Molecular markers in thyroid cancer: current role in clinical practice

BRAF as a prognostic marker in papillary thyroid cancer

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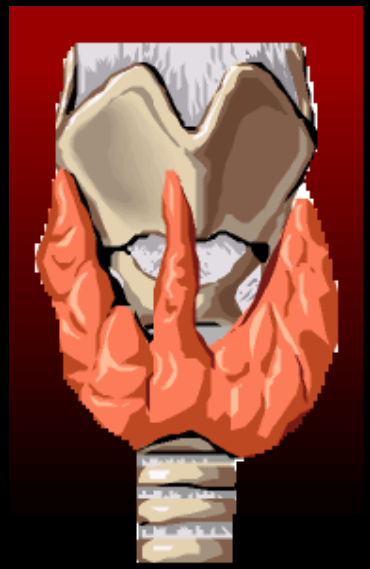
THYROID CANCER

Bari,
7-10 novembre 2013



√ from thyroid follicular cells:

- Papillary → **The most prevalent histotype**
- Follicular
- Anaplastic
- Poorly differentiated

√ from parafollicular C cells:

- Medullary

THYROID TUMORIGENESIS

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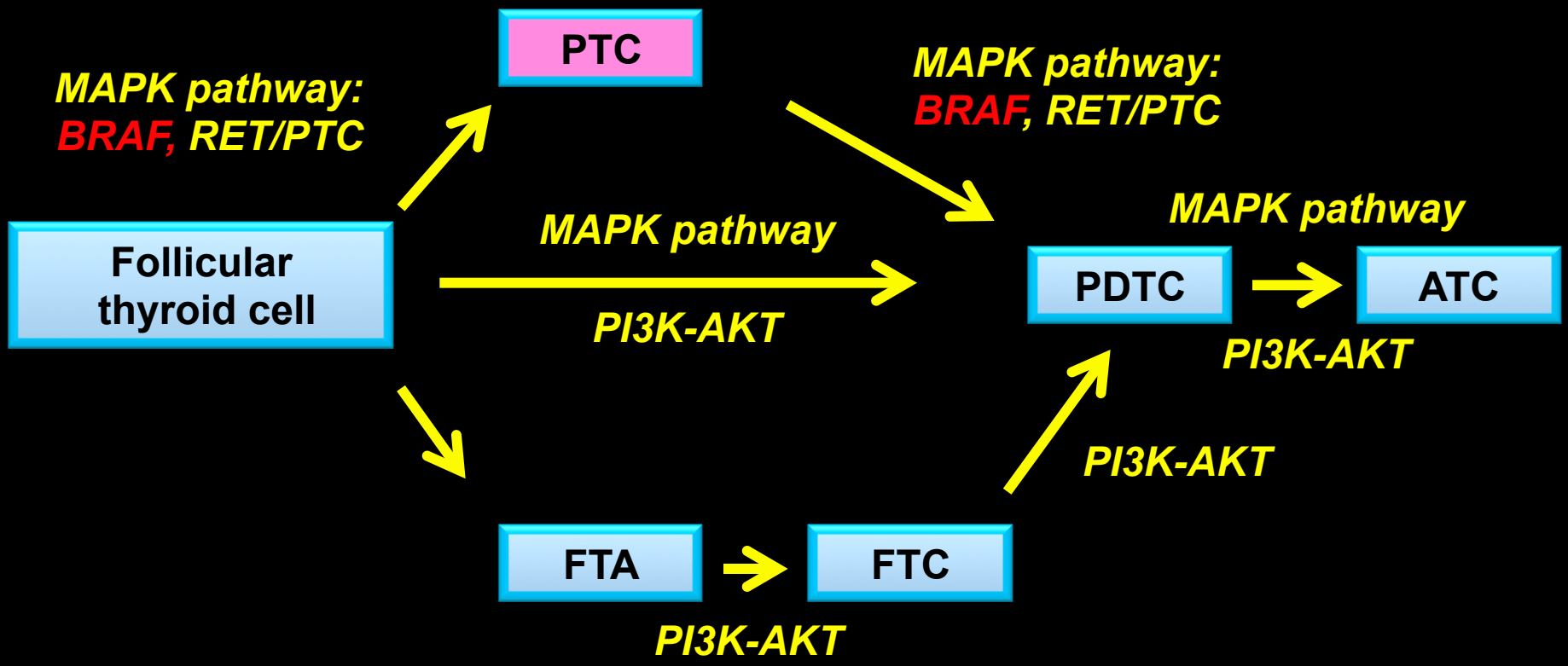

