Convegno AME-FADOI Veneto

LA PATOLOGIA NODULARE TIROIDEA: PERCORSO DIAGNOSTICO-TERAPEUTICO

LA BIOLOGIA MOLECOLARE

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Indicazioni

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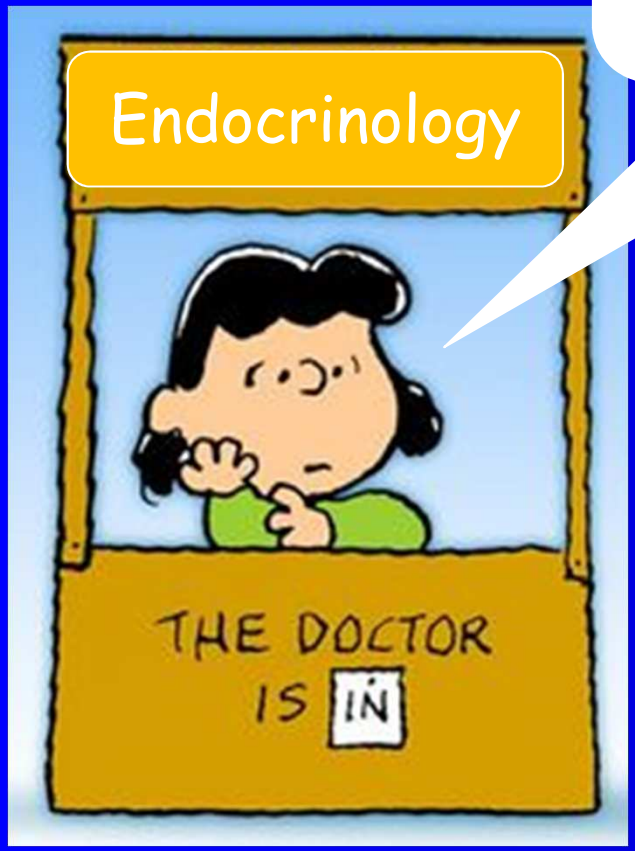
Sezione di Endocrinologia e Medicina Interna
Direttore: Prof. Ettore degli Uberti
Dipartimento di Scienze Mediche
Università degli Studi di Ferrara

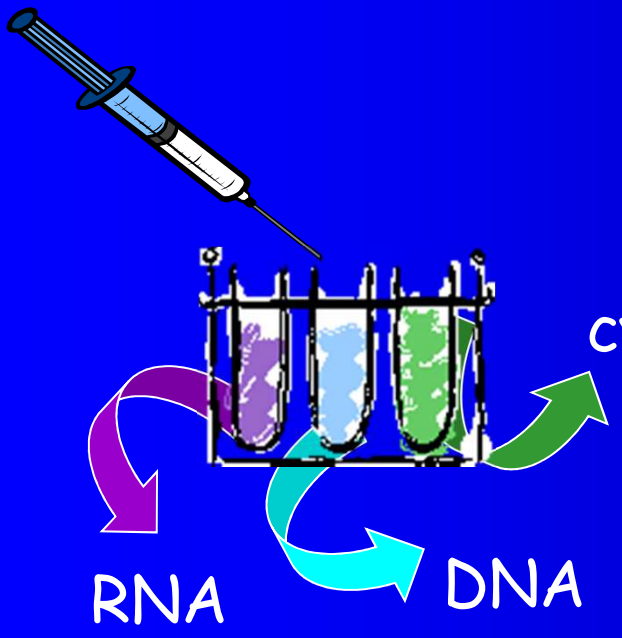
Molecular diagnostics in thyroid nodules

Overview of genetic markers



Molecular markers

Molecular diagnostics in thyroid nodules



rearrangement studies

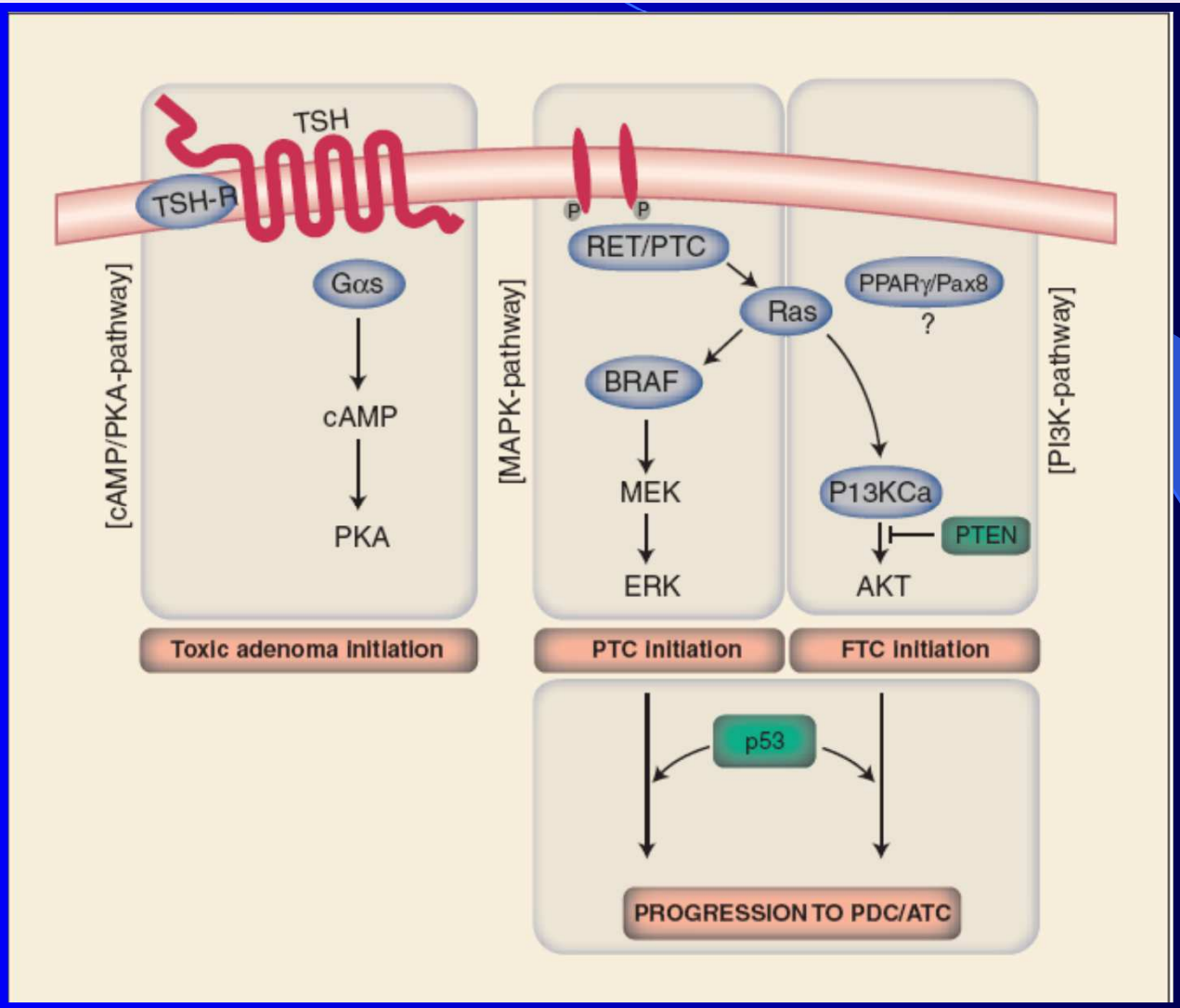
somatic mutation analysis

15-20% FNAB inconclusive or unable to discriminate between follicular adenoma and carcinoma

need for partial or total thyroidectomy for diagnostic purposes

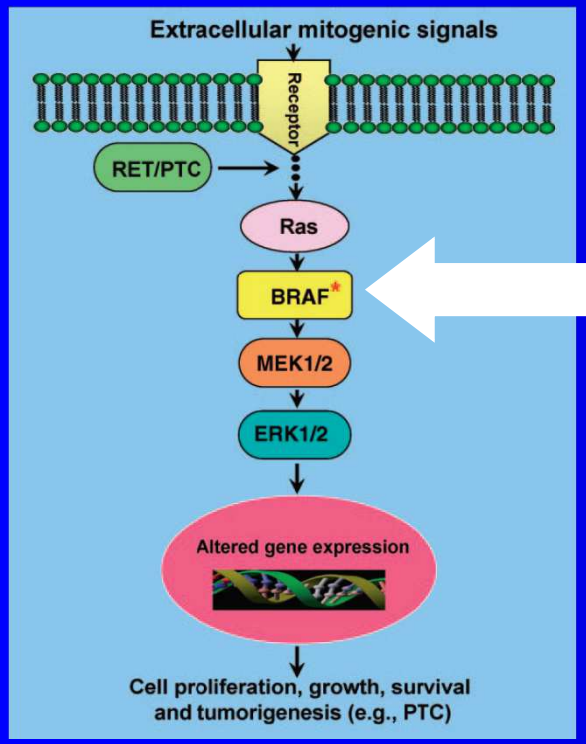
Riesco-Eizaguirre et al. Clin Transl Oncol 2007, 9:686-693

