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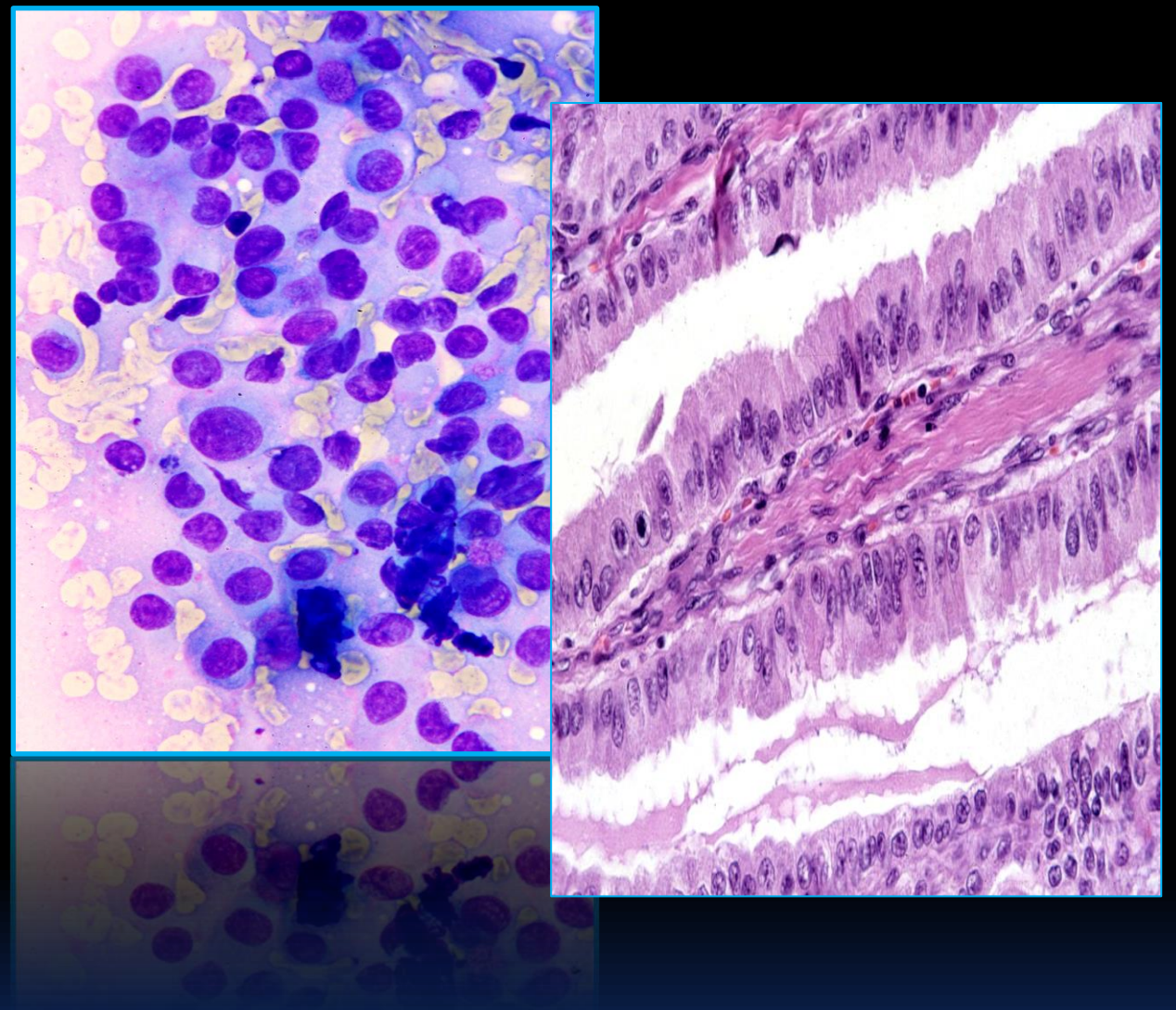
@Bfaquin



Updates in Thyroid Cytology And More....

Selected 5th Edition WHO Updates for Thyroid

- A few changes including:
  - Follicular tumors divided into:
    - Benign
    - Low-risk
    - Malignant
  - Mitoses per 2 mm^2 instead of per 10 hpf
  - Oncocytic instead of Hurthle cell
  - Aside from FVPTC, other types of PTC are referred to as subtypes

5th Edition WHO UPDATES FOR THYROID

- Thyroid Follicular Cell-Derived Neoplasms:
- 1. Benign tumors
  - Thyroid follicular nodular disease
  - Follicular adenoma
  - Follicular adenoma with papillary architecture
  - Oncocytic adenoma
- 2. Low-risk neoplasms
  - NIFTP
  - Thyroid tumor of uncertain malignant potential
  - Hyalinizing trabecular tumor
- 3. Malignant neoplasms
  - Follicular thyroid carcinoma
  - Invasive encapsulated FVPTC
  - PTC – classic type and subtypes
  - Oncocytic thyroid carcinoma
  - Follicular-derived thyroid carcinoma, high-grade
    - Differentiated high-grade thyroid carcinoma
    - Poorly differentiated thyroid carcinoma
  - Anaplastic thyroid carcinoma

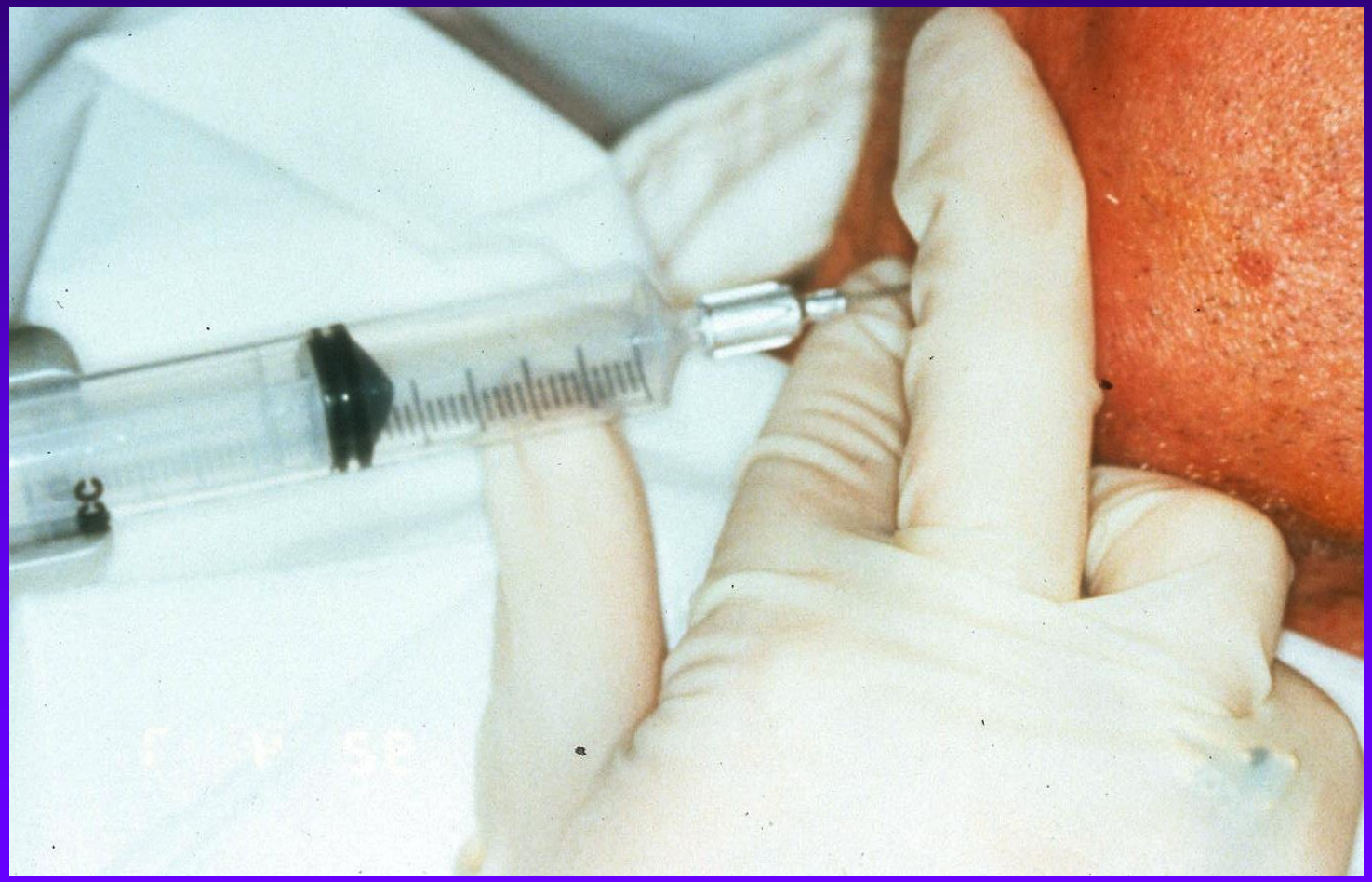
THYROID CYTOLOGY UPDATE

Even if you do not signout cytopathology, a working knowledge of basic thyroid cytology is valuable (e.g. interpreting thyroid cytology reports)

Overview of Thyroid FNA:

Each year over 550,000 thyroid FNAs are performed in the U.S. !!!

Among the most common Non-GYN cytology specimen



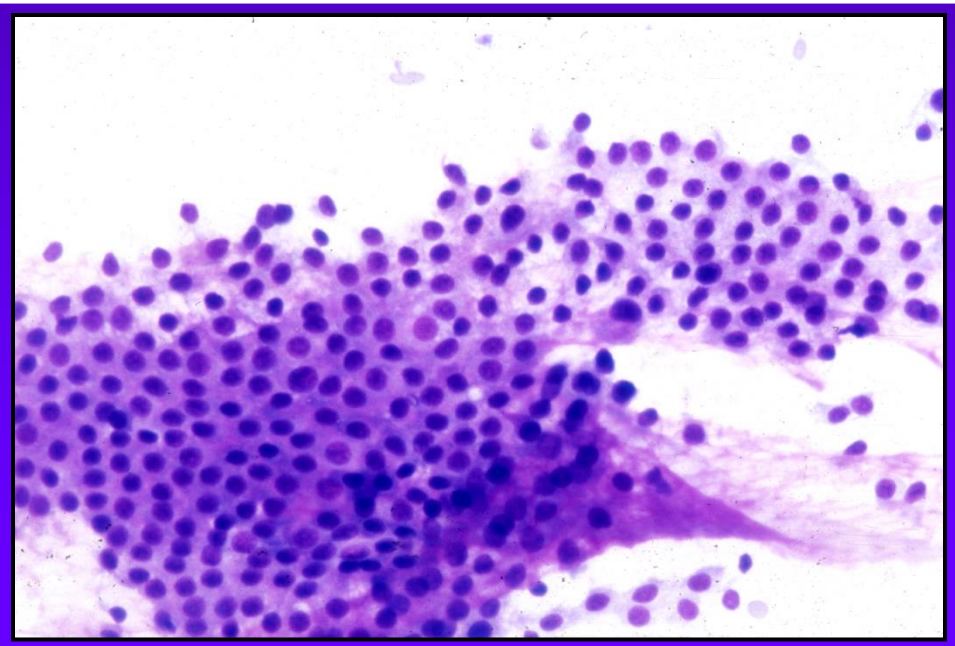
THYROID FNA: THE GOOD NEWS…

FNA has led to fewer thyroid surgeries

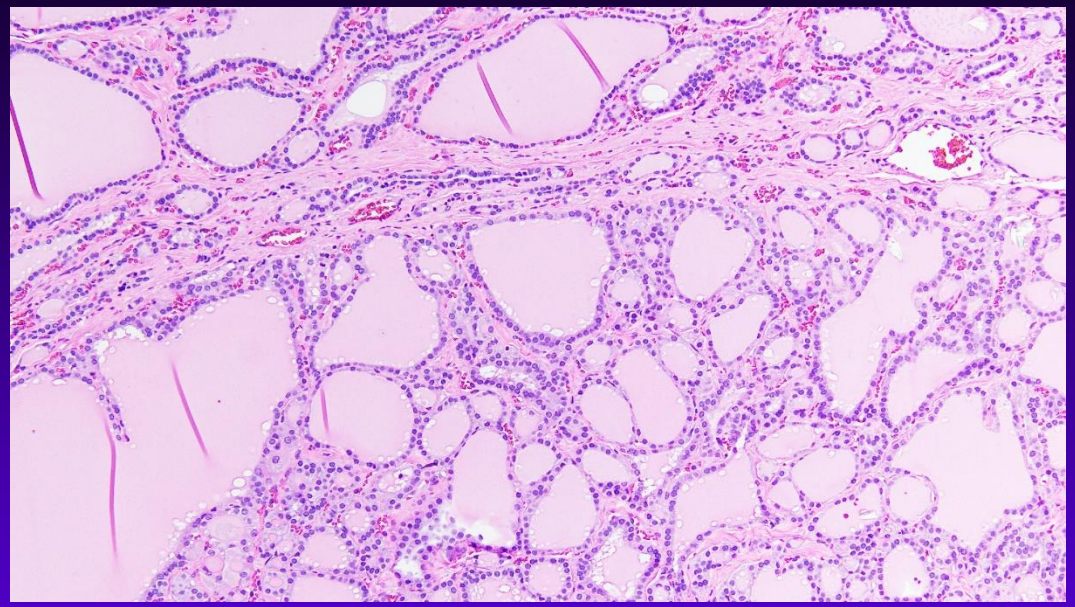
- **Reduced the number of surgeries by 50% [benign result in 60-70% of FNAs]**
- **Increased the yield of malignancies by 2-3X**
- **Decreased the costs of management by over 25%**
- **With Bethesda System and molecular testing, FNA plays a KEY ROLE in the triage of patients with a radiologically abnormal thyroid nodule!**

Cytologic Reporting of Follicular Lesions

- **BENIGN**
  - **Macrofollicles and colloid, consistent with a benign thyroid nodule.**

