Molecular testing of thyroid nodules, practical considerations

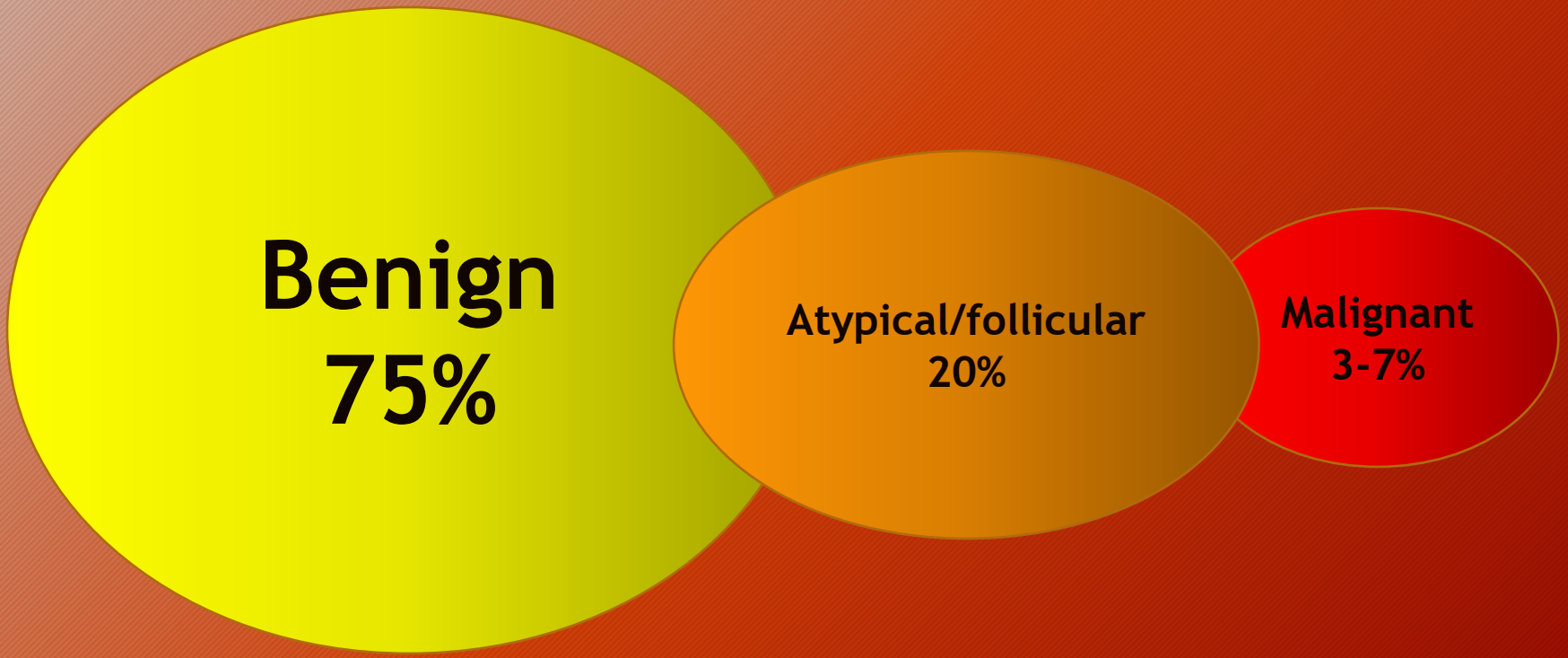
Víctor J. Carlo Chévere, MD, FCAP, ECNU

Chief of Cytology Service & FNA Clinic

Puerto Rico Pathology

No disclosures

Thyroid Aspiration Cytology



Malignancy risk,… and NIFTP

TABLE 2. THE 2017 BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLOGY: IMPLIED RISK OF MALIGNANCY AND RECOMMENDED CLINICAL MANAGEMENT

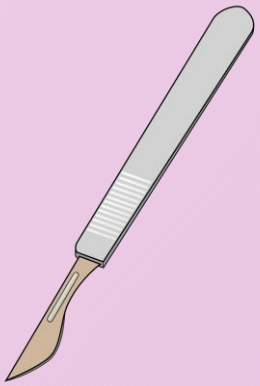
Diagnostic category	*Risk of malignancy if NIFTP ≠ CA (%)*	*Risk of malignancy if NIFTP = CA (%)*	*Usual management*[a]
Nondiagnostic or unsatisfactory	5–10	5–10	Repeat FNA with ultrasound guidance
Benign	0–3	0–3	Clinical and sonographic follow-up
Atypia of undetermined significance or follicular lesion of undetermined significance	6–18	~10–30	Repeat FNA, molecular testing, or lobectomy
Follicular neoplasm or suspicious for a follicular neoplasm	10–40	25–40	Molecular testing, lobectomy
Suspicious for malignancy	45–60	50–75	Near-total thyroidectomy or lobectomy[b,c]
Malignant	94–96	97–99	Near-total thyroidectomy or lobectomy[c]

Adapted with permision from Ali and Cibas (7).
[a]Actual management may depend on other factors (e.g., clinical, sonographic) besides the FNA interpretation.
[b]Some studies have recommended molecular analysis to assess the type of surgical procedure (lobectomy vs. total thyroidectomy).
[c]In the case of "suspicious for metastatic tumor" or a "malignant" interpretation indicating metastatic tumor rather than a primary thyroid malignancy, surgery may not be indicated.
NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features; CA, carcinoma; FNA, fine-needle aspiration.

Cibas, Ali. Thyroid 2017.

What should we do?