Molecular diagnostics in thyroid nodules



Riesco-Eizaguirre et al. Clin Transl Oncol 2007, 9:686-693

Molecular diagnostics in thyroid nodules



DNA

BRAF V600E point mutation

[K601E and V599Ins]

- ✓ 45-80% of PTC, mainly tall cell and classic hystology
- ✓ ↑ extrathyroidal invasion
- ✓ higher stage
- ✓ ↑ recurrence (with reduced I up-take)

Lupi et al. J Clin Endocrinol Metab. 2007;92:4085

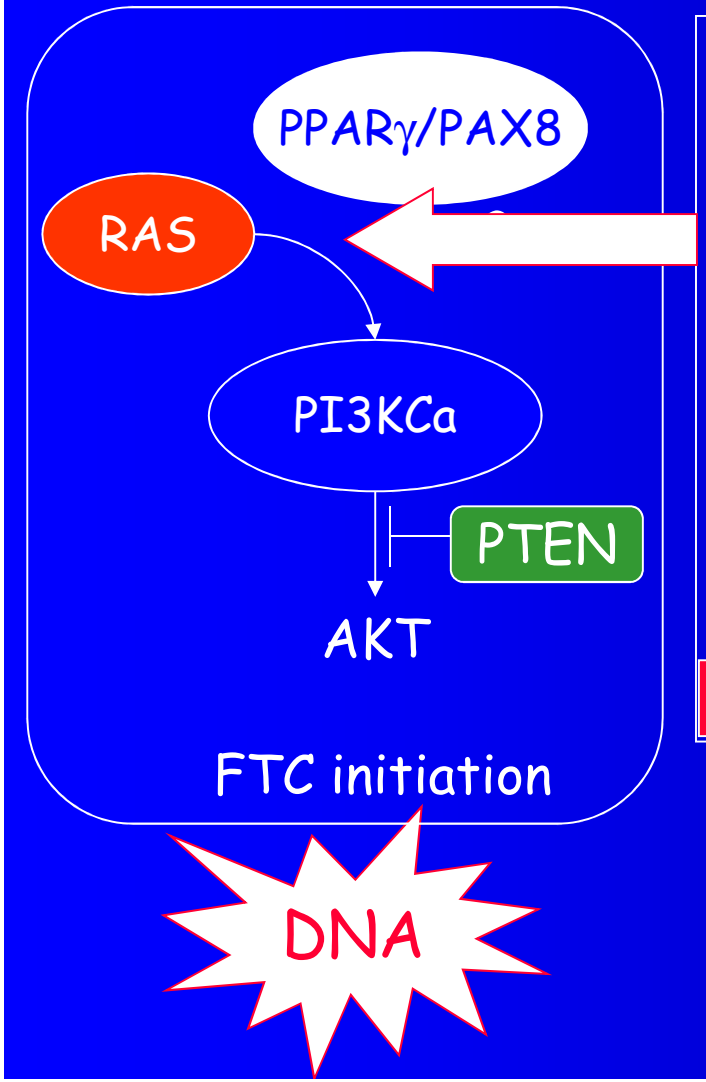
- ✓ ↑ de-differentiation

resticted to PTC

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

Molecular diagnostics in thyroid nodules

FOLLICULAR CARCINOMA



NRAS and HRAS mutations

- ✓ 30% FA and 45% of FTC
- ✓ large tumor size
- ✓ ↑distant metastases
- ✓ ↑de-differentiation and poor prognosis

Not specific !

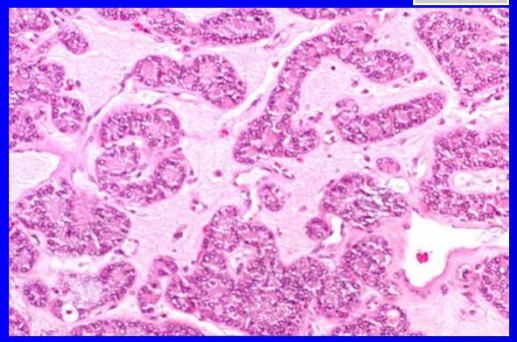
Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

Molecular diagnostics in thyroid nodules

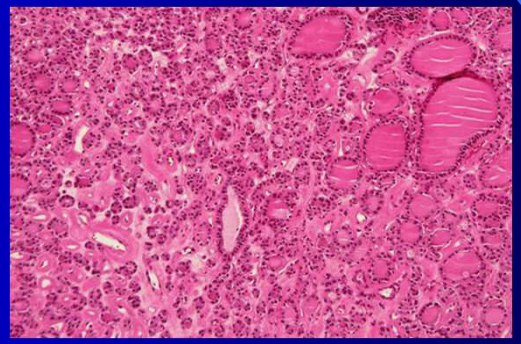
FOLLICULAR CARCINOMA

NRAS and HRAS mutations

+



Follicular adenoma



Follicular carcinoma

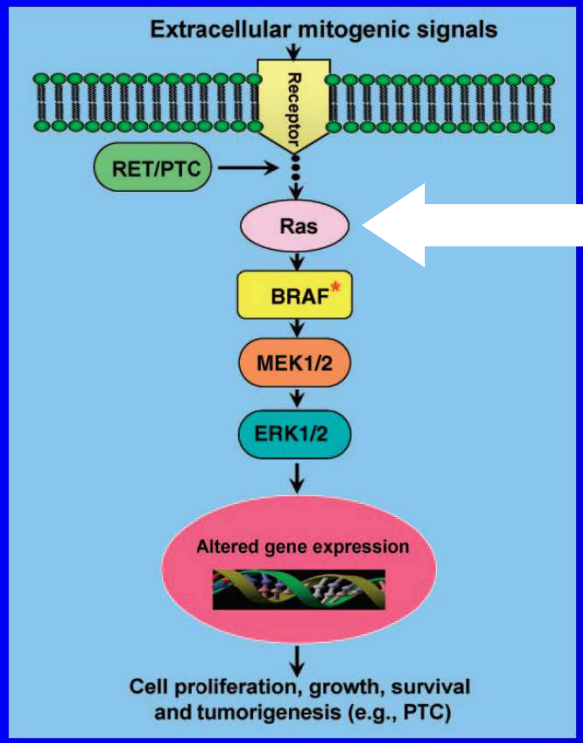
prophylactic surgery?

Improved diagnostic accuracy in samples with negative or insufficient cytology

Nikiforova et al. Mod Pathol 2004, 17 (suppl. 1):77A EFE 2015

Molecular diagnostics in thyroid nodules

PAPILLARY CARCINOMA



<u>NRAS, HRAS or KRAS point mutations</u>

- ✓ 15-20% of PTC, mainly follicular variant
- ✓ ↑encapsulation
- ✓ ↓lymph node metastases

Adeniran et al. Am J Surg Pathol 2006, 30: 216

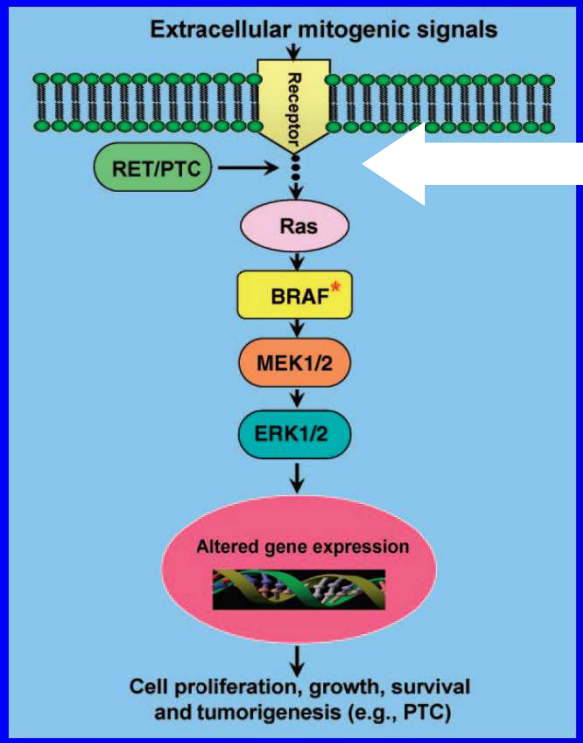
- ✓ also found in benign lesions

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

DNA

Molecular diagnostics in thyroid nodules

PAPILLARY CARCINOMA



RET/PTC1 and RET/PTC3 paracentric inversions

- ✓ 20% of PTC, mainly classical histology
- ✓ younger age
- ✓ radiation exposure
- ✓ ↑ lymph node metastases
- ✓ lower stage (micro)
- ✓ found in adenomas and benign lesions

RNA!