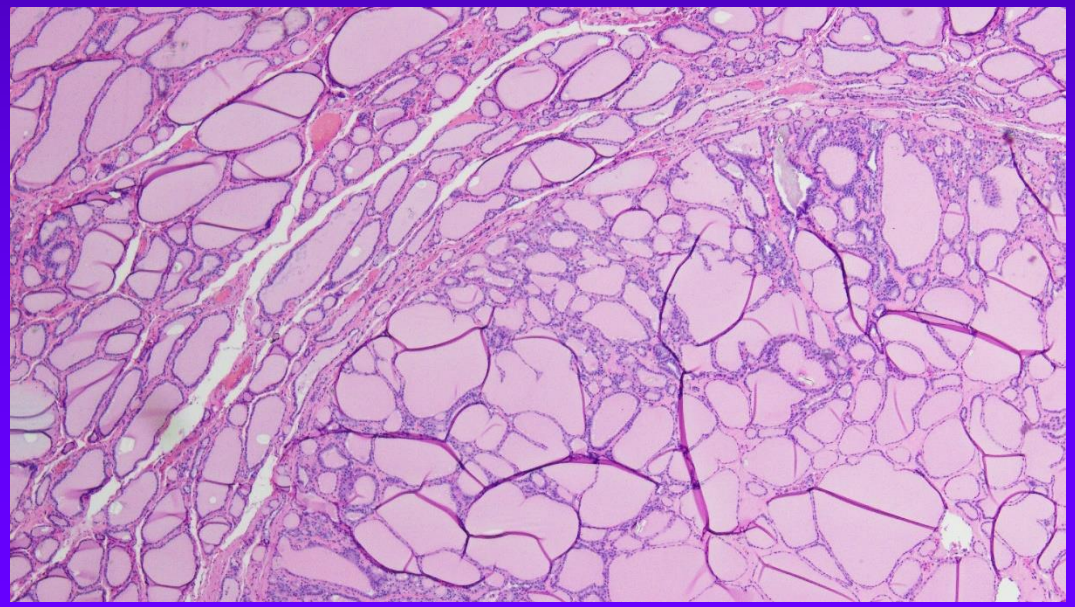


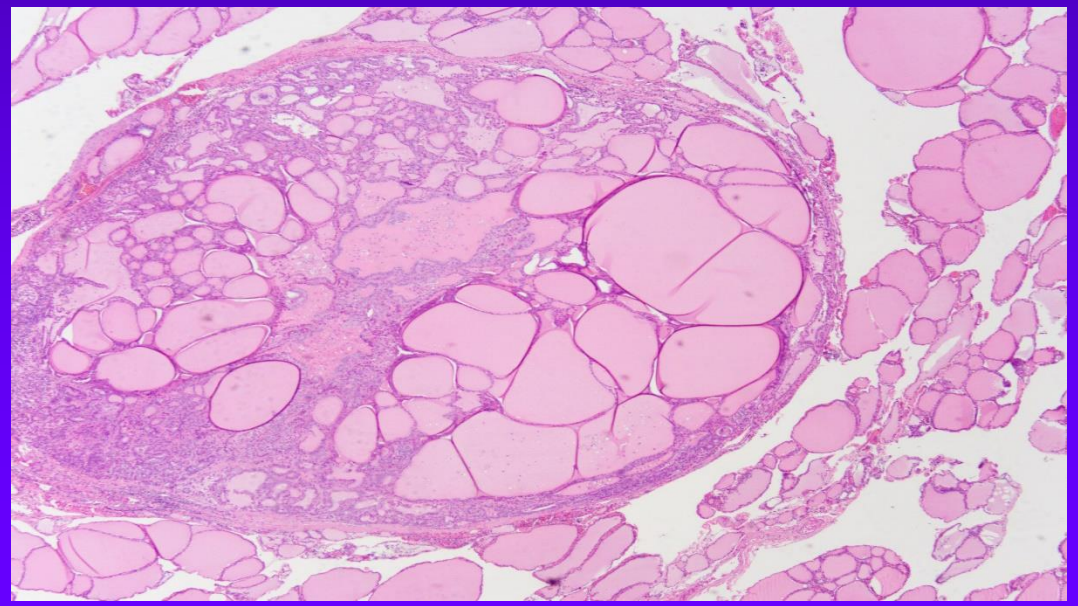
WHO: Benign Follicular Nodular Disease



Optional Names:

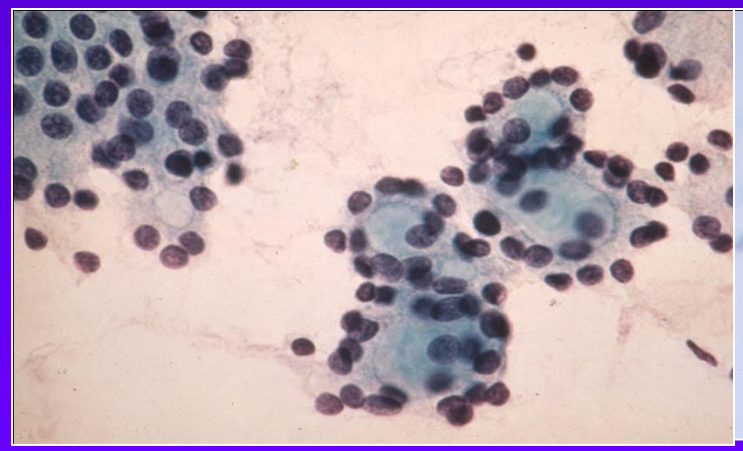
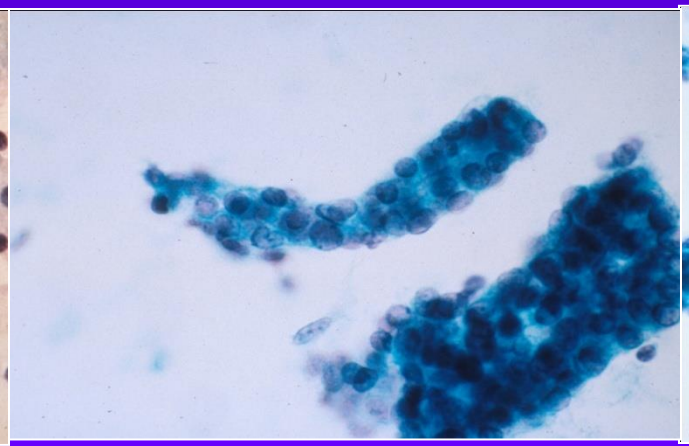
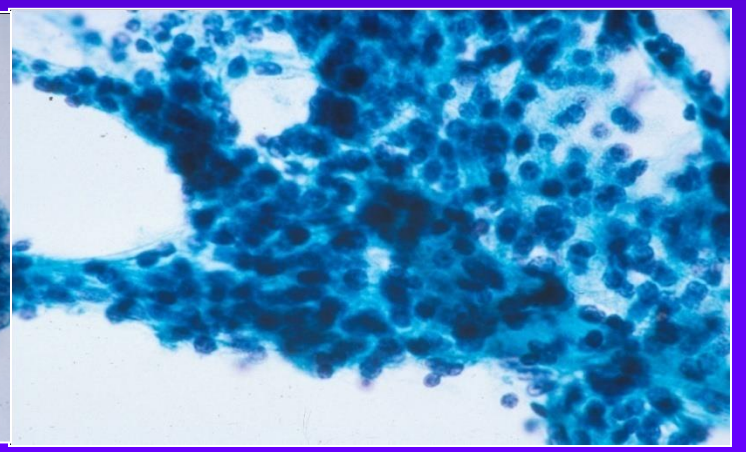
- Adenomatous nodules
- Adenomatoid nodules
- Hyperplastic nodules
- Nodular goiter

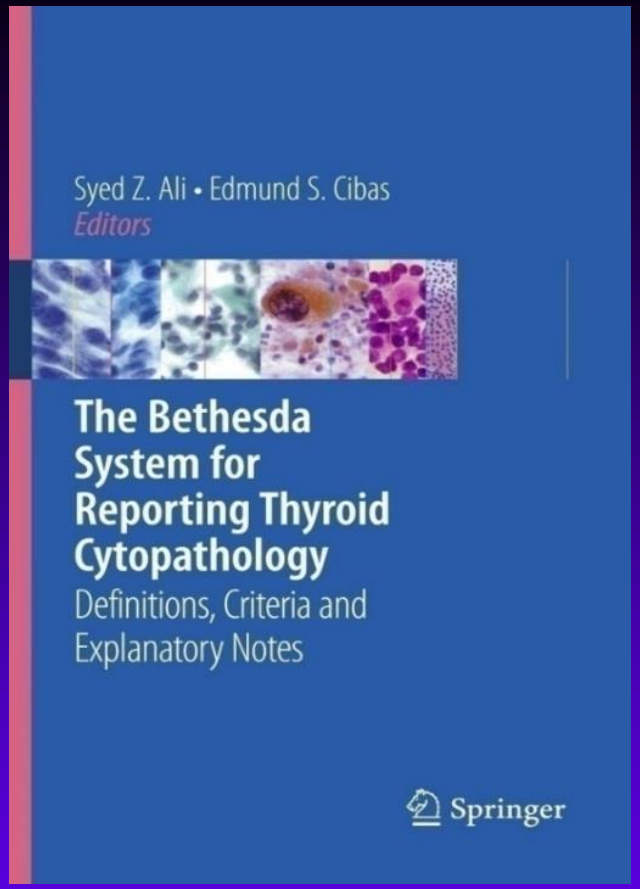




Cytologic Reporting of Follicular Lesions

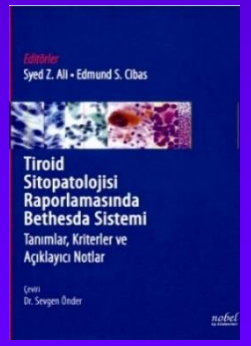
- **FOLLICULAR NEOPLASM**
  - ***Note: Distinction between a follicular adenoma and follicular carcinoma is not possible based upon cytologic material.***

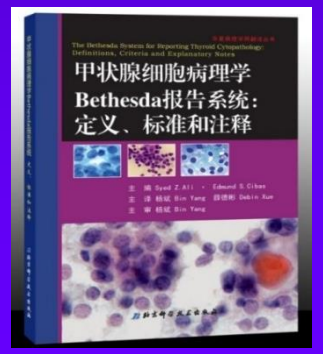




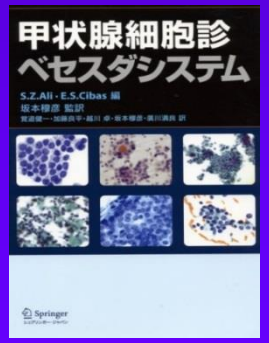


The Bethesda System for Reporting Thyroid Cytopathology: 2009-2022

- Most widely used reporting system for thyroid cytopathology in the world
- Translated into 4 languages
- Has revolutionized the practice of thyroid cytopathology and adapted it for the application of molecular diagnostics







The 3rd Edition of the Bethesda System for Thyroid Cytopathology will be Published in Mid-2023

ROM IN BETHESDA 2nd & 3rd Editions

2nd Ed Category	2nd Ed. ROM
Non-Diagnostic	5-10%
Benign	0-3%
AUS/FLUS	10-30%
Susp for Follicular Neoplasm	25-40%
Susp for Hurthle Cell Neoplasm	25-40%
Suspicious for Malignancy	50-75%
Malignant	97-99%

3rd Ed Category	3rd Ed. ROM
Non-Diagnostic	13% (5-20%)
Benign	4% (2-7%)
AUS	22% (13-30%)
Follicular Neoplasm	30% (23-34%)
Follicular Neoplasm:: Oncocytic FN	30% (23-34%)
Suspicious for Malignancy	74% (67-83%)
Malignant	97% (97-100%)

3rd Edition ROM for Pediatric Patients ROM is higher

TABLE 1.3. The Bethesda System for Reporting Thyroid Cytopathology in Pediatric Patients with implied risk of malignancy (ROM) and possible management recommendations.[10, 12-18, 64-69]

Diagnostic category	ROM Ave% (range)	Possible Management Recommendations
Nondiagnostic	**14** (0-33)	Repeat FNA with ultrasound guidance
Benign[a]	**6** (0-27)	Clinical and sonographic follow-up
Atypia of Undetermined Significance	**28** (11–54)	Repeat FNA or surgical resection
Follicular Neoplasm[b]	**50** (28-100)	Surgical resection
Suspicious for Malignancy	**81** (40–100)	Surgical resection
Malignant	**98** (86–100)	Surgical resection

[a] ROM is skewed by selection bias since a majority of thyroid nodules classified as benign do not undergo surgical excision.

[b] Includes cases of follicular neoplasm with oncocytic features (Hürthle cell neoplasm).

The Indeterminate Thyroid FNA Categories: Many are NIFTP

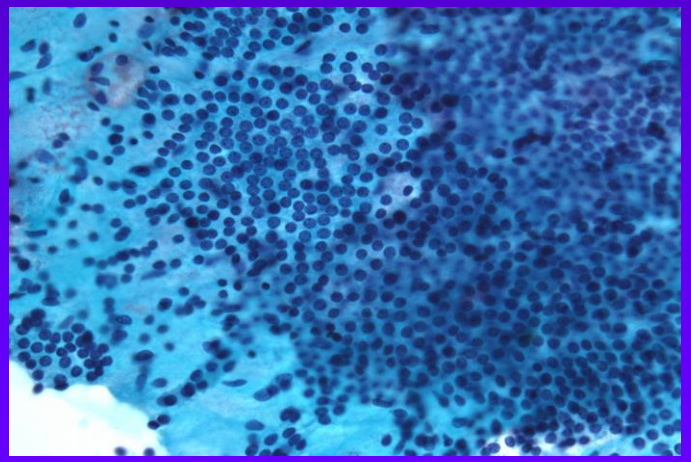
The Indeterminate Thyroid FNA Comprises 15-30% of All Thyroid FNAs

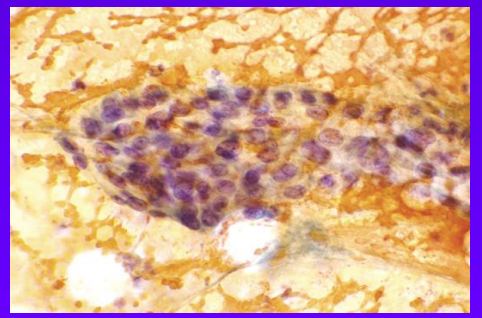
Category	Management	Implied Risk of Malignancy (%)
Non-Diagnostic	Repeat FNA with U/S	13%
Benign	Clinical and U/S F/U	4%
AUS	Repeat FNA, Molecular Testing, Lobectomy, or Surveillance	22%
Follicular Neoplasm	Lobectomy, Molecular Test	30%
Follicular Neoplasm: Oncocytic FN	Lobectomy, Molecular Test	30%
Suspicious for Malignancy	Lobectomy/ Total Thyroid Molecular Test	74%
Malignant	Lobectomy, Total Thyroid	97%

TBSRTC: AUS in 2022

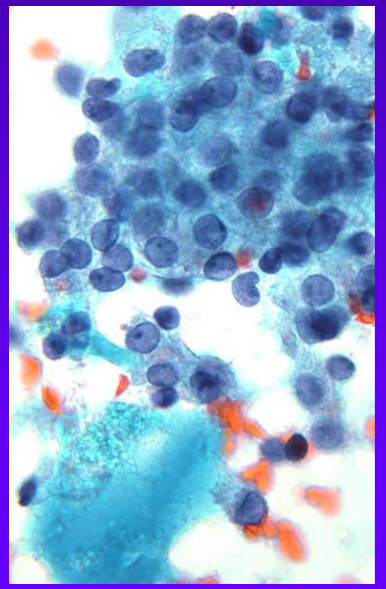
14 Years Old Now!!!

