MOLECULAR TECHNIQUES ON CYTOLOGY: READY FOR PRIME TIME?

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CYTOLOGY IS THE MOST INTRIGUING DIAGNOSTIC CHALLENGE OF THE 21st CENTURY

APPLICATIONS OF CYTOLOGY

- SCREENING
- PREVENTION
- DIAGNOSIS
- PROGNOSTIC PARAMETERS
- PREDICTION OF THERAPEUTIC RESPONSE
- FOLLOW-UP

DRAWBACKS OF THYROID CYTOLOGY

Amount of inadequate diagnoses which may sometimes exceed 15%.

Unpredictable rate of indeterminate diagnoses (AUS-FN) which may represent as many as 25% of all thyroid diagnoses.

SIAPEC/IAP CLINICAL ITALIAN CONSENSUS ON THE CLASSIFICATION OF THYROID LESIONS ON FNAB (L'ENDOCRINOLOGO, 2008, PATHOLOGICA, 2010)

DIAGNOSTIC CODE	DIAGNOSTIC CATEGORY	HISTOLOGIC CORRESPONDANCE
TIR 1 (Thy 1 BTA/ RCPath)	Nondiagnostic/ non representative	Inadequate. Cyst/hemorrhage
TIR 2 (Thy 2 BTA/ RCPath)	Negative for malignant cells	Nodular goiter; thyroiditis
TIR 3 (Thy 3 BTA/ RCPath)	Inconclusive/indeterminate (follicular proliferation)	Follicular adenoma; Hurthle cell neoplasm; follicular carcinoma; follicular variant of papillary carcinoma
TIR 4 (Thy 4 BTA/ RCPath)	Suspicious of malignancy	Follicular variant of papillary carcinoma
TIR 5 (Thy 5 BTA/ RCPath)	Diagnostic of malignancy	Malignant neoplasia

THE BETHESDA CLASSIFICATION (Baloch ZW et al. Diagn Cytopathol 2008)

Roma,
9-11 novembre 2012

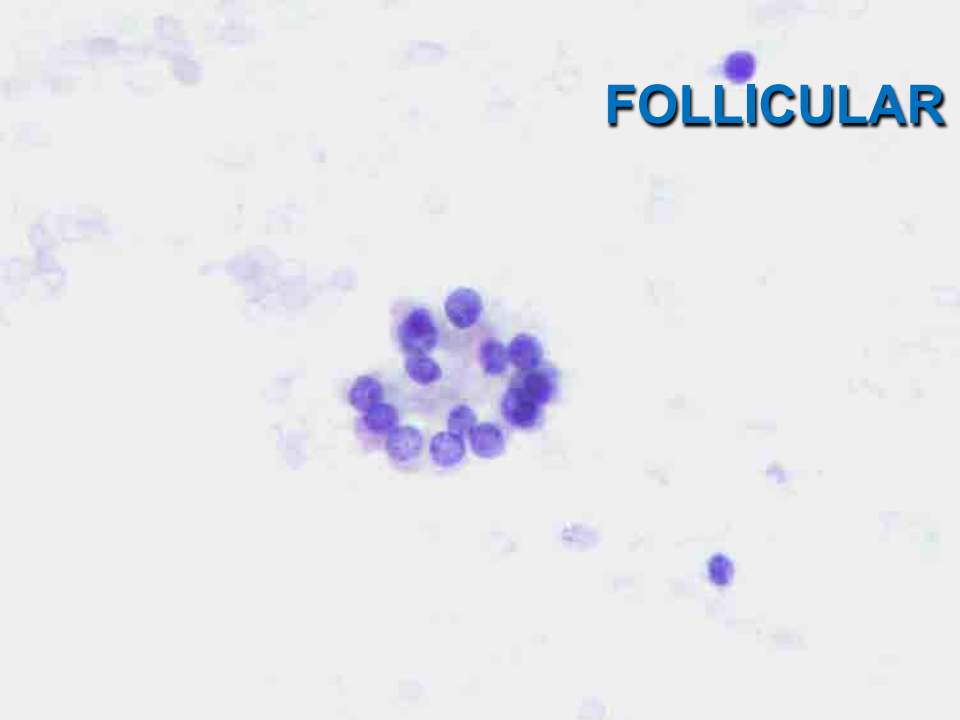
Suggested Categories	*Alternate Term (s)**	*Risk of Malignancy***
Non-diagnostic (TIR 1)	Unsatisfactory	
Benign (TIR 2)		<1%
Indeterminate Follicular lesion	Atypical cells of undetermined significance (ACUS)	5-10%
Neoplasm (TIR/Thy 3) 1. Follicular Neoplasm 2. Hurthle cell Neoplasm	Suspicious for: 1. Follicular Neoplasm 2. Hurthle cell Neoplasm	20-30%
Suspicious for Malignancy (TIR 4)		50-75%
Malignant (TIR 5)		100%