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

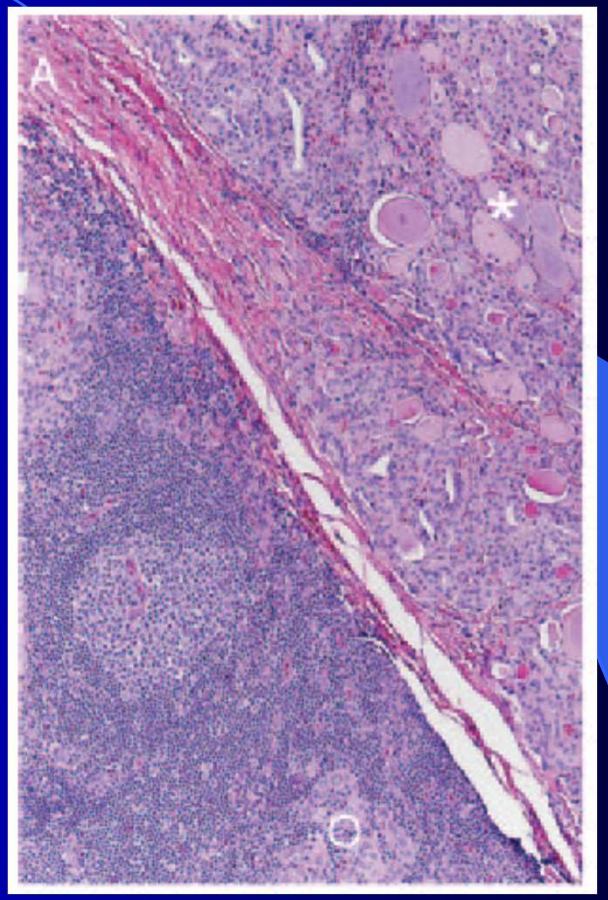
Molecular diagnostics in thyroid nodules

RET/PTC rearrangement

Found in 62% of HT

Sheils OM et al. Int J Surg Pathol 2000 ,8:185–189
Wirtschafter A et al. Laryngoscope 1997, 107:95–100
Rhoden KJ et al. J Clin Endocrinol Metab 2006, 91: 2414–2423

occult neoplasm ?

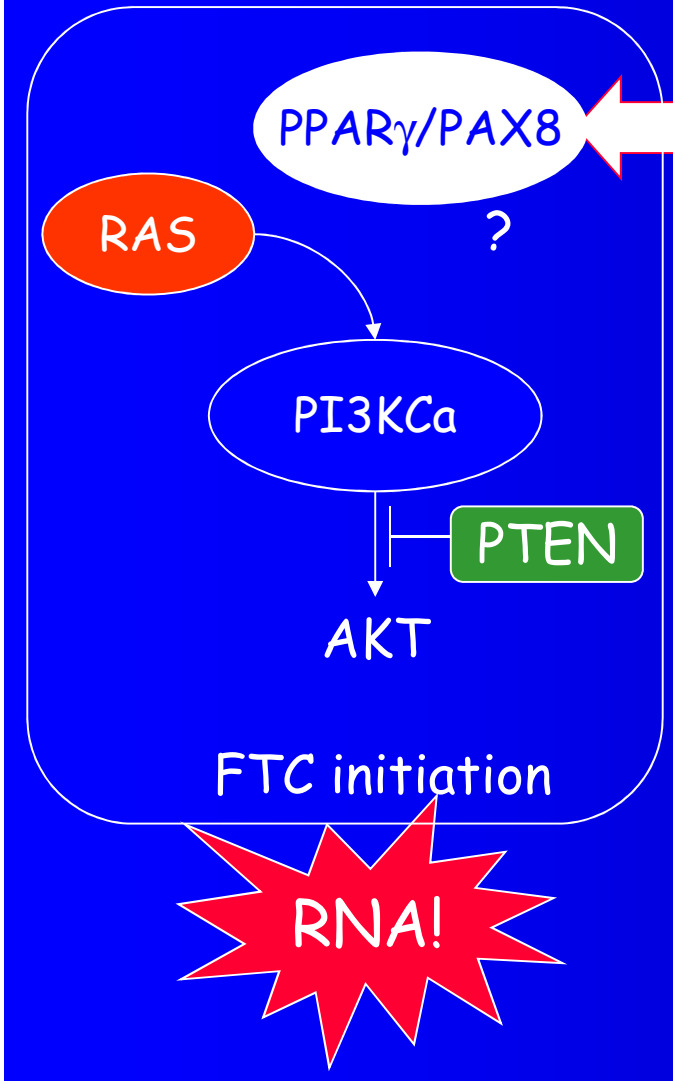


Bias!

technical limitations
high false positive results
lack of reproducibility

Molecular diagnostics in thyroid nodules

FOLLICULAR CARCINOMA



PPARγ/PAX8 rearrangement

t(2;3) (q13;p25)

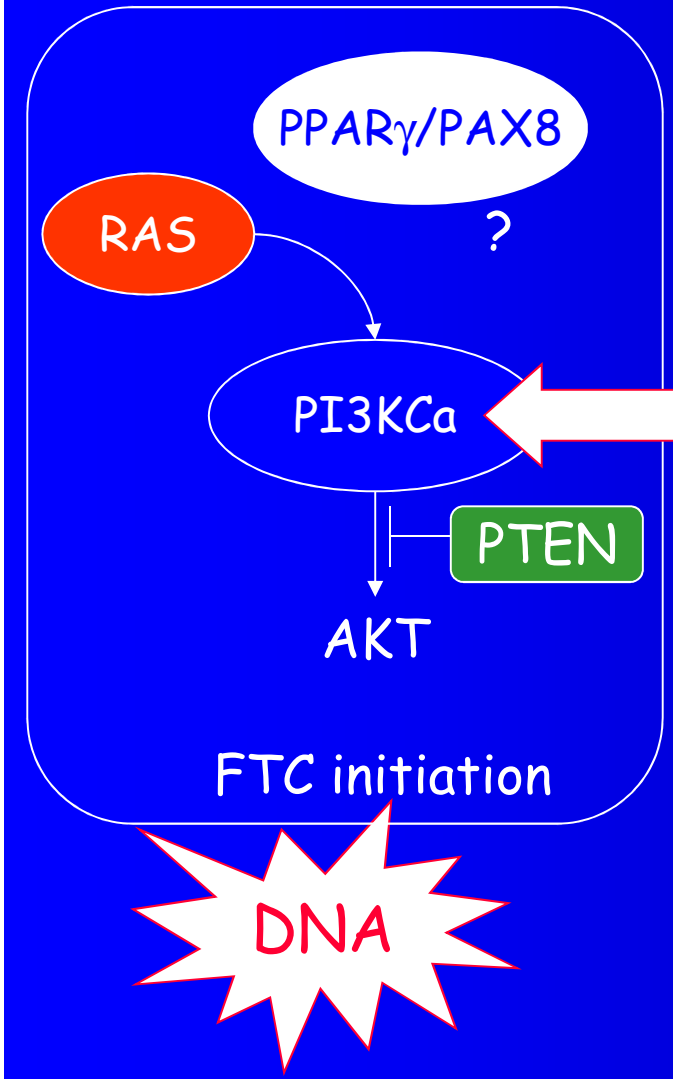
- ✓ 45% of FTC
- ✓ younger age
- ✓ small tumor size
- ✓ ↑ vascular invasion