THE MAPK KINASE PATHWAY

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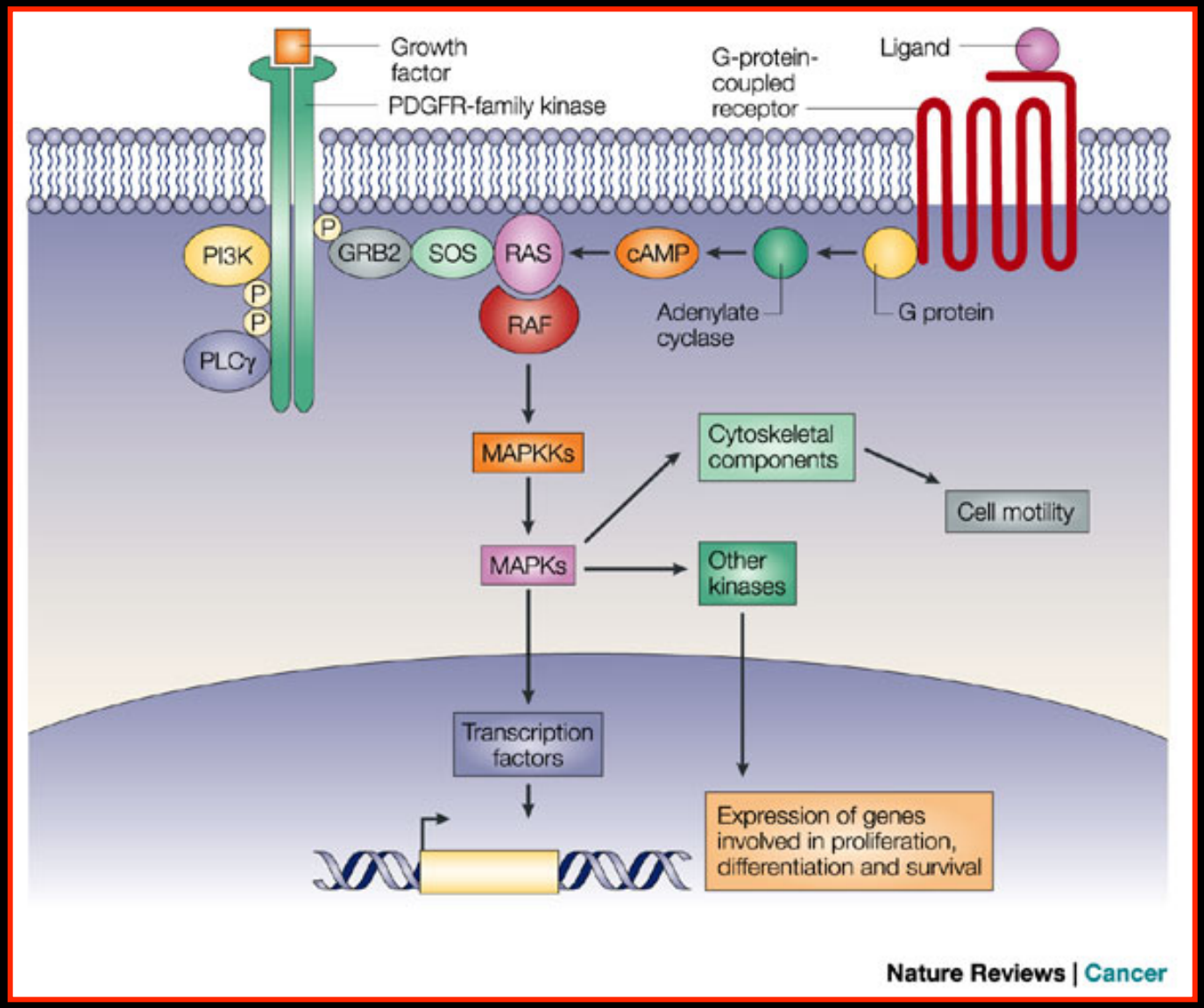

COSMIC somatic mutations in human cancer

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V600E Exon 15

Papillary thyroid carcinoma

Gene Name	Sample Number	Positive Samples	Percent Mutated
BRAF	13706	6549	48%

http://www.sanger.ac.uk/genetics/CGP/

BRAF as a prognostic marker in papillary thyroid cancer

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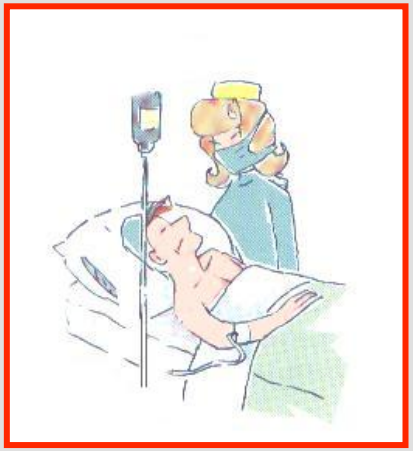
The BRAFV600E mutation has been correlated with worse clinical and pathological features in PTC

BRAFV600E mutation



Targeted Expression of BRAF^{V600E} in Thyroid Cells of Transgenic Mice Results in Papillary Thyroid Cancers that Undergo Dedifferentiation

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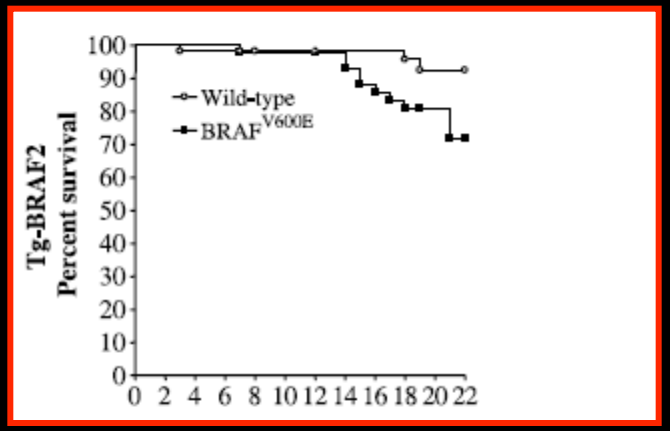
Transgenic mice with the Braf mutation show aggressive features …….

Line	Age (wk)	PTC prevalence (%)	Histologic characterization (%)				
			Tall cell	Poorly differentiated	Invasion		
					Capsular	Vascular	Muscle
Tg-BRAF2	12	14/15 (93)	87	47	73	47	27
Tg-BRAF2	22	13/14 (93)	79	50	93	50	36

….. and incresed rate of mortality



Knauf et al Cancer Res 2005

Positive correlation with clinical/pathological features

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Clinical features	p	Authors
Age	<0.0001	Nikiforova et al. JCEM 2003
	0.01	Fugazzola et al. End Rel Can 2006
Male gender	0.006	Kim et al. Clin Endocrinol 2006
Size	0.006	Kim et al. Clin Endocrinol 2006
	0.01	Jo et al. JCEM 2006
Multifocality	0.04	Trovisco et al. Virchows Arch 2005
Node metastases	0.001	Xing et al. JCEM 2005
Extrathyoid extension	0.001	Xing et al. JCEM 2005
	0.03	Nikiforova et al. JCEM 2003
	0.003	Riesco-Eizaguirre et al. End Rel Can 2006
Distant metastases	0.003	Namba et al. JCEM 2003
Stage (I+II vs III +IV)	0.002	Xing et al. JCEM 2005
	0.01	Riesco-Eizaguirre et al. End Rel Can 2006
	0.003	Nikiforova et al. JCEM 2003

BRAF^{V600E} and clinical/pathological features in low risk PTC (T1-T2, N0, M0)

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Clinical-pathological features	BRAF + (n=106)	BRAF – (n=213)	P value
Age, mean	41±13.9	44.6±13.4	0.03
Male gender	25/106	56/213	NS
Tumor size	1.4±0.8	1.6±1	NS
Multifocality	37/106	47/213	0.02
Aggressive variant	24/106	18/213	0.0004
Capsule infiltration	63/98	65/170	<0.0001
Intrathyroidal vascular invasion	9/96	10/168	NS
Stage II	6/106	27/213	NS
Treatments 131-I, n	14/97	8/185	0.003