Risk of malignancy high enough to do something

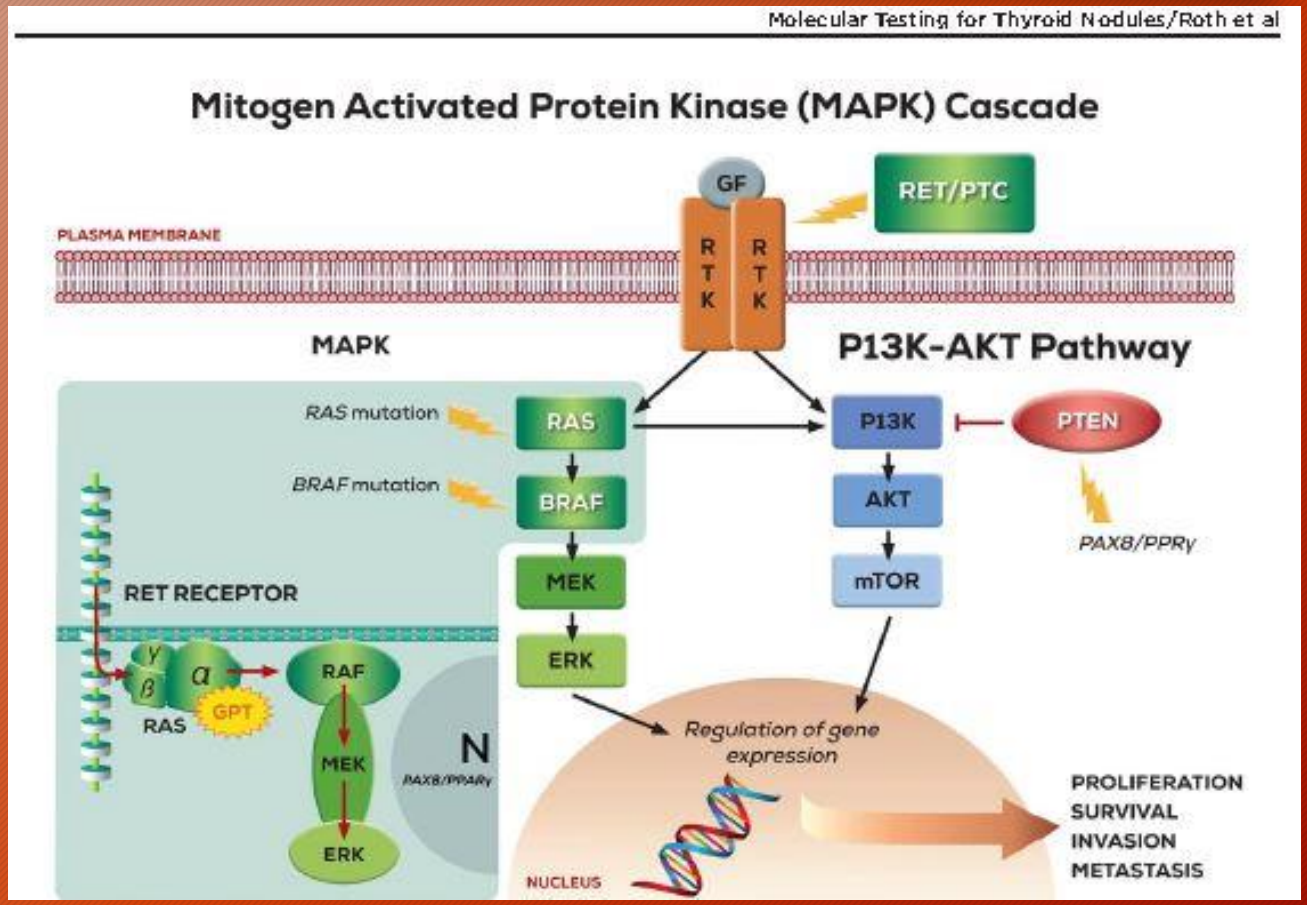


Risk of malignancy low enough to be concerned about overtreatment

So far, follow up, repeat biopsy

- Up to 50% will have a negative repeat biopsy
  - Increased risk even if negative in repeat biopsy? (VanderLaan, 2011; Renshaw 2010)
- Repeated indeterminate biopsy up to 27% risk of malignancy (Faquin, 2009)

Cancer is a molecular disease



Roth et al. 2018

First idea

- Let's test for mutations and see if we can identify the cancers in those indeterminate biopsies so we can identify those who need surgery (rule in approach)
- First panels based in 7 genes testing

Molecular findings in thyroid cancer

- Papillary CA: BRAF (45%), RET/PTC (20%), RAS (10%)
- Follicular CA: RAS (40%), PAX8/PPARG1 (30%)
- Medullary CA: RET (95% familial, 50% sporadic)
- But:
  - Mutations may be present in benign nodules (RAS)
  - Mutations may not be identified in malignant nodules
- Result: Mutations were not detected in most nodules, many cancers were missed by this approach and not all detected mutations led to a final cancer diagnosis

Hassell L., Gillies E., Terence S. *Cytologic and Molecular Diagnosis of Thyroid Cancers.* Cancer Cytopathology, 2011.