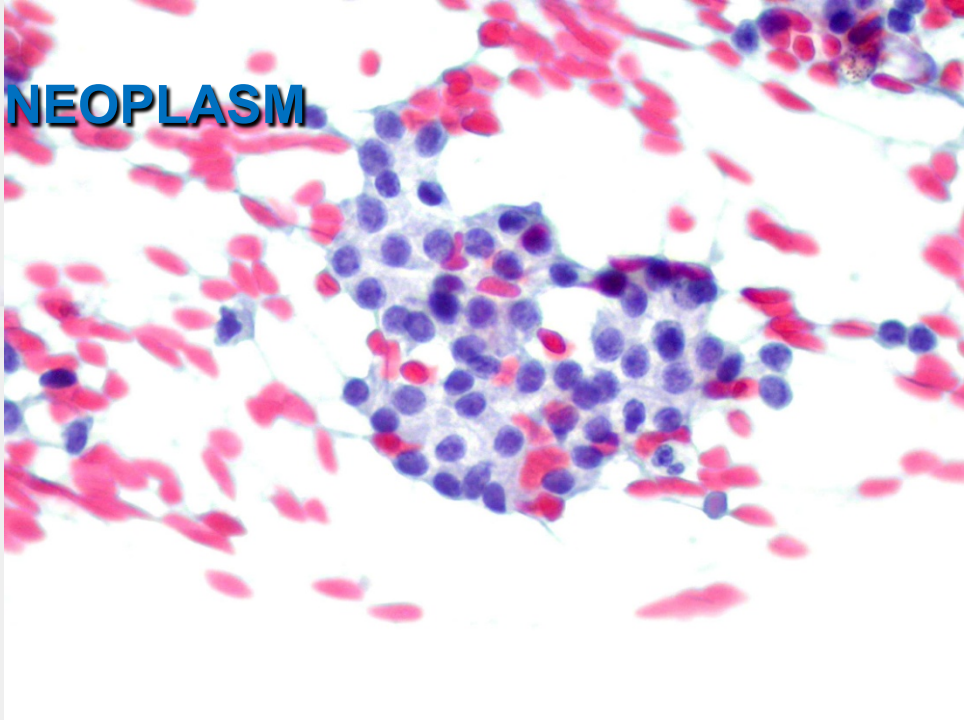
*These terms can be used instead of the suggested terms (based on website responses and NCI meeting attendees); ** Data collected from literature (from Baloch ZW, modified)