Also found in follicular adenomas and in Hurtle cell carcinomas

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

Molecular diagnostics in thyroid nodules

FOLLICULAR CARCINOMA



PI3K/Akt pathway alterations

PIK3CA mutations: 23% ATC and 8% FTC

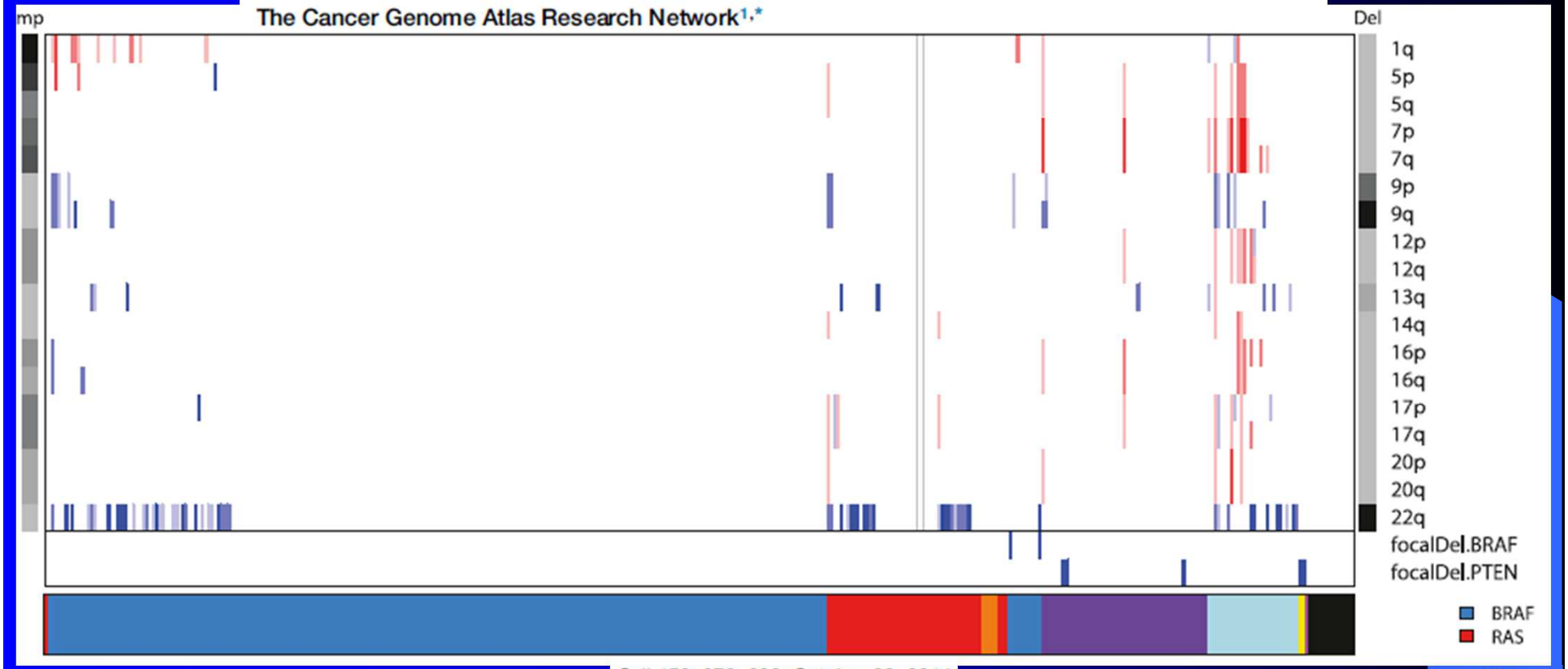
PTEN LOH: 7% FA and 27% of FTC

Lack of large studies

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

Molecular diagnostics in thyroid nodules

Integrated Genomic Characterization of Papillary Thyroid Carcinoma



Cell *159*, 676–690, October 23, 2014

Molecular diagnostics in thyroid nodules

"Our results propose a reclassification of thyroid cancers into molecular subtypes that better reflect their underlying signaling and differentiation properties, which has the potential to improve their pathological classification and better inform the management of the disease"

Cell *159*, 676–690, October 23, 2014

Molecular diagnostics in thyroid nodules

somatic mutation analysis

pyrosequencing
Kim et al. J Clin Endocrinol Metab, 2011, 96:658

MASA
Pelizzo et al. Clin Chem Lab Med. 2011;49:325

RFLP
Zatelli et al. Eur J Endocrinol 2009, 161:467

direct sequencing
Zatelli et al. Eur J Endocrinol 2009, 161:467

allelic discrimination
Rossi et al. J Clin Endocrinol Metab 2012;97:2354

specific colorimetric mutation detection assay
(Mutector; TrimGen, Sparks, MD)

Xing et al. J Clin Oncol. 2009;27:2977-82

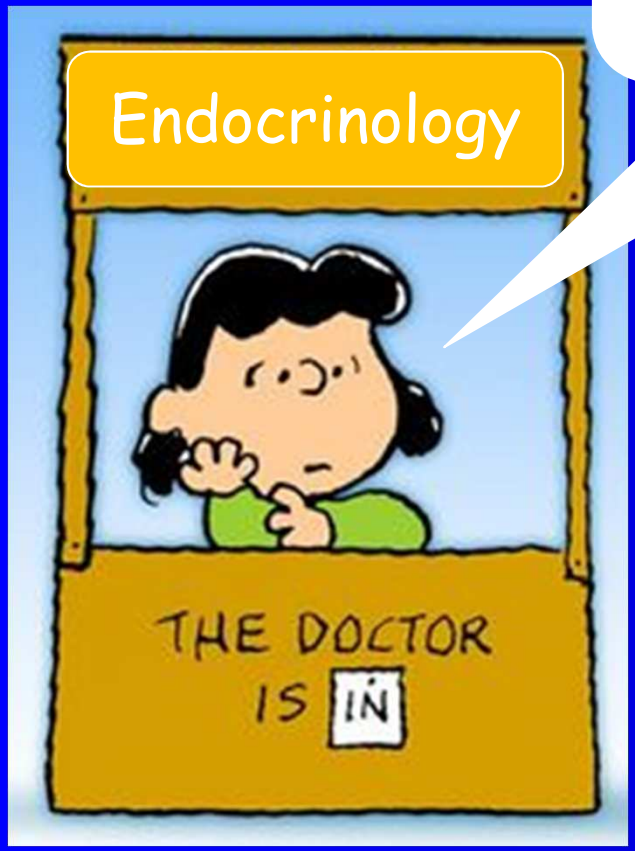
Affordable costs

Dedicated instruments

Experienced personnel

Molecular diagnostics in thyroid nodules

Diagnostic importance?



Relevant!!

Molecular diagnostics in thyroid nodules

BRAFV600E molecular test

	Cytology		BRAF		Cytology + BRAF	
Sensitivity	100	77.3	89.6	64.0	89.6	86.7
Specificity	36.4	98.8	95.5	100	95.5	98.8
PPV	92.9	92.1	99.4	100	99.4	92.9
NPV	100	95.9	52.5	93.7	52.5	97.5
Accuracy	93.3	95.4	90.2	95.4	90.2	96.9
K value	0.51±0.11	0.81±0.02	0.63±0.07	0.76±0.05	0.63±0.07	0.88±0.01

Kim et al. J Clin Endocrinol Metab, 2011, 96:658

Zatelli et al . Eur J Endocrinol 2009, 161:467

EFE 2015

Molecular diagnostics in thyroid nodules

Number of clinical/US findings suspected for malignancy in nodules diagnosed as cancer at histology	
None	4
< 1 cm	3
> 1 cm	1
One	59
< 1 cm	41
> 1 cm	18
Two	79
< 1 cm	62
> 1 cm	17
More than two	91
< 1 cm	34
> 1 cm	57

Even nodules lacking clinical/US findings suspected for malignancy may underlie a thyroid cancer!!!

None of the clinical/US findings suspected for malignancy predict BRAF status

Rossi ….Zatelii JCEM 2012;97:2354

Molecular diagnostics in thyroid nodules

BRAFV600E molecular test	Cytology		BRAF		Cytology + BRAF	
	S	NS	S	NS	S	NS
Sensitivity	76,8	69,4	56,6	51	92,9	84,7
Specificity	99,7	99,9	100	100	99,7	99,9
PPV	97,7	98,6	100	100	98,1	98,8
NPV	96,5	98	93,6	96,9	98,9	99
Accuracy	96,6	98,1	94,1	97	98,8	99

BRAF testing significantly increases FNAB sensitivity also in nodules clinically non suspected

15 PTC patients "rescued" by BRAF analysis

Rossi ….Zatelii JCEM 2012;97:2354

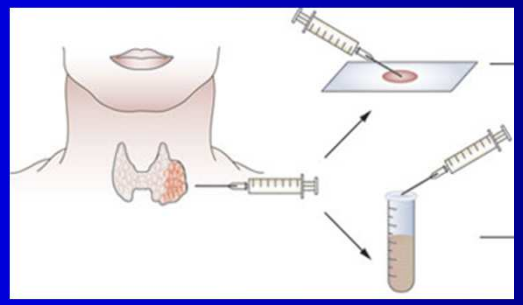
Molecular diagnostics in thyroid nodules

Cancer prevalence

nodules >1 cm	nodules <1 cm
7.2%	12.2%

p<0.001

140 microPTC
- 13 multifocal
- 17 with lymphnode metastases



Rossi ….Zatelii JCEM 2012;97:2354

EFE 2015

Molecular diagnostics in thyroid nodules

BRAFV600E molecular test	S	%	NS	%
ACUS	26	3,1	78	4,9
PTC	6	75,0	10	37,0
BRAF +	5	19,2	0	0,0
FN	35	4,2	62	1,2
PTC	5	25,0	9	23,7
BRAF +	2	5,7	6	9,7

BRAF testing identifies as malignant 10% of FN

Indication to total thyroidectomy

Rossi ….Zatelii JCEM 2012;97:2354

EFE 2015

Molecular diagnostics in thyroid nodules

Frequencies of genetic alterations according to BSTRC classes

Genetic alteration (n)	BSTRC classes						
	I	II	III	IV	V	VI	Total
BRAF V600E	4	3	7	4	6	10	34
BRAF and RET/PTC 1	0	0	2	0	1	1	4
BRAF and RET/PTC 3	0	0	0	0	1	1	2
RAS	1	21	4	2	1	2	31
RAS and RET/PTC 3	0	1	0	0	0	0	1
RET/PTC-1	2	25	3	0	1	1	32
RET/PTC-3	4	19	3	1	1	1	29
RET/PTC-1 and -3	0	0	0	0	0	1	1
Total samples with genetic alteration(s)	11	69	19	7	11	17	134
None	22	699	33	30	11	11	806
All samples	33	768	52	37	22	28	940
Genetic alteration frequence (%)							
BRAF V600E	12.1	0. 4	17.3	10.8	36.3	42.8	4.2
RAS	3	2.8	7.7	5.4	4.5	7.1	3.4
RET/PTC-1 and RET/PTC-3	18.2	5.8	15.4	2.7	18.2	17.8	7.3
Total (s)	33.3	9	36.5	18.9	50.0	60.7	14.2