Second idea

- Let's test for mutations and see if we can exclude cancer in those indeterminate biopsies and identify those who do not need surgery (rule out approach)

And then the market race

- Tests with high specificity and PPV worked to improve sensitivity and NPV
- Tests with high sensitivity and NPV worked to improve specificity and PPV

High PPV test (rule in) + High NPV test (rule out)

=

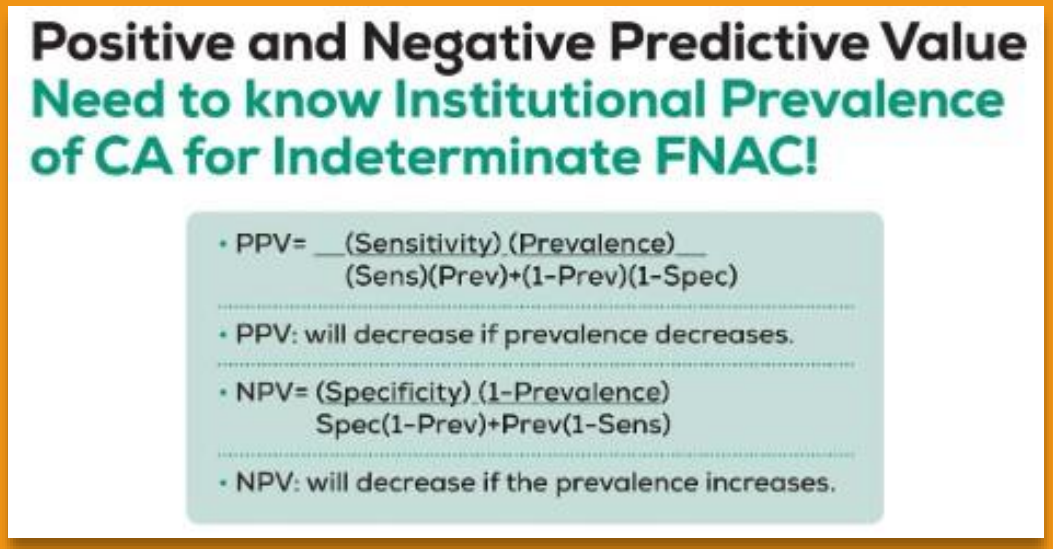
Today's market

Molecular testing in 2021

- To stratify risk of malignancy
  - Molecular testing in in category III & IV biopsies
- To tailor the surgical procedure
  - Categories V & VI
- To predict risk of progression
  - Select patients to treat vs patients to monitor in small thyroid tumors in selected patients

Stratifying risk in indeterminates

- I want to use a test to identify those who have a disease from those who don't in a given population
- Sensitivity & specificity are constant, but predictive values depend on prevalence



Roth, et al; 2018.

Example

- The categories III & IV in lab A have a risk of malignancy of 16%
- The categories III & IV in lab B have a risk of malignancy of 38%
- Therefore, in a group of 100 cases:
  - Lab A will have 16 carcinomas
  - Lab B will have 38 carcinomas

Example

- If both labs use the same test, with 91% sensitivity and 68% specificity
  - Lab A will get a positive result in 15 of the 16 patients, with 1 false negative
  - Lab B will get a positive result in 35 of the 38 patients, with 3 false negatives

Lab A			
	Pos	Neg	Total
Cancer	15	1	16
No cancer	40	44	84

Lab B			
	Pos	Neg	Total
Cancer	35	3	38
No cancer	30	32	62

Example

NPV = TN / (TN + FN)

In Lab A: NPV = 44 / (44 + 1) = 98%

In Lab B: NPV = 32 / (32 + 3) = 91%

Same test will have different NPV in populations with different prevalence!

Afirma

- The idea: *With a very high negative predictive value, the chance of malignancy in a negative case is so low that surgery can be avoided*
- New version: Genomic Sequencing Classifier
  - Uses NGS, RNA test
  - Interrogates > 10,000 genes (nuclear and mitochondrial)
  - Special tests for Hürthle cells, medullary CA, parathyroid, and metastatic lesions
  - Analysis performed by algorithms
  - Validated with the same specimens than first version