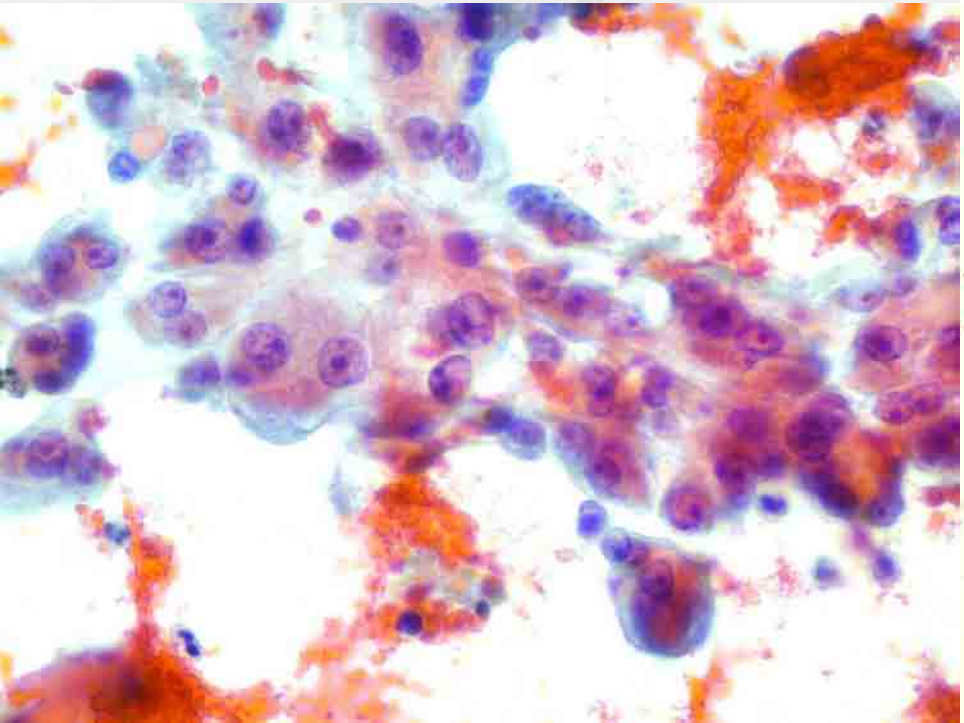
TIR 3: FOLLICULAR NEOPLASM/ HURTHLE CELL NEOPLASM

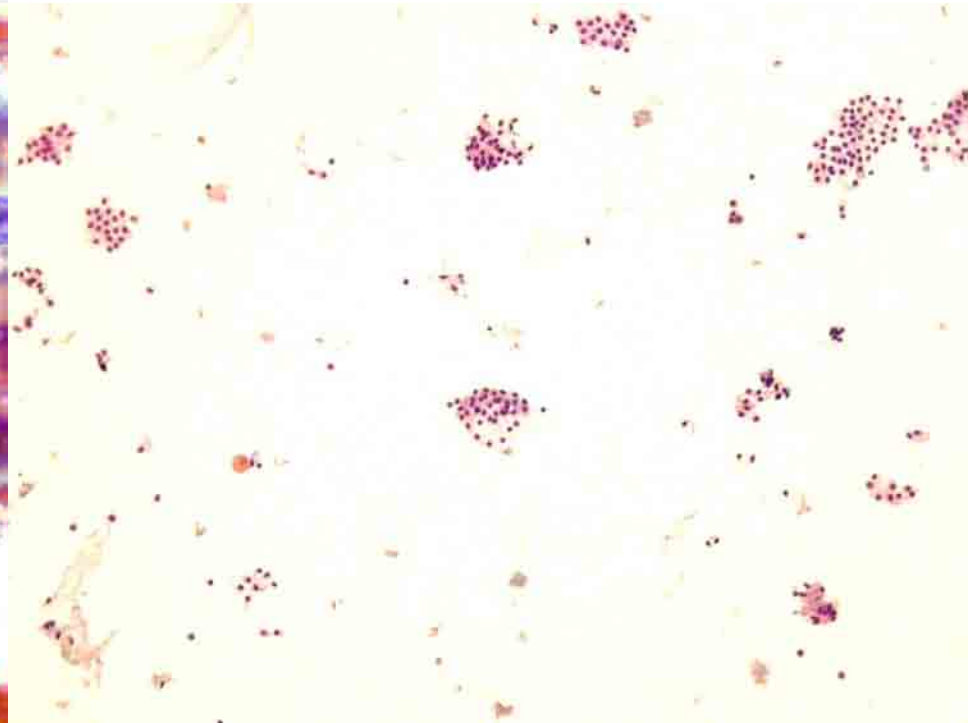
- Two subcategories have been recently introduced (TIR 3A corresponding to the FLUS/AUS of TBS and TIR 3B)
- The morphologic criteria are less reproducible
- Action: Follow-up for low-risk lesions (TIR 3A) and surgery for high-risk nodules (TIR 3B)
- Molecular techniques may be helpful in identifying subgroups with different risk of malignancy

FOLLICULAR NEOPLASM





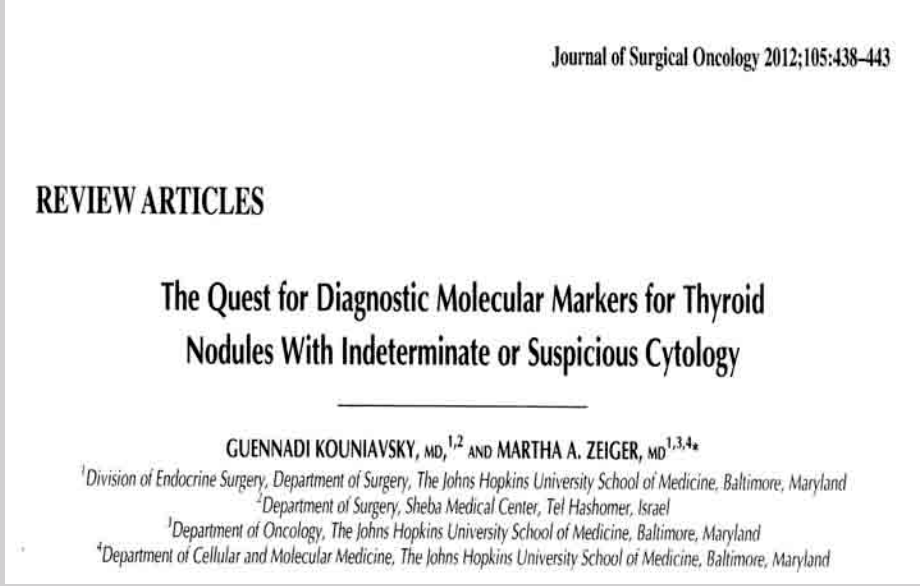




Is morphology alone enough?

HOW CAN WE IMPROVE THE DIAGNOSTIC ACCURACY OF THYROID FNAB?

MOLECULAR MODELS



Journal of Surgical Oncology 2012;105:438–443

REVIEW ARTICLES

The Quest for Diagnostic Molecular Markers for Thyroid Nodules With Indeterminate or Suspicious Cytology

GUENNADI KOUNIAVSKY, MD,[1,2] AND MARTHA A. ZEIGER, MD[1,3,4]*

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[2]Department of Surgery, Sheba Medical Center, Tel Hashomer, Israel
[3]Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland
[4]Department of Cellular and Molecular Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland

PROTEIN-BASED ASSAYS

- Protein-based panels

DNA-BASED STUDIES

- Panels of molecular markers

RNA-BASED STUDIES

- UbcH10
- HMGA2
- Alternative splicing patterns
- MicroRNA (including miRNA-based panels)
- RNA-based panels

Table 4 Distribution of markers used in preoperative FNA of thyroid by other methods for detection of marker and the average values of sensibility, specificity, positive predictive value, negative predictive value, diagnose accuracy obtained.