RAS mutations and RET/PTC rearrangements are present in benign lesions

Molecular diagnostics in thyroid nodules

Diagnostic value of cytology + genetic analyses

	Cytology combined with			
	BRAF	RAS	RET/PTC	All genetic analyses
PPV	100	76,3	61,1	66,7
NPV	72,6	45,6	38,1	51,9
sensitivity	76,4	40,3	45,8	82,2
specificity	100	80	53,3	31,8
accuracy	85,5	55,6	48,7	63,2

BRAF alone is sufficient to detect malignant lesions

Rossi ….Zatelii Thyroid. 2015;25:221-8

Molecular diagnostics in thyroid nodules

Cancer risk in thyroid nodules with indeterminate cytology according to Bethesda classification and genetic alteration

N° (%)	Class III (n°=52*)	Class IV (n°=37)	Class V (n°=22)	Indeterminate cytology (n°=111)
Cytology alone	19.2%	21,6%	90,9%	27,1 %
Any mutation	47,3%	71,4%	90,9%	63,1%
BRAF	100%	100%	100%	100%
RAS	0%	50%	0%	14,2%
RET/PTC-1	40%	-	100%*	57,1%
RET/PTC-3	0%	0%	100%*	33,3%
No mutations	3%	10%	90,9%	13,5%

Rossi ….Zatelii Thyroid. 2015;25:221-8

Molecular diagnostics in thyroid nodules

PTC distribution according to TNM stages and the presence/absence of a genetic alteration

Thyroid cancers			
TNM staging (AJCC/UICC)	Genetic alteration		Total
	positive	negative	
I	28	19	**47**
II	0	0	0
III	13	6	19
IV	6	0	6
Total	47	25	72

Rossi ….Zatelii Thyroid. 2015;25:221-8

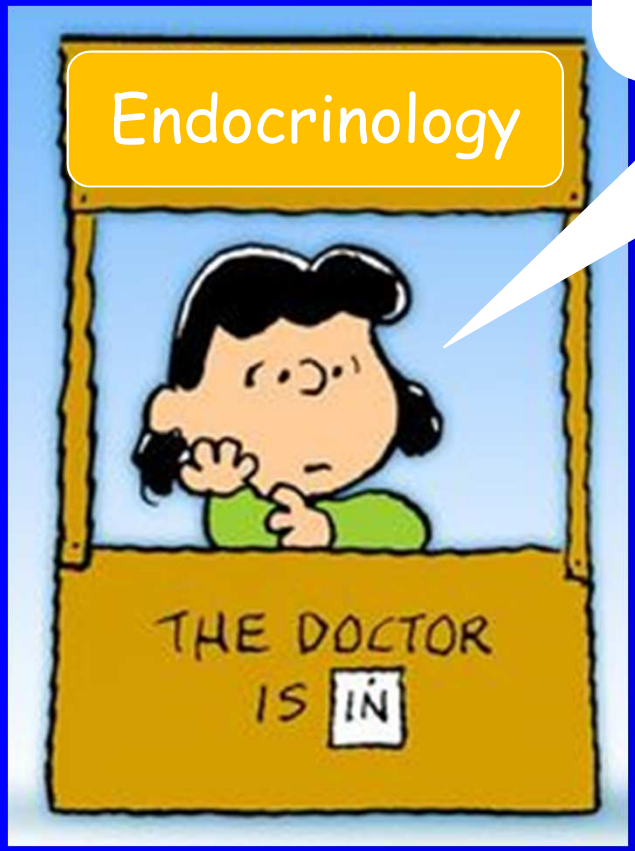
Molecular diagnostics in thyroid nodules

CONCLUSION -1

BRAF molecular analysis increases diagnostic sensitivity of cytology for PTC

Molecular diagnostics in thyroid nodules

Prognostic relevance?

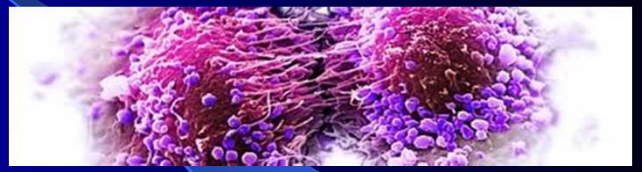


Not so sure...

Molecular diagnostics in thyroid nodules

PAPILLARY CARCINOMA

BRAF mutation(s)