Benign

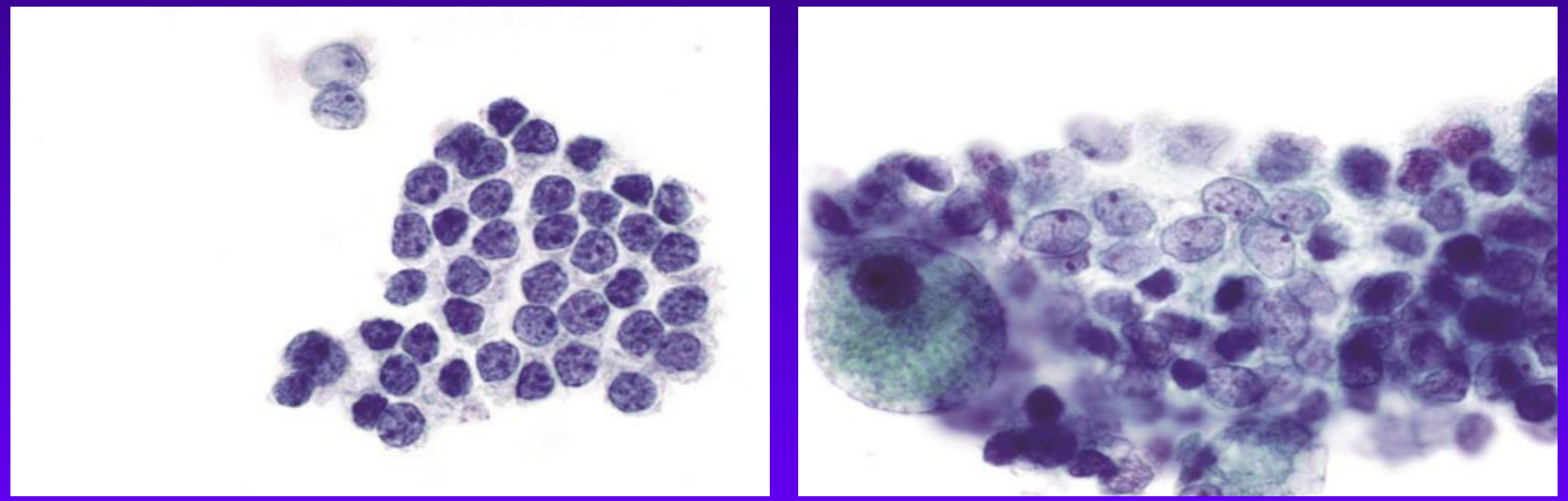




Follicular Neoplasm

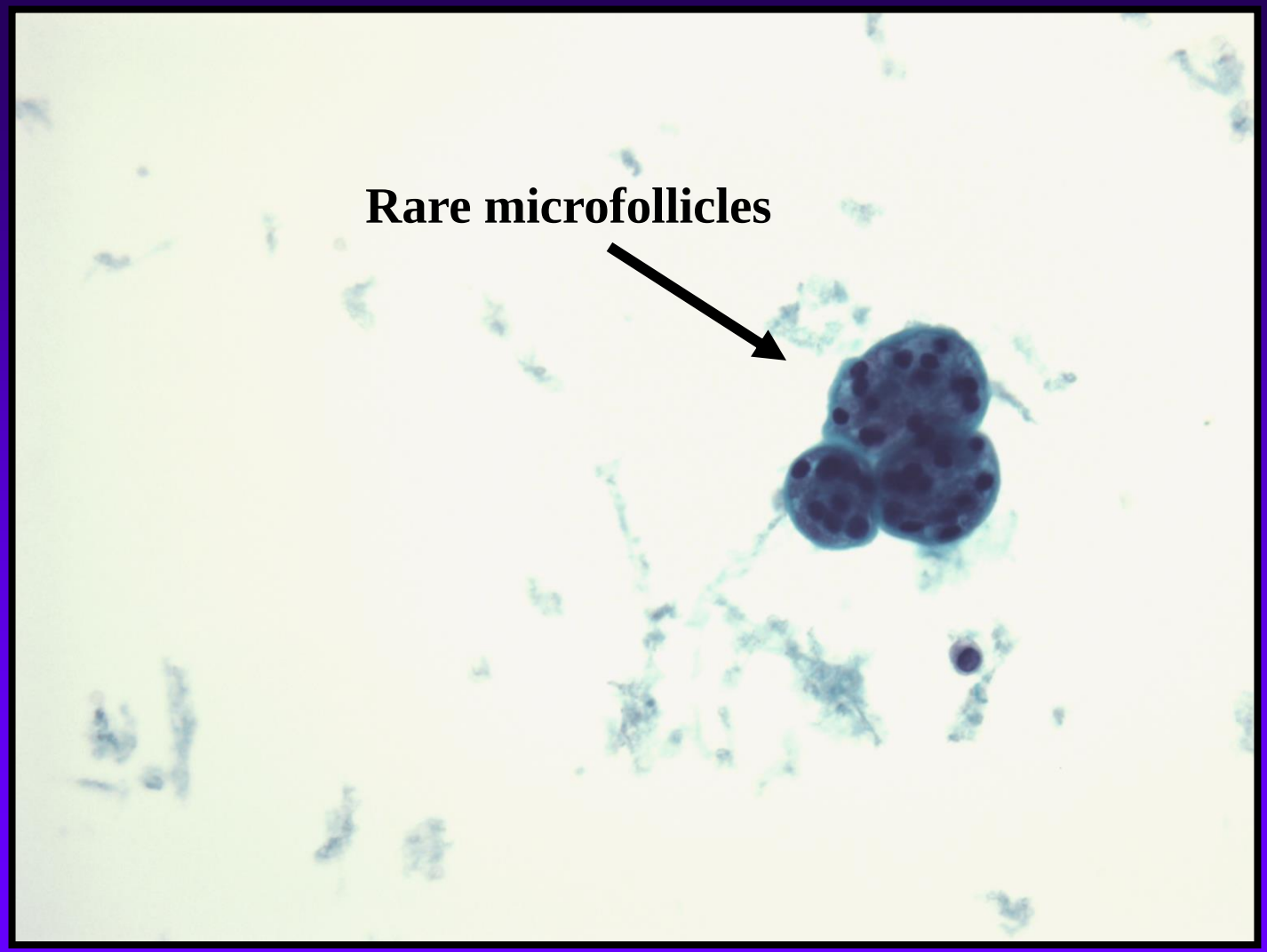


AUS – Nuclear Atypia Scenario: Focal Features of Papillary Carcinoma



Figs. 4.5 A and B, The Bethesda atlas

AUS- Other:
Hypocellular but Microfollicular



3rd Edition Bethesda Updates for AUS

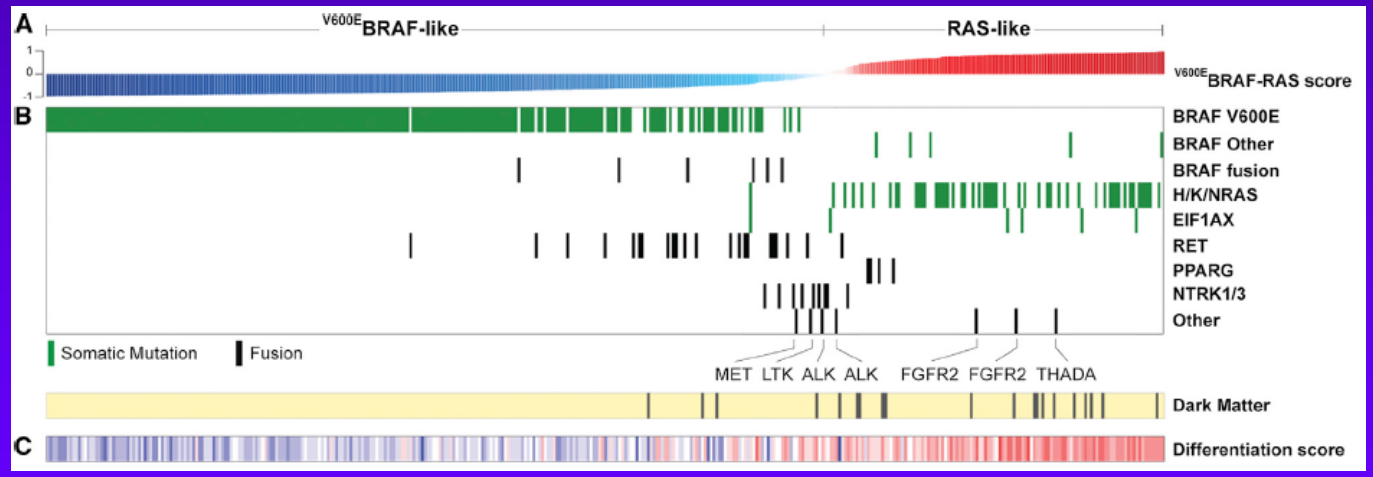
- **FLUS will no longer be included**
- **<10% (range: 3-20% in lit.)**
- **Potential for overuse/abuse –**
  - **Role for intralab monitoring (QA metric)**
- **Recommended management:**
  - **Repeat FNA, molecular testing, lobectomy, or surveillance**
- **Subclassification to help guide management**
  - **AUS with Nuclear atypia = increased risk of PTC or NIFTP**
  - **AUS Other = lower risk of PTC or NIFTP**

How is molecular testing helping to triage patients with a thyroid nodule?

ATA Guidelines-
Include Molecular Testing Option for the Indeterminate Categories

The Cancer Gene Atlas Project

- **TCGA Study: 71 gene expression profile**
- **Two broad categories:**
  - **BRAF-like: Classic PTC and most subtypes of PTC**
  - **RAS-like: Follicular carcinoma, follicular adenoma, FVPTC, NIFTP**
- ***"RAS-like PTCs and BRAF-like PTCs are fundamentally different in their genomic, epigenomic, and proteomic profiles."***



The Cancer Gene Atlas Research Network Cell (2014) Cell 2014;159:676-690.

Molecular Markers for Cancer Risk Prediction

Risk of Structural Disease Recurrence

(In patients without structurally identifiable disease after initial therapy)

High Risk

Gross extrathyroidal extension, incomplete tumor resection, distant metastases, or lymph node >3cm

Intermediate Risk

Aggressive histology, minor extrathyroidal extension, vascular invasion, or > 5 involved lymph nodes (0.2-3 cm)

Low Risk

Intrathyroidal DTC ≤ 5 LN micrometastases (< 0.2 cm)

FTC, extensive vascular invasion (≈ 30-55%)
pT4a gross ETE (≈ 30-40%)
pN1 with extranodal extension, >3 LN involved (≈ 40%)
PTC, >1 cm, TERT mutated ± BRAF mutated* (>40%)
pN1, any LN > 3 cm (≈ 30%)
PTC, extrathyroidal, BRAF mutated* (≈ 10-40%)
PTC, vascular invasion (≈ 15-30%)
Clinical N1 (≈20%)
pN1, > 5 LN involved (≈20%)
Intrathyroidal PTC, < 4 cm, BRAF mutated* (≈10%)
pT3 minor ETE (≈ 3-8%)
pN1, all LN < 0.2 cm (≈5%)
pN1, ≤ 5 LN involved (≈5%)
Intrathyroidal PTC, 2-4 cm (≈ 5%)
Multifocal PMC (≈ 4-6%)
pN1 without extranodal extension, ≤ 3 LN involved (2%)
Minimally invasive FTC (≈ 2-3%)
Intrathyroidal, < 4 cm, BRAF wild type* (≈ 1-2%)
Intrathyroidal unifocal PTMC, BRAF mutated*, (≈ 1-2%)
Intrathyroidal, encapsulated, FV-PTC (≈1-2%)
Unifocal PMC (≈ 1-2%)

Molecular Signature

BRAF+TERT, RAS+TERT
Multiple driver mutations (eg. NRAS and PIK3CA or TP53)
TERT

ALK fusions
NTRK1 fusions
NTRK3 fusions
BRAF V600E
RET/PTC

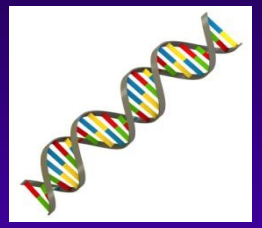
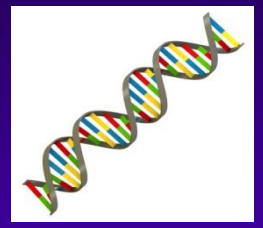
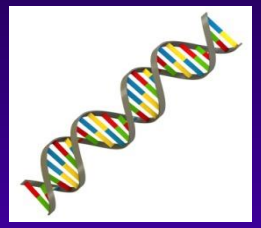
→ BRAF V600E- like mutations

RAS
BRAF K601E
PAX8/PPARG

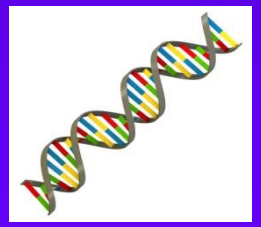
→ RAS-like mutations

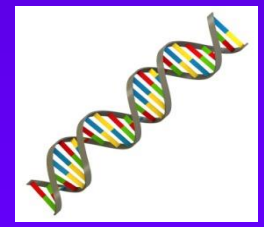
Haugen BR et al. *Thyroid*. 2016, 26:1-133

TBSRTC and Improved Diagnostic Accuracy Using Commercial Molecular Tests

- Afirma, Thyroseq V3, ThyGenX-ThyraMIR
- Especially useful for “indeterminate” thyroid FNAs
- Increase the pre-operative diagnostic accuracy of thyroid FNA
- **Helps to address NIFTP**





ThyroSeq v.3 & Afirma

	ThyroSeq GC[20]	Afirma GSC[25]
Study type	Multicenter, prospective, double-blind	Multicenter, retrospective, double-blind
Total number, samples	247	191
Nodule size by ultrasound, median (range), cm	2.1 (0.5-7)	2.6 (1.0-9.1)
Disease prevalence, %	27.5	23.7
Sensitivity, % (95%CI)	94.1 (86-98)	91.1 (79-98)
Specificity, % (95%CI)	81.6 (75-87)	68.3 (60-76)
NPV	97.3 (93-99)	96.1 (90-99)
PPV	65.9 (56-75)	47.1 (36-58)
Benign call rate	61%	54%
Avoidable surgeries for histologically benign nodules with indeterminate cytology	82%	68%

*Adapted from Steward, DL, et al.[20]

Steward et al, JAMA Oncol 2018

Afirma GSC and Xpression Atlas

Analytical and Clinical Validation of Expressed Variants and Fusions From the Whole Transcriptome of Thyroid FNA Samples

Trevor E. Angell[1], Lori J. Wirth[2], Maria E. Cabanillas[3], Maisie L. Shindo[4], Edmund S. Cibas[5], Joshua E. Babiarz[6], Yangyang Hao[6], Su Yeon Kim[6], P. Sean Walsh[6], Jing Huang[6], Richard T. Kloos[7], Giulia C. Kennedy[8] and Steven G. Waguespack[3]*

- GSC Benign = 4% ROM
- GSC Suspicious = 50% ROM
- Xpression Atlas can provide additional specific information to inform clinical decision making

