

BRAF: quali evidenze sul suo significato diagnostico e prognostico?

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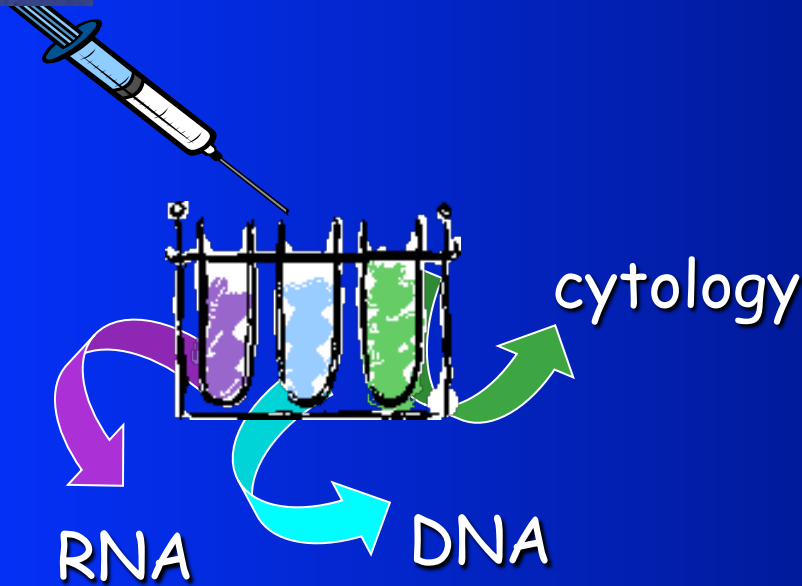
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BRAF: diagnostic and prognostic significance



rearrangement studies

somatic mutation analysis

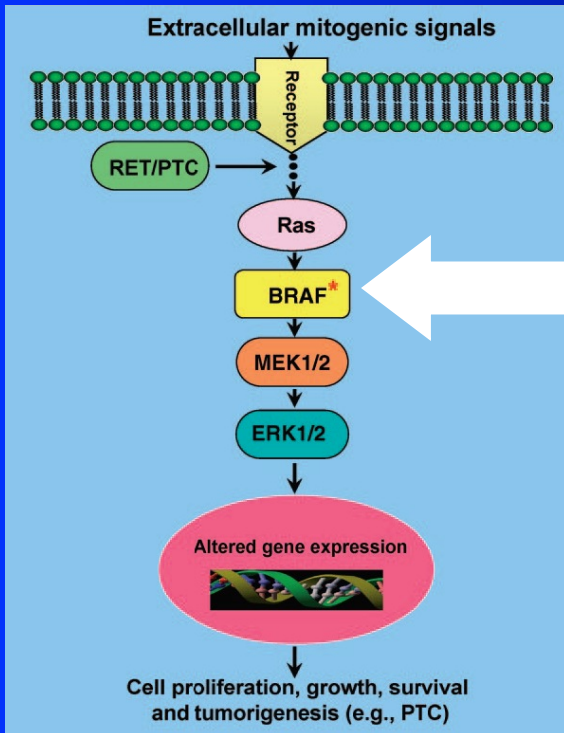
15-20% FNAB inconclusive or unable to discriminate between follicular adenoma and carcinoma

need for partial or total thyroidectomy for diagnostic purposes

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



BRAF: diagnostic and prognostic significance

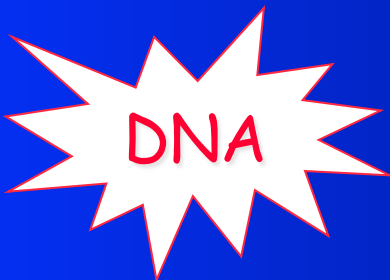


BRAF V600E point mutation

[K601E and V599Ins]

