Molecular pathology of thyroid cancers

Peter Lakatos

1st Department of Medicine

Semmelweis University

Thyroid nodules

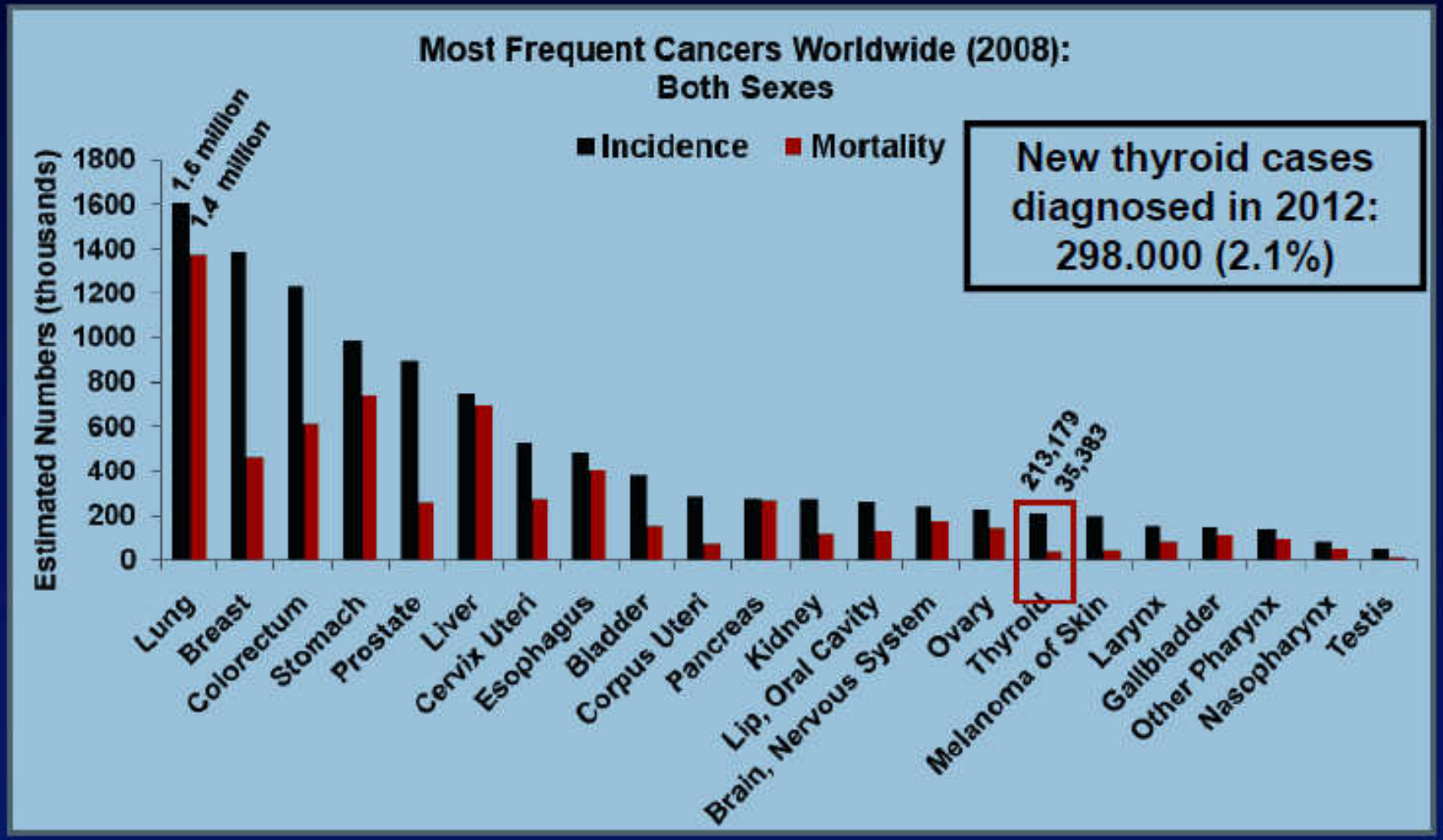
- 4-7% of population with palpable nodules
- More frequent in women
- Incidence increasing with age
- 5-10% of solitary cold nodules are malignant

The question is which one will become malignant?