Thyroseq v3 – Multigene Classifier

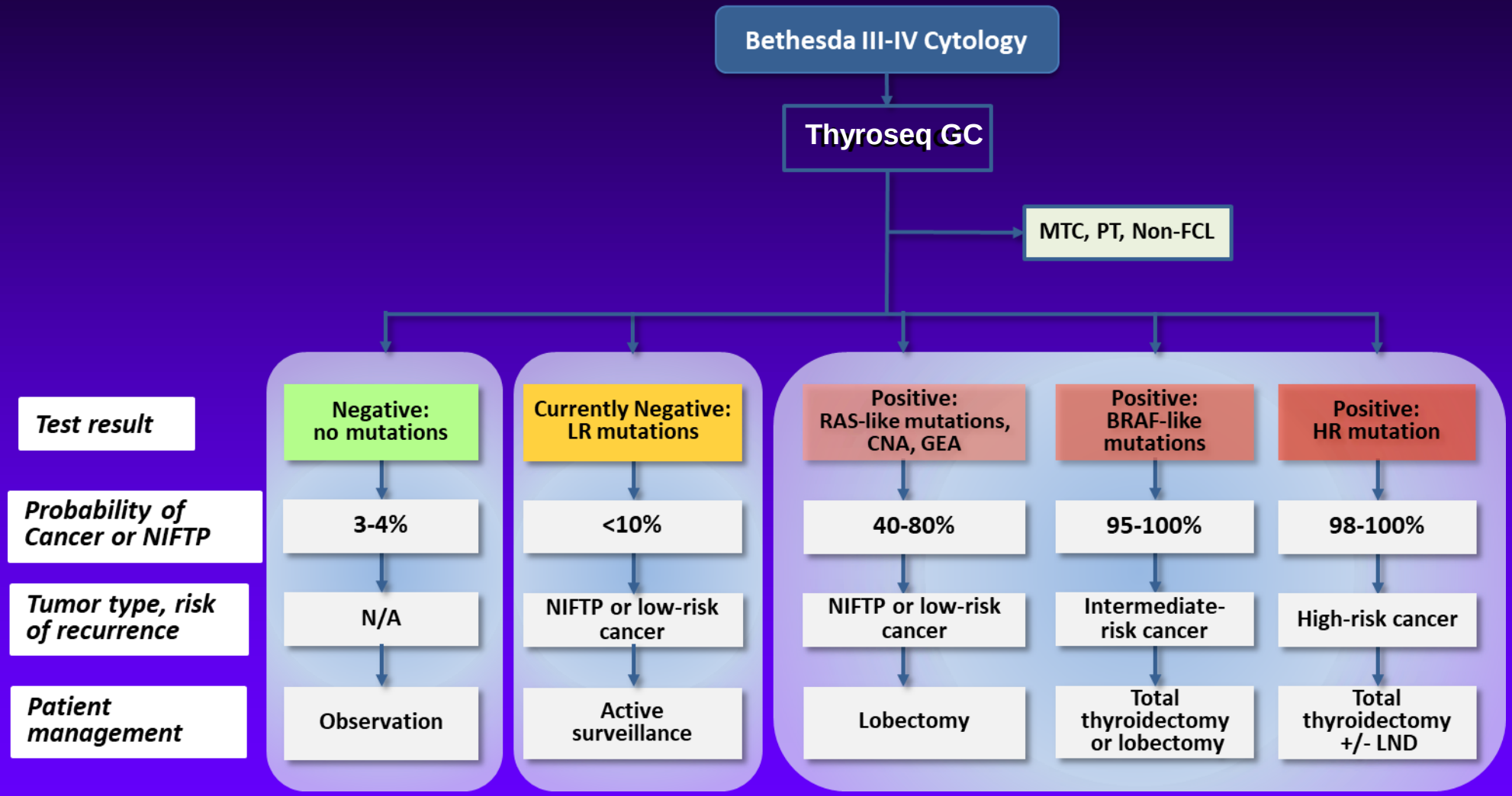
JAMA Oncology | Original Investigation

Performance of a Multigene Genomic Classifier in Thyroid Nodules With Indeterminate Cytology
A Prospective Blinded Multicenter Study

David L. Steward, MD; Sally E. Carty, MD; Rebecca S. Sippel, MD; Samantha Peiling Yang, MBBS, MRCP, MMed; Julie A. Sosa, MD, MA; Jennifer A. Sipos, MD; James J. Figge, MD, MBA; Susan Mandel, MD, MPH; Bryan R. Haugen, MD; Kenneth D. Burman, MD; Zubair W. Baloch, MD, PhD; Ricardo V. Lloyd, MD, PhD; Raja R. Seethala, MD; William E. Gooding, MS; Simion I. Chiosea, MD; Cristiane Gomes-Lima, MD; Robert L. Ferris, MD, PhD; Jessica M. Folek, MD; Raheela A. Khawaja, MD; Priya Kundra, MD; Kwok Seng Loh, MBBS; Carrie B. Marshall, MD; Sarah Mayson, MD; Kelly L. McCoy, MD; Min En Nga, MBBS; Kee Yuan Ngiam, MBBS, MRCS, MMed; Marina N. Nikiforova, MD; Jennifer L. Poehls, MD; Matthew D. Ringel, MD; Huaitao Yang, Md, PhD; Linwah Yip, MD; Yuri E. Nikiforov, MD, PhD

- DNA and RNA of 112 thyroid-related genes:
- Mutations, fusions, copy number, and gene expression
- Can obviate surgery in 61-67% of patients with Bethesda III/IV nodules
- Information on specific genetic alterations can help inform management: Follow, lobectomy, total thyroid.

Application of Thyroseq GC



Nikiforov YE. *Endocr Pract*. 2017:979

ThyroSeq V3 GC Report

Result summary with specific cancer probability

Interpretation of results with cancer risk assessment

Detailed Results

RIGHT MID THYROID FNA

Test Result	Probability of Cancer	Potential Management
POSITIVE	High (>95%)	Surgical excision * *See interpretation below for details

INTERPRETATION

- Mutations in the NRAS and TERT genes were identified.
- RAS mutations are early oncogenic events in follicular-type thyroid tumors (follicular variant PTC, follicular carcinoma, NIFTP), 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 >95% risk of cancer and increases likelihood of more aggressive disease.
- Because of the molecular signature of high-risk cancer, total thyroidectomy could be considered for many of these 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	41%
	NRAS	p.Q61R	c.182A>G	36%
Gene fusions	Negative			
Copy number alterations	Negative			
Gene expression profile	Negative			
Parathyroid	Negative			
Medullary/C-cells	Negative			

AF=Variant Allele Frequency

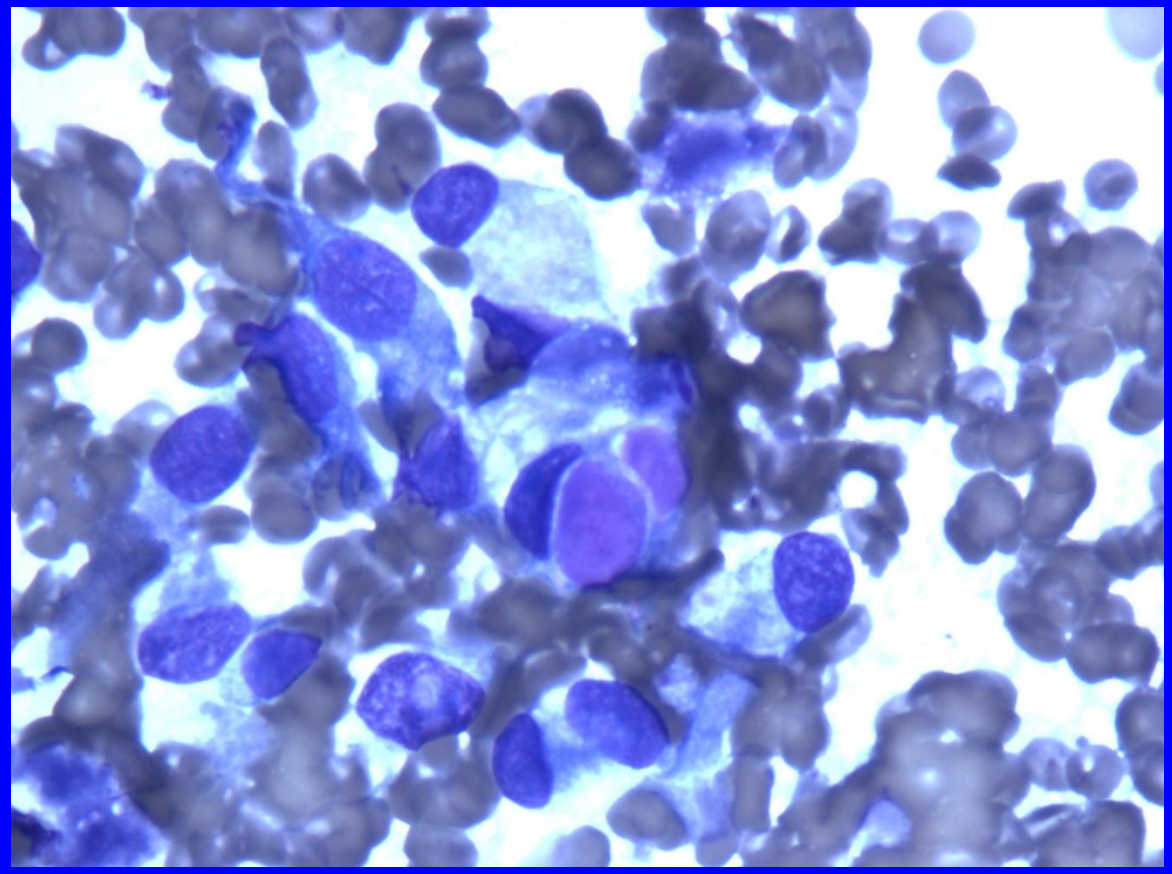
TERT + RAS (high-risk signature)

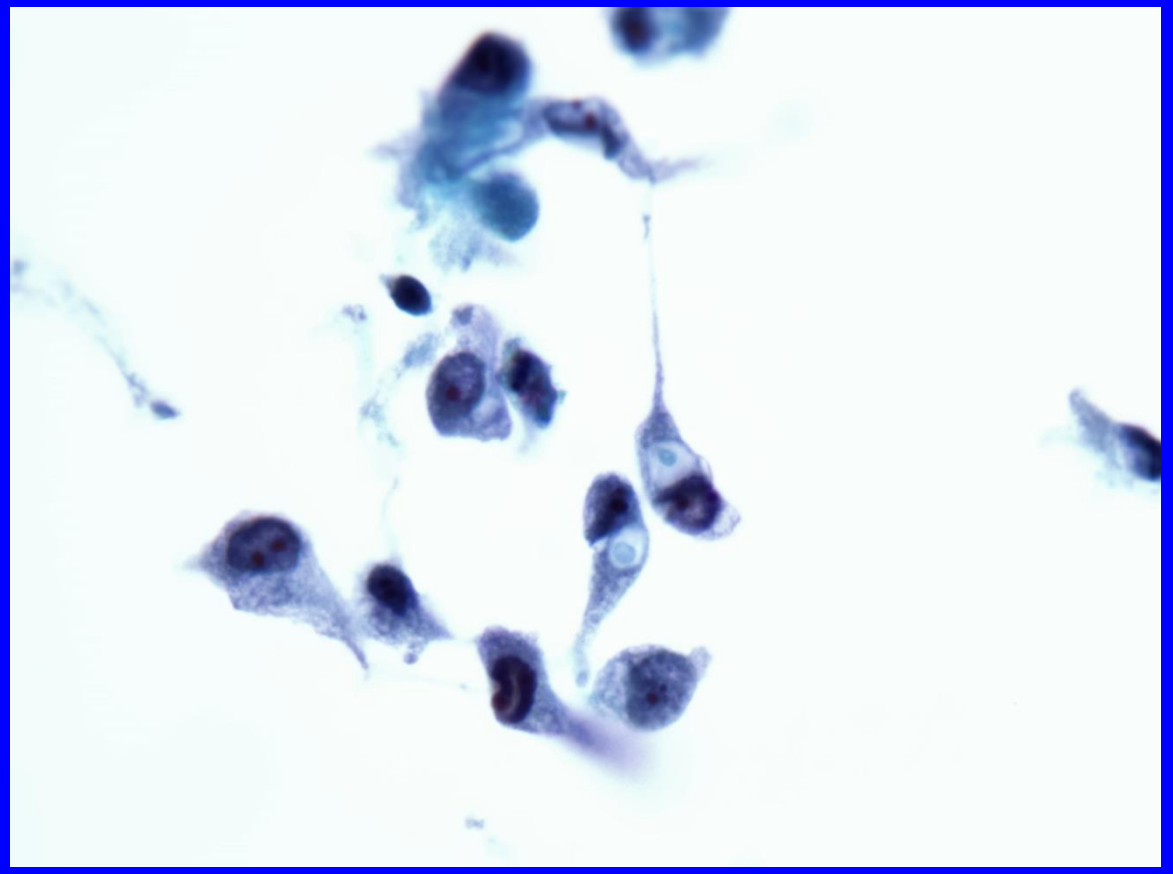
Kinase Fusions are Sometimes Identified by FNA Molecular Testing

NTRK & Other Kinase Fusion-Related Thyroid Carcinomas

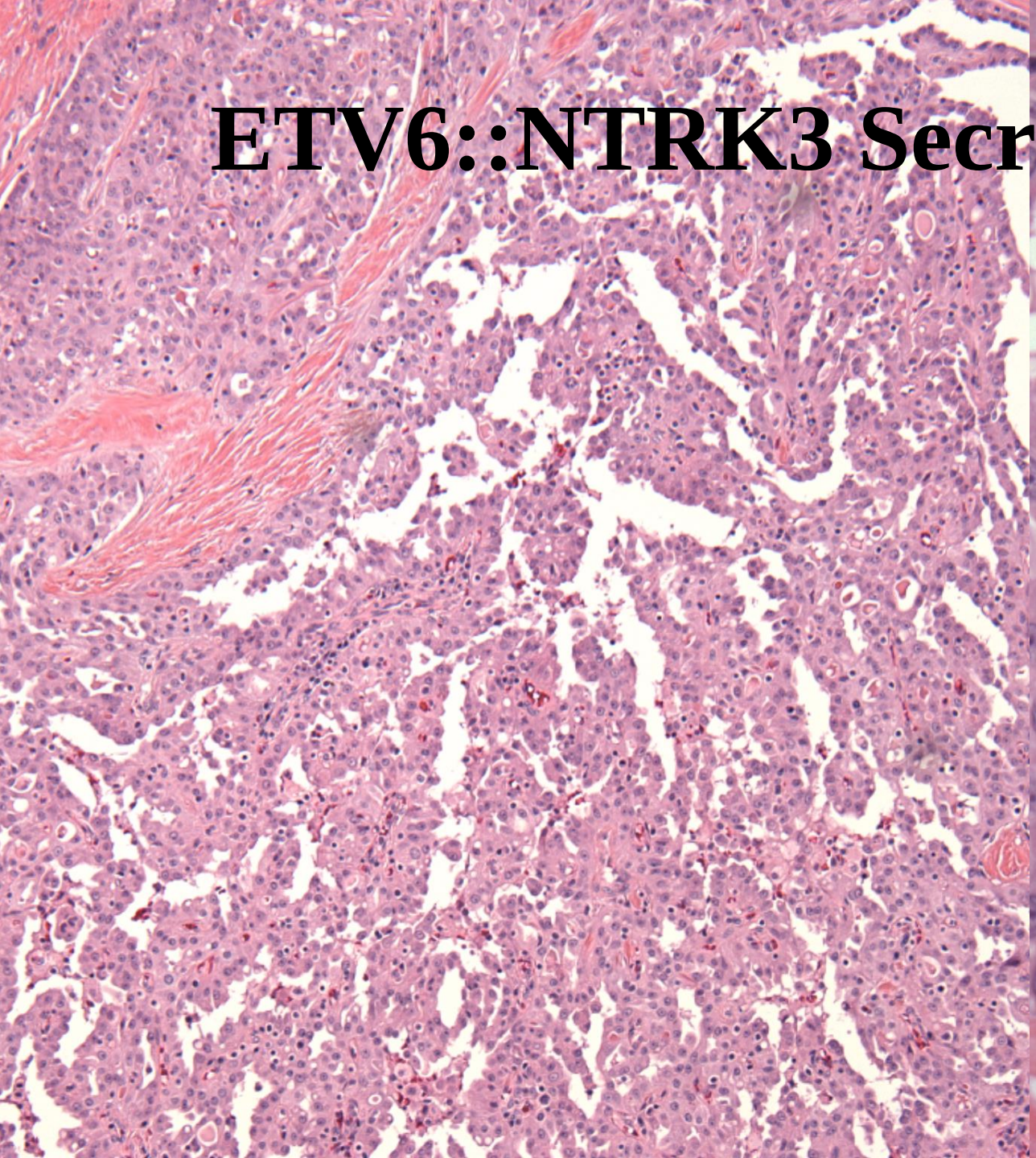
- A unique subtype of aggressive thyroid cancer that includes both PTC, FC, and secretory carcinoma
- Unusual histologic patterns in thyroid cancers should prompt consideration of additional testing
- Opportunity for specialized targeted therapeutics