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

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



restricted to PTC





BRAF: diagnostic and prognostic significance

**BRAFV600E
molecular test**

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

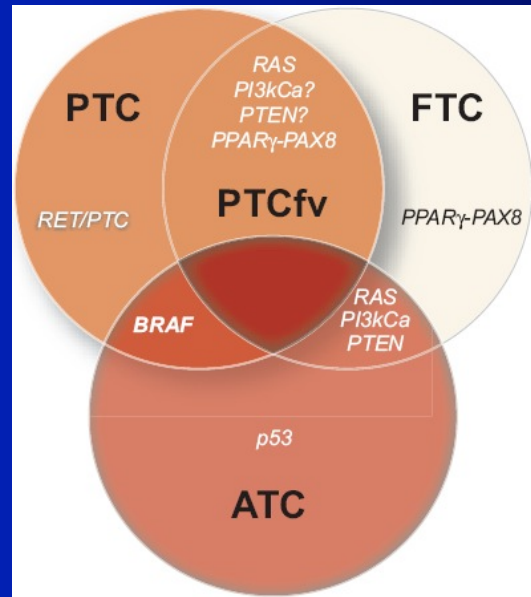


**Affordable costs
Dedicated instruments
Experienced personnel**



BRAF: diagnostic and prognostic significance

- does it improve diagnosis?



BRAF: diagnostic and prognostic significance

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



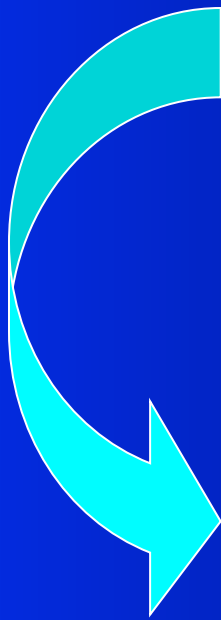
BRAF: diagnostic and prognostic significance

**BRAFV600E
 molecular test**



	BRAF ^{V600E} (+)	BRAF ^{V600E} (-)	<i>p</i> value
n	154 (91.1)	15 (8.9)	
Age at diagnosis (yr)	48.8 ± 12.1	40.1 ± 12.7	0.01
<45	56 (36.4)	8 (53.3)	0.31
≥45	98 (63.6)	7 (46.7)	
Gender			
Male	22 (14.3)	2 (13.3)	0.77
Female	132 (85.7)	13 (86.7)	
Tumor size (mm)	10.90 ± 9.63	11.07 ± 5.78	0.95
<5	26 (16.9)	3 (20.0)	0.02
5 to <10	74 (48.1)	2 (13.3)	
10 to <15	19 (12.3)	6 (40.0)	
15 to <20	17 (11.0)	3 (20.0)	
≥20	18 (11.7)	1 (6.7)	
Extrathyroidal invasion	83 (53.9)	6 (40.0)	0.44
Lymph node metastasis	55 (35.7)	4 (26.7)	0.67

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



**BRAF mutation analysis
 may help especially in
 small nodules**

BRAF: diagnostic and prognostic significance

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!!!

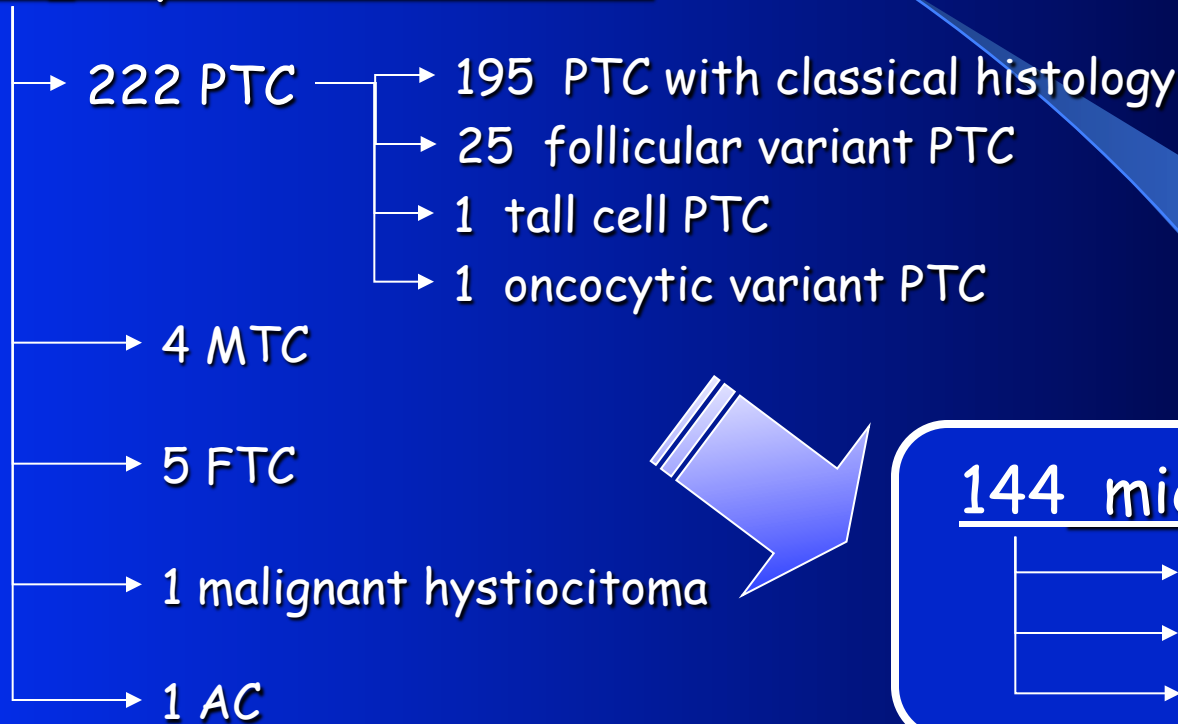
None of the clinical/US findings suspected for malignancy predict BRAF status



