Extending Expressed RNA Genomics From Surgical Decision Making for Cytologically Indeterminate Thyroid Nodules to Targeting Therapies for Metastatic Thyroid Cancer

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INTRODUCTION

Challenges in the management of thyroid nodules and cancers include: 1) differentiating benign from malignant thyroid disease when cytopathology is indeterminate; 2) defining the extent of initial thyroid surgery; and 3) identifying targeted treatments for patients with thyroid cancers that are refractory to standard treatment.

For patients with thyroid nodules, clinical findings and serology (thyroid-stimulating hormone with or without calcitonin) are rarely sufficient to exclude malignancy. Although noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP) are considered to have a low risk of malignant behavior after surgical excision, we group them with malignant nodules to indicate their recommended surgical treatment in contrast to benign nodules.[1] Neck ultrasound and thyroid fine-needle aspiration biopsy (FNAB) to acquire samples for cytological assessment are required for most nodules measuring >1.0 to 1.5 cm to differentiate the benign majority from the malignant minority. Various ultrasound scoring systems define features that identify approximately 17% to 53% of nodules as being reliably benign,[2] but for the remainder that demonstrate imaging characteristics associated with a cancer risk of ≥5%, FNAB typically is performed. Definitive benign cytological findings (Bethesda category II) are found in approximately 60% to 75% of nodules and findings of suspicious for malignancy (Bethesda category V) or malignant (Bethesda category VI) are found in <10%,[3,4] but at least 15% of nodules are deemed cytologically indeterminate, portending a risk of malignancy/NIFTP of 11% to 29% based on surgical pathology.[3] Consequently, even with ultrasound and cytological examination, the character of thyroid nodules remains uncertain in approximately 1 in 7 thyroid patients. When a cytologically indeterminate nodule has been identified by an expert cytopathologist, doubt exists that the risk of cancer can be sufficiently reduced to avoid surgery through repeat biopsy,[5-9] review by a second cytopathologist,[10,11] further subclassification of the indeterminate category,[12] or correlation with thyroid nodule ultrasound classification.[12-16]

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Historically, the majority of patients with cytologically indeterminate thyroid nodules were advised to undergo surgical resection given their cancer risk. However, approximately 80% of these operated nodules proved to be histologically benign.[17,18] Consequently, most of these surgeries generated unnecessary direct and indirect medical costs, patient anxiety and diminished productivity during recovery, and complications. Although high-volume thyroid surgeons report relatively low surgical complication rates of 1% to 3%, approximately 26% to 81% of patients undergo surgery with low-volume surgeons whose complication rates generally are much higher,[19-22] including hypothyroidism,[23] clinically severe hypocalcemia/hypoparathyroidism,[24,25] recurrent laryngeal nerve injury,[26,27] and, less commonly, infection and bleeding.[23,28-30] Higher surgical complication rates have been reported among the elderly,[31,32] who are heavily represented in the thyroid nodule population. These findings highlight the burden of diagnostic surgery among patients with cytologically indeterminate nodules and the need for change.

Gene Expression Classifier Development, Validation, and Real-World Performance

The traditional practice of observing cytologically benign nodules, despite recognition that approximately 1% to 5% were malignant,[33] helped to set the acceptable threshold for a rule-out test to avoid diagnostic surgery at a negative predictive value (NPV) of 95% (ie, a cancer risk of 5% when the test was negative). The first rule-out test to meet this threshold was the Afirma Gene Expression Classifier (GEC) (Veracyte, South San Francisco, California). The GEC quantified messenger RNA (mRNA) expression among 167 genes for the main benign versus suspicious classifier, and 6 specialized mRNA expression cassettes, including one designed to strictly limit false-negative results among patients with Hurthle neoplasms, and others for the specific identification of medullary thyroid carcinoma, parathyroid tissue, renal cell carcinoma, breast cancer, and malignant melanoma.