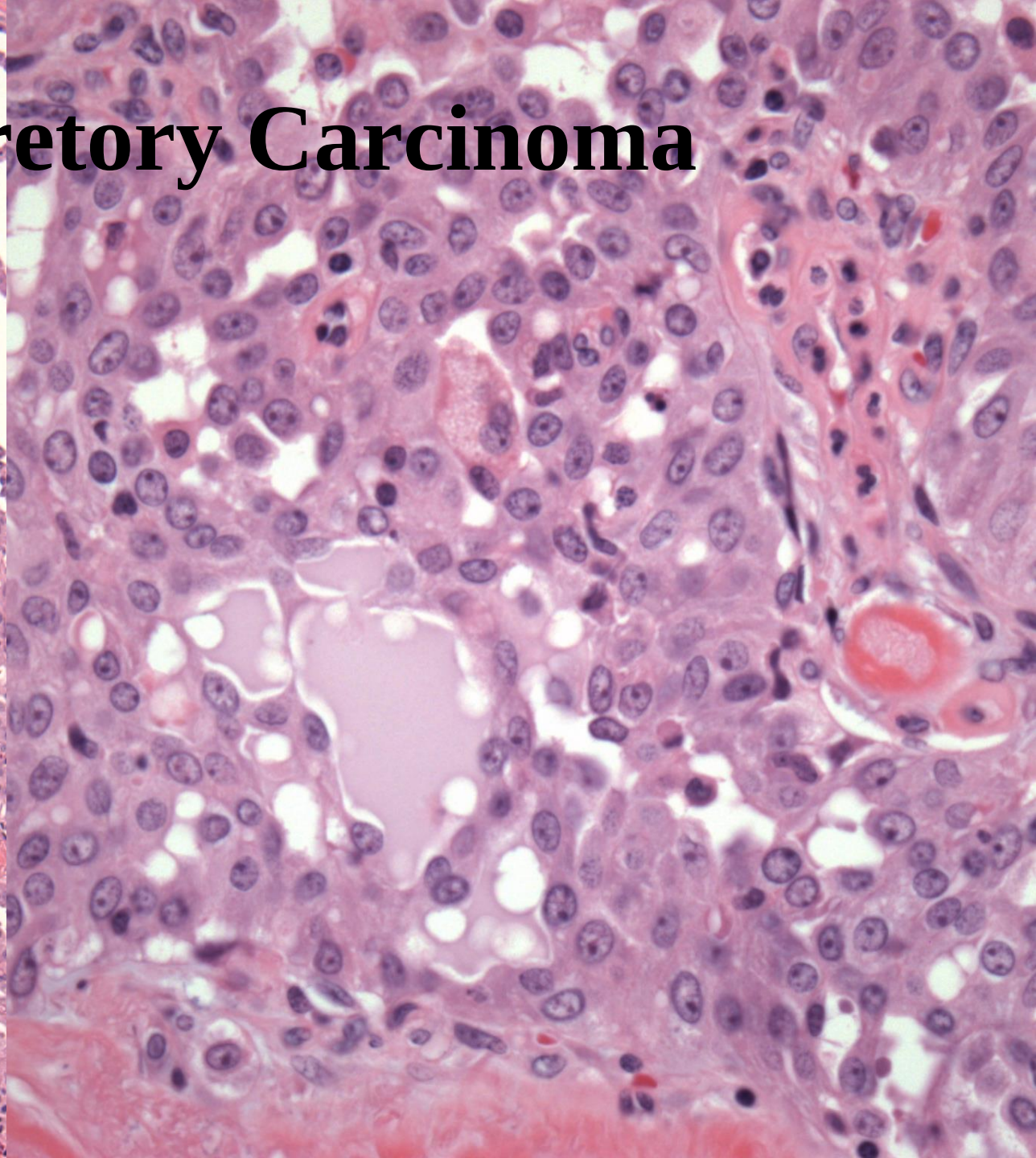
Kinase Fusion-Related Thyroid Cancer
FNA of Secretory Carcinoma





ETV6::NTRK3 Secretory Carcinoma





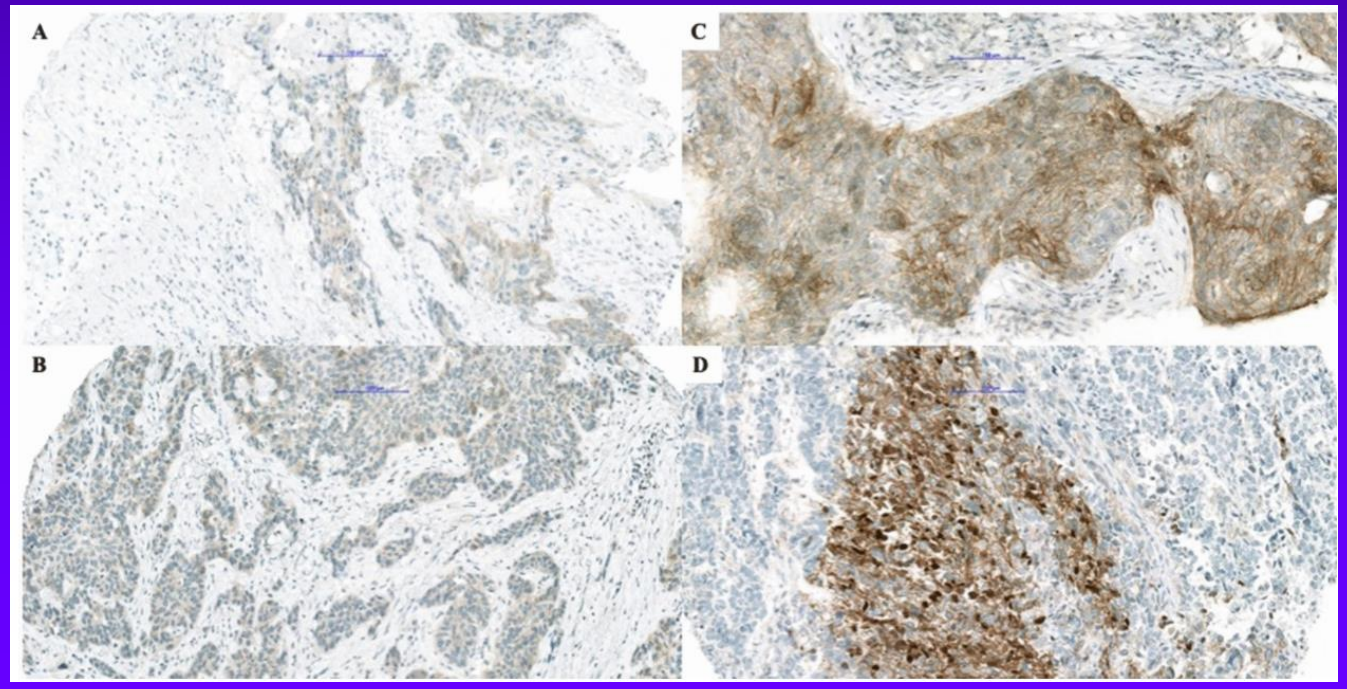
Pan-TRK Immunohistochemistry

Applicability of pan-TRK immunohistochemistry for identification of *NTRK* fusions in lung carcinoma

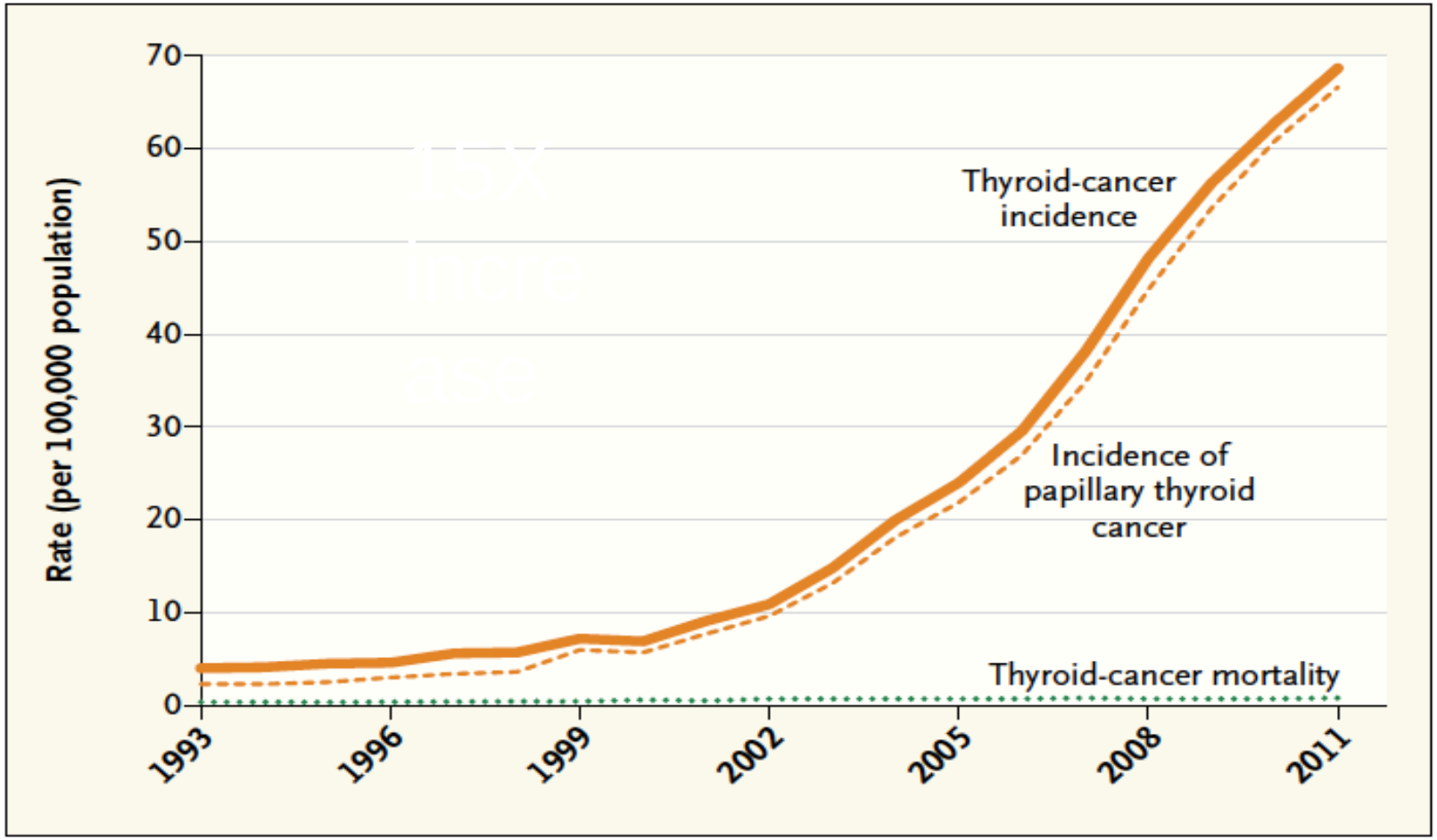
Simon Strohmeier, Iva Brcic, Helmut Popper, Bernadette Liegl-Atzwanger, Jörg Lindenmann & Luka Brcic

2021

- **Useful as a screening for TRK overexpression; molecular is gold standard.**
- **Multiple Ab's including EPR17341 (Ventana and Abcam) and A7H6R (Cell Signaling)**
- **75% sensitivity and 96% specificity**



The Overdiagnosis & Overtreatment of Thyroid Cancer

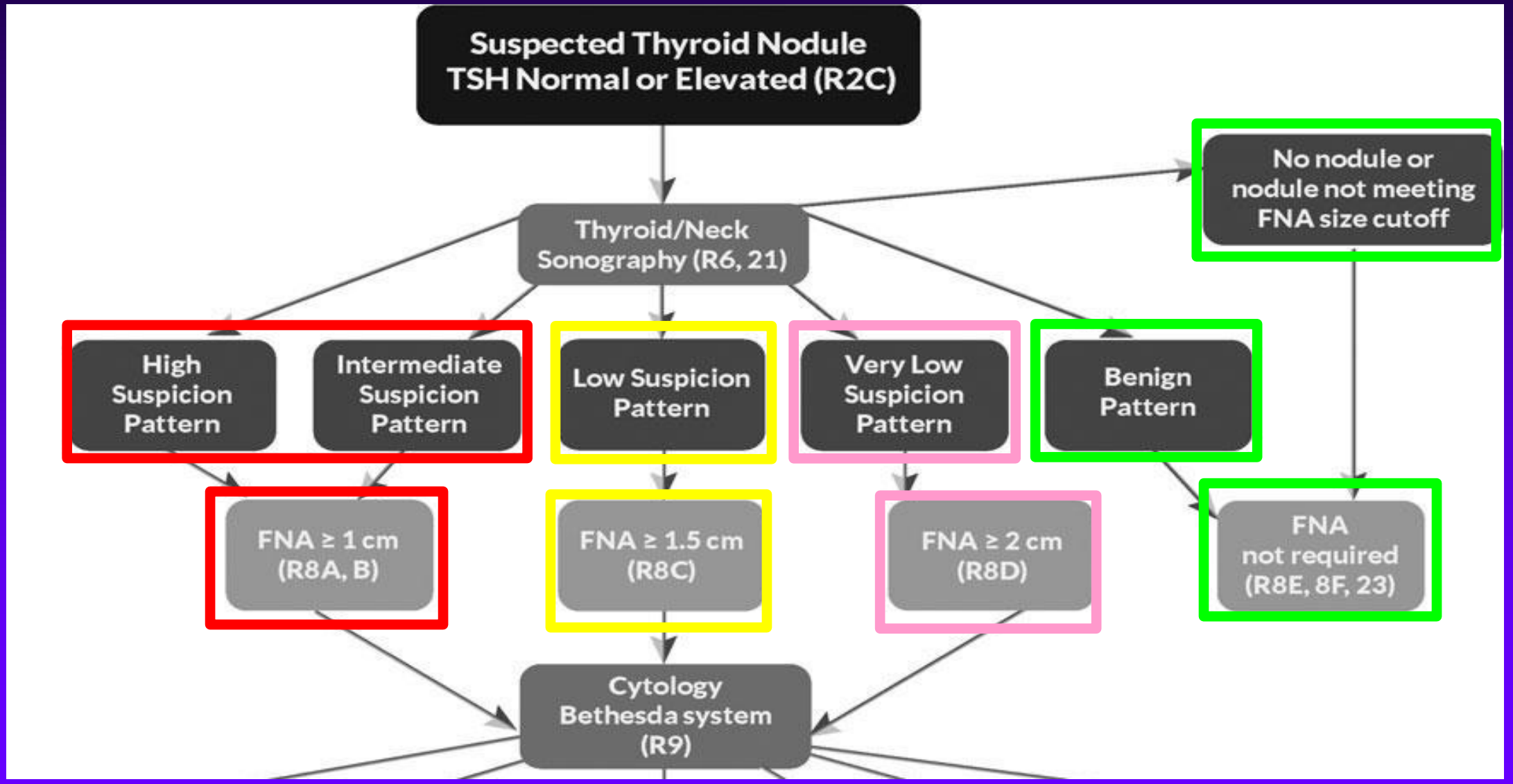


Thyroid-Cancer Incidence and Related Mortality in South Korea, 1993–2011.