BRAF: diagnostic and prognostic significance



233 thyroid carcinomas

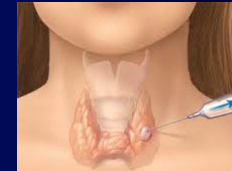


144 microcarcinomas

- 140 PTC
- 3 MTC
- 1 FTC



BRAF: diagnostic and prognostic significance



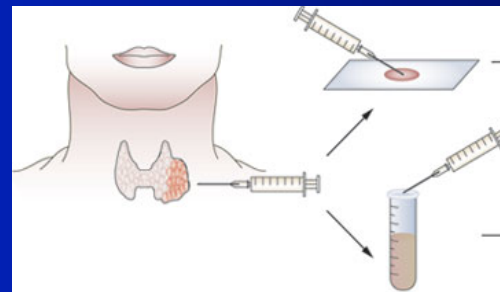
Cancer prevalence

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

$p < 0.001$

140 microPTC

- 13 multifocal
- 17 with lymphnode metastases



BRAF: diagnostic and prognostic significance



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



BRAF: diagnostic and prognostic significance

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

BRAF: diagnostic and prognostic significance



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





BRAF: diagnostic and prognostic significance

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%



BRAF: diagnostic and prognostic significance

CONCLUSION -1



BRAF molecular analysis increases diagnostic sensitivity of cytology for PTC and may influence clinical management

BRAF: diagnostic and prognostic significance

- any prognostic value?

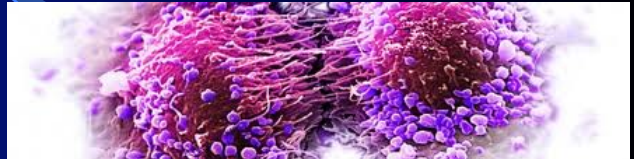


BRAF: diagnostic and prognostic significance

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



⊕

MMP, vimentin, osteopontin

epithelial-mesenchymal transition

little growth advantage

BUT

genomic instability

Mitsutake et al. Cancer Research 2005;65: 2465



BRAF: diagnostic and prognostic significance

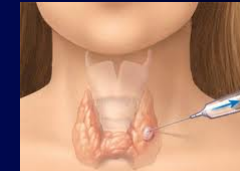


Table 1. Association of *BRAF* Mutation Status Detected on Thyroid Fine-Needle Aspiration Biopsy With Poorer Clinicopathologic Characteristics of Papillary Thyroid Cancer

Characteristic	<i>BRAF</i> Positive (n = 73)		<i>BRAF</i> Negative (n = 117)		P*
	No.	%	No.	%	
Age at diagnosis, years					.28
Median	43		46		
Range	24-77		12-83		
Sex, male	24	32.9	29	24.8	.25
Tumor size, cm					.35
Median	1.8		1.5		
Range	0.6-10.0		0.6-6.0		
Extrathyroidal extension	17	23.3	13	11.1	.039
Capsular invasion	21	28.8	19	16.2	.045
Lymph node metastasis	28	38.4	21	18.0	.002
I	54	74.0	89	76.1	
II	4	5.5	15	12.8	
III	9	12.3	9	7.7	
IV	6	8.2	4	3.4	
III/IV	15	20.6	13	11.1	.093
Multifocality	34	46.6	47	40.2	.45

Abbreviation: AJCC, American Joint Committee on Cancer.