Elisei et al JCEM 2012

Negative correlation with clinical/pathological features

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Extrathyroidal Invasion (p)	Lymph node metastasis (p)	Stages III and IV (p)	Ref.
0.283	0.522	0.093	Namba, 2003
	0.165	0.931	Xu, 2003
0.427		0.578	Puxeddu, 2004
	0.237	0.581	Fugazzola, 2004
	0.933		Sedliarou, 2004
0.182		0.143	Trovisco, 2005
0.414	0.206 /0.513	0.752	Kim, 2005
0.472	0.398	0.349	Liu, 2005
0.087	0.106		Jin, 2006
0.928	0.426		Park, 2006
0.313	0.378		Abrosimov, 2007
		0.514	Mitsiades, 2007
0.386	0.733	0.221	Durante, 2007

BRAFV600E Mutation Does Not Mean Distant Metastasis in PTC *Sancisi et al JCEM 2012*

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Clinical pathological features	Distantly metastatic PTC Total (n = 47)	Distantly metastatic PTC BRAFV600E (n = 14)	Control PTC Total (n = 75)	Control PTC BRAFV600E (n = 33)
Extrathyroidal extension[a]				
0	4 (8.5%)	0 (–)	42 (56.0%)	21 (63.6%)
+	22 (46.8%)	5 (35.7%)	25 (33,3%)	8 (24.3%)
++	17 (36.2%)	7 (50.0%)	8 (10.7%)	4 (12.1%)
NA	4 (8.5%)	2 (14.3%)	0	0
Vascular invasion[b]				
0	17 (36.2%)	6 (42.8%)	53 (70.7%)	22 (66.7%)
+	16 (34.0%)	4 (28.6%)	18 (24.0%)	10 (30.3%)
++	9 (19.1%)	1 (7.2%)	4 (5.3%)	1 (3.0%)
NA	7 (14.9%)	3 (21.4%)	0	0
pT				
1a	2 (4.3%)	0	0	0
1b	0	0	26 (34.7%)	12 (36.4%)
2	0	0	16 (21.3%)	7 (21.2%)
3	37 (78.7%)	9 (64.3%)	33 (44.0%)	14 (42.4%)
4a	4 (8.5%)	3 (21.4%)	0	0
4b	1 (2.1%)	0	0	0
X	3 (6.4%)	2 (14.3%)	0	0
pN				
0	6 (12.8%)	1 (7.1%)	35 (46.7%)	18 (54.5%)
1a	8 (17.0%)	3 (21.5%)	10 (13.3%)	3 (9.1%)
1b	32 (68.0%)	9 (64.3%)	28 (37.3%)	12 (36.4%)
X	1 (2.1%)	1 (7.1%)	2 (2.7%)	0
Stage				
1	9 (19.1%)	2 (14.3%)	50 (66.7%)	22 (66.7%)
2	0	0	6 (8.0%)	1 (3.0%)
3	10 (21.3%)	2 (14.3%)	7 (9.3%)	4 (12.1%)
4	25 (53.2%)	10 (71.4%)	12 (16.0%)	6 (18.2%)
NA	3 (6.4%)	0	0	0

Follow-up 7 years

p=NS

No evidence for a role of BRAF mutation in tumor progression

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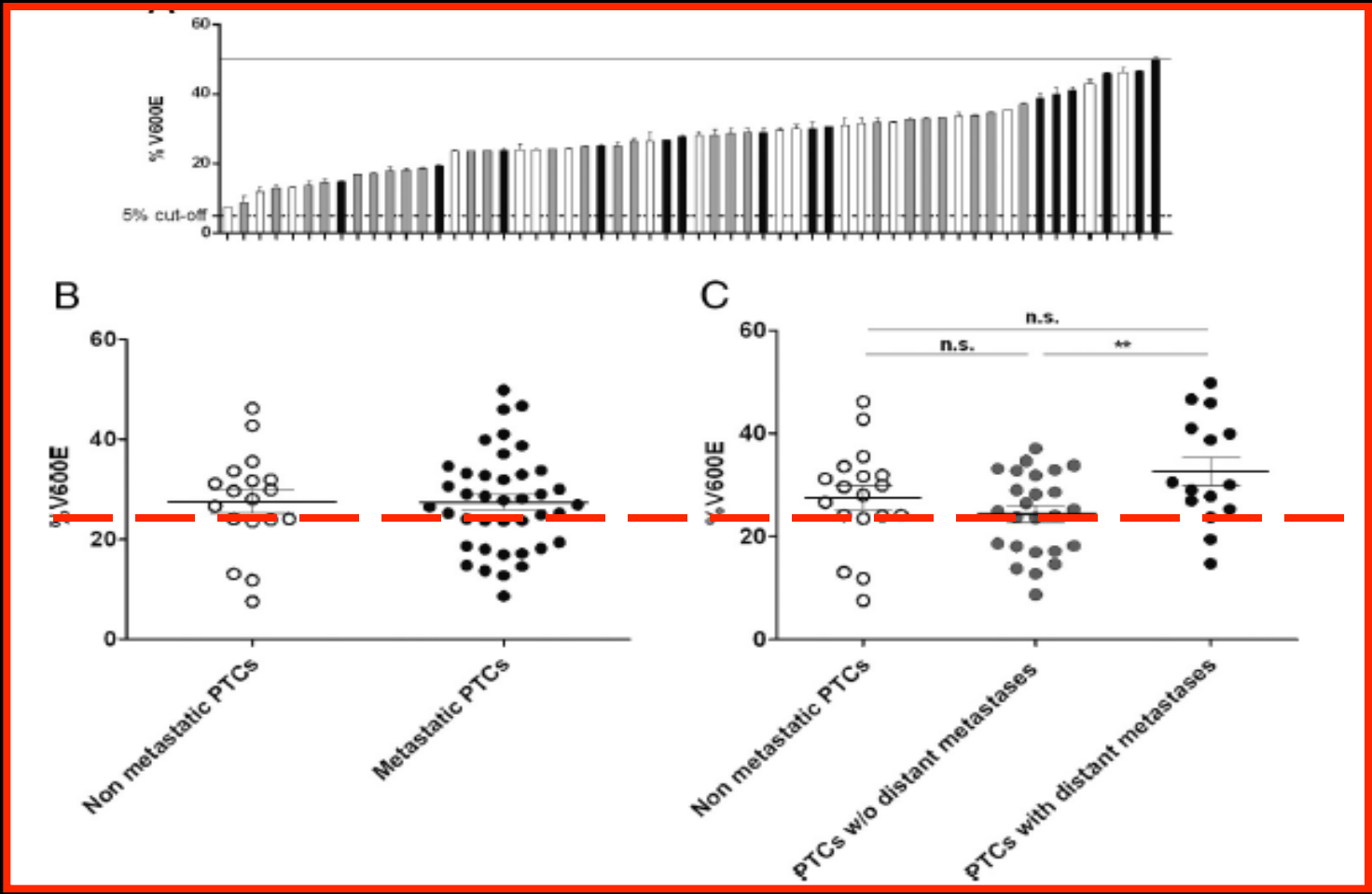
Gandolfi et al JCEM 2013