Thyroid cancer

- 1.5-2.1% of all newly diagnosed cancers
- Increasing in number during the last 25 years:
  - From 4.8 to 8.0 persons per 100,000
  - 11.7 females, 4.2 males /100,000

Worldwide Incidence and Mortality by Cancer Type



GLOBOCAN 2008 Fast Stats; World Cancer Research Fund International. Available at: http://www.wcrf.org/cancer_statistics/world_cancer_statistics.php. Accessed 4 September 2014.

Classification of thyroid cancers

Of follicular origin:

- Differentiated
  - Papillary 80%
  - Follicular 10%
  - Hürthle cell 3-5%
- Non-differentiated
  - Anaplastic 1-2%

Of parafollicular origin:

  - Medullary 5%

Thyroid cancer and US

- hypodensity
- microcalcification
- hypervascularization
- solitary
- irregular borders
- lack of halo sign

The more of the above signs present, the more likely the nodule is malignant.

FNAB

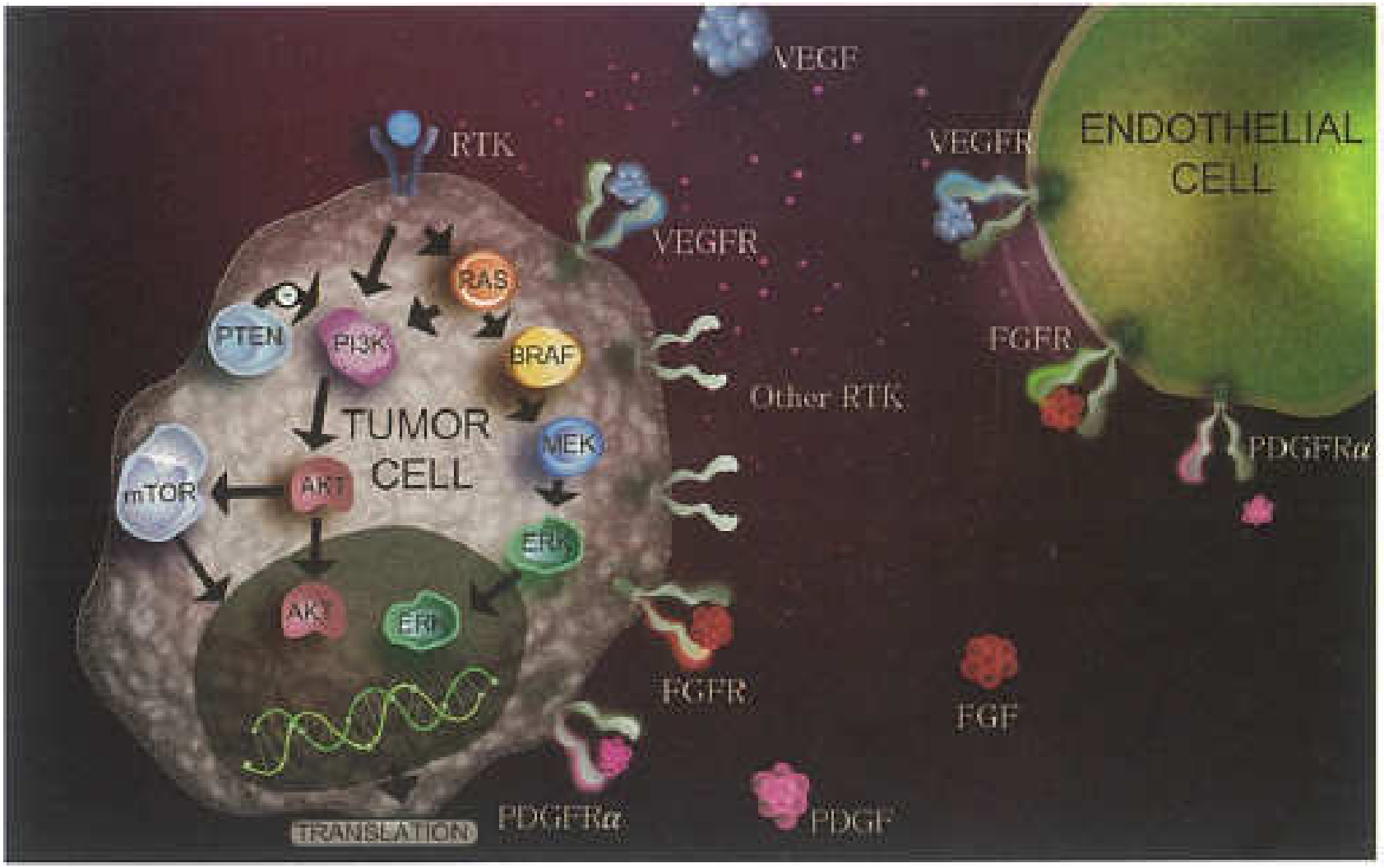
- FNAB is the most realiable in determining dignity
- 4 result categories:
  - non-diagnostic
  - benign
  - uncertain: AUS/FLUS; FN/SFN; SMC **– 10-40%!**
  - malignant
- Limitations of FNAB:
  - No differentiation between benign follicular or Hürthle cell adenoma or malignant versions
  - False negative: < 5%