*P value from Fisher's exact test for categorical data and Wilcoxon rank sum test for continuous data.



BRAF: diagnostic and prognostic significance



BRAFV600E molecular test	PTC persistence/recurrence prediction	
	All PTC	Conventional PTC
sensitivity	68%	79%
specificity	66%	60%
PPV	36%	34%
NPV	88%	92%

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

BRAF mutation-positive patients are significantly more likely to have PTC persistence/recurrence



BRAF: diagnostic and prognostic significance

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 ...Zatelli submitted

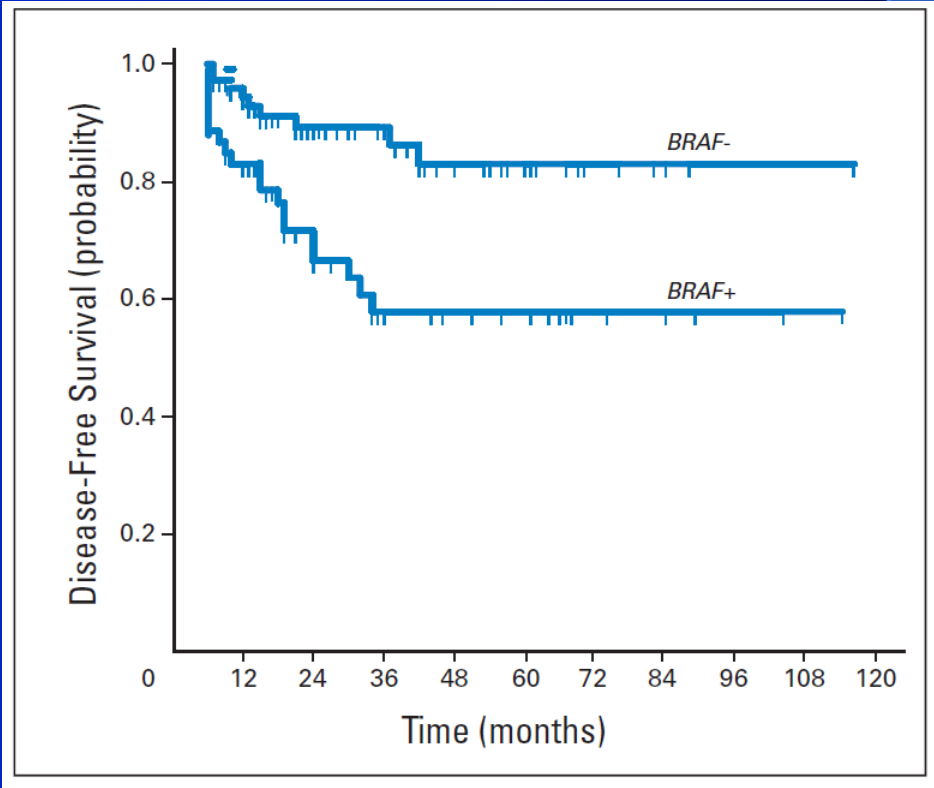




BRAF: diagnostic and prognostic significance

**BRAFV600E
molecular test**

**significantly reduced disease-free probability
in BRAF+ patients**

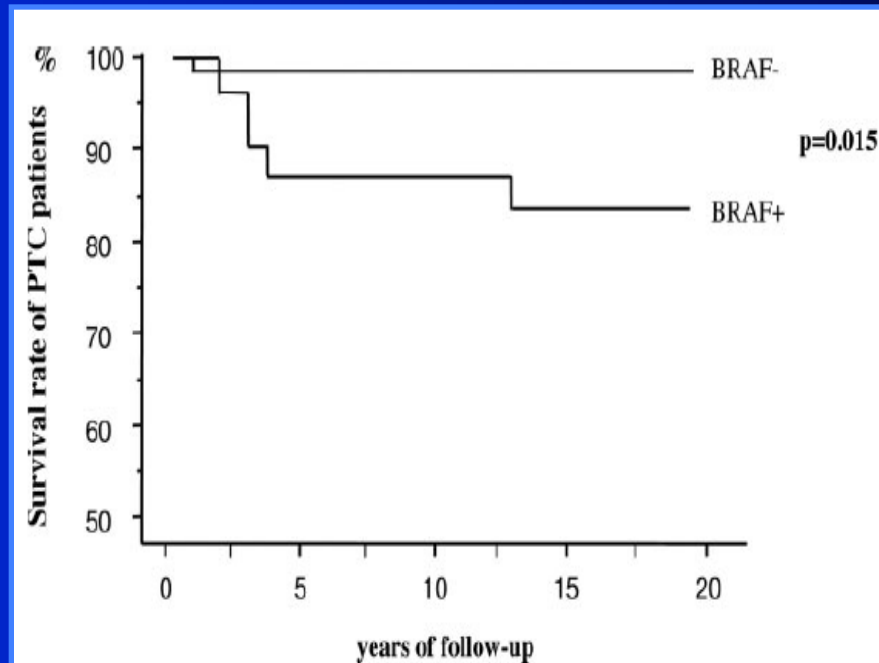




BRAF: diagnostic and prognostic significance

**BRAFV600E
molecular test**

**significantly increased mortality
in BRAF+ patients**



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



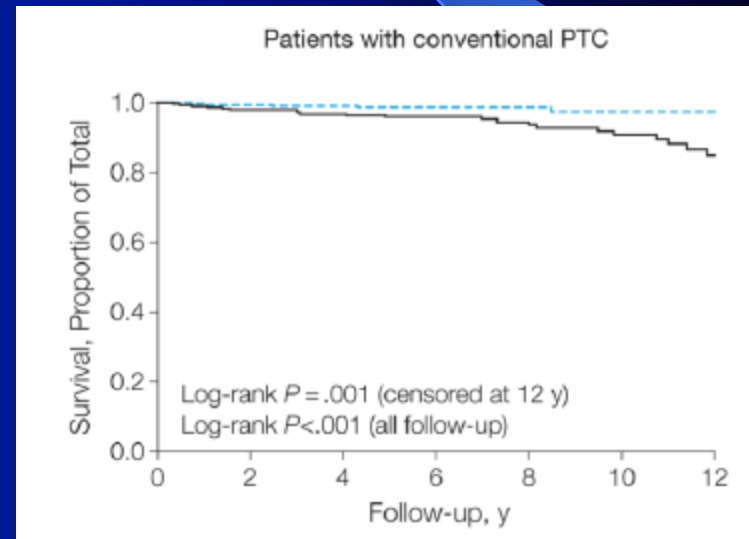
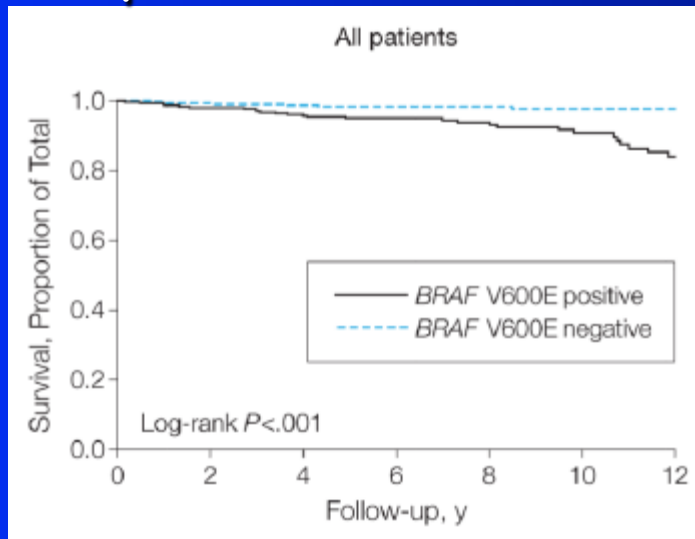