(−) → NIS expression

(−) → NIS trafficking to the membrane

Riesco-Eizaguirre et al. Endocrine-Related Cancer 2006, 13: 257

(+) → DNA synthesis and apoptosis

→ little growth advantage

BUT

genomic instability

Mitsutake et al. Cancer Research 2005;65: 2465

(+) → MMP, vimentin, osteopontin

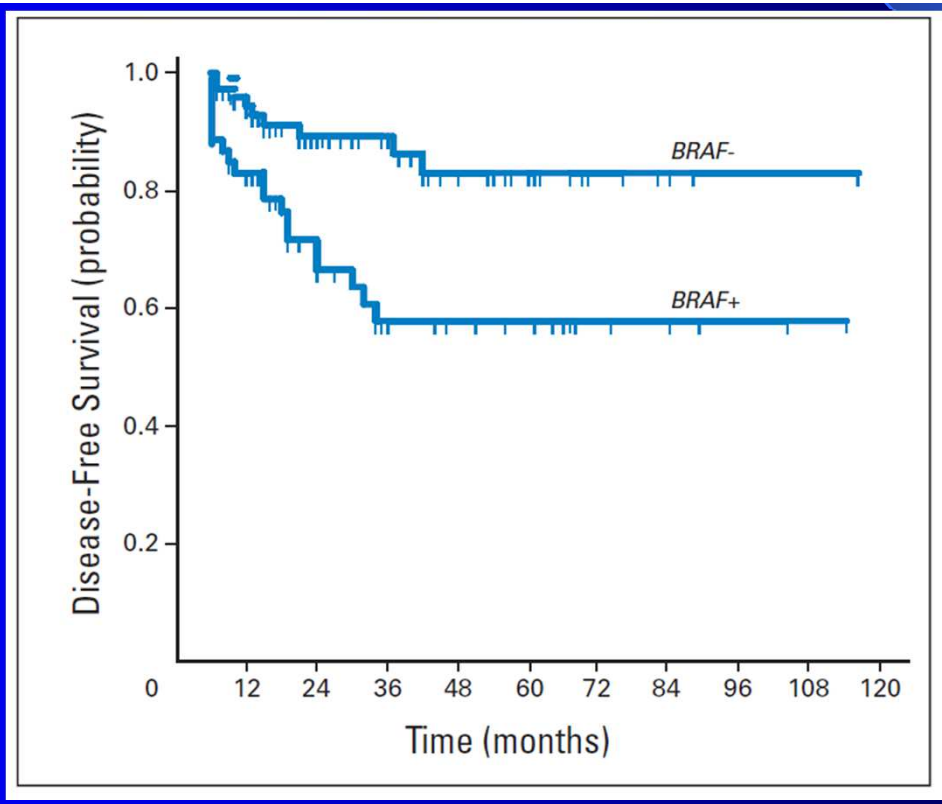
epithelial-mesenchimal transition

Vasko et al. Curr Opin Oncol 2007;19: 11

Molecular diagnostics in thyroid nodules

BRAFV600E molecular test

significantly reduced disease-free probability in BRAF+ patients

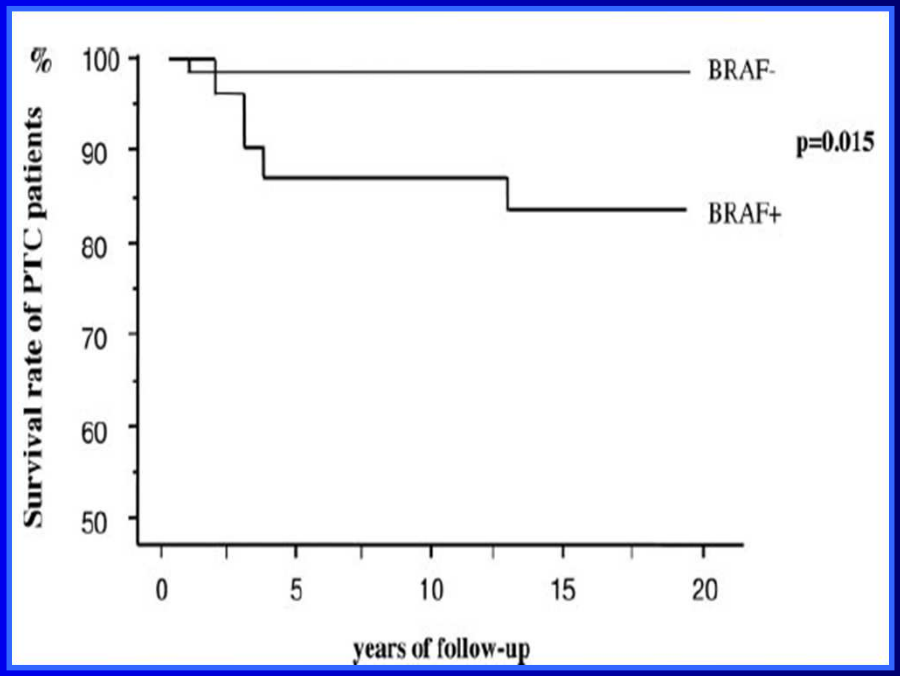


Xing et al. J Clin Oncol. 2009;27:2977-82

Molecular diagnostics in thyroid nodules

BRAFV600E molecular test

significantly increased mortality in BRAF+ patients



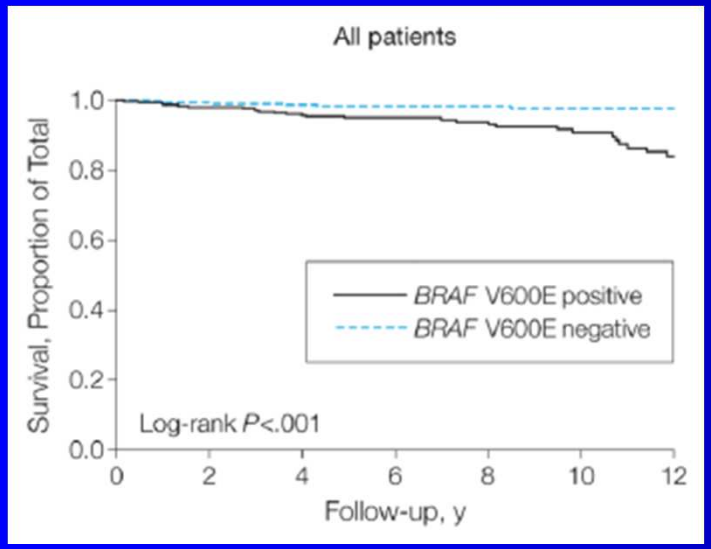
Elisei et al. J Clin Endocrinol Metab. 2008;93:3943

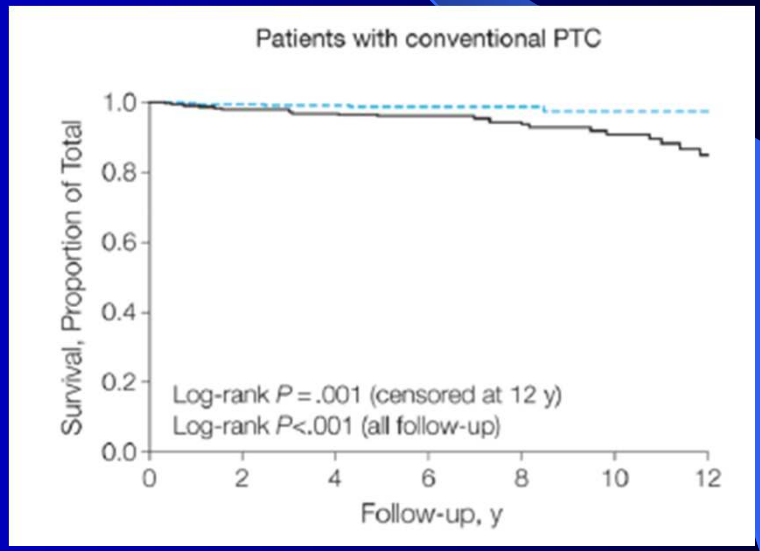
Molecular diagnostics in thyroid nodules

Association Between *BRAF* V600E Mutation and Mortality in Patients With Papillary Thyroid Cancer

JAMA. 2013 April 10; 309(14): 1493–1501. doi:10.1001/jama.2013.3190.

1849 patients



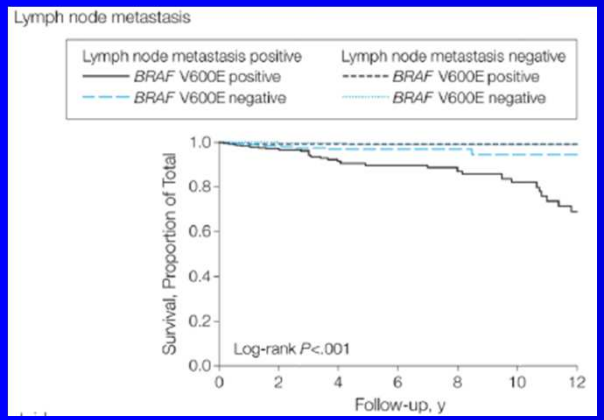


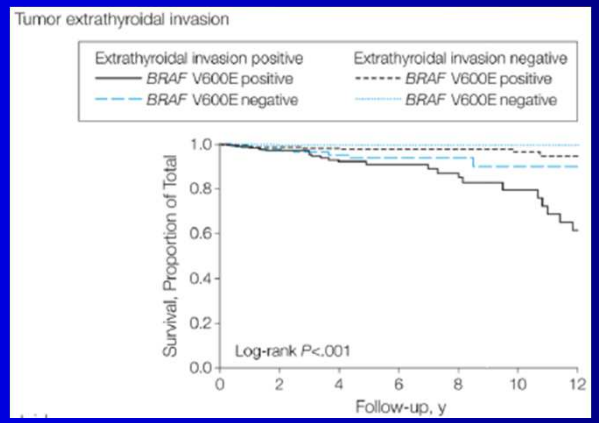
Greater mortality in BRAF+ (5.3%) vs BRAF- (1.1%) patients

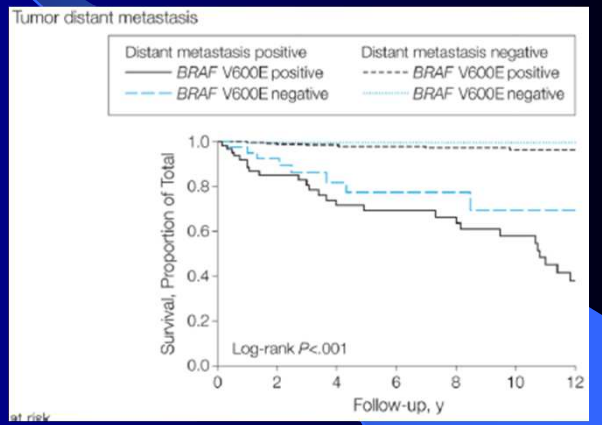
Molecular diagnostics in thyroid nodules

Association Between *BRAF* V600E Mutation and Mortality in Patients With Papillary Thyroid Cancer

JAMA. 2013 April 10; 309(14): 1493–1501. doi:10.1001/jama.2013.3190.







"When lymph node metastasis, extrathyroidal invasion, and distant metastasis were also included in the model, the association of BRAF V600E with mortality for all PTC was no longer significant"

Molecular diagnostics in thyroid nodules

Prospective study

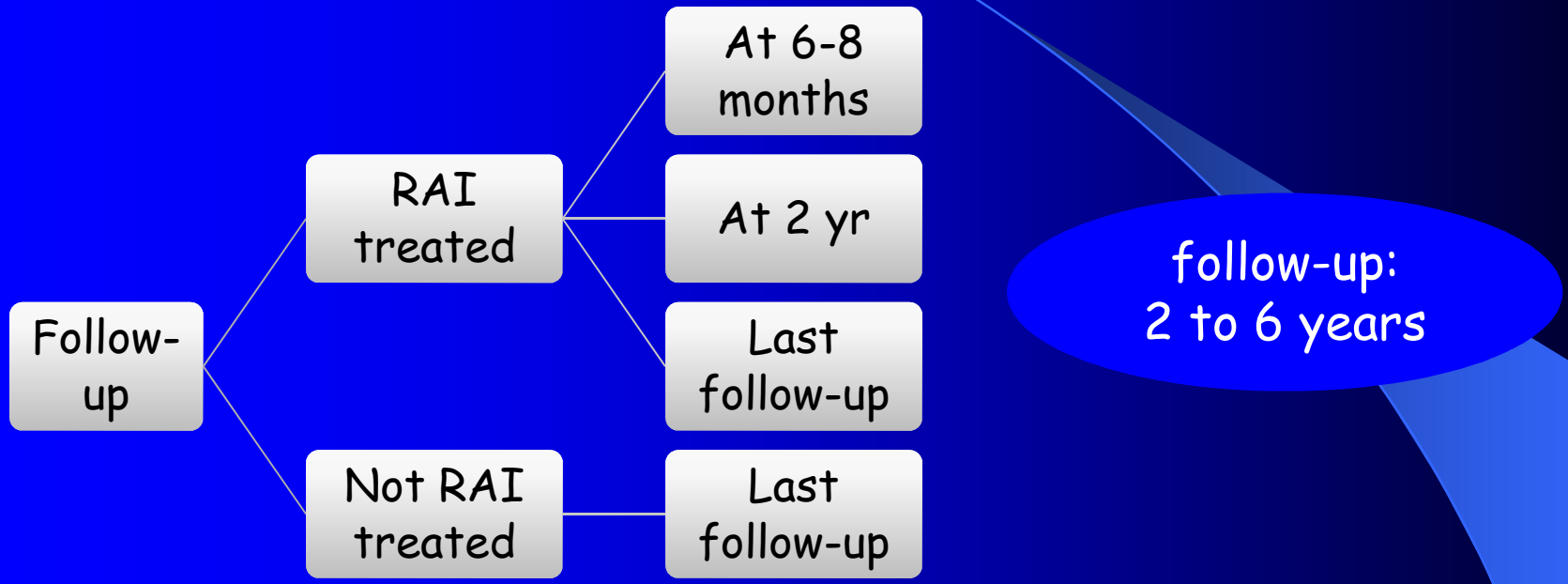
156 PTC

- 80 BRAF V600E + PTC
  - 40 N_1
  - 40 N_0
- 76 BRAF V600E - PTC
  - 36 N_1
  - 40 N_0

Case-control study, matching for risk factors such as :