The average mutated allele percentage is not different between non metastatic and metastatic PTC



BRAF as a prognostic marker in papillary thyroid cancer

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BRAFV600E MUTATION AND WORSE OUTCOME

Clinical-pathological features of PTC and Outcome:

Elisei et al JCEM 2008

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Clinical features	Free of desease (n=83)	Persistent disease and dead patients (n=19)	P value
Age, mean±SD (yrs) > 60 (yrs)	41.5±13.9 9/83 (10.8%)	48.7±20.2 7/19 (36.8%)	0.06 *0.005*
Male sex	15/83 (18.1%)	5/19 (26.3%)	0.4
Tumor size, mean±SD (cm) >3 cm	1.8±1.2 8/80 (10%)	2.6±1.8 8/18 (44%)	*0.009* *0.0004*
Multifocality	28/80 (35%)	9/16 (56.3%)	0.1
Limphnode Metastases	27/82 (32.9%)	13/18 (72.2%)	*0.002*
Extrathyroid Extension	5/81 (6.2%)	7/16 (43.8%)	*<0.0001*
Distant Metastasis	1/82 (1.2%)	5/18 (27.8%)	*<0.0001*
De Groot' Class (III and IV)	23/76 (30.2%)	14/17 (82.4%)	*<0.0001*
TNM Stage (III and IV)	10/80 (12.5%)	10/18 (55.5%)	*<0.0001*
VEGF expression (score 2 and 3)	40/73 (54.8%)	13/15 (86.6%)	*0.02*
Vascular invasion	18/73 (24.6%)	9/15 (60%)	*0.006*
$BRAF^{V600E}$ mutation	25/83 (30.1%)	13/19 (68.4%)	*<0.002*

BRAF as a prognostic marker in papillary thyroid cancer

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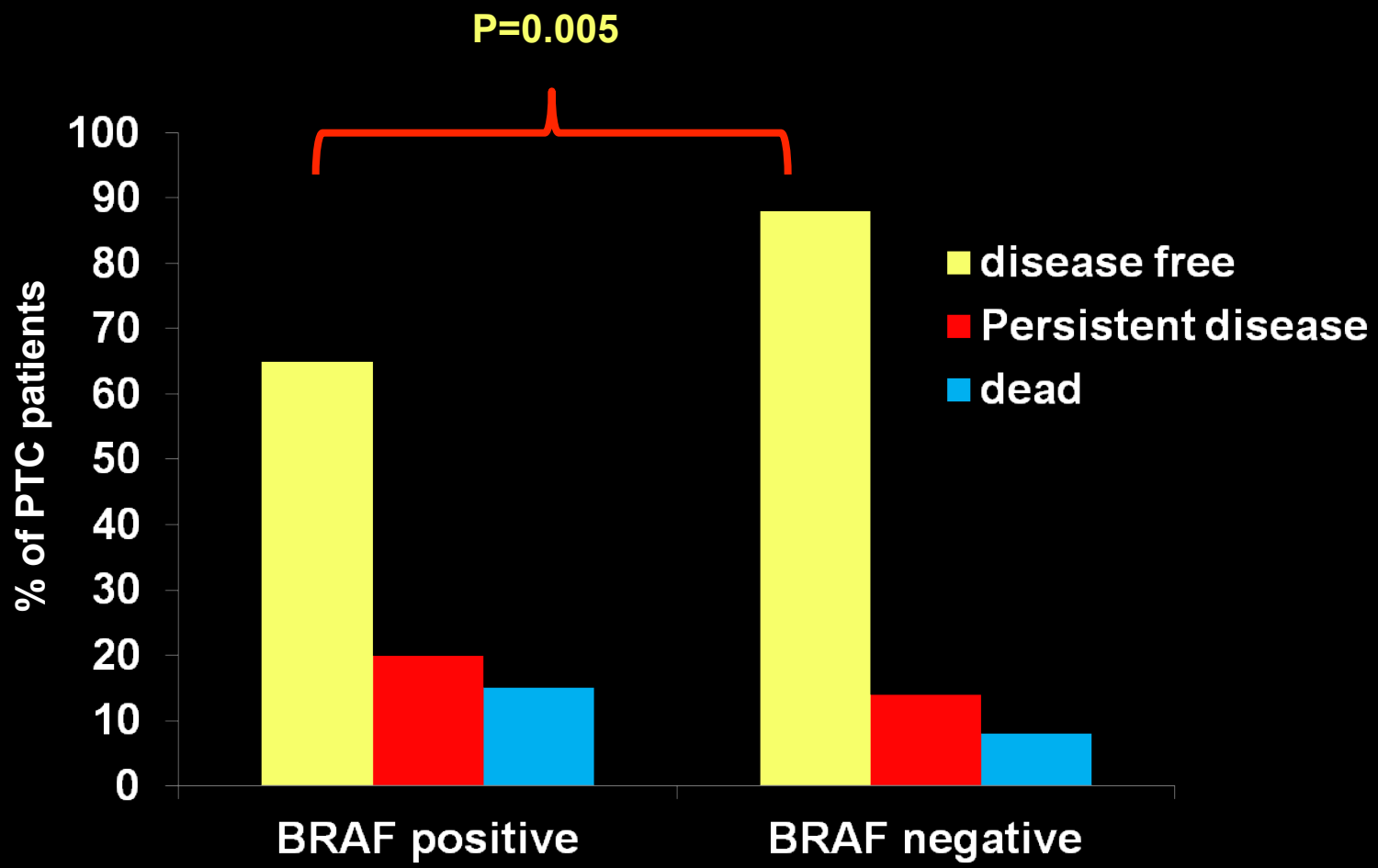