BRAF: diagnostic and prognostic significance

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

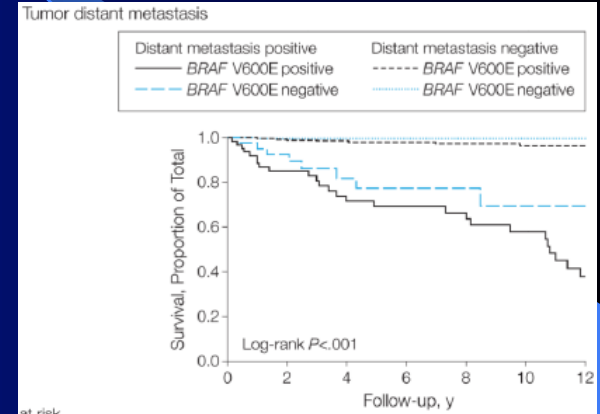
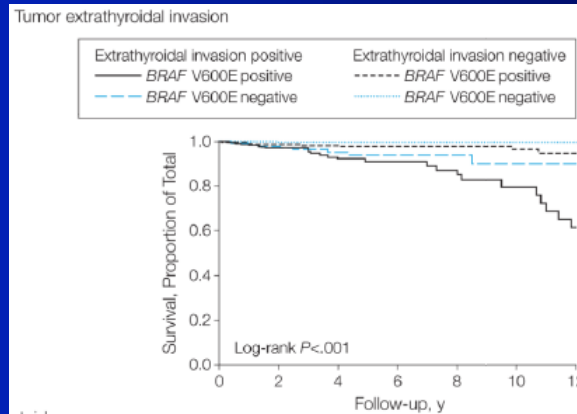
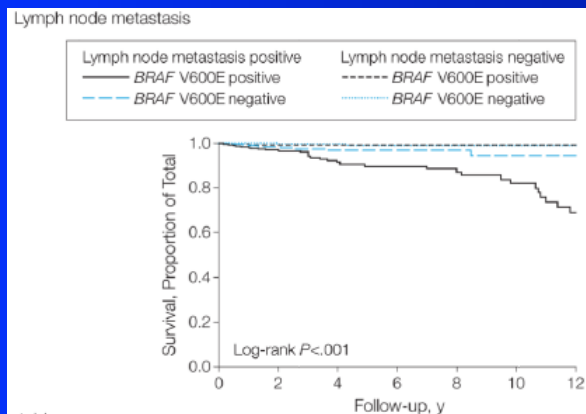




BRAF: diagnostic and prognostic significance

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”





BRAF: diagnostic and prognostic significance

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.

A higher BRAF V600E-associated patient mortality was observed in several clinicopathological subcategories, but statistical significance was lost with adjustment for patient age, sex, and medical center.





BRAF: diagnostic and prognostic significance

CONCLUSION -2

BRAF status may predict patients outcome



BRAF: diagnostic and prognostic significance

- any surgical relevance ?



BRAF: diagnostic and prognostic significance



**BRAFV600E
molecular test**

→ surgical strategy — lobectomy
 — total thyroidectomy

↓ lymph node dissection ↓ no dissection

better staging

High prognostic impact

- extrathyroidal invasion
- lymph node metastasis
- local neck recurrence
- PTC recurrence
- complications