Marker	Method	Number of experiments	Average SN	Average SP	Average PV +	Average PV -	Average AC
BRAF	Nucleic acids extraction and PCR	26	52.35	97.92	99.85	51.62	70.54
RET		11	18.20	88.73	87.00	59.60	55.30
RAS		5	23.00	97.20	82.20	63.20	65.00
HMGA2		2	75.00	96.00	94.00	83.50	87.50
MUC-1		2	74.50	95.50	91.50	85.50	87.50
GAL3		1	100.00	17.00	44.00	100.00	50.00
FIBRONECTIN		1	81.00	100.00	100.00	63.00	89.00
HMGI		1	100.00	100.00	100.00	100.00	100.00
FRA-1		1	100.00	25.00	57.00	100.00	62.00
TELOMERASE	Nucleic acids extraction and PCR for hTERT gene expression	3	84.00	63.00	73.00	79.00	74.00
	TRAP PCR-ELISA	4	52.30	81.00	77.00	72.00	68.00
DAP IV	Cytoenzymology	2	91.00	78.50	74.00	92.50	83.50
	Nucleic acids extraction and PCR	1	87.00	33.00	46.00	80.00	55.00
PPARgamma	FISH	1	20.00	100.00	100.00	46.00	60.00

SN, sensibility; SP, specificity; PV +, predictive positive value; PV -, predictive negative value; AC, accuracy; PCR, Polymerase chain reaction; ELISA, Enzyme-Linked Immunoabsorbent Assay; hTERT, Human Telomerase Reverse Transcriptase; TRAP, Telomere Repeat Amplification Protocol; FISH, Fluorescence *in situ* hybridization.

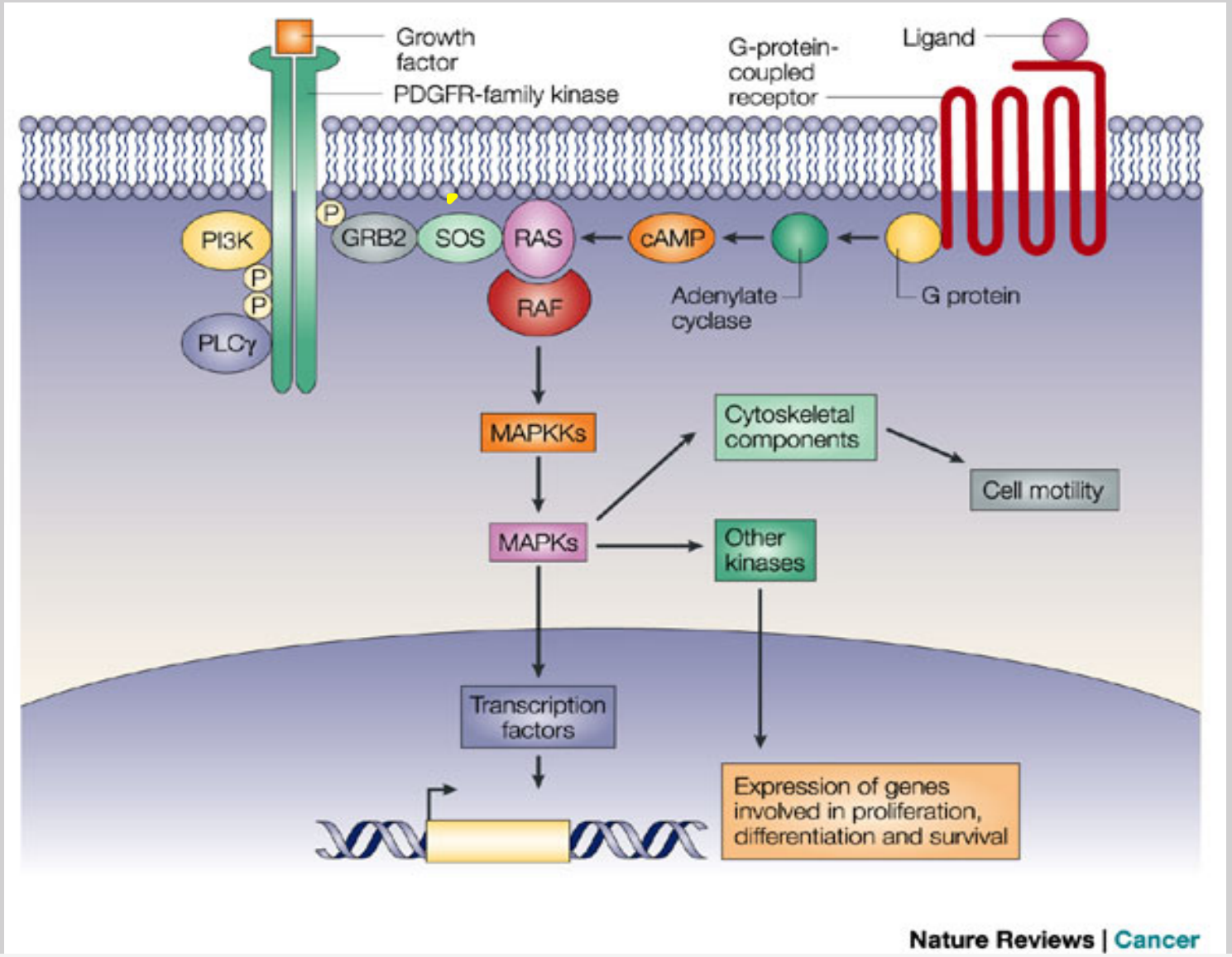
Correia Rodrigues* and *coll. Endocr Journal 2012; 59: 417-424

BACKGROUND

The diagnostic application of molecular biology is one of the most intriguing challenge of these last years

***BRAF, RET/PTC* and *RAS* mutations are mutually exclusive and are found in approximately 80% of thyroid neoplasms and in more than 70% of PC but only in 20% of the follicular variants of PC**