Elisei et al JCEM 2008

Clinical-pathological features and Outcome (Multivariate Logistic Regression Analysis)

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Clinical features	Odds ratio	95% CI	P-value
Age at diagnosis > 60	1.25	0.08-19.28	0.87
Tumor size	0.51	0.25-1.04	0.06
De Groot' s classes	10.97	0.72-166.84	0.08
TNM Stage	1.02	0.89-1.16	0.73
VEGF (score 2-3)	1.20	0.95-1.51	0.12
Vascular Invasion	0.97	0.10-9.00	0.98
BRAF mutation	14.63	1.28-167.29	*0.03*

Elisei R et al, JCE&M 2008

$BRAF^{V600E}$ and outcome in low risk PTC (T1-T2, N0, M0)

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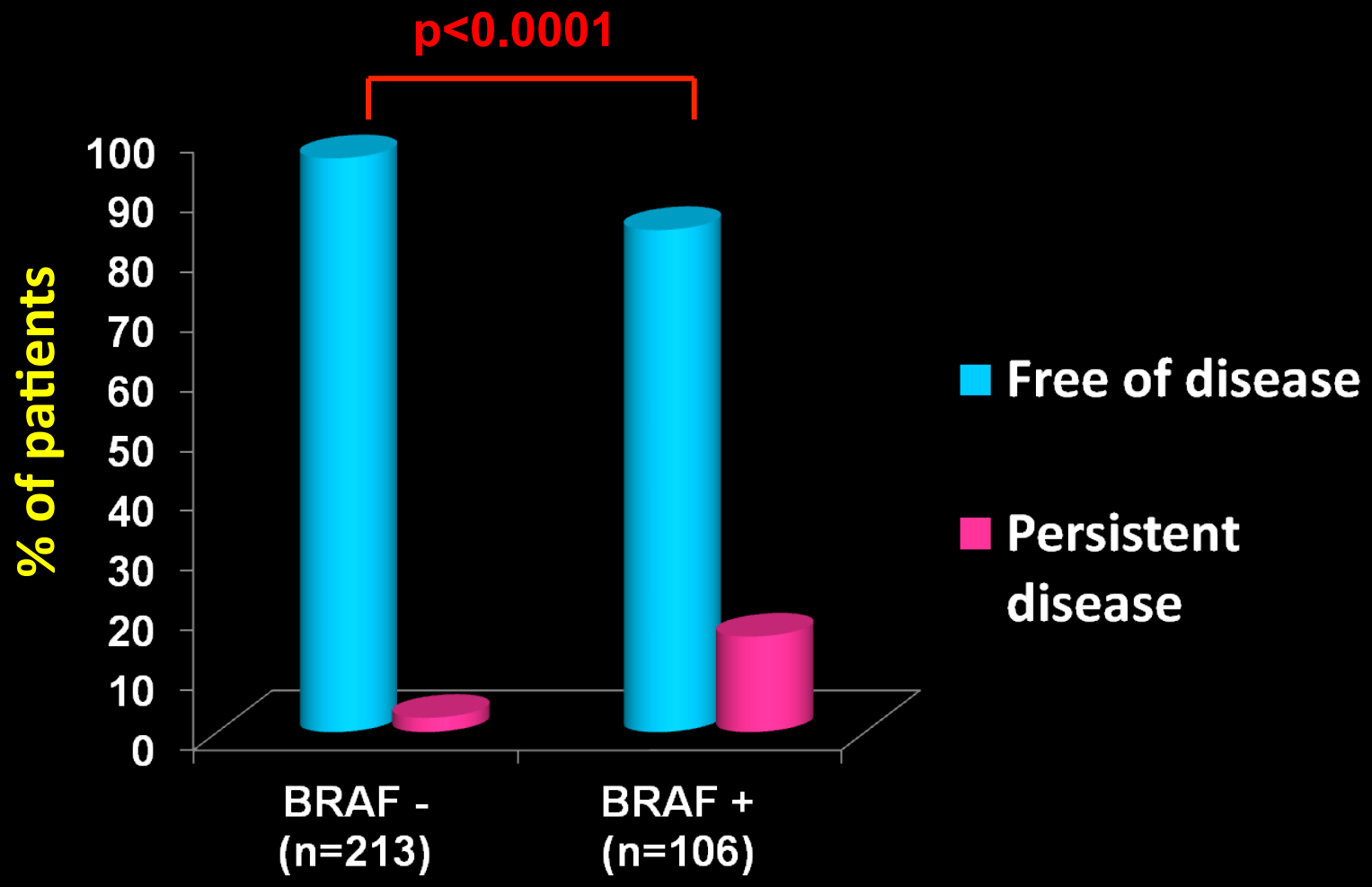

Elisei et al JCEM 2012

BRAF as a prognostic marker in papillary thyroid cancer

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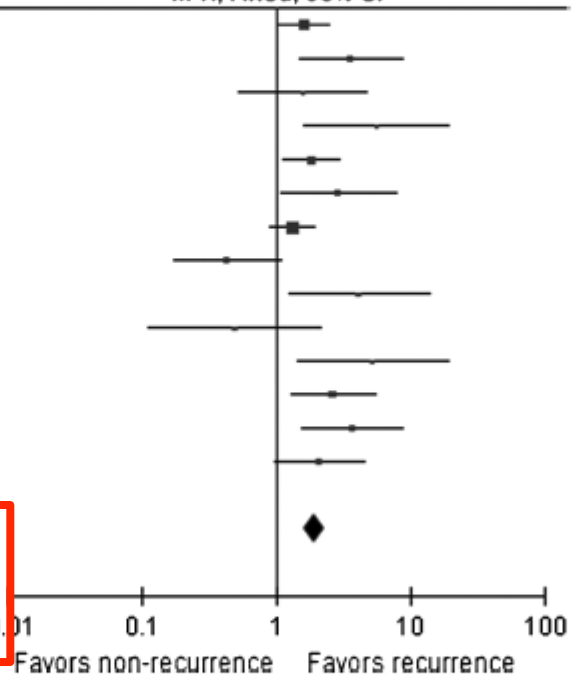
Study or Subgroup	braf+/rec+ Events	Total	braf-/rec+ Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Abubaker 2008	44	153	25	143	19.2%	1.64 [1.07, 2.54]
Elisei 2008	13	38	6	64	3.3%	3.65 [1.51, 8.80]
Fugazzola 2006	5	18	5	29	2.8%	1.61 [0.54, 4.80]
Howell 2011	11	86	3	133	1.8%	5.67 [1.63, 19.74]
Kebebew 2007	38	111	18	98	14.2%	1.86 [1.14, 3.04]
Kim 2006	32	149	4	54	4.4%	2.90 [1.08, 7.82]
Musholt 2010	35	87	38	126	23.1%	1.33 [0.92, 1.93]
O'Neill 2010	6	55	10	40	8.6%	0.44 [0.17, 1.10]
Riesco-Eizaguirre 2006	9	28	3	39	1.9%	4.18 [1.24, 14.06]
Sapio 2006	2	11	4	11	3.0%	0.50 [0.11, 2.19]
Sykorova 2010	8	67	3	133	1.5%	5.29 [1.45, 19.31]
Xing 2005	23	92	9	96	6.6%	2.67 [1.30, 5.45]
Xing 2009	15	40	6	60	3.6%	3.75 [1.59, 8.84]
Yip 2009	18	106	8	100	6.1%	2.12 [0.97, 4.66]
Total (95% CI)		**1041**		**1126**	**100.0%**	**1.93 [1.61, 2.32]**
Total events	259		142			