**what
lymphnode
dissection?**

Xing Endocrine Reviews 2007; 28: 742



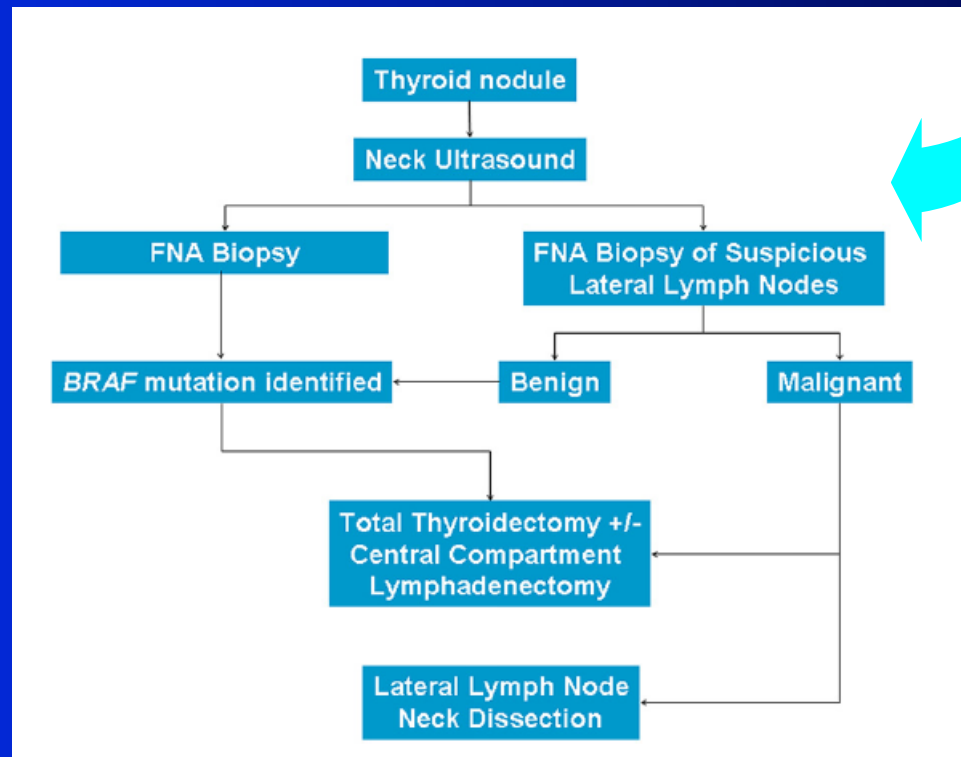
BRAF: diagnostic and prognostic significance

**BRAFV600E
 molecular test**



PREDICTOR OF LYMPHNODE METASTASES

Indication for sentinel lymphnode





BRAF: diagnostic and prognostic significance

CONCLUSION -3

BRAF V600E may influence surgical approach



BRAF: diagnostic and prognostic significance

**BRAFV600E
molecular test**

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





BRAF: diagnostic and prognostic significance

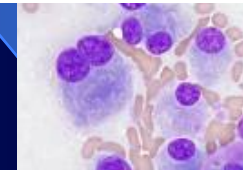
THEREFORE

**BRAFV600E
molecular test**

- ✓ increases cytology diagnostic sensitivity for PTC
- ✓ may predict patients outcome
- ✓ influences surgical approach
- ✓ allows detection of minimal disease metastatic to cervical lymph nodes



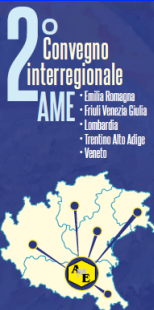
FNAB material



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



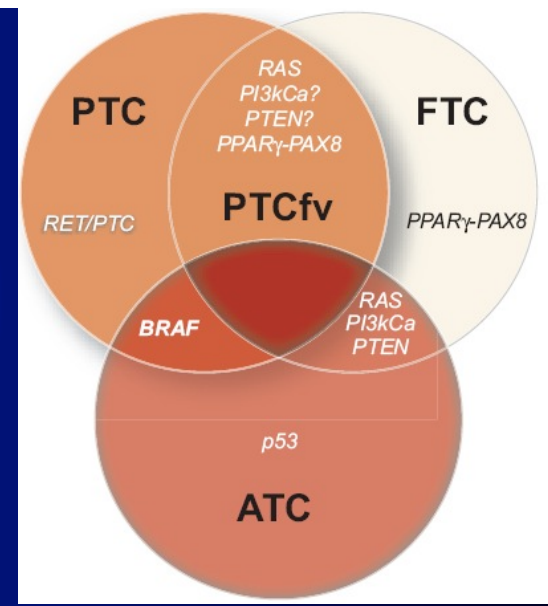


BRAF: diagnostic and prognostic significance

	BRAF	RET/PTC	RAS	PI3KCa*	PTEN	PPAR γ /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





BRAF: diagnostic and prognostic significance

BUT

Molecular testing
is not sufficient
to detect all malignant cases

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





BRAF: diagnostic and prognostic significance

...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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