Ahn et al N Engl J Med (2014)

NIFTP is one solution…
ATA Guidelines Have Also Helped

FNA-U/S Strategy with TIRADS



Surgical Management for Malignancy: A dramatic change

- **For low-risk papillary or follicular carcinoma (<4 cm) lobectomy is acceptable**

Size	Other	Surgery
<1 cm	cN0, no gross ETE	Lobectomy
>1 cm and <4 cm	cN0, no gross ETE	Lobectomy or bilateral thyroidectomy
>4 cm	N1, gross ETE, M1	Bilateral thyroidectomy

- **Completion thyroidectomy offered if bilateral thyroidectomy would have been recommended had the diagnosis been available before the initial surgery**

Less Radioactive Iodine Therapy

- No benefit for low-risk thyroid cancers
- Minimal rationale for use includes any of the following:
  - >4 cm
  - Microscopic ETE
  - Unfavorable histology (e.g., tall cell, columnar, hobnail PTC, widely invasive FC, HG/PDTC)
  - N1 or >5 micromets (<0.2 cm)
- Lower doses (30 mCi) for low-intermediate risk cancer

So, what is the story behind NIFTP???

And what does the practicing pathologist/cytologist need to know??

Reclassifying Thyroid Cancer

The New York Times | http://nyti.ms/1qrFSYT

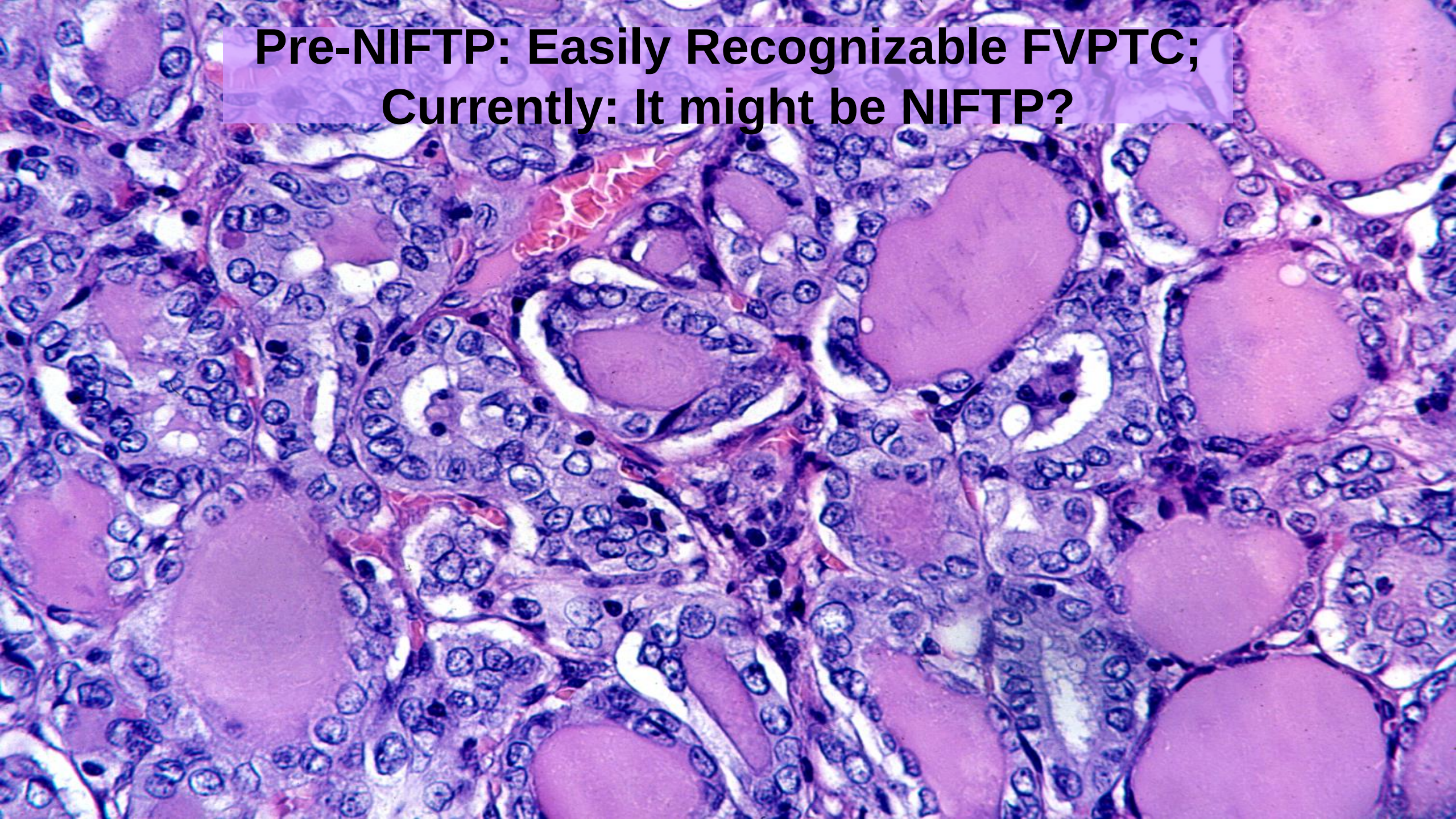
HEALTH

It's Not Cancer: Doctors Reclassify a Thyroid Tumor

By GINA KOLATA APRIL 14, 2016

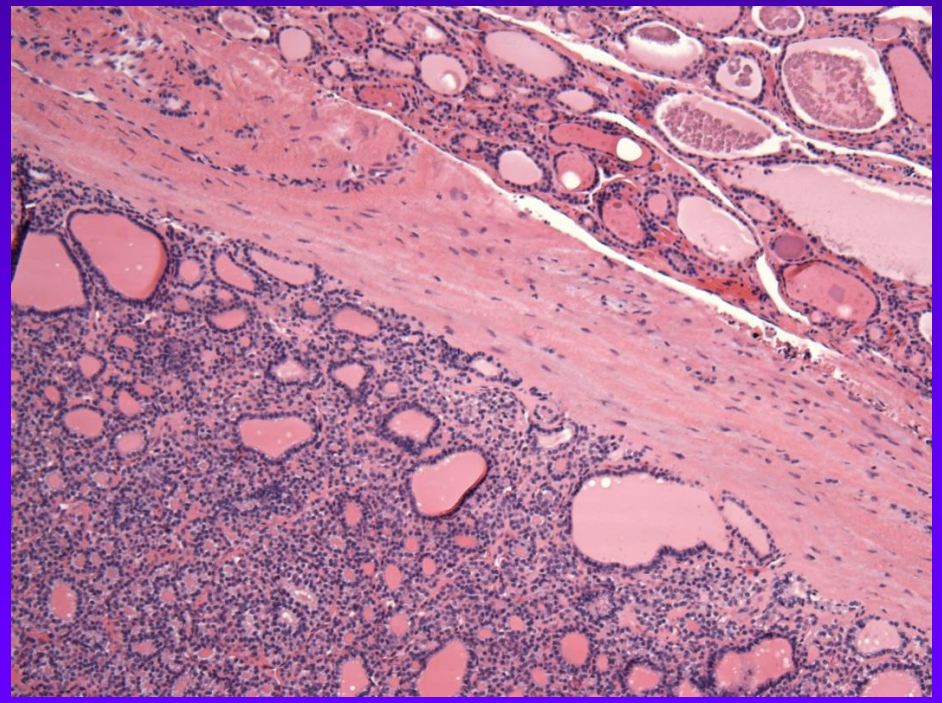
An international panel of doctors has decided that a type of tumor that was classified as a cancer is not a cancer at all.

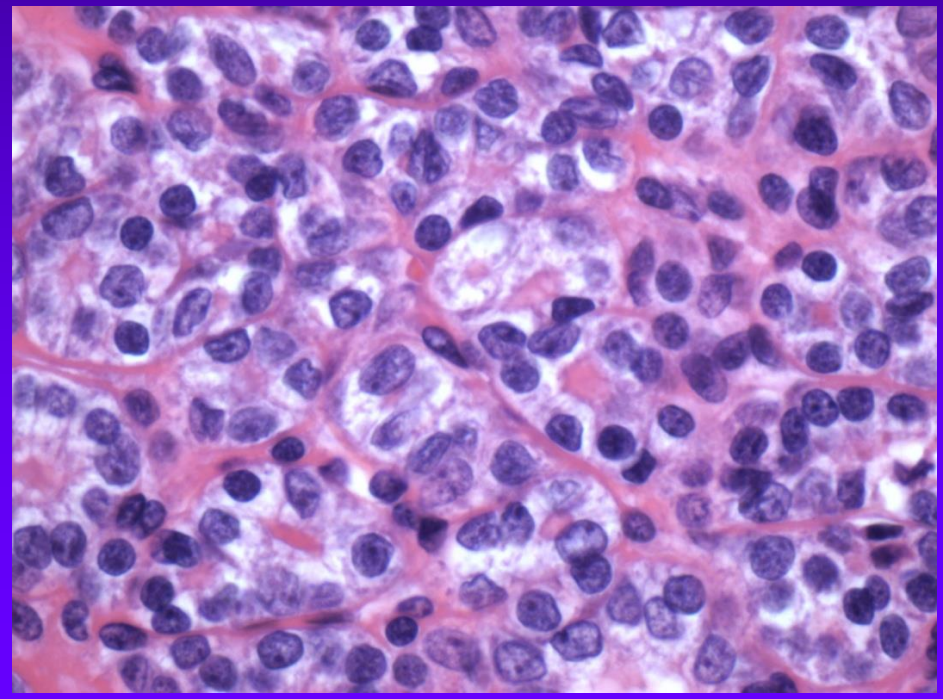
As a result, they have officially downgraded the condition and thousands of patients will be spared removal of their thyroid, treatment with radioactive iodine and regular checkups for the rest of their lives, all to protect against a tumor that was never a threat.


Pre-NIFTP: Easily Recognizable FVPTC;
Currently: It might be NIFTP?

NIFTP

- Solves an important thyroid pathology issue
  - Redefines this set of low-risk cancers as “neoplasms” similar to follicular adenomas, and managed like adenomas –
  - No completion thyroidectomy, and no RAI.





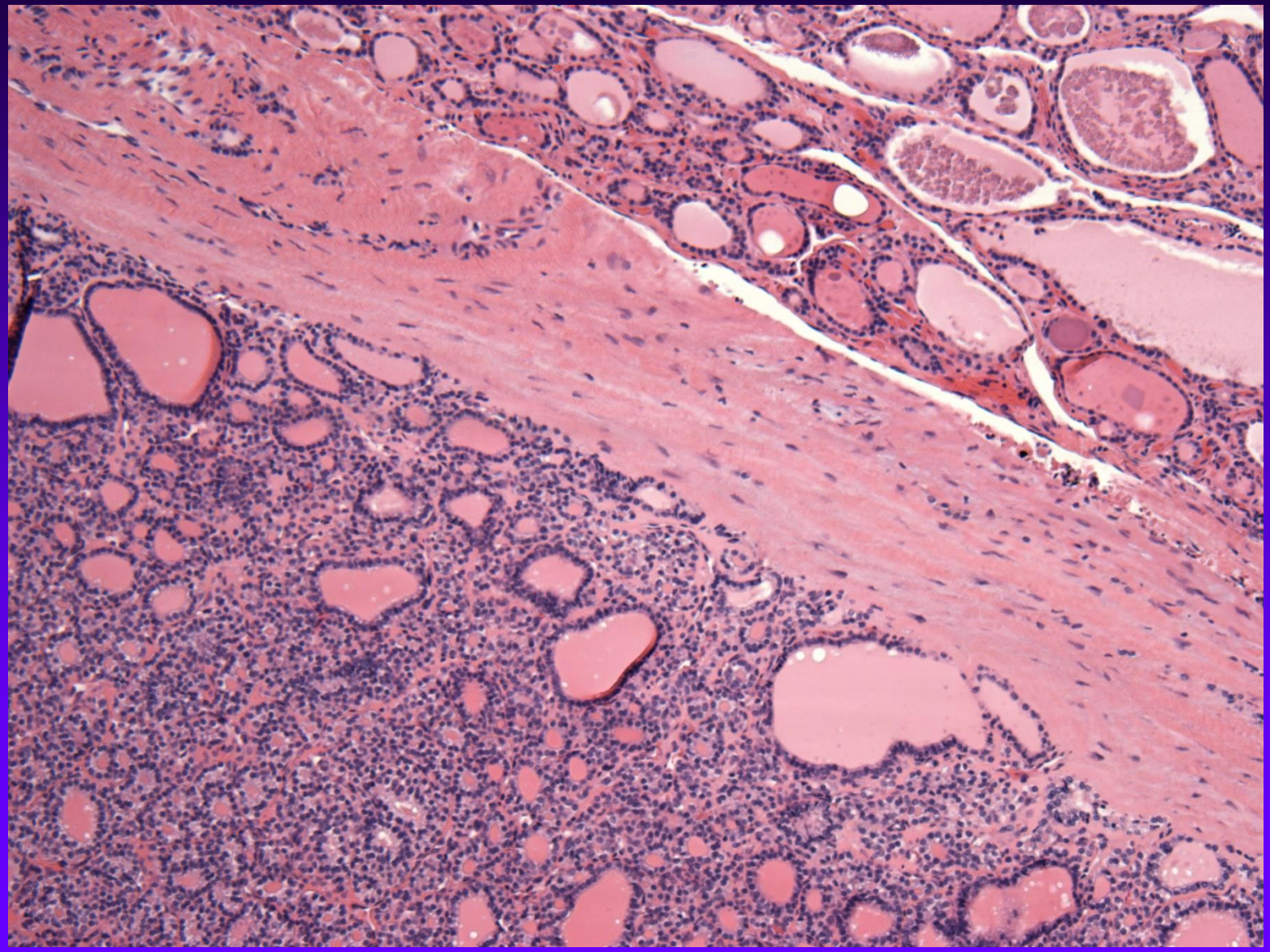
WHO: Histologic Criteria for Diagnosing NIFTP