Genetic tests

- Genetic alterations are causative factors
- Genetic alterations are consequences of cancer

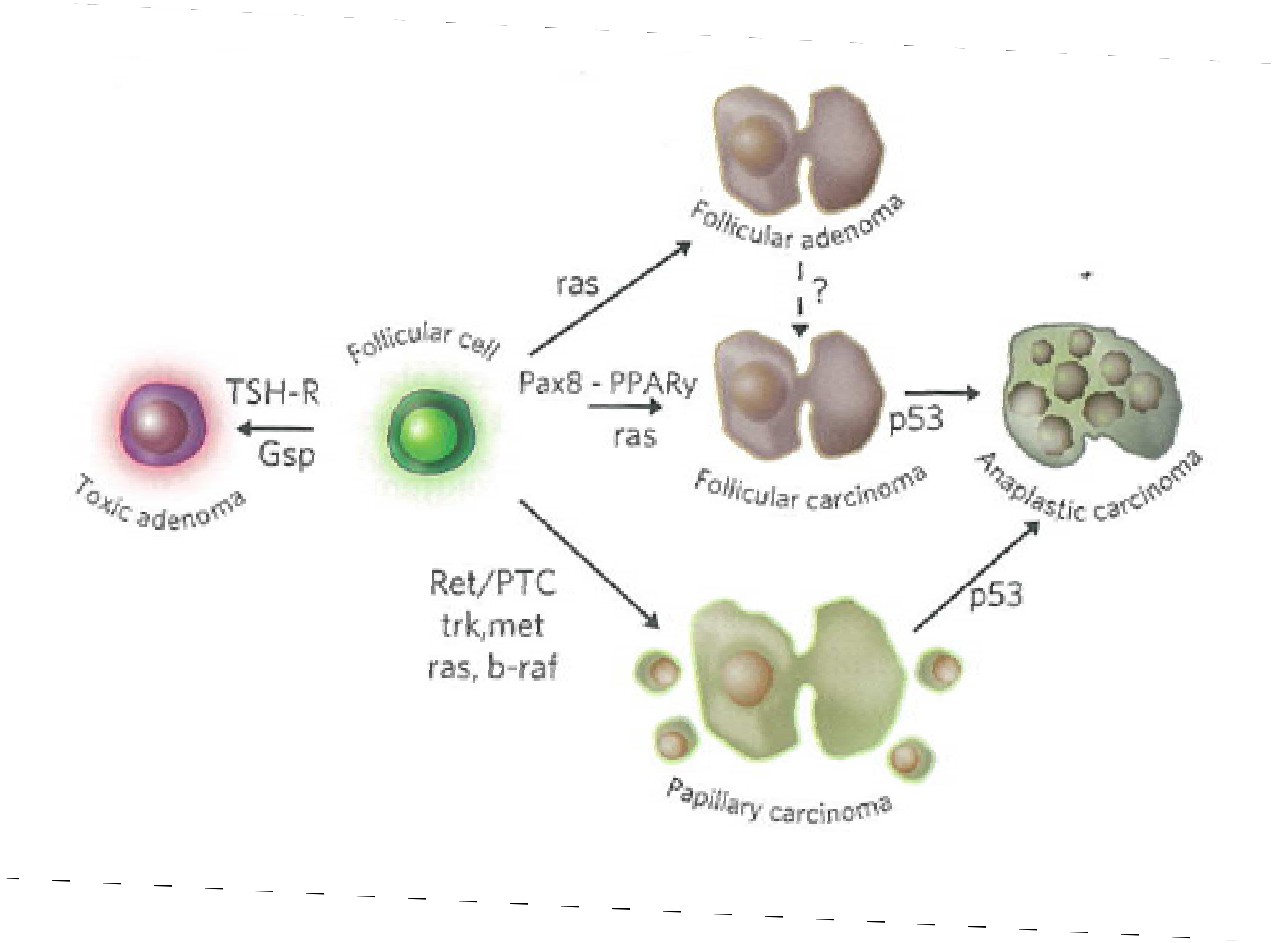
Is genetic testing a good marker for malignancy?