Heterogeneity: $Chi^2 = 30.16$, $df = 13$ ($P = 0.004$); $I^2 = 57\%$

Test for overall effect: $Z = 7.01$ ($P < 0.00001$)



BRAFV600E is strongly associated with tumor recurrence

Tufano et al Medicine 2012

BRAF as a prognostic marker in papillary thyroid cancer

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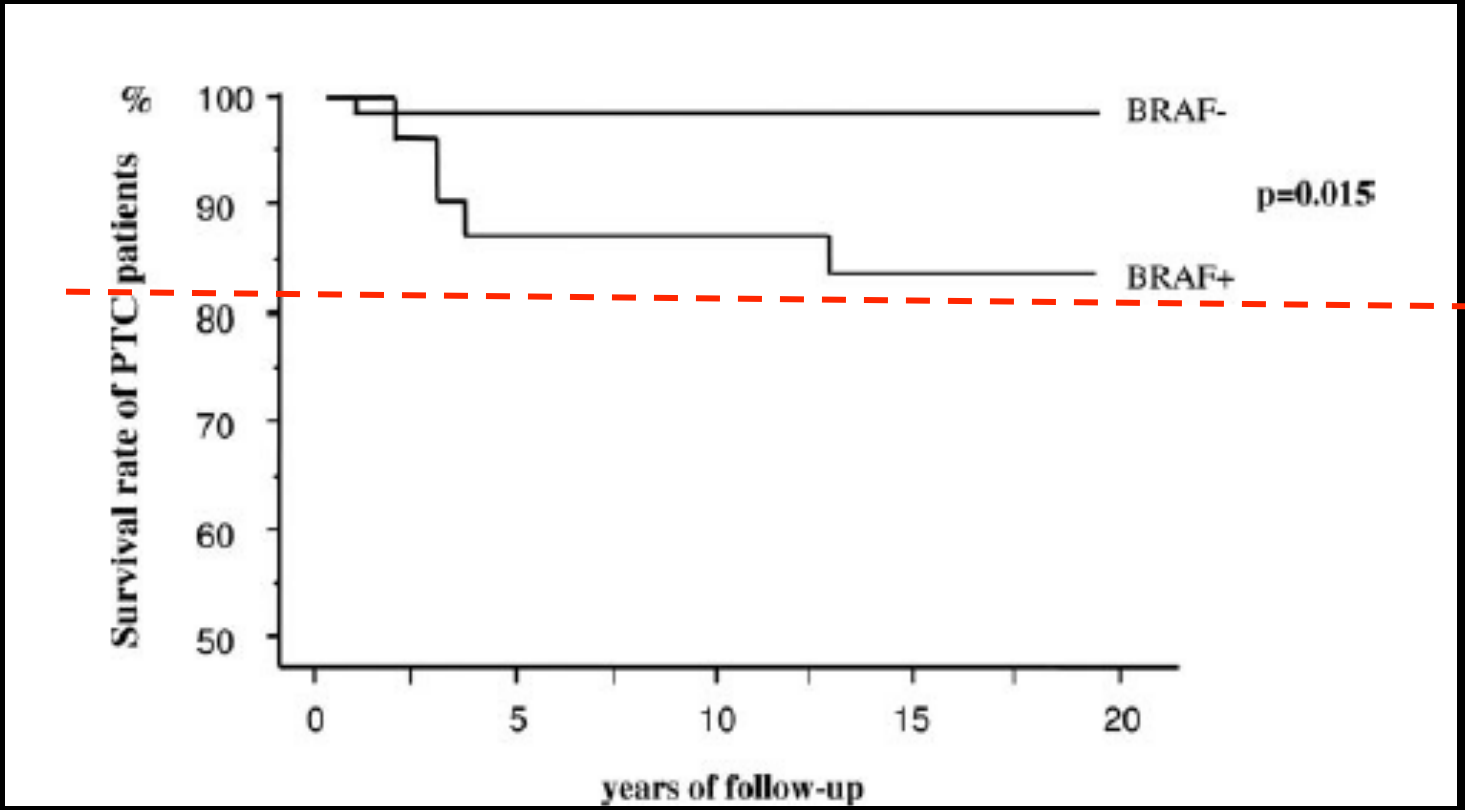

Elisei et al JCEM 2008

Association Between *BRAFV600E Mutation and* Mortality in PTC *Xing et al JAMA 2013*

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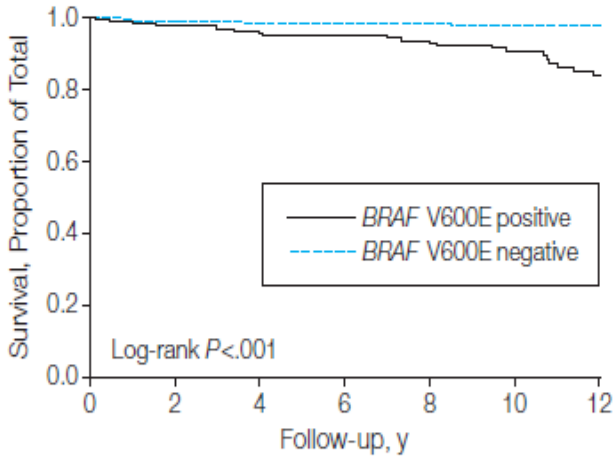
a multicentric study