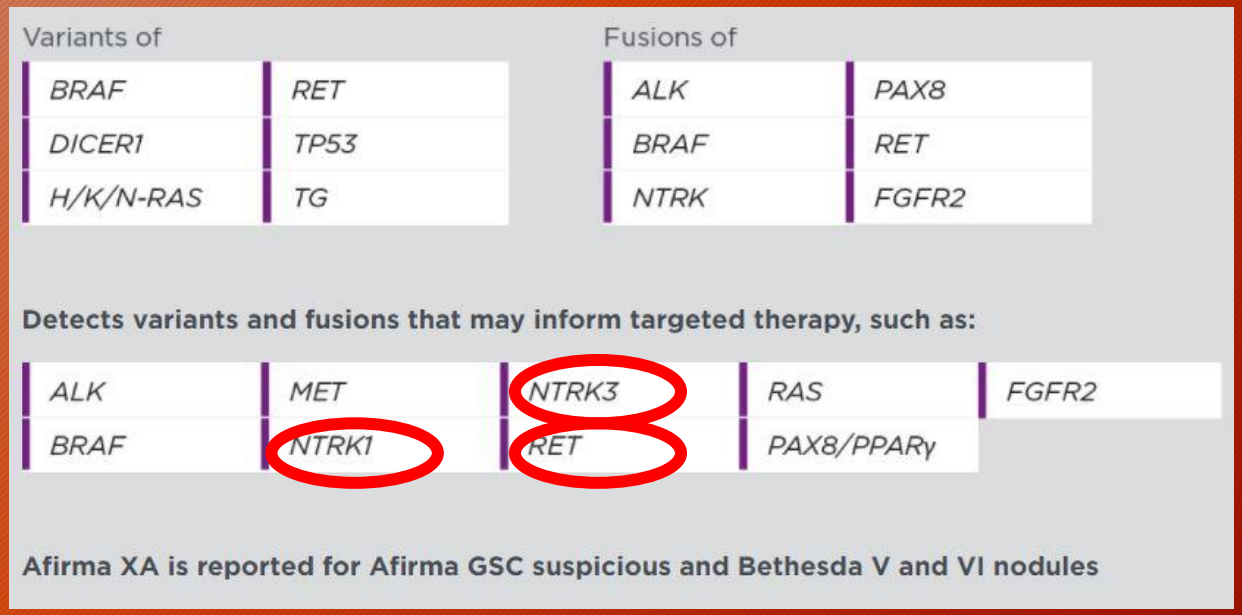
Afirma

- Valuidated in multicenter, retrospective double-blind study with 191 samples; molecular result not considered for surgery
- Alleges 96% NPV, 47% PPV, 91% Sensitivity, 68% Specificity, with 66% Benign call rate (NIFTP not included in final dx. Patel, et al. JAMA. 2018).
- Later studies reporting 76%* benign call rate, 60% PPV*, 94% specificity (Endo, et al; 2019)
- Benign call rate in 2/3 Hürthle cell lesions with 89% sensitivity

* Really? These higher numbers also reported with Thyroseq in later studies (Ohori et al; 2019)

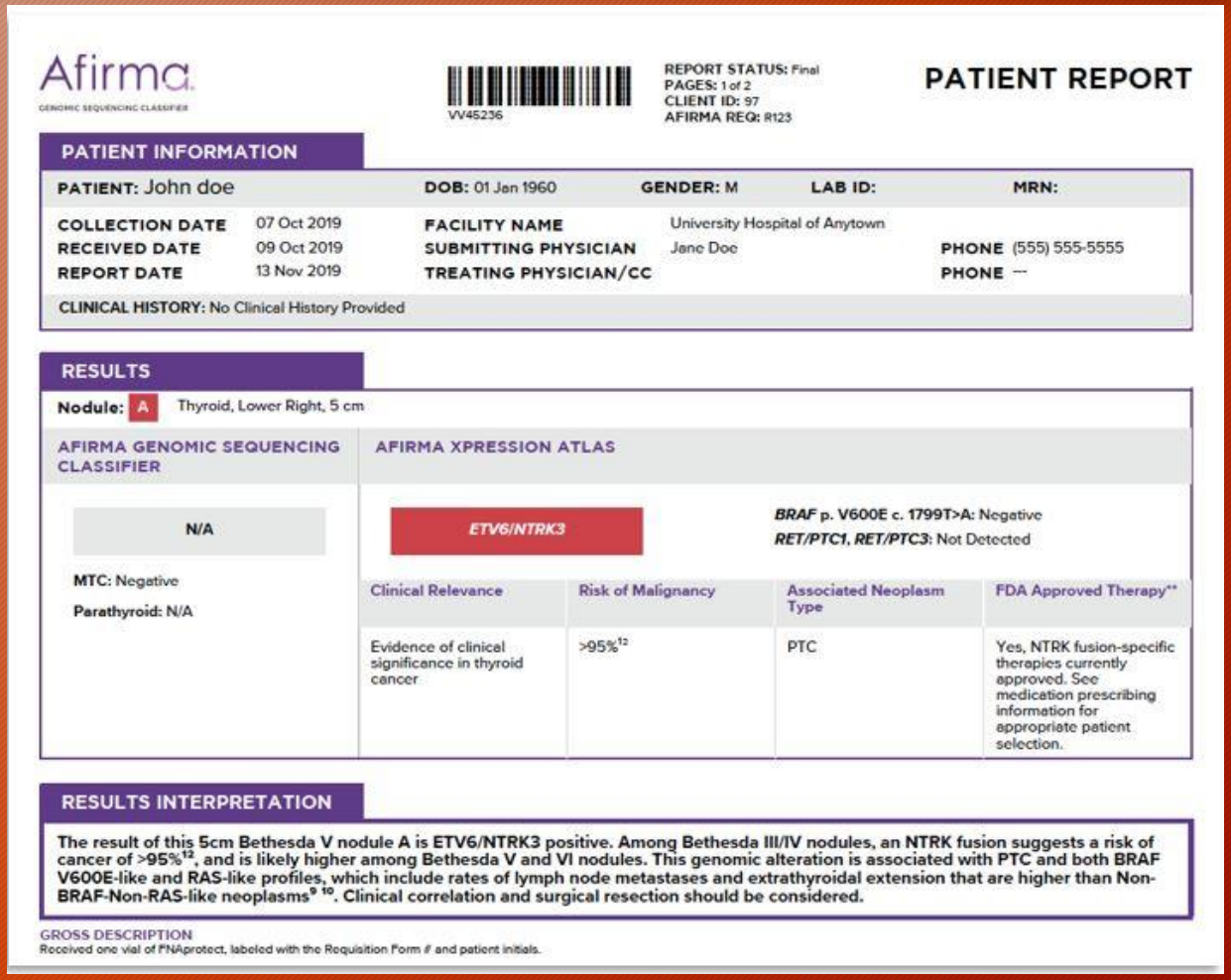
Afirma Xpression Atlas

- Panel can be reflexed for “Suspicious” or requested in category V or VI diagnoses
- Panel of 593 genes, 905 variants, 235 fusions



From: Veracyte website.