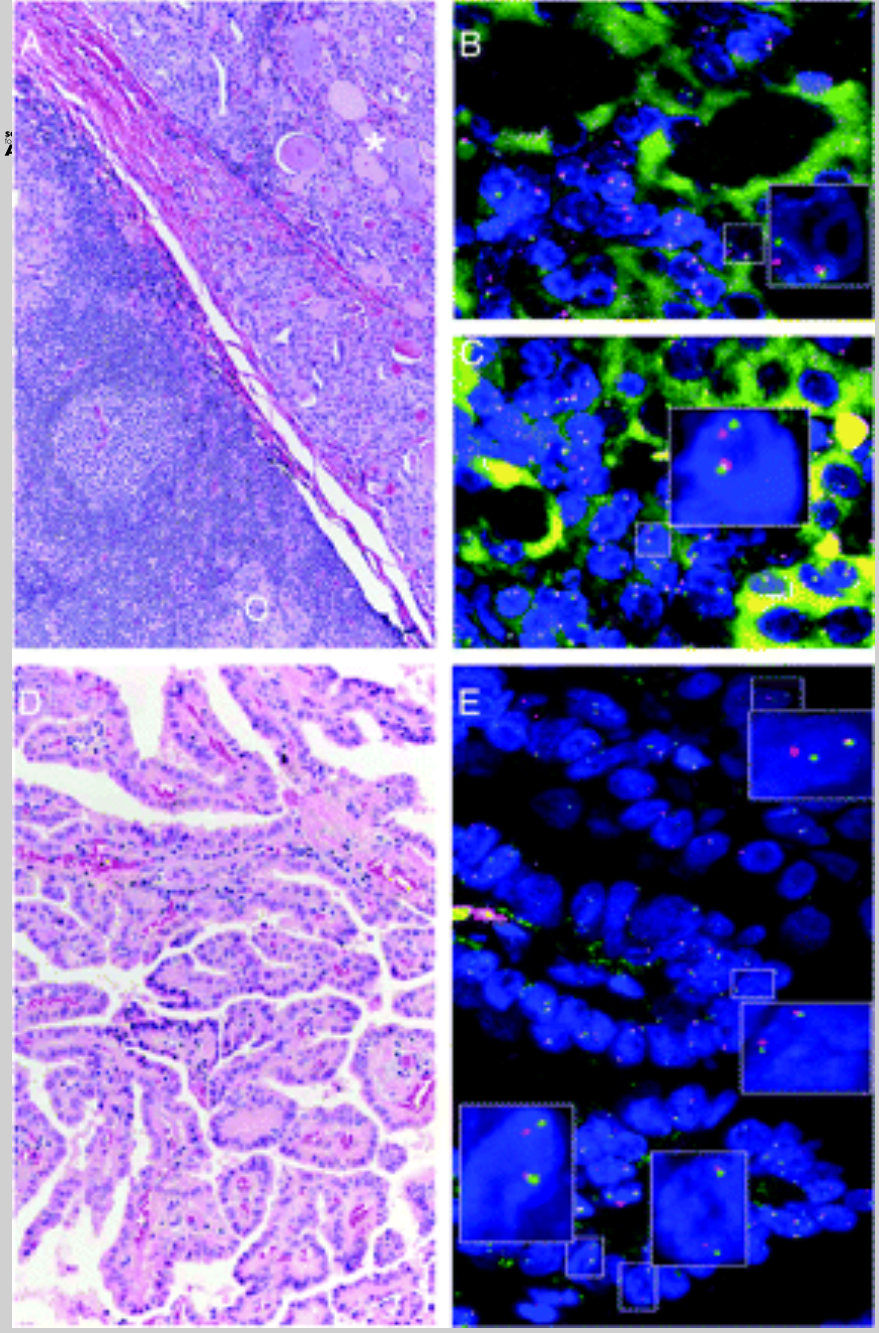
They may represent diagnostic markers applied to FNAB samples



Courtesy Dr. Maurizio Martini-Catholic University Rome

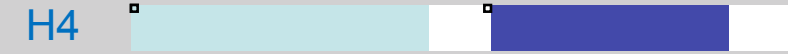
RET/PTC

Roma,
9-11 novembre 2012



Wild-type ret

***RET*/PTC-1**



***RET*/PTC -2**

***RET*/PTC -3**

Rhoden KJ, Unger K, Salvatore G et al. JCEM 2006; 91: 2414-2423

Courtesy of Dr.S. Ezzat, Univ. of Toronto


BRAF WORLD AND FNAB

CLINICAL SIGNIFICANCE OF BRAF MUTATIONS

- **BRAF V600E is the most prevalent oncogenic mutation in PTC**
- **BRAF V600E mutation is an early event in thyroid tumorigenesis (Puxeddu, JCEM, 2004) and is observed in 39% of microPTC (Lupi, JCEM, 2007)**
- **BRAF mutations are mostly related to a more aggressive tumor behaviour (extracapsular invasion, lymphnode metastases and a worst outcome of patients with PTC, Basolo et al, JCEM 2008)**

ROLE OF BRAF MUTATIONAL ANALYSIS

- **BRAF Analysis**

DIAGNOSIS OF CARCINOMA

PROGNOSIS

ORIGINAL ARTICLE
Endocrine Care

Molecular Testing for Mutations in Improving the Fine-Needle Aspiration Diagnosis of Thyroid Nodules

Yuri E. Nikiforov, David L. Steward, Toni M. Robinson-Smith, Bryan R. Haugen, Joshua P. Klopper, Zhaowen Zhu, James A. Fagin, Mercedes Falciglia, Katherine Weber, and Marina N. Nikiforova