Main transduction pathways in thyroid cancer



Schlumberger M, Pacini F, Tuttle RM, Thyroid tumors. (4th ed), Institut Médico-Educatif, Paris, 2015.

General scheme of thyroid tumorigenesis



Schlumberger M, Pacini F, Tuttle RM, Thyroid tumors. (4th ed), Institut Médico-Educatif, Paris, 2015.

Differentiated thyroid cancers

- PTC and FTC comprise 90% of all thyroid malignancies.
- PTC is the most common histological type of all thyroid malignancies (60-80%). Somatic mutations are found in more than 40-70% of papillary carcinoma cases.
- FTC is the second most common histological type with a frequency is 10-15%. Mutation is present in 30-50%.

Benvenga, S., Horm Metab Res, 2008. 40(5): p. 323-8.
Woodruff, S.L., et al., Am J Surg, 2010. 200(4): p. 462-6.
Schlumberger, M., Ann Endocrinol (Paris), 2007. 68(2-3): p. 120-8.
Cheng, S.P., et al., Langenbecks Arch Surg, 2008. 393(5): p. 729-32.

Genetic alterations in PTC

PTC:

- **BRAF (v-raf murine sarcoma viral oncogene homolog B1)**
- **RET/PTC (RET tyrosine-kinase protooncogene / papillary thyroid carcinoma)**
- **RAS (rat sarcoma viral oncogene homolog) mutációk**

BRAF mutation is thought to correlate with tumor aggressivity:

- **extrathyroidal growth**
- **lymphnode involvement**
- **radioiodine resistance**
- **tumor reoccurence**

Albarel F, Conte-Devolx B, Oliver C.,Ann Endocrinol (Paris). 2012. Apr. 13. [Epub ahead of print]

Genetic alterations in FTC

FTC:

- RAS

Albarel F, Conte-Devolx B, Oliver C.,Ann Endocrinol (Paris). 2012. Apr. 13. [Epub ahead of print]

Risk of Cancer in nodules positive for RAS mutations

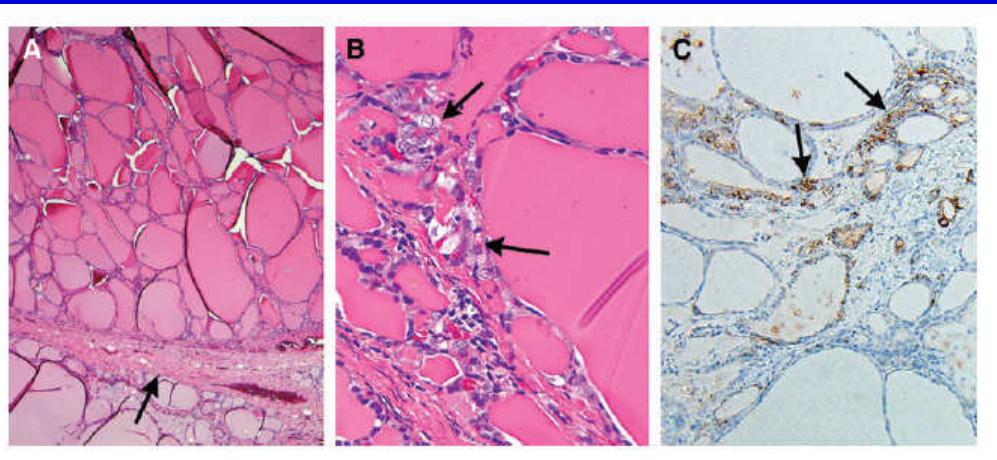
TABLE 1. MOLECULAR ABNORMALITIES DETECTED IN AUS/FLUS FNA SAMPLES AND ASSOCIATED CANCER RISK

	Number of mutation-positive samples	*Nodules with surgical outcome*	*Cancer on surgery (cancer risk)*
Point mutation			
NRAS	7	5	3 (60%)
HRAS	7	6	5 (83%)
KRAS	3	3	3 (100%)
PTEN	2	1	0
EIF1AX	2	2	1 (50%)
BRAF^{V600E}	1	1	1 (100%)
BRAF^{K601E}	1	1	1 (100%)
TSHR	1	0	0
Gene fusions			
THADA	3	3	2 (67%)
PPARG	1	1	1 (100%)
NTRK3	1	1	1 (100%)
NTRK1	1	1	1 (100%)
ALK	1	1	1 (100%)

79%

AUS/FLUS, atypia of undetermined significance/follicular lesion of undermined significance; FNA, fine-needle aspiration.