Sample report



Afirma
GENOMIC SEQUENCING CLASSIFIER

VV45236

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

PATIENT REPORT

PATIENT INFORMATION

PATIENT: John doe | **DOB:** 01 Jan 1960 | **GENDER:** M | **LAB ID:** | **MRN:**

COLLECTION DATE	07 Oct 2019	**FACILITY NAME**	University Hospital of Anytown		
RECEIVED DATE	09 Oct 2019	**SUBMITTING PHYSICIAN**	Jane Doe	**PHONE**	(555) 555-5555
REPORT DATE	13 Nov 2019	**TREATING PHYSICIAN/CC**		**PHONE**	---

CLINICAL HISTORY: No Clinical History Provided

RESULTS

Nodule: A Thyroid, Lower Right, 5 cm

AFIRMA GENOMIC SEQUENCING CLASSIFIER

N/A

MTC: Negative

Parathyroid: N/A

AFIRMA XPRESSION ATLAS

ETV6/NTRK3

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

Clinical Relevance	Risk of Malignancy	Associated Neoplasm Type	FDA Approved Therapy**
Evidence of clinical significance in thyroid cancer	>95%[12]	PTC	Yes, NTRK fusion-specific therapies currently approved. See medication prescribing information for appropriate patient selection.

RESULTS INTERPRETATION

The result of this 5cm Bethesda V nodule A is ETV6/NTRK3 positive. Among Bethesda III/IV nodules, an NTRK fusion suggests a risk of cancer of >95%[12], and is likely higher among Bethesda V and VI nodules. This genomic alteration is associated with PTC and both BRAF V600E-like and RAS-like profiles, which include rates of lymph node metastases and extrathyroidal extension that are higher than Non-BRAF-Non-RAS-like neoplasms[9] [10]. Clinical correlation and surgical resection should be considered.

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

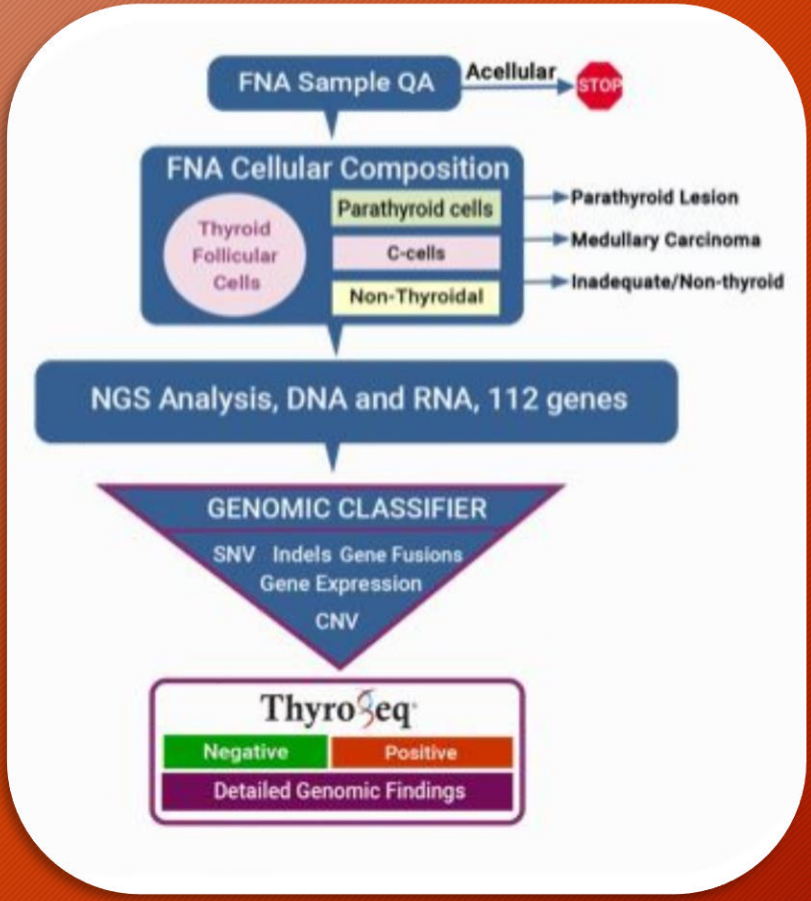
Our experience

	Puerto Rico Pathology	Literature
Malignant cases	5.5%	3-7%
AUS/FLUS (III)	8%	8-12%
Follicular neoplasm (IV)	7%	2-8%
All indeterminates (III & IV)	15%	14-26%
Risk malignancy in cat III	12.5%	5-15%
Risk malignancy in cat IV	14%	15-30%
Risk malignancy III & IV	13.6%	
Afirma Benign call rate	64% (48/75 cases)	66%
Benign call rate in cat II with prior III or IV	74% (14/19)	

ThyroSeq v3

- First with Next generation sequencing and specific mutation reporting
- Original validation studies heavily criticized (one center, biased pathologic diagnosis)
- New version tested in a double blinded multicenter trial
  - Can test samples collected in their media and FFPE tissue
  - Negative call rate 61% (Steward et al, 2018)