Promising results from a small GEC validation study[34] led to classifier finalization and clinical validation in a pivotal 49-center prospective and blinded study. The performance of GEC testing in 210 nodules categorized as atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS; Bethesda category III) or suspicious for follicular neoplasm (SFN; Bethesda category IV), all of which subsequently had definitive surgical pathology diagnoses, demonstrated a test sensitivity of 90%, specificity of 52%, NPV of 94%, and positive predictive value (PPV) of 37% at a cancer prevalence of 24%.[18] Subsequently, 28 real-world clinical experience studies have cumulatively reported that only 13% of nodules with GEC benign results underwent surgical resection,[6,7,35-59] a marked reduction compared with the historical treatment of patients with cytologically indeterminate thyroid nodules.[17,60] In 26 of these studies, only 3% of nodules (50 of 1934 nodules) with GEC benign results were found to be malignant.[6,7,18,35,38-42,44,45,47-54,56-59,61] Investigators who assessed GEC test performance only in operated cases with surgical histology systematically underestimated test specificity and NPV by excluding many unoperated GEC benign nodules that were very likely true-negative results.[62] The broader clinical impact of GEC testing can be concluded from the observation that among the first 90,140 consecutive adequate nodule samples analyzed, approximately 44% received a benign result.[63] In addition, the routine collection of material for molecular testing at the time of the initial FNAB may avoid the need for repeat biopsy along with its inconvenience and discomfort for the patient and additional health care costs.[64]

Genomic Sequencing Classifier Development, Validation, and Real-World Performance

Advances in genomic analysis and machine-learning techniques presented the opportunity to migrate Afirma to a more comprehensive whole RNA transcriptome sequencing platform and to enhance the test's performance (Fig. 1A). The Afirma Genomic Sequencing Classifier (GSC) interrogates >10,000 nuclear and mitochondrial genes for the measurement of gene expression counts, sequence variants, and changes in genomic copy number, including loss of heterozygosity.[65] GSC architecture includes a set of initial classifiers to identify parathyroid tissue, medullary thyroid cancer, and oncogenic mutations strongly linked to papillary thyroid cancer, *BRAF* V600E variant, and *RET/PTC1* and *RET/PTC3* fusions. Samples with negative results in these initial classifiers then are tested with a follicular cell content adequacy classifier in anticipation of the subsequent benign versus suspicious categorization by the core GSC classifier.