Type of Papillary Thyroid Cancer	Mortality, No./Total (%)				Person-Years of Follow-up	Deaths per 1000 Person-Years (95% CI)		Hazard Ratio (95% CI)	
	Overall	*BRAF* V600E–Positive	*BRAF* V600E–Negative	*P* Value		*BRAF* V600E–Positive	*BRAF* V600E–Negative	Unadjusted	Adjusted[a]
All types	56/1849 (3.0)	45/845 (5.3)	11/1004 (1.1)	<.001	7856.75	12.87 (9.61-17.24)	2.52 (1.40-4.55)	5.31 (2.74-10.30)	2.66 (1.30-5.43)
Conventional	39/1233 (3.2)	33/659 (5.0)	6/574 (1.0)	<.001	5466.75	11.80 (8.39-16.60)	2.25 (1.01-5.00)	5.63 (2.34-13.51)	3.53 (1.25-9.98)
Follicular variant	6/411 (1.5)	4/82 (4.9)	2/329 (0.6)	.02	1572.25	11.21 (4.21-29.86)	1.65 (0.41-6.58)	6.02 (1.10-32.96)	1.67 (0.06-47.49)

[a]Proportional hazards regression model adjusted for patient sex and age at diagnosis and stratified by medical center.

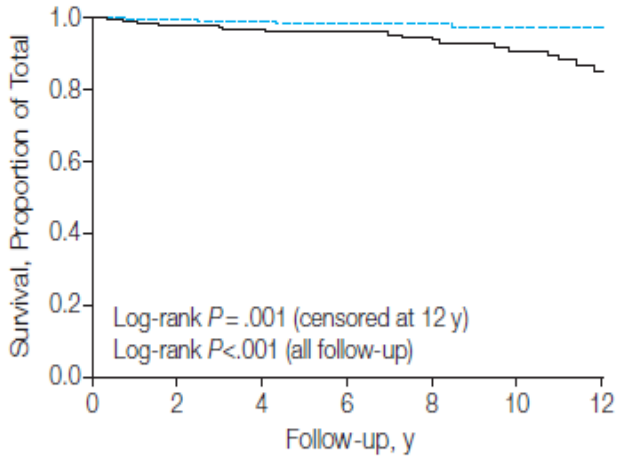
All patients



No. at risk

BRAF V600E positive	845	530	309	197	147	95	65
BRAF V600E negative	1004	640	393	243	177	127	87

Patients with conventional PTC



No. at risk

BRAF V600E positive	659	411	246	162	121	79	56
BRAF V600E negative	574	375	240	143	101	80	61

Correlation between BRAF mutation and advanced disease: *molecular reasons*

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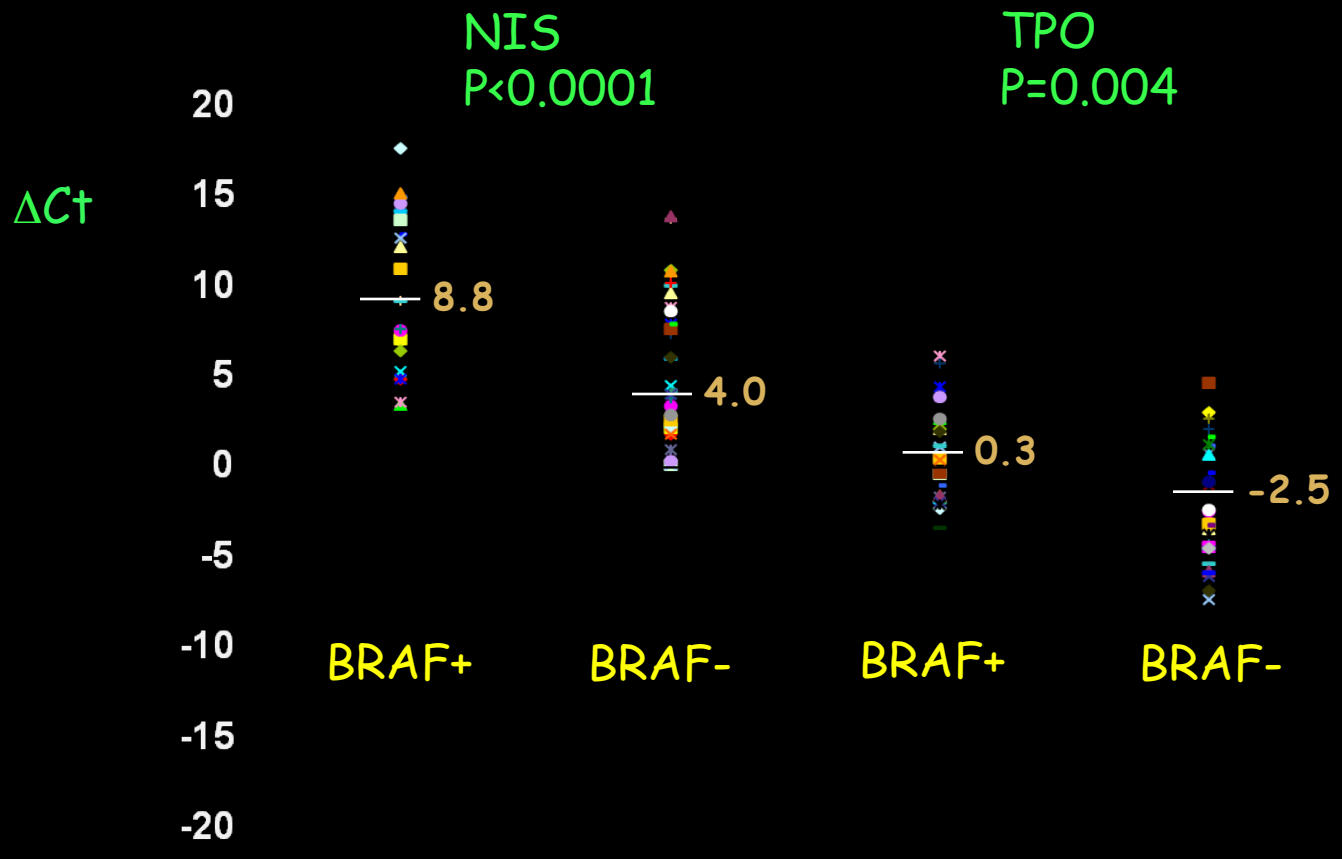
NIS and TPO mRNA expression is significantly lower in $BRAF^{V600E}$ positive PTC