ThyroSeq v3

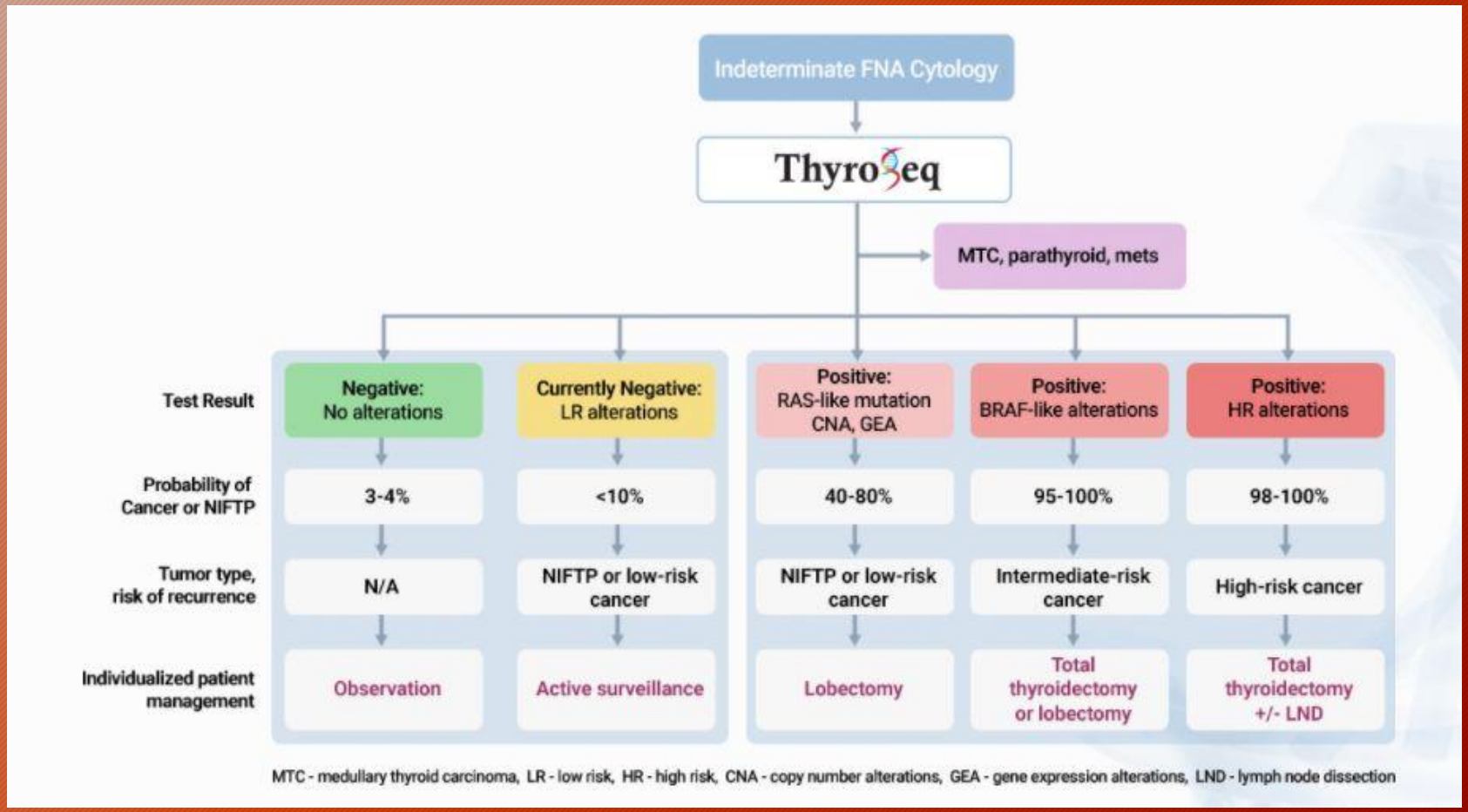

FNA Sample QA
Acellular
STOP
FNA Cellular Composition
Thyroid Follicular Cells
Parathyroid cells
C-cells
Non-Thyroidal
Parathyroid Lesion
Medullary Carcinoma
Inadequate/Non-thyroid
NGS Analysis, DNA and RNA, 112 genes
GENOMIC CLASSIFIER
SNV Indels Gene Fusions
Gene Expression
CNV
ThyroSeq
Negative
Positive
Detailed Genomic Findings

ThyroSeq v3

Bethesda category of cytology	Bethesda III	Bethesda IV	Bethesda III + IV
	cytology (95% CI)	cytology (95% CI)	cytology (95% CI)
No of cases	154	93	247
Disease Prevalence	23%	35%	28%
ThyroSeq v3 performance:			
Sensitivity	91% (77-97%)	97% (85-100%)	94% (86-98%)
Specificity	85% (77-90%)	75% (63-84%)	82% (75-87%)
PPV *	64% (50-77%)	68% (54-80%)	66% (56-75%)
NPV	97% (92-99%)	98% (89-100%)	97% (93-99%)

Steward, DL et al. JAMA Oncol. 2018.

ThyroSeq v3


Indeterminate FNA Cytology
ThyroSeq
MTC, parathyroid, mets
Test Result
Negative: No alterations
Currently Negative: LR alterations
Positive: RAS-like mutation CNA, GEA
Positive: BRAF-like alterations
Positive: HR alterations
Probability of Cancer or NIFTP
3-4%
<10%
40-80%
95-100%
98-100%
Tumor type, risk of recurrence
N/A
NIFTP or low-risk cancer
NIFTP or low-risk cancer
Intermediate-risk cancer
High-risk cancer
Individualized patient management
Observation
Active surveillance
Lobectomy
Total thyroidectomy or lobectomy
Total thyroidectomy +/- LND
MTC - medullary thyroid carcinoma, LR - low risk, HR - high risk, CNA - copy number alterations, GEA - gene expression alterations, LND - lymph node dissection

Patient			Accession #: **MGP17-1505**
DOB/Age/Sex	07/04/1984 (Age: 32) F		Client Accession #:
Client Identifier		Client	CBLPATH, INC
Collection Date	06/23/2017	Requesting Physician	
Accession Date	06/26/2017	Ordering Physician	
Reported Date	07/03/2017	T	914-698-5706
		F	914-251-1306