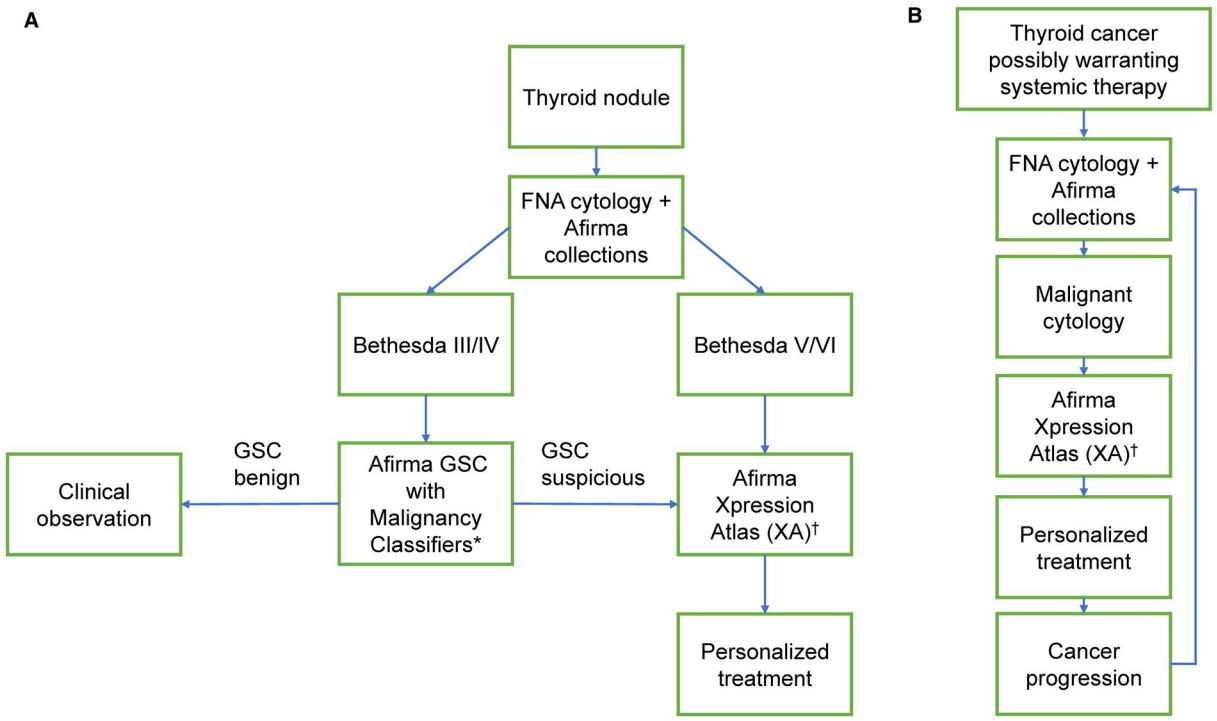

Figure 1. Clinical use flow diagram of the Afirma Genomic Sequencing Classifier (GSC) and Xpression Atlas (XA) in (A) thyroid nodules and (B) thyroid cancer warranting systemic therapy. *Malignancy classifiers include the medullary thyroid cancer classifier, *BRAF* V600E classifier, parathyroid classifier, and *RET/PTC1* plus *RET/PTC3* fusion detection. †Malignancy classifiers are included with XA. FNA indicates fine-needle aspiration.

Two additional specialized classifiers coordinate with the core GSC classifier to preserve high test sensitivity and improve test specificity among Hurthle cell–dominant samples. First, all adequate samples are tested with the Hurthle cell index (HI) classifier to detect molecularly those samples with Hurthle cell features. HI-negative samples are assessed by the core GSC classifier, whereas HI-positive samples are subjected to further analysis. To permit the application of distinct criteria for benignancy to nonneoplastic and neoplastic Hurthle cell–dominant FNAB samples, HI-positive samples are evaluated further with a Hurthle cell neoplasm index (NI) classifier to identify samples that are neoplastic. NI-positive samples (ie, neoplastic samples) are scored by the core GSC classifier using the same threshold as that for HI-negative samples. However, NI-negative samples (ie, those deemed nonneoplastic) are scored by the core GSC classifier using a less stringent cutoff value to permit more of these samples to be accurately characterized as GSC benign.[66]

A total of 634 FNAB samples were used to build the GSC core ensemble model, consisting of 12 independent classifiers.[65] To minimize overfitting and accurately reflect classifier performance incorporating random noise, hyperparameter tuning and model selections were performed using repeated nested cross-validation.[67] Hyperparameter tuning was performed within the inner layer of the cross-validation, and the classifier performance was summarized using the outer layer of the 5-fold cross-validation repeated 40 times. For each classifier, the decision boundary was chosen to optimize specificity with a minimum requirement of 90% sensitivity to detect malignancy.

The locked Afirma GSC system was validated using independent FNAB samples with sufficient remaining RNA from the pivotal GEC validation study (among patients aged ≥21 years and with nodules measuring ≥1 cm).[18] This cohort was unbiased by the current widespread use of molecular testing to avoid surgery and allowed for a direct comparison of the GSC with its predecessor, the GEC. The use of a locked, multicenter, blinded, and prospectively collected cohort enrolled prior to FNAB fulfills the key goal sought in an ideal prospective validation design: the minimization of bias. This validation strategy should not be diminished by equating it with a retrospective study design. Clinical validation