RAS mutation-positive „benign” nodules



Evidence for clonal neoplasm and early transformation to cancer

Nikiforov et al. Thyroid 2015. Sept 10. [Epub]

Genetic alterations in FTC

FTC:

- **RAS**
- **RET/PTC**
- **PAX8/PPAR-gamma mutations**

RAS family influencing 3 signaling patways:

- **MAP-kinase**
- **phosphatidylinositol-3-kinase/protein-kinase-B (PI3K/AKT)**
- **adhesion and migration**

Mutated RAS protein elicits GTP-ase effect and a consequent constitutive activation of follicular cell proliferation (genomic instability, increased growth potential, tumor development).

Albarel F, Conte-Devolx B, Oliver C.,Ann Endocrinol (Paris). 2012. Apr. 13. [Epub ahead of print]

Other genetic alterations in thyroid cancer

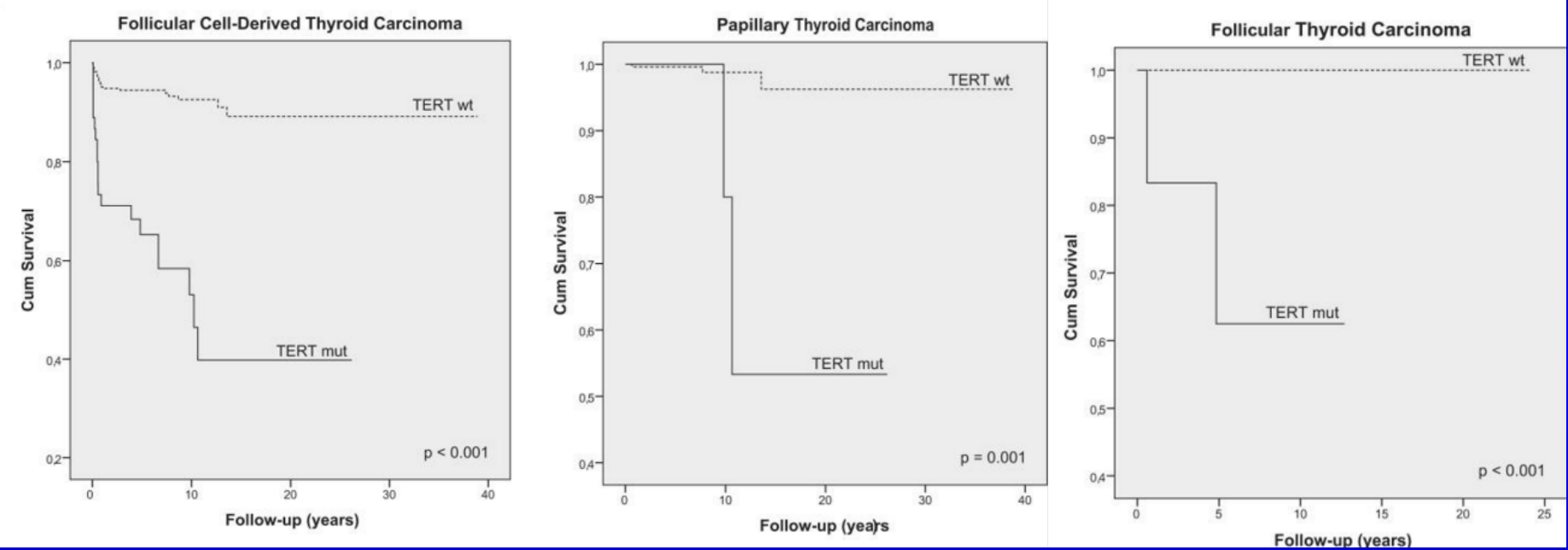
A RET proto-oncogene encodes a thyrosine-kinase transmembrane receptor.
PTC:

- **RET/PTC1 fusion protein results in reduced malignancy**
- **RET/PTC2 rare**
- **RET/PTC3 fusion protein results in enhanced malignancy**

A PAX8 (paired box 8) gene encodes a transcriptional factor that has a role in the tissue differentiation.
The PAX8/PPAR-gamma-1 gene rearrangements has been observed in FTC.

Albarel F, Conte-Devolx B, Oliver C.,Ann Endocrinol (Paris). 2012. Apr. 13. [Epub ahead of print]