JCEM 2009; 94: 2092-2098

Original Article

Contribution of Molecular Testing to Thyroid Fine-Needle Aspiration Cytology of "Follicular Lesion of Undetermined Significance/Atypia of Undetermined Significance"

N. Paul Ohori, MD[1]; Marina N. Nikiforova, MD[1]; Karen E. Schoedel, MD[1]; Shane O. LeBeau, MD[2]; Steven P. Hodak, MD[2]; Raja R. Seethala, MD[1]; Sally E. Carty, MD[3]; Jennifer B. Ogilvie, MD[3]; Linwah Yip, MD[3]; and Yuri E. Nikiforov, MD, PhD[1]

Cancer Cytopathol 2010; 118: 17-23

TABLE 2. SPECIFICITY OF *BRAF* DETECTION IN THYROID FINE-NEEDLE ASPIRATION SAMPLES

	Samples (n)	BRAF *positive*	*Final diagnosis in* BRAF-*positive samples*
Thyroid nodule FNA, prospective studies	1814	159	PTC = 159 (100%)
Thyroid nodule FNA, retrospective studies	685	291	PTC = 291 (100%)
Research FNA of surgically removed thyroid	267	131	PTC = 130 (99.2%) HN[a] = 1 (0.8%)
Total	2766	581	PTC = 580 (99.8%) HN[a] = 1 (0.2%)

[a]HN reported as atypical nodular hyperplasia (91).
FNA, fine-needle aspiration; HN, hyperplastic nodule; PTC, papillary thyroid carcinoma.

TABLE 3. ROLE OF MOLECULAR TESTING OF FINE-NEEDLE ASPIRATION WITH INDETERMINATE CYTOLOGY IN REFINING CANCER PROBABILITY IN THYROID NODULES

Category of indeterminate cytology	*Molecular testing result*	*Cancer probability (%)*
Follicular lesion of indeterminate significance ($n = 21$)	Mutation positive ($n = 3$)	100
	Mutation negative ($n = 18$)	0
Follicular of Hürthle cell neoplasm ($n = 23$)	Mutation positive ($n = 9$)	100
	Mutation negative ($n = 14$)	21
Suspicious for malignancy ($n = 7$)	Mutation positive ($n = 3$)	100
	Mutation negative ($n = 4$)	50
Total ($n = 51$)	Mutation positive ($n = 15$)	100
	Mutation negative ($n = 36$)	14

Based on the data reported by Nikiforov *et al.* (74).

Nikiforova MN and Nikiforov YE Thyroid 2009

How can we break through these negative mutational results?

2010

- 1,402 THYROIDECTOMIES
- 3,071 THYROID FNABs (>90% LIQUID-BASED CYTOLOGY)
- 102 MALIGN. NEOPL. (3,2%)

2001-2010

- 12,293 THYROIDECTOMIES
- 22,762 THYROID FNABs (>60% LIQUID-BASED CYTOLOGY)
- 643 MAL. NEOPL. (2,8%)

2010-2012

270 *BRAF* analysis on LBC thyroid FNAB

IMMUNOCYTOCHEMICAL PANEL (HBME-1 AND GALECTIN-3) + BRAF ANALYSIS ON INDETERMINATE CASES (TIR 3) PROCESSED BY LIQUID-BASED CYTOLOGY

ICC panel (HBME-1 and Gal-3) and BRAF analysis in Follicular Neoplasms (Fadda et al. USCAP Abs. 358 Mod Pathol 2012; 25: 88A)

TIR 3 (FN/SFN)

NPV ICC: 100%

	ICC -	ICC +	BRAF -	BRAF+
BENIGN (9)	9	0	9	0
PTC (3)	0	3	3	0
FVPC (8)	0	8	8	0

Comparison between immunocytochemistry (ICC) and BRAF analysis with the histological outcome for SM/TIR 4 (SIAPEC Firenze 2012)

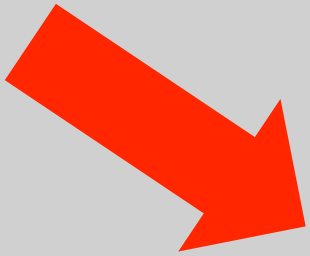
Roma,
9-11 novembre 2012

	ICC(-)	ICC(+)	BRAF (-)	BRAF (+)
BL (7)	7	0	7	0
PTC (20)	1	19	6	14
FVPC (10)	3	7	9	1

ROLE OF BRAF MUTATIONAL ANALYSIS

- BRAF Analysis

DIAGNOSIS OF CARCINOMA



PROGNOSIS

J Clin Endocrin Metab. First published ahead of print July 14, 2010 as doi:10.1210/jc.2010-0337

SPECIAL FEATURE

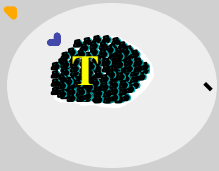
Extensive Clinical Experience

Correlation between the *BRAF* V600E Mutation and Tumor Invasiveness in Papillary Thyroid Carcinomas Smaller than 20 Millimeters: Analysis of 1060 Cases