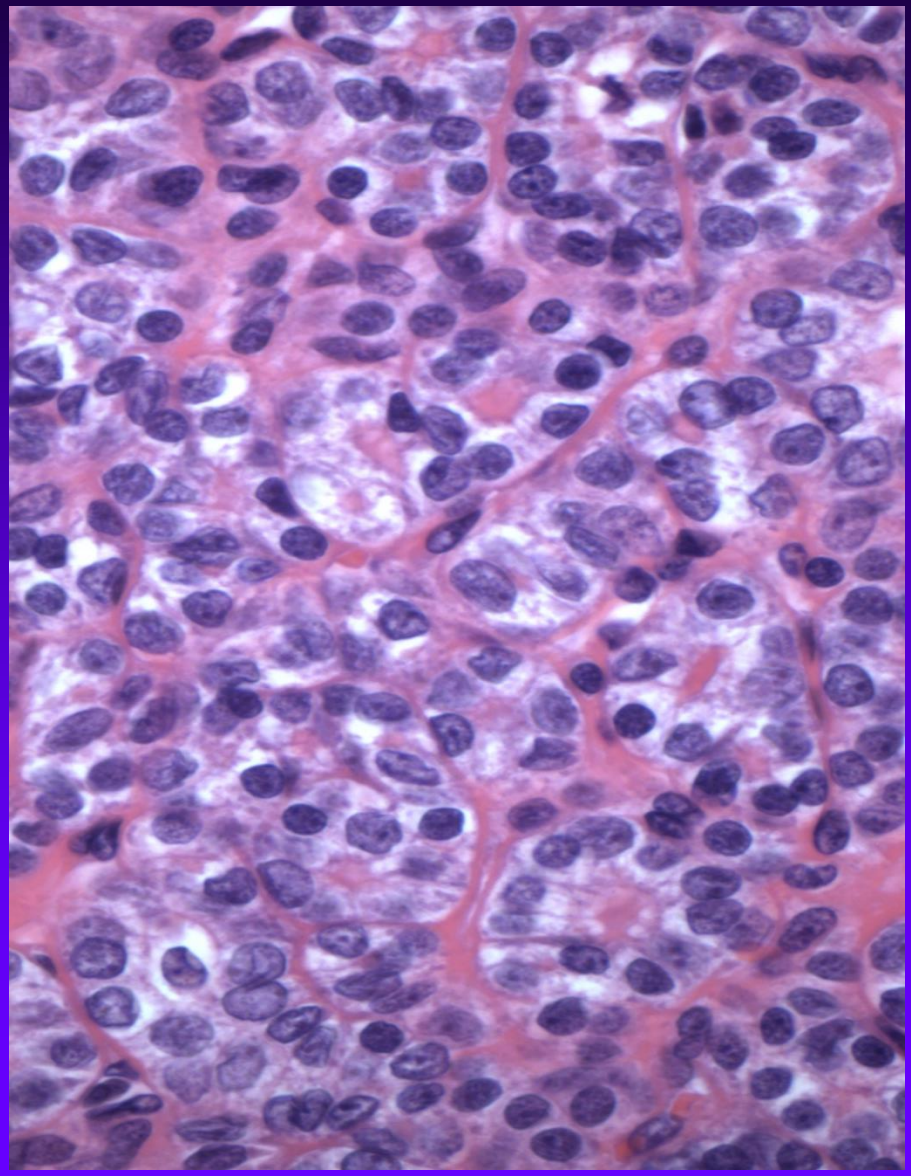
1. Encapsulation or clear demarcation[a]
2. Follicular growth pattern[b] with
   <1% Papillae
   No psammoma bodies
   < 30% Solid/trabecular/insular growth pattern
3. Nuclear score 2-3
4. No vascular or capsular invasion[c]
5. No tumor necrosis
6. No high mitotic activity[d]

Key Implications for Diagnosing NIFTP

- Must submit and evaluate the ENTIRE capsule
- Any cases suggestive of invasive growth should be excluded
- Nuclear atypia should be diffuse or multifocal
- Tumor should be predominantly follicular-patterned
- Oncocytic versions are now recognized by WHO
- Micro (<1 cm) versions are now recognized by WHO

NIFTP





NIFTP Can be Large!

THYROID
Volume 27, Number 4, 2017

DOI: 10.1089/thy.2016.0649

Outcome of Large Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features

Bin Xu,[1] Giovanni Tallini,[2] Theresa Scognamiglio,[3] Benjamin R. Roman,[4] R. Michael Tuttle,[5] and Ronald A. Ghossein[6]

- **79 cases of NIFTP, >4.0 cm (median 4.5 cm)**
- **No LN mets or recurrences**
- **33% had lobectomy alone**
- **Lobectomy alone appears adequate for NIFTP even when large**

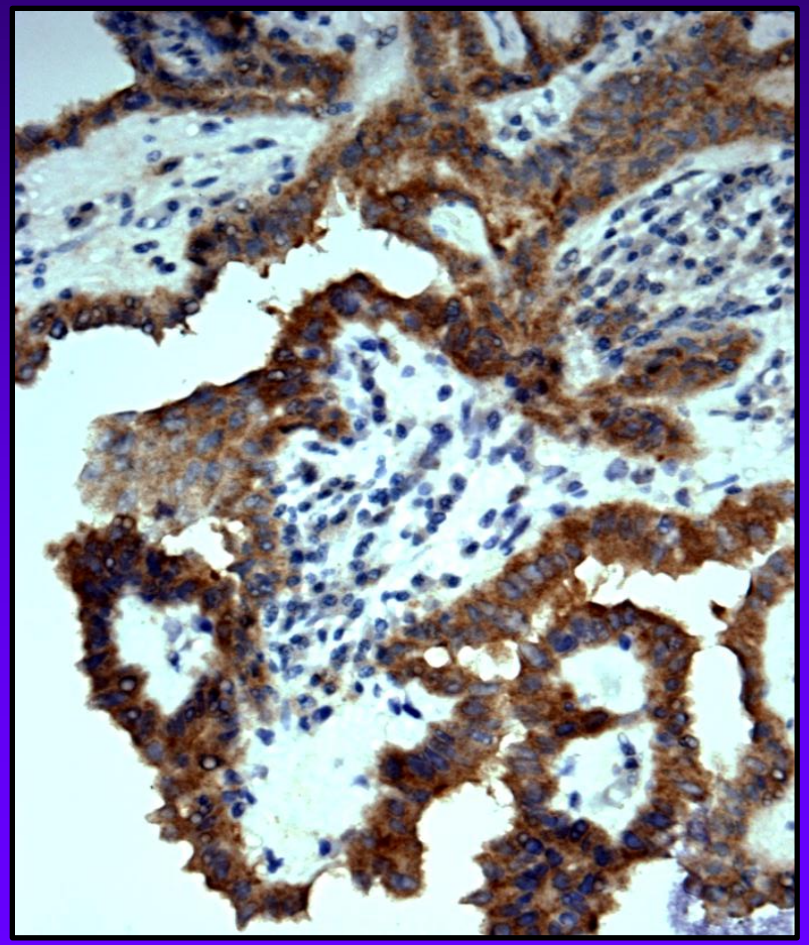
NIFTP: Low-Risk Molecular Profile

- RAS mutations
- BRAF K601E mutation
- PPARgamma fusion
- THADA fusion
- BRAF V600E is absent

$BRAF^{v600}$ IHC: A Very useful Tool

IHC for BRAF

- Shows strong correlation with molecular profile
  - Mouse monoclonal antibody: IHC600
    - Cell Signalling #29002
  - May be useful for small PTCs and for FNA
  - Useful to assess possible NIFTP cases
  - We use it routinely for all of our PTC and NIFTP



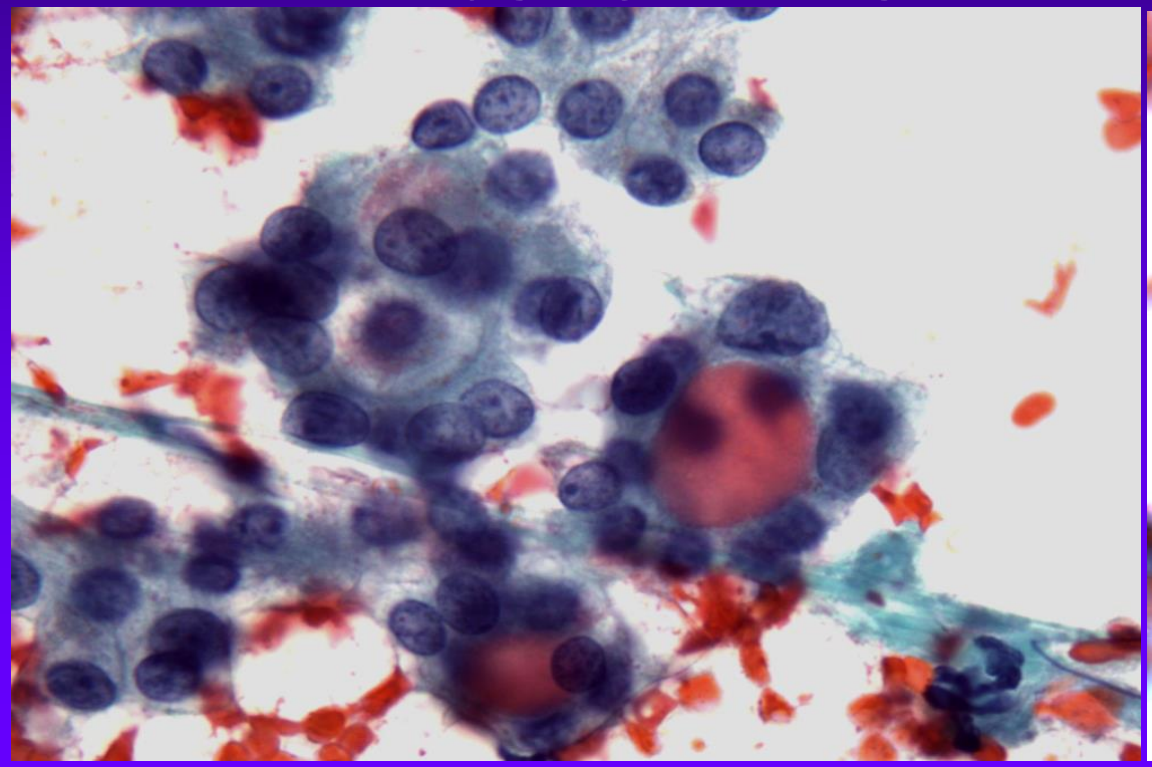
Suggested Revisions to NIFTP

- Cases with 3+ nuclear grade should be entirely sampled to exclude classical PTC features
- Molecular testing to exclude BRAF, TERT, P53, or other high-risk molecular features for grade 3 nuclei cases

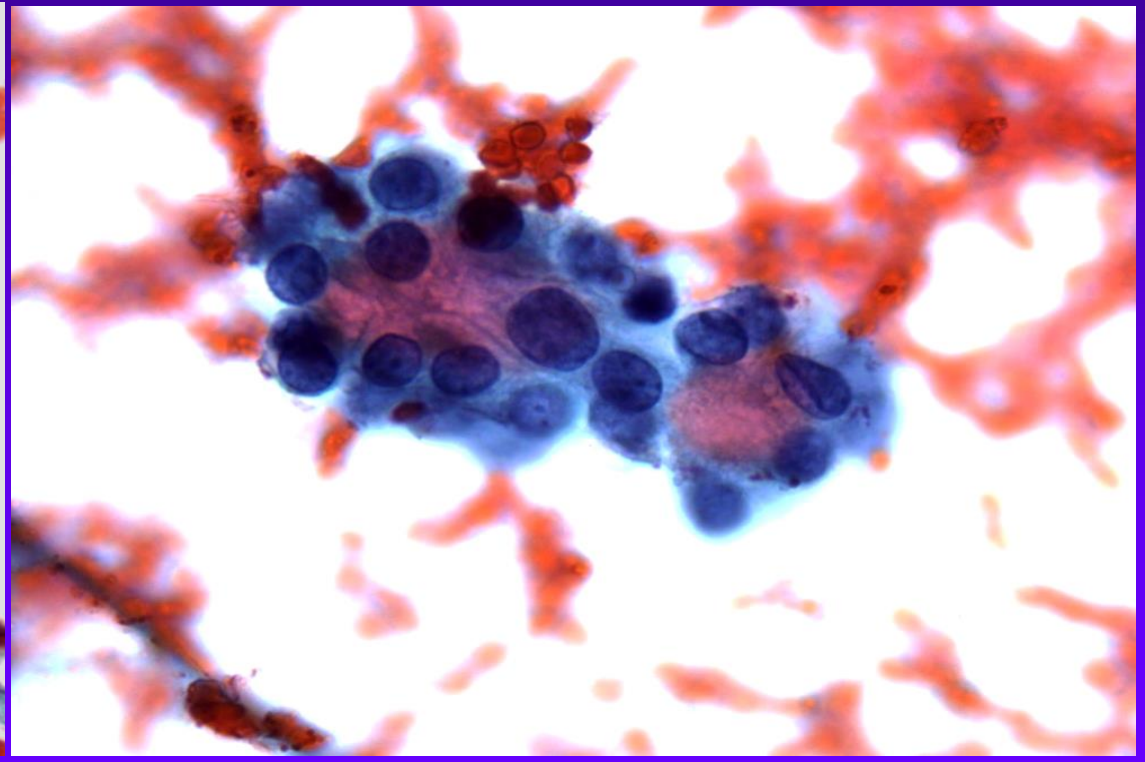
Thyroid FNA: NIFTP has created problems for cytology!

