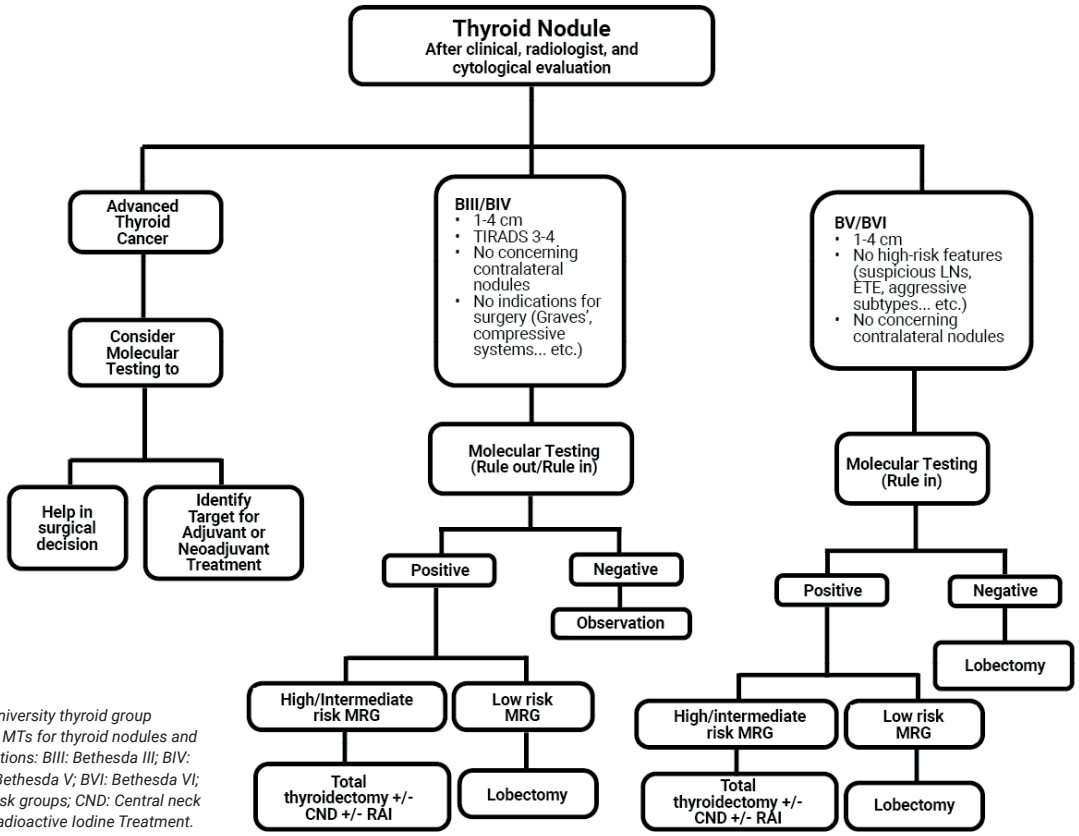


Figure 4: McGill University thyroid group protocol for using MTs for thyroid nodules and cancers. Abbreviations: BIII: Bethesda III; BIV: Bethesda IV; BV: Bethesda V; BVI: Bethesda VI; MRG: molecular risk groups; CND: Central neck dissection; RAI: Radioactive Iodine Treatment.

and clinical experience studies provided similar BCR ranges, demonstrating the capacity of molecular tests to prevent many patients with ITNs from undergoing needless diagnostic surgery [3]. Ongoing improvements in molecular testing sequencing and classifier systems have enhanced its specificity and positive predictive value (PPV), leading to an expanded recommended application to detect specific thyroid-related molecular changes. Various studies have linked certain mutations with a higher risk of aggressive features in histopathologically confirmed cancers, while another study found a significant correlation between molecular risk groups (MRGs) and the American Thyroid Association (ATA) Risk Stratification System in assessing recurrence risk [2,4]. This suggests that preoperative molecular tests are valuable not only for avoiding unnecessary surgery but also to help guide the extent of the surgery. Molecular tests also aid in identifying actionable molecular alterations in advanced thyroid cancers (Figure 4). Additionally, molecular testing has the potential to guide the decision for other management options, including active surveillance and minimally invasive procedures.

Conclusion

Molecular testing is a significant adjunct to clinical, sonographic and cytologic findings for risk stratification of thyroid nodules and cancers, and has significant potential for improving management decision-making and clinical outcomes when incorporated into routine clinical settings. Numerous studies have shown its efficacy in reducing unnecessary surgeries for ITNs and its ability to influence extent of surgery. However, additional research is needed to monitor long-term outcomes for patients with negative molecular results and to assess the impact of various management strategies (including active surveillance, minimally invasive procedures and diverse surgical options) on patients with different MRGs.

References

1. Rajab M, Payne RJ, Forest VI, Pusztaszeri M. Molecular Testing for Thyroid Nodules: The Experience at McGill University Teaching Hospitals in Canada. *Cancers* (Basel) 2022;**14(17)**:4140.
2. Pusztaszeri M, Rajab M, Payne RJ. Molecular Testing for Thyroid Nodules: The Experience at McGill University Teaching Hospitals in Canada. In: Kakudo K, Liu Z, Jung CK, et al (Eds.). *Thyroid FNA Cytology*. Singapore; Springer; 2023
3. Patel J, Klopper J, Cottrill EE. Molecular diagnostics in the evaluation of thyroid nodules: Current use and prospective opportunities. *Front Endocrinol* (Lausanne) 2023;**14**:1101410.
4. Liu JB, Ramonell KM, Carty SE, et al. Association of comprehensive thyroid cancer molecular profiling with tumor phenotype and cancer-specific outcomes. *Surgery* 2023;**173(1)**:252–9.

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Declaration of competing interests:
None declared.