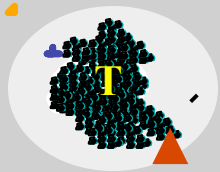
Fulvio Basolo, Liborio Torregrossa, Riccardo Giannini, Mario Miccoli, Cristiana Lupi, Elisa Sensi, Piero Berti, Rossella Elisei, Paolo Vitti, Angelo Baggiani, and Paolo Miccoli

Departments of Surgery (F.B., L.T., R.G., C.L., E.S., P.B., P.M.), Experimental Pathology B.M.I.E., Biostatistics Research Unit (M.M., A.B.), and Endocrinology (R.E., P.V.), University of Pisa, 56126 Pisa, Italy

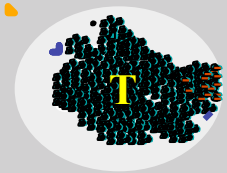
1,047 CASES OF ≤2 cm PTCs



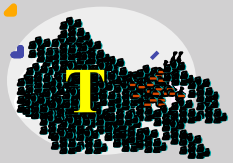
A, totally encapsulated



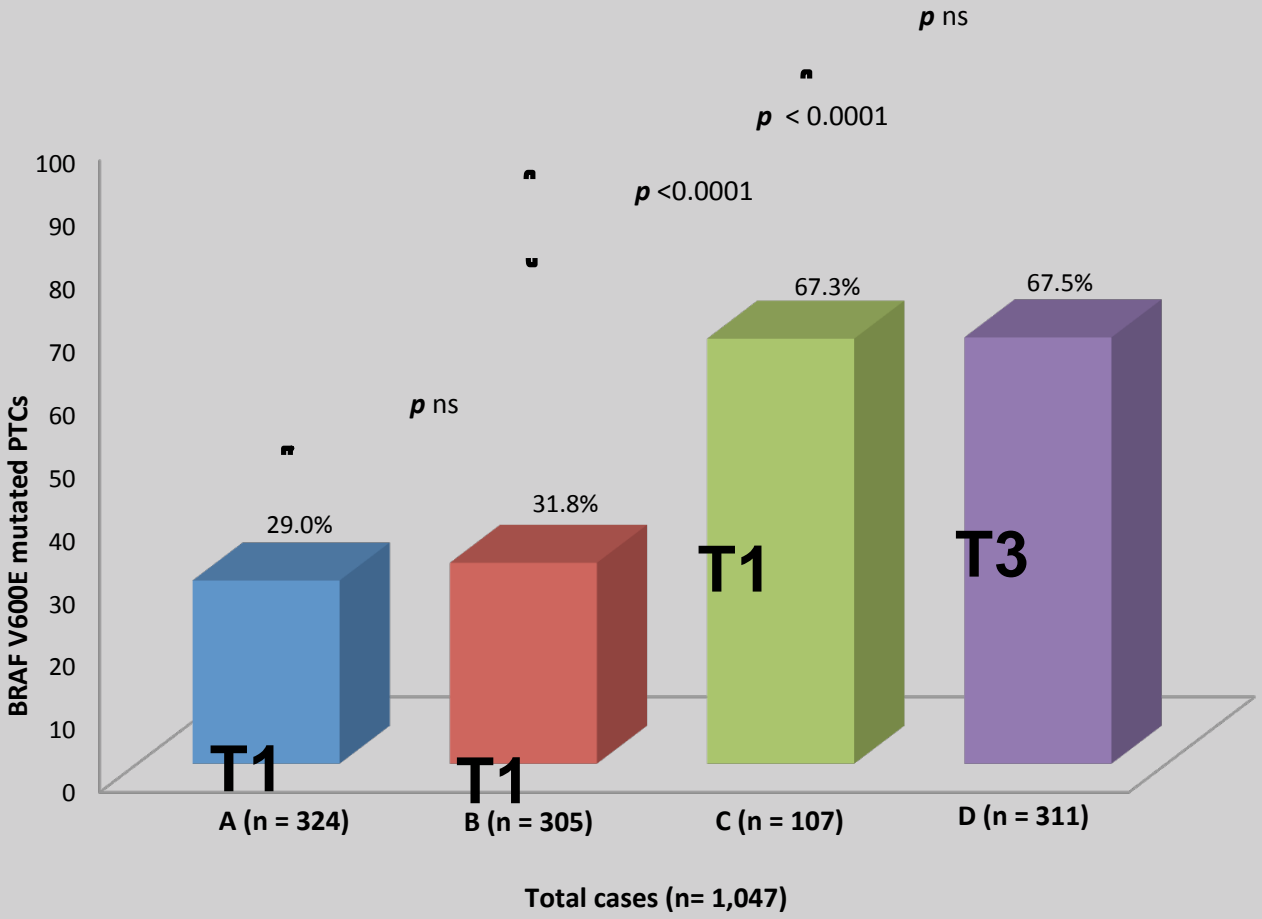
B, not encapsulated without thyroid capsule invasion