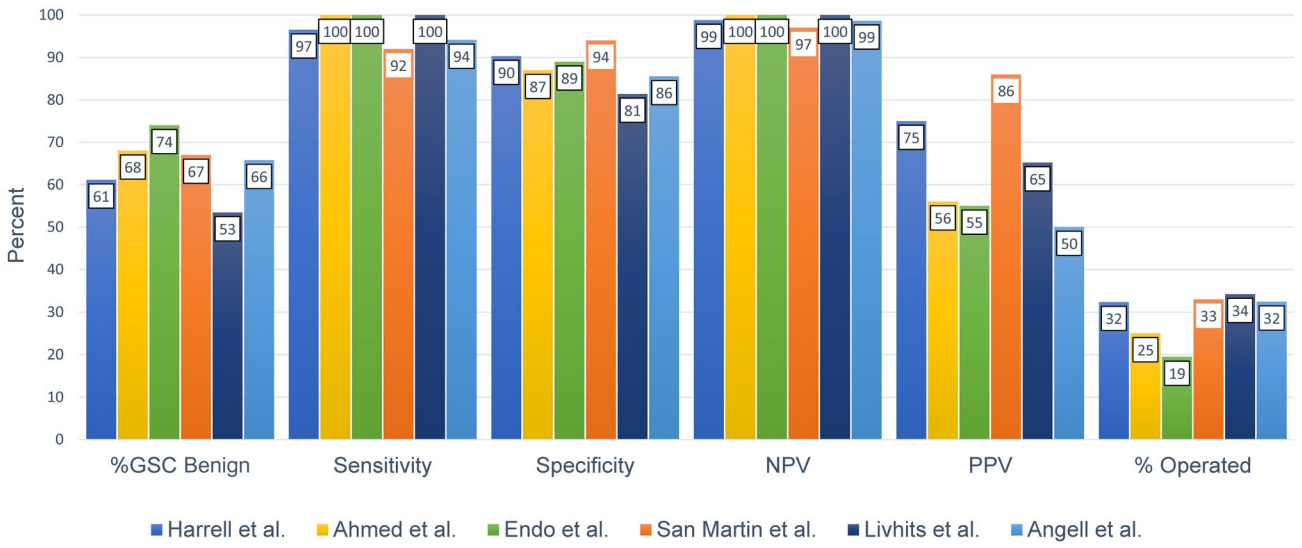

Figure 2. Clinical experience with the Afirma Genomic Sequencing Classifier (GSC) from multiple centers. Unoperated GSC benign nodules were counted as true-negative results. Unoperated GSC suspicious nodules were excluded. Data were obtained from Harrell et al,[59] Ahmed et al,[68] Endo et al,[69] San Martin et al,[70] Livhits et al,[71] and Angell et al.[72]

for the GSC in 190 AUS/FLUS and SFN nodules with blinded consensus surgical histology diagnoses demonstrated 91% sensitivity, 68% specificity, 47% PPV, and 96% NPV in a cohort with a 24% cancer prevalence.[65] Among the subgroup of Hurthle cell histologies, specificity was markedly improved from 12% with GEC to 59% with GSC, and sensitivity was maintained at 89%.[65] Overall, these pivotal GSC clinical validation data predicted an increase in how often a benign result occurs, an NPV of ≥95% in the majority of clinical settings, decreased diagnostic surgery, and an increased rate of cancer among nodules with GSC suspicious results.[65]

The GSC entered routine clinical use in July 2017. Similar to the GEC, GSC results among patients aged <21 years or from nodules measuring <1 cm are provided; however, they are notated as outside of indication because to the best of our knowledge test performance among such samples has not been established. To our knowledge to date, 6 independent studies have been reported at national conferences or have been published (Fig. 2).[59,68-72] These real-world experiences demonstrate that approximately two-thirds of test results are classified as GSC benign, approximately two-thirds of the GSC suspicious nodules are proven malignant or NIFTP, and two-thirds of all tested patients go on to clinical observation in lieu of diagnostic surgery. Using surgical histology when available and otherwise assuming that unoperated GSC benign nodules are truly benign, these 6 experiences demonstrate an actual GSC upper limit of NPV as 97% to 100%. Two centers have reported their experience among Hurthle cell–dominant AUS/FLUS and SFN nodules.[59,72] Whereas historically approximately 1 of 5 GEC tests returned as benign, the GSC benign rate increased to 2 of 3 tests. This markedly improved benign call rate extends the cost-effectiveness of GSC to Hurthle cell–dominant cytologically indeterminate specimens.[42]

Beyond the GSC: Xpression Atlas Development, Validation, and Real-World Performance

A challenge for gene panel testing strategies for cytologically indeterminate thyroid nodules has been that to achieve high sensitivity and high NPV, many variants with suboptimal specificity must be included. Consequently, relatively few specimens result as negative, and the number of specimens identified with high PPV variants is low.[73,74] Nevertheless, decision making may be different among the few patients whose thyroid nodule genotype predicts a cancer risk >95% compared with when it is just 50%.