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

THYROSEQ® GC RESULTS SUMMARY

RIGHT UPPER THYROID FNA

Test Result	Probability of Cancer or NIFTP	Potential Management
POSITIVE	**High (~99%)**	**Surgical excision*** **See interpretation below for details*

INTERPRETATION

- BRAF V600E mutation was identified in this sample without other high-risk mutations.
- BRAF V600E is associated with a very high (~99%) probability of papillary thyroid carcinoma or related cancers.
- Risk of cancer recurrence associated with an isolated BRAF V600E mutation is intermediate for tumors >1cm and may be low for tumors <1cm.
- Surgical management may include total thyroidectomy or lobectomy, depending on tumor size and other clinical factors.
- 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	BRAF	p.V600E	c.1799T>A	23%
Gene fusions	Negative			
Gene expression profile	Positive			
Parathyroid	Negative			
Medullary/C-cells	Negative			

AF=Variant Allele Frequency

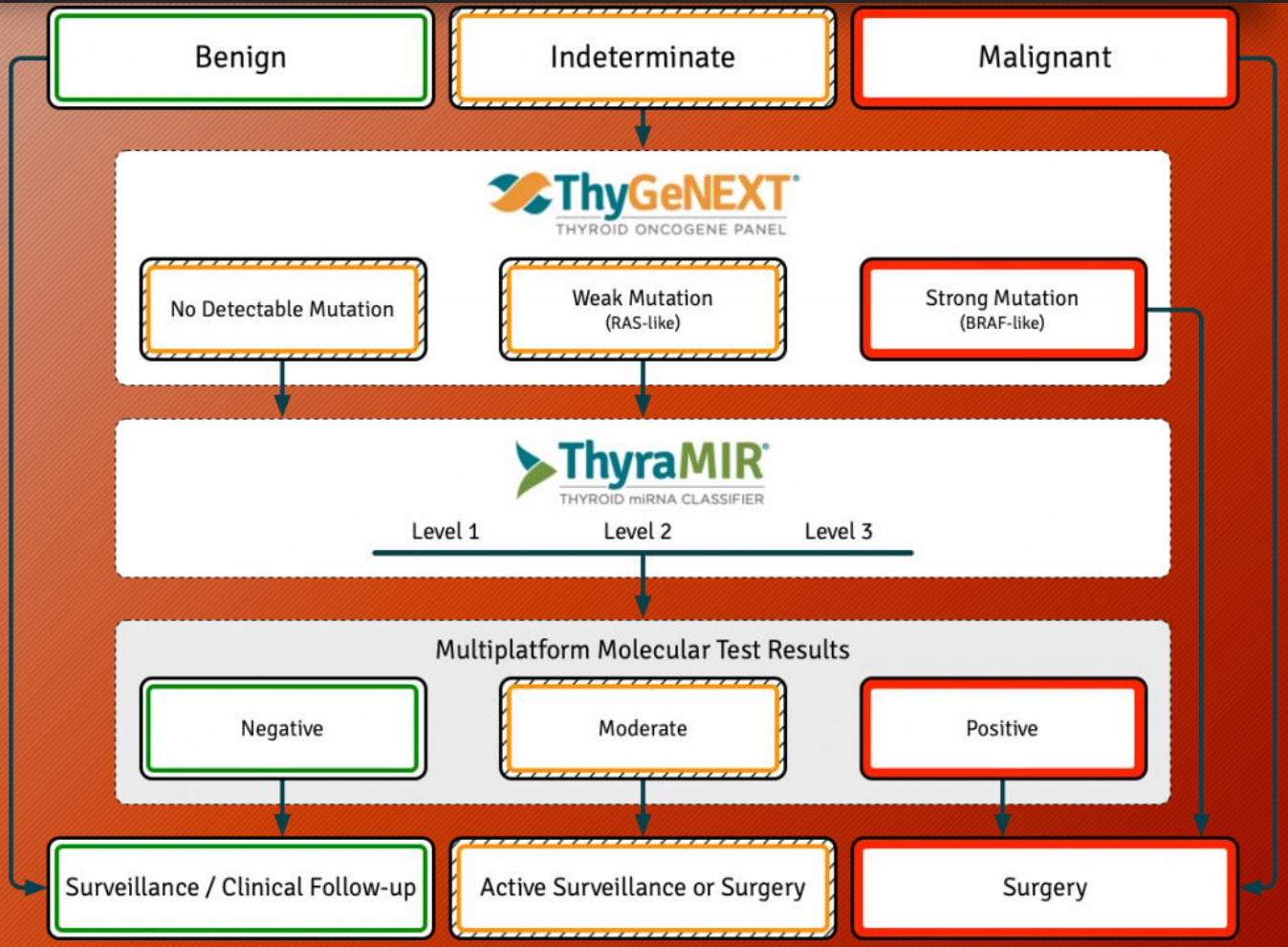
ThygeNEXT/ThyraMIR

- First tests for DNA and RNA markers with high specificity using NGS
  - Also targets mutations with prognostic/therapeutic implications
- If negative, tests for miRNA
  - Non coding RNA implicated in gene expression regulation
  - Their expression profiles have been implicated in pathophysiology of cancer

ThygeNEXT/ThyraMIR

ThyGeNEXT® NGS Panel		ThyraMIR® miRNA classifier
DNA mutation panel	RNA panel (# fusions)	
ALK	ALK (2)	miR-29b-1-5p
BRAF	BRAF (3)	miR-31-5p
GNAS	NTRK (8)	miR-138-1-3p
HRAS	PPARg (5)	miR-139-5p
KRAS	RET (14)	miR-146b-5p
NRAS	THADA (5)	miR-155
PIK3CA	mRNA controls: NKX2-1, PAX8, TBP, USP33	miR-204-5p
PTEN		miR-222-3p
RET		miR-375
TERT		miR-551b-3p