C, thyroid capsule invasion



D, extrathyroidal extension

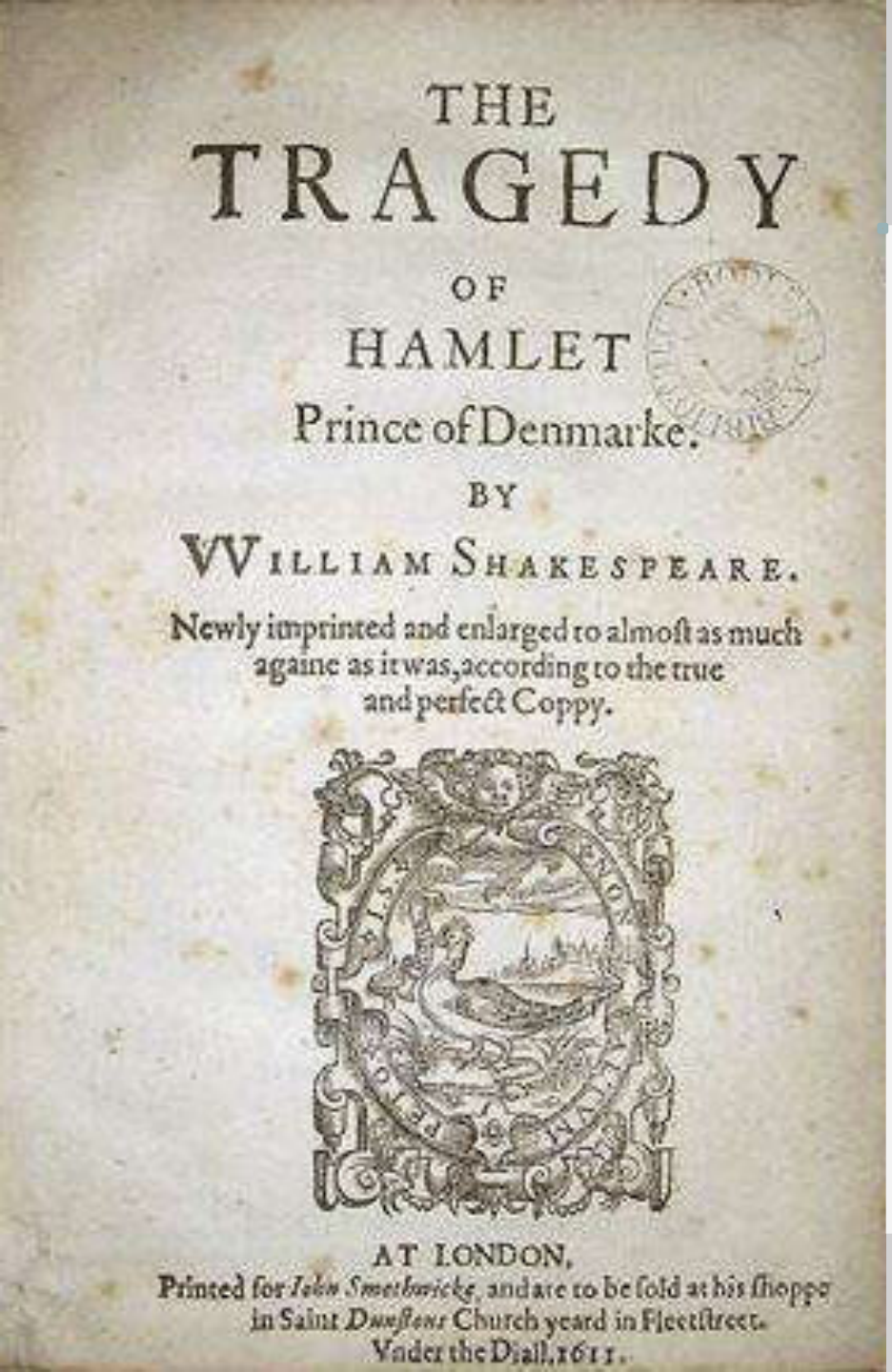


Courtesy Dr. Fulvio Basolo – University of Pisa (Italy)

OUR EXPERIENCE

BRAF AND PROGNOSTIC AGGRESSIVE PARAMETERS IN MICROPTC (Rossi ED et al. Cancer Cytopathol, in press)

	BRAF +	BRAF-	P value
MONOLATERAL CANCER	13	10	
BILATERAL CANCER	12	6	P=0,020
INTRATHYROID CANCER	23	14	
EXTRATHYROID CANCER	2	2	NS (0.847)
NODES NEGATIVE	15	15	
NODES POSITIVE	10	1	P=0,018


THE
TRAGEDY
OF
HAMLET
Prince of Denmarke.
BY
WILLIAM SHAKESPEARE.
Newly imprinted and enlarged to almost as much
againe as it was, according to the true
and perfect Coppy.
AT LONDON,
Printed for *Iohn Smethwicke*, and are to be sold at his shoppe
in Saint *Dunstons* Church yeard in Fleetstreet.
Vnder the Diall. 1611.

Can these techniques improve the diagnostic accuracy for thyroid FNAB?

THIS IS STILL A WORK-IN-PROGRESS…

- Immunocytochemical markers are more sensitive than molecular tests but less specific
- ICC expression is more useful than BRAF analysis for indeterminate lesions (where FVPC are more frequent)
- A significant BRAF correlation with aggressive parameters (nodal mets, multifocality and extracapsular invasion) is observed in suspicious lesions and PTC
- BRAF and ICC can be accurately carried out on LBC thyroid FNAB

The balance between costs and benefits is against the use of molecular markers for the diagnosis of follicular neoplasms (TIR 3) (at least for the time being…)

PATOLOGISTS SHOULD BE PART OF THE OPERATIVE TEAM WITH SURGEONS, ONCOLOGISTS, NUCLEAR PHYSICIANS, RADIOLOGISTS AND CLINICIANS

FOR YOUR ATTENTION