The emerging evidence of correlation between genomic variants and neoplasm histology, behavior, predicted clinical course, and therapeutic options has generated interest in increasingly larger genomic panels.[74-78] To provide this information for GSC suspicious AUS/FLUS and SFN nodules, when cancer or NIFTP is not ruled out with high NPV, the Afirma

Xpression Atlas (XA) can be requested to report findings from the transcriptome across 511 genes, including 761 variants and 130 fusion pairs (Fig. 1A). The malignancy classifiers plus XA also can assess FNAB specimens from nodules classified as suspicious for malignancy and malignant and metastases without initial GSC testing (Fig. 1A). Nonexpressed variants are not measured by transcriptional sequencing. However, because the transcriptome reflects the nodule's active genomic activity, its signaling pathways, and their interactions with environmental signals, it has hypothetical advantages over DNA-based genomic findings that may occur in transcriptionally silent genes but not impact nodule biology.

The potential usefulness of genomic insights from XA may be proven to include consideration of initial surgical treatment options based on a variant's risk of malignancy and metastatic potential.[75,78] However, for neoplasms clinically confined to the thyroid, to our knowledge data are lacking to demonstrate improved clinical outcomes based on the extent of surgery because randomized trials based on mutational status have not been performed.

Beyond initial surgical treatment decisions, XA may help to guide treatment decisions for patients with thyroid cancer that is refractory to standard treatment options, with sample collection performed via FNAB rather than surgical resection (Fig. 1B). In addition, subsequent FNAB assessment may prove helpful when disease sites break through the current treatment (Fig. 1B). For example, the combination of dabrafenib plus trametinib recently was approved by the US Food and Drug Administration for *BRAF* V600E–mutated anaplastic thyroid cancer, and larotrectinib recently was approved for refractory solid tumors harboring a neurotrophic receptor tyrosine kinase (*NTRK*) gene fusion (without a known acquired resistance mutation) regardless of the cancer type (tissue agnostic). Beyond these approved drugs, multiple other recent clinical trials have investigated therapies for specific targets relevant for thyroid cancer and reported with XA, including *BRAF, NTRK, EGFR, RET, MET, ROS1, ALK, PAX8/PPARG*, and *HRAS*. Initial data related to 2 compounds targeting RET alterations have demonstrated highly encouraging treatment activities.[79,80] The assessment of variants for targeted therapy from a large genomic panel allows for the simultaneous evaluation of multiple targetable genomic changes, many of which are rare.

Summary

Thyroid nodule cytopathology plays a critical role in thyroid nodule management. The Afirma GSC, which uses genomic data from >10,000 genes used in an ensemble of complex machine-learned algorithms, extends the usefulness of cytopathology among cytologically indeterminate samples by returning a GSC benign result in approximately two-thirds of all tested samples (Fig. 2). The GSC was rigorously developed and independently validated to address the broad spectrum of thyroid pathology encountered in both the laboratory and real-world practice settings. The GSC is a malignancy/NIFTP rule-out test whose highly accurate benign result allows clinicians to recommend clinical observation over diagnostic surgery (Fig. 1A). Conversely, a suspicious GSC result, with an increased risk of malignancy over the FNAB result alone, may give a patient greater reassurance in proceeding with surgery. To complement the GSC, the XA provides genomic insights from a curated panel of 511 genes. Emerging data have suggested that XA may provide insights regarding tumor histology and behavior and the potential effectiveness of targeted therapy options among patients with GSC suspicious AUS/FLUS and SFN nodules, nodules that are suspicious for malignancy and malignant, and thyroid cancer metastases (Figs. 1A and 1B). Routine collection of the Afirma sample as part of all thyroid FNAB procedures streamlines workflows and the patient experience to expedite informed patient management decisions from the time of the first FNAB. Afirma facilitates personalized treatment decisions based on genomic insights derived from the transcriptome of the biopsied target and extends the diagnostic and therapeutic reach of cytopathologists and FNAB sample collection.

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Peter M. Sadow has acted as a pathology case reviews consultant for Veracyte Inc for work performed outside of the current study. Giulia C. Kennedy and Richard T. Kloos are employees of and equity owners in Veracyte Inc, which developed and sells the molecular tests used in the current study. Paul W. Ladenson has acted as a paid consultant for and received speaker's honoraria from Veracyte Inc for work performed outside of the current study. The other authors made no disclosures.

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