FVPTC vs NIFTP:
Cannot be accurately distinguished by FNA

Invasive FVPTC



NIFTP



3rd Edition Revised ROM Based on NIFTP
ROM is lower when NIFTP is subtracted

Diagnostic category	% Decrease in ROM if excluding NIFTP[a] Ave% (range)	Estimated Final ROM if excluding NIFTP[b] Ave%
Nondiagnostic	**1.3** (0-2)	**12**
Benign	**2.4** (0-4)	**2**
Atypia of Undetermined Significance	**6.4** (6-20)	**16**
Follicular Neoplasm	**7.1** (0.2-30)	**23**
Suspicious for Malignancy	**9.1** (0-40)	**65**
Malignant	**2.6** (0-13)	**94**

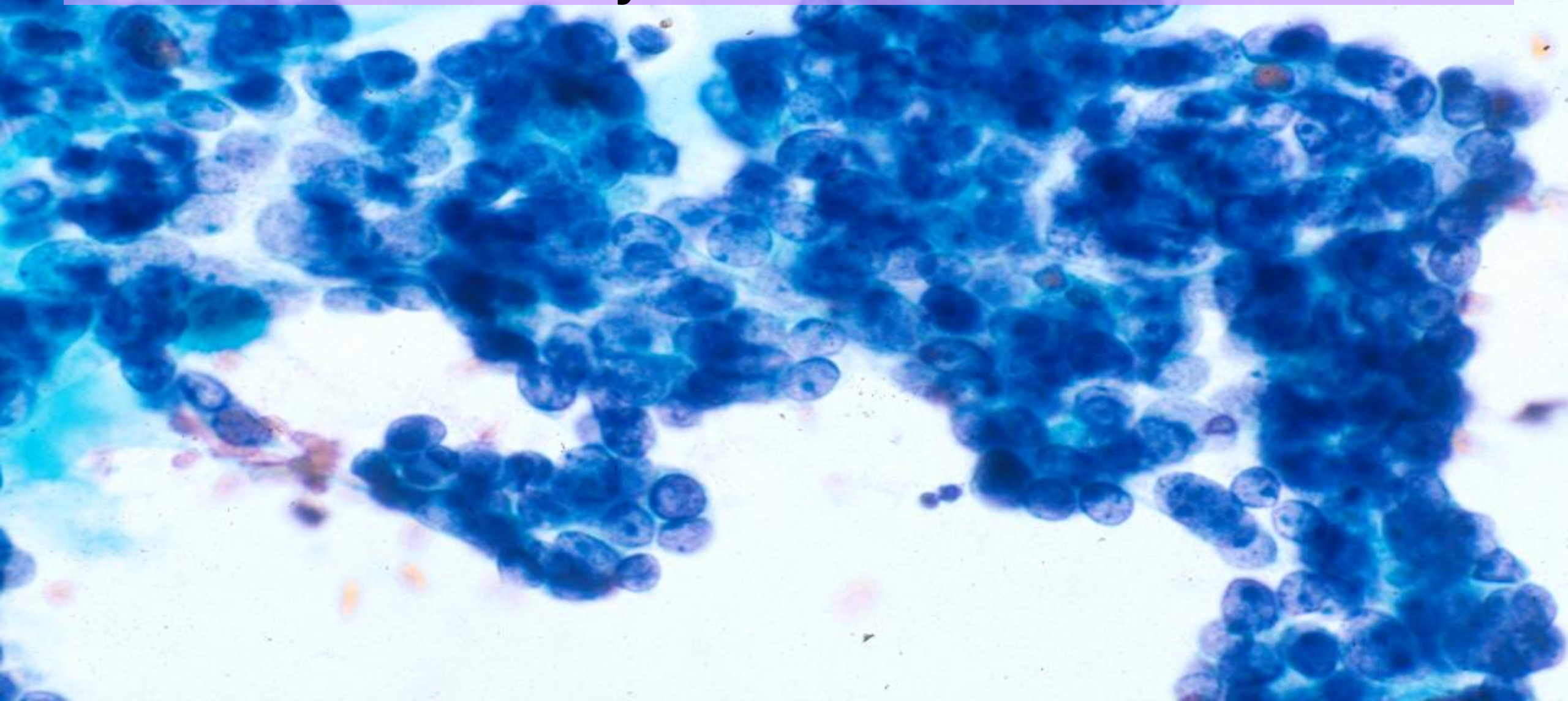
[a] Based on weighted average (mean) reduction in malignancy with expected ranges calculated from refs [21, 23, 70-78]

[b] Based on estimated average ROM values from Table 1.2 minus values presented in this table

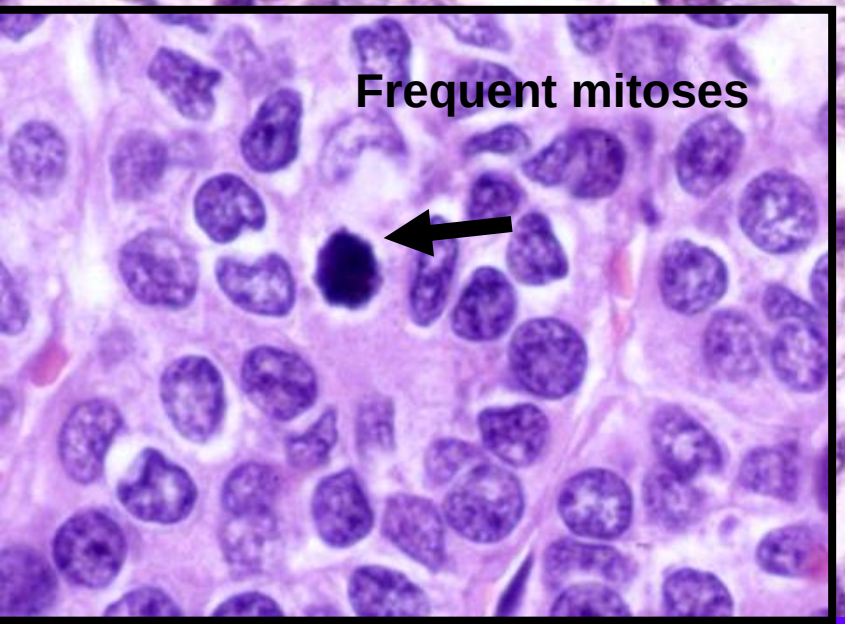
WHO: Follicular-Derived Thyroid Carcinoma with High-Grade Features– i.e. The “Bad” Thyroid Cancer

1) Poorly differentiated thyroid carcinoma (PDTC)
2) Differentiated high-grade thyroid carcinoma (DHGTC)

FNA and Thyroseq: TERT+-RAS+ Poorly Differentiated Thyroid Carcinoma



Poorly Differentiated Carcinoma with Insular Pattern



WHO: Poorly Differentiated Thyroid Carcinoma

- Insular type is the classic form
- Approx. 5-10% of thyroid carcinomas
- Mean survival = 3.9 years
- Metastasis to LN, lung, bone, liver, brain
- Poor prognosis even when encapsulated or focal
- Most have TERT or other “high-risk” mutations

TERT Mutations –

Indicator of Aggressive Cancer?

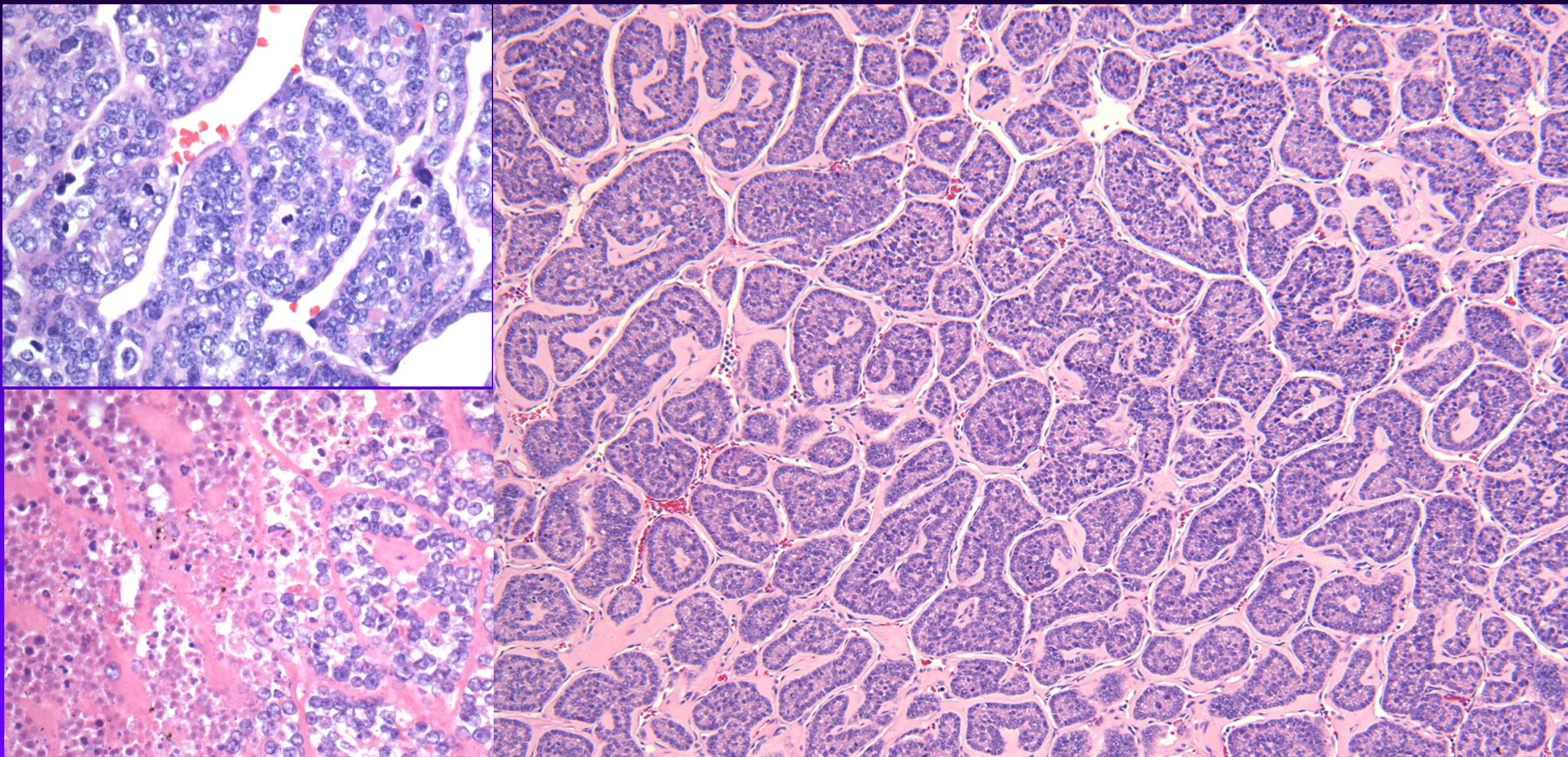
- ***TERT:*** **Telomerase reverse transcriptase**
- ***TERT*** **promoter activating mutations**
  - **Seen in any type of follicular-derived carcinoma**
    - **PTC-7.5%**
    - **Follicular -17.1%**
    - **Poorly differentiated -29.0%**
    - **Anaplastic-33.3%**
  - **Associated with aggressive behavior especially when paired with other mutations such as RAS and BRAF**

Liu et al Endocr Relat Cancer (2013), Landa et al J Clin Endocrinol Metab (2013), Melo et al J Clin Endocrinol Metab (2014)

WHO: Histologic Criteria for PDTC

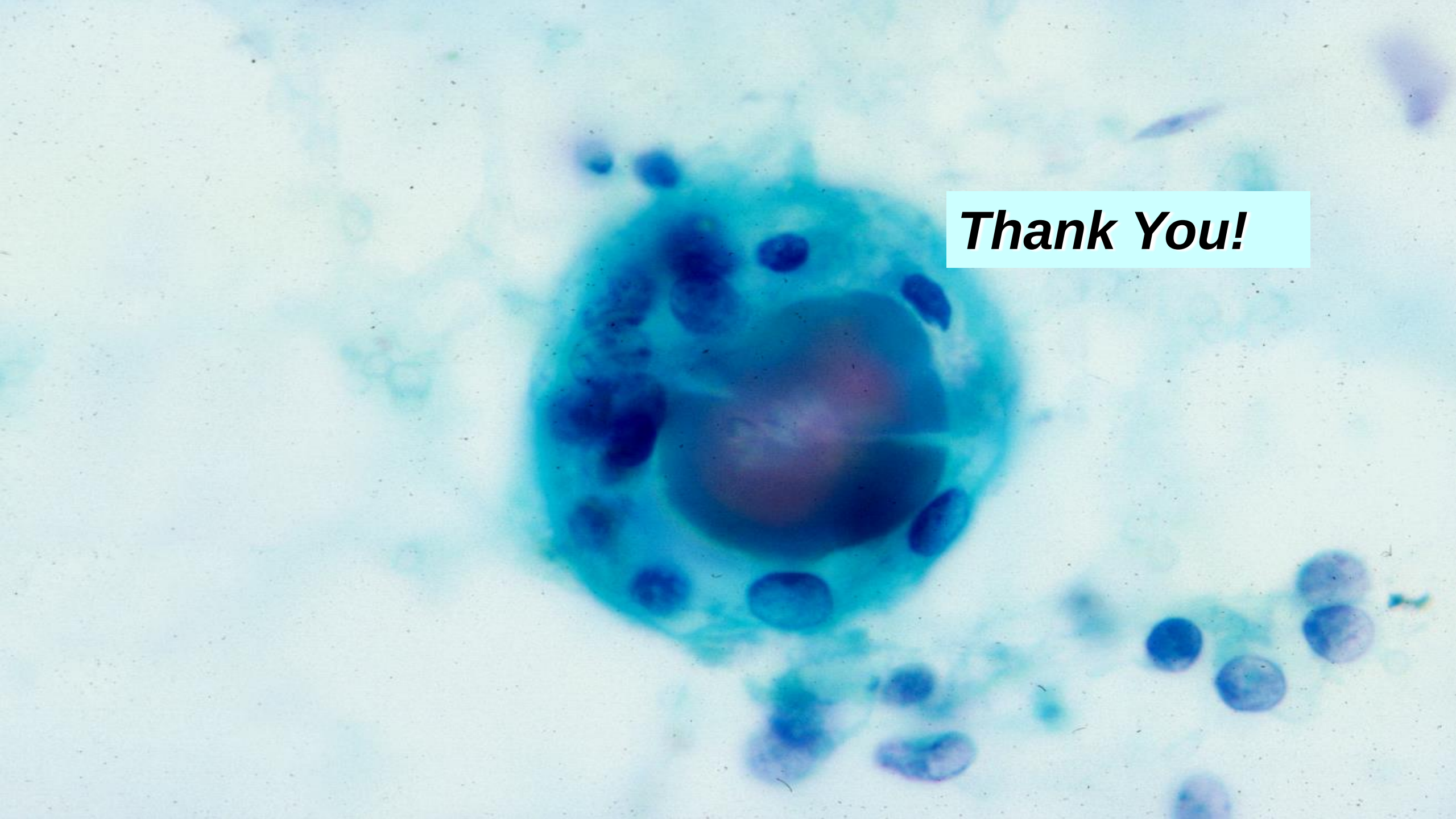
- Malignant follicular neoplasm = invasive
- Solid, trabecular or insular growth pattern
- Absence of conventional nuclear features of papillary carcinoma
- Presence of at least <u>one of the following:</u>
  - Convoluted nuclei
  - Mitotic activity $\geq$ 3 per 2 mm^2
  - Tumor Necrosis
- Absence of anaplastic morphology

PDTC



SUMMARY OF KEY POINTS

- **The Bethesda FNA System has revolutionized thyroid management**
- **The 3rd Edition is in sync with WHO updates and has improved ROMs**
- **Molecular testing paired with FNA can be helpful when used judiciously!**
  - **Beware of tumors with high-risk mutations, double mutations, and certain gene fusions!**


Thank You!