1. gender
2. age
3. hystotype
4. disease stage

Molecular diagnostics in thyroid nodules



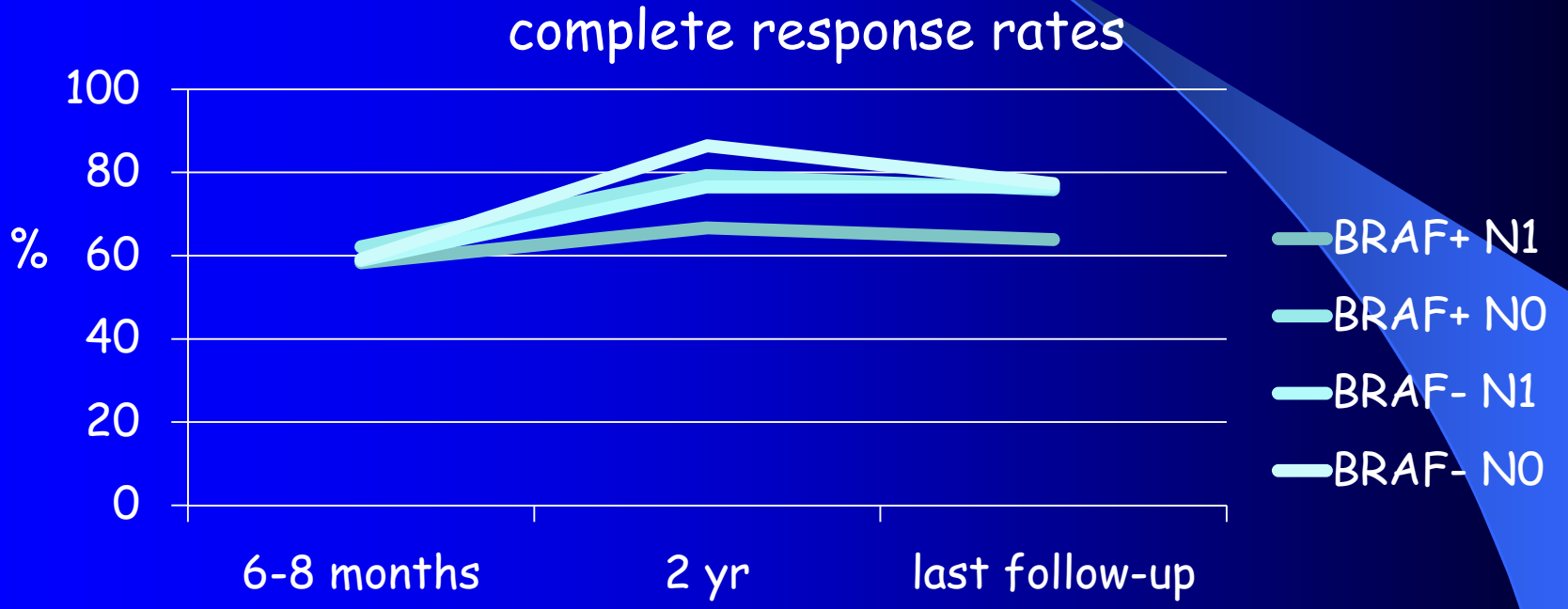
Evaluations:

- basal Tg levels
- rhTSH-stimulated Tg
- neck US
- I^{131} Total body scan

1. COMPLETE RESPONSE
2. LOCOREGIONAL DISEASE
3. BIOCHEMICAL PESISTENCE

Molecular diagnostics in thyroid nodules

PATIENTS SUBMITTED TO RAI



complete response rates
do not depend on BRAF mutation

no impact on prognosis

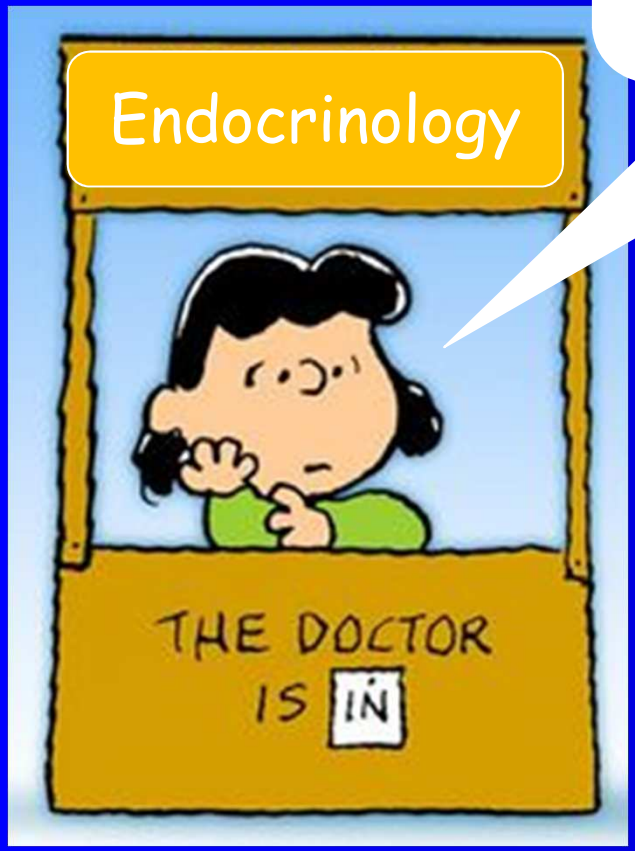
Molecular diagnostics in thyroid nodules

CONCLUSION -2

BRAF status may not predict
patients outcome

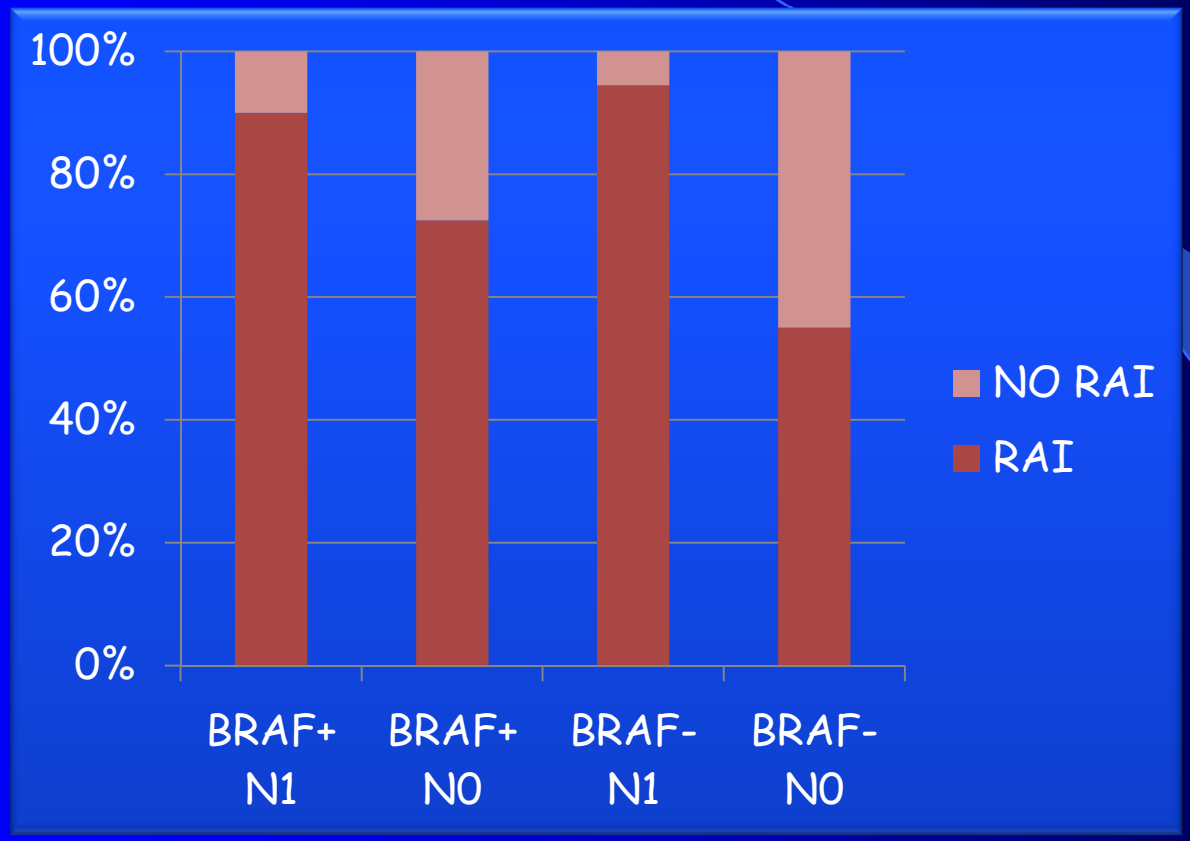
Molecular diagnostics in thyroid nodules

What about therapy?