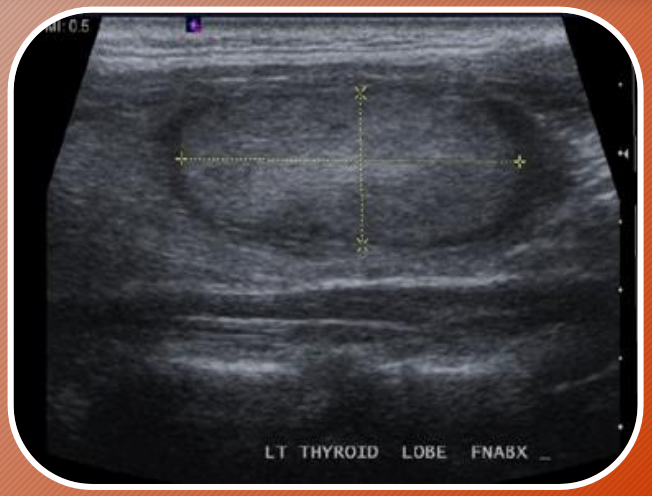
ThyGeNEXT / ThyraMIR



ThygeNext/ThyraMIR

- Can test from specimen in their transport media or from slides
- Claims 95% sensitivity and 90% specificity, with NPV of 97% and PPV of 75% with the combination testing (adjusted prevalence of disease, Lupo et al, 2020)
- Negative call rate 46% and moderate call rate 28% in recent clinical validation (Lupo et al, 2020)
- Test with less supporting literature

In practice, not all AUS are equal



Solid nodule: PPV 15-27%

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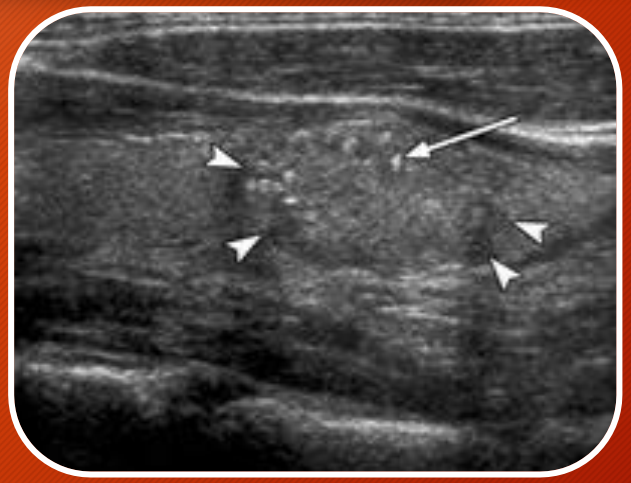

Microcalcifications: PPV 42-94%

- These two nodules have different pre-test probabilities of malignancy, both for the FNA and for molecular testing.
- If both have indeterminate cytology, a negative molecular test may NOT have the same NPV for each nodule.

P.W. Rosario. *Thyroid Nodules with Atypia or FLUS (Bethesda Category III): Importance of Ultrasonography and Cytological Subcategory*. Thyroid. 24: 115-1120. July 2014.

In practice, not all AUS are equal

- AUS/FLUS cases with nuclear atypia – higher risk of PTC
- AUS/FLUS cases with architectural atypia only – lower risk of PTC

In practice - for the clinician

- What do I want?
  - Reassurance that the nodule is benign to *avoid* surgery?
    - Need a test with high sensitivity and high NPV
    - All commercially available claim to do this
  - Confirmation that it is malignant for *definitive* surgery?
    - Need a test that identifies high risk mutations
- What do I need?
  - Know the risk of malignancy in the indeterminate results I get
  - Other factors affecting the pre-test probability of malignancy

In practice - for the pathologist

- What is my proportion of indeterminate cases?
  - Am I dumping suspicious or positive cases in the indeterminate category?
    - Will decrease my NPV for molecular testing
  - Am I dumping negative cases in the indeterminates?
    - Some of those will get positive molecular test and then unnecesary surgey, will also increase costs.
- Do I have an idea of the risk of malignancy of my indeterminates?

Other uses for molecular

- Prognosis:
  - Coexistence of BRAF with PIK3CA, AKT1, TERT, or TP53 marker for increased aggressiveness
  - May use to select patients with microcarcinomas for surgery vs monitoring? (ATA 2015)
- Diagnosis!:
  - BRAF V600E mutation excludes NIFTP; maybe ETV6-NTRK3?
- Mutations for which specific therapies are available (currently three FDA approved)

Considerations / take home notes

- Growing literature that molecular testing can help in triaging indeterminate thyroid nodules
- Specific higher risk mutations are now reported by most commercially available tests, but some require it to be requested
- Molecular tests are NOT perfect, false positives and false negatives do occur, correlate with other data, F/U patients according to guidelines
- As a rule, molecular tests should not be repeated in the same nodule (cytology in a previously tested nodule may, in certain circumstances)

Considerations / take home notes

- Patients requesting molecular in benign nodules
  - NPV of category II is 97%
  - Benign molecular will only add 2.5% certainty
  - But will have 32% false positives (at 68% specificity)
- Availability of molecular testing may increase the indeterminate dx by the pathologists, which will increase the false positive molecular results and number of surgeries
- Xpression Atlas of Afirma in category V or VI: a negative results does not mean benign pathology or reduced risk!
- Possibility of a NIFTP diagnosis, effect in different validation studies, patient education

Thank You