(Romei C et al, Endoc Relat Cancer 2008)

Correlation between BRAF mutation and advanced disease: *molecular reasons*

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BRAF mutation is associated with tumor-promoting

Increased expression of cMET, VEGF, MMP, NFK

BRAFV600E

methylation of tumor-suppressor genes

Decreased expression of p27

BRAF as a prognostic marker in papillary thyroid cancer

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CONCLUSIONS I

- THE CORRELATION OF BRAFV600E WITH AGGRESSIVE CLINICAL-PATHOLOGICAL FEATURES OF PTC IS CONTROVERSIAL

BRAF as a prognostic marker in papillary thyroid cancer

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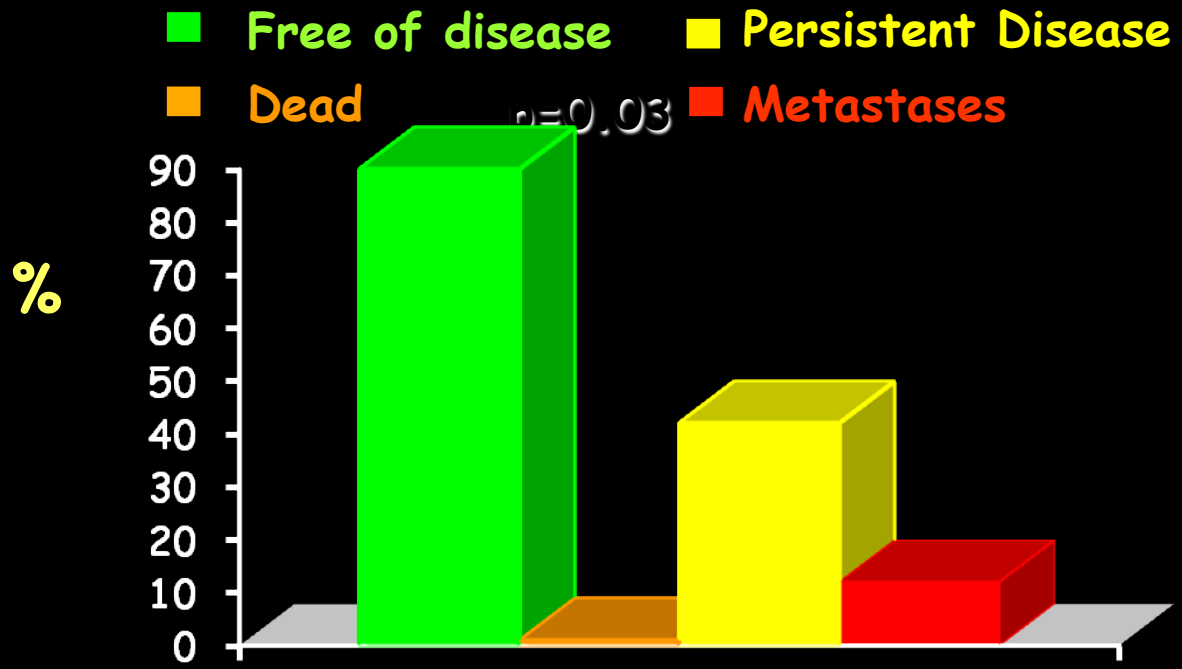
Papillary thyroid cancer in children:

- low prevalence of BRAFV600E mutations
- more aggressive behavior
- low prevalence of tumor related death



Our serie
n=78

One patient died
pulmonary fibrosis

BRAF as a prognostic marker in papillary thyroid cancer

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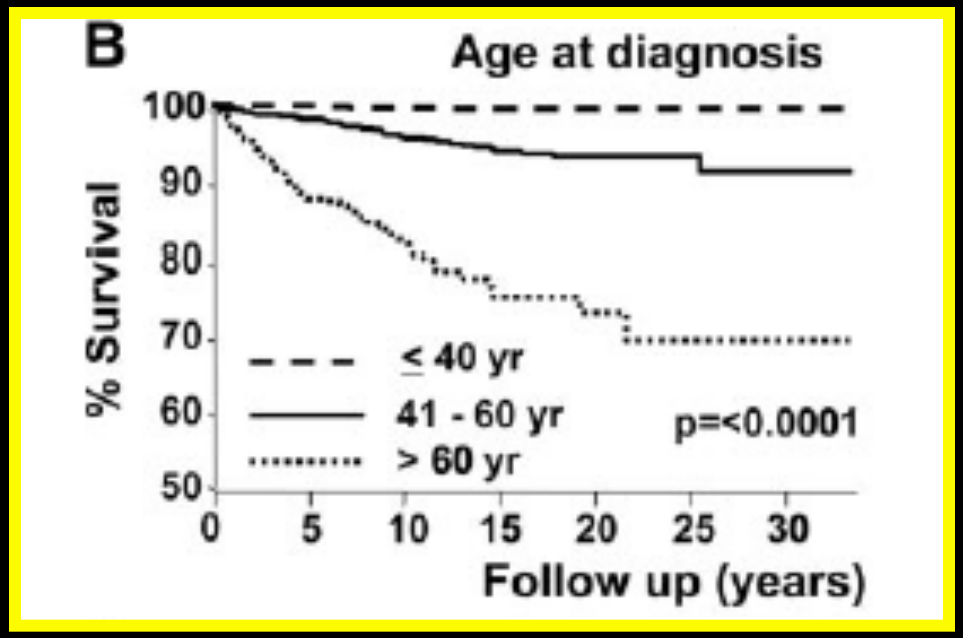
Papillary thyroid cancer in older age:

- high prevalence of BRAFV600E mutations
- more aggressive behavior
- higher prevalence of tumor related death



Elisei et al JCEM 2010

BRAF as a prognostic marker in papillary thyroid cancer

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CONCLUSIONS II

- THE ROLE OF THE BRAFV600E IS STRONGLY ASSOCIATED WITH TUMOR RECURRENCE AND DEATH

- THE BAD PROGNOSTIC ROLE OF THIS MUTATION COULD BE RELATED TO THE CORRELATION WITH AN ALTERED EXPRESSION OF TUMOR PROMOTING GENES

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thank you