Well…

Molecular diagnostics in thyroid nodules



BRAF mutation did not influence the indication for radiometabolic therapy

Molecular diagnostics in thyroid nodules

PTC distribution according to TNM stages and the presence/absence of a genetic alteration

Thyroid cancers			
TNM staging (AJCC/UICC)	Genetic alteration		Total
	positive	negative	
I	28	19	**47**
II	0	0	0
III	13	6	19
IV	6	0	6
Total	47	25	72

Rossi ….Zatelii Thyroid. 2015;25:221-8

Molecular diagnostics in thyroid nodules

Thyroid cancer in Ferrara

	Years	Patients number	I^{131} therapy (no)	% I^{131} therapy/ thyroid cancer
pre-BRAF	2000-2006	467	402	86,08%
post-BRAF	2007-2013	738	524	71,00%

↑ 36.7% in DTC diagnosis (+39 new cases/year)

> 50% stage I and II

Molecular diagnostics in thyroid nodules

BRAFV600E
molecular test

May address patients with
persistent/recurrent disease
to therapy with
BRAF-specific inhibitors

Cantwell-Dorris et al. Mol Cancer Ther 2011, 10: 385

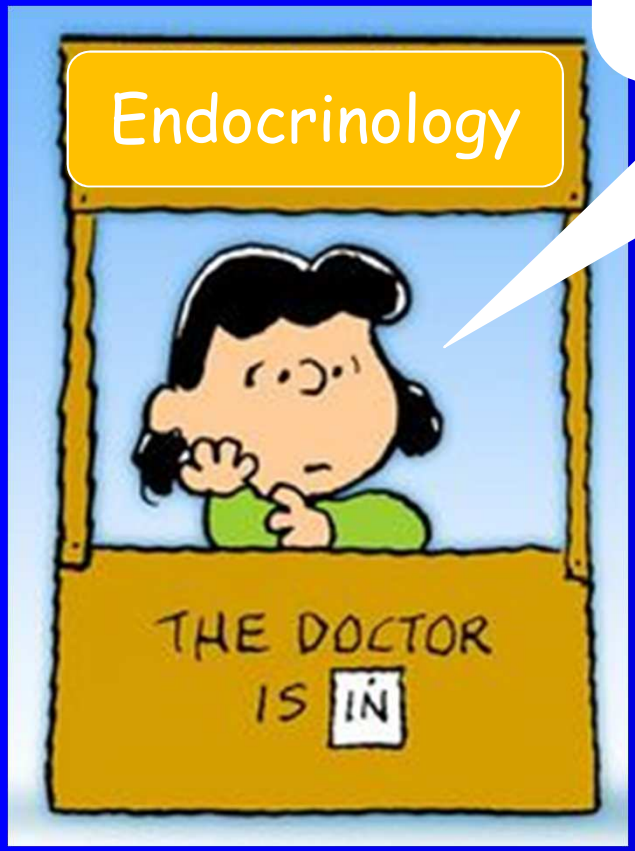
Molecular diagnostics in thyroid nodules

CONCLUSION -3

BRAF V600E may influence therapeutic approach

Molecular diagnostics in thyroid nodules

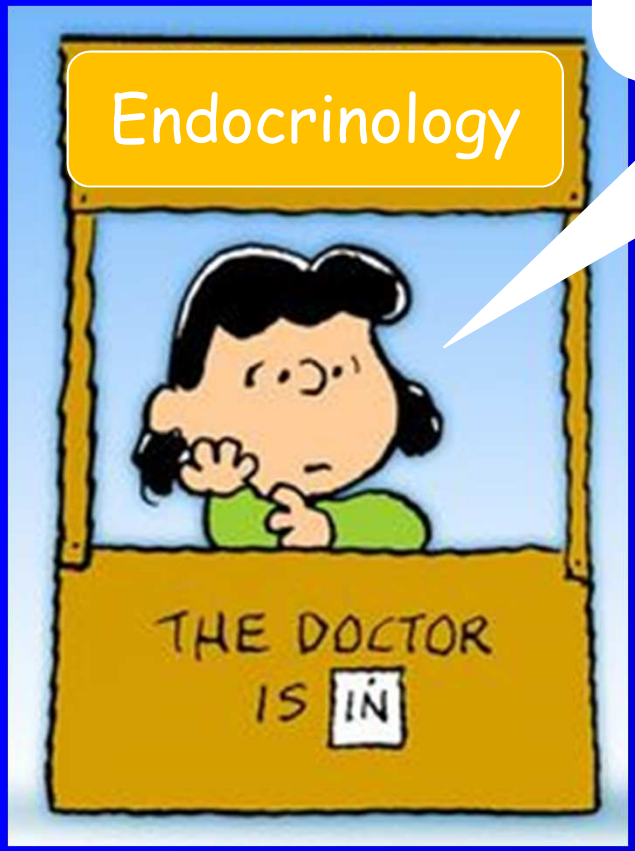
When testing in cytology?



For diagnostic purposes

Molecular diagnostics in thyroid nodules

When testing in histology?



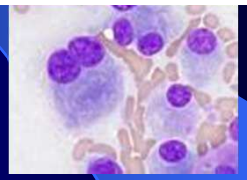
Search for somatic RET mutations

Molecular diagnostics in thyroid nodules

THEREFORE

BRAFV600E molecular test

FNAB material



- ✓ increases cytology diagnostic sensitivity for PTC
- ✓ does not seem to affect prognosis
- ✓ may influences therapeutic approach

Xing et al. 2004 J Clin Endocrinol Metab 89:2867
Cohen et al. 2004 Clin Cancer Res 10:2761
Domingues et al. 2005 Cytopathology 16:27
Zatelli et al 2009 J Clin Endocrinol Metab
Nikiforov et al. 2009 J Clin Endocrinol Metab 94:2092
Rossi et al 2012 Clin Endocrinol Metab
Kim et al. 2006 Ann Surg 244:799

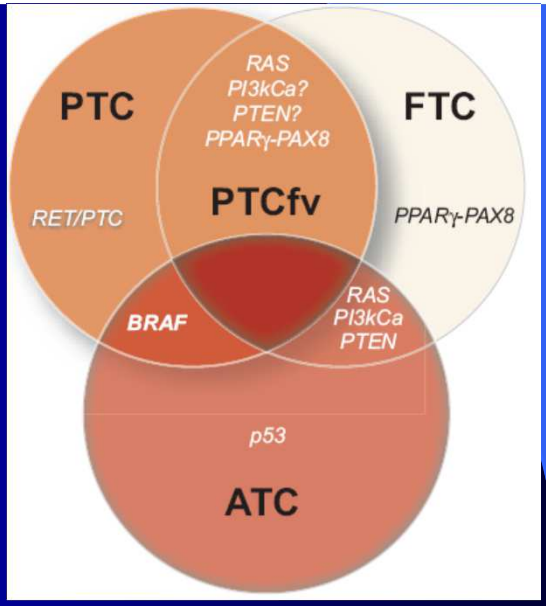
Kim et al. 2006 Clin Endocrinol 65:364
Xing 2007 Endocr Rev 28:742
Nikiforova et al 2008 Expert Rev Mol Diagn 8:83
Riesco-Eizaguirre et al. 2006. Endocr Rel Cancer 13:257
Xing et al. 2005 J Clin Endocrinol Metab 90:6373
Mojica et al 2006 Endocr Pathol 17:183

Molecular diagnostics in thyroid nodules

	BRAF	RET/PTC	RAS	PI3KCa*	PTEN	PPARγ/PAX8
PTC	29–83%	2.5–59%**	–	–	–	–
PTCfv	–	–	5–15%	15%	2%	37.5%
FTC	–	–	7–62%	8–42%	6–7%	36–45%
FA	–	14%	9–11%	8–23%	–	4–33%
ATC/PDC	10–35%	–	50–55%	54%	16%	–
Extrathyroid extension	Yes	No	–	–	–	–
Increased recurrence risk	Yes	No	–	–	–	–
Poor survival	?	No	Yes	–	–	–

Riesco-Eizaguirre et al. Clin Transl Oncol 2007; 9:686

molecular biology can help



Molecular diagnostics in thyroid nodules

BUT

Molecular testing
is not sufficient
to detect all malignant cases

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

Molecular diagnostics in thyroid nodules

…in realtà…

Analisi	Utilità			Erogabilità SSR
	diagnostica	prognostica	predittiva	
BRAF (V600E)	POSITIVA FORTE	POSITIVA FORTE	NEGATIVA DEBOLE	SI (condizionata)

Per la diagnosi di neoplasia maligna contestualmente al primo FNA in noduli con forte sospetto clinico-US (es . ipoecogenicità, margini sfumati, microcalcificazioni) e/o sospetto/dubbio citologico di carcinoma.
Pazienti con BRAFV600E avrebbero prognosi peggiore.

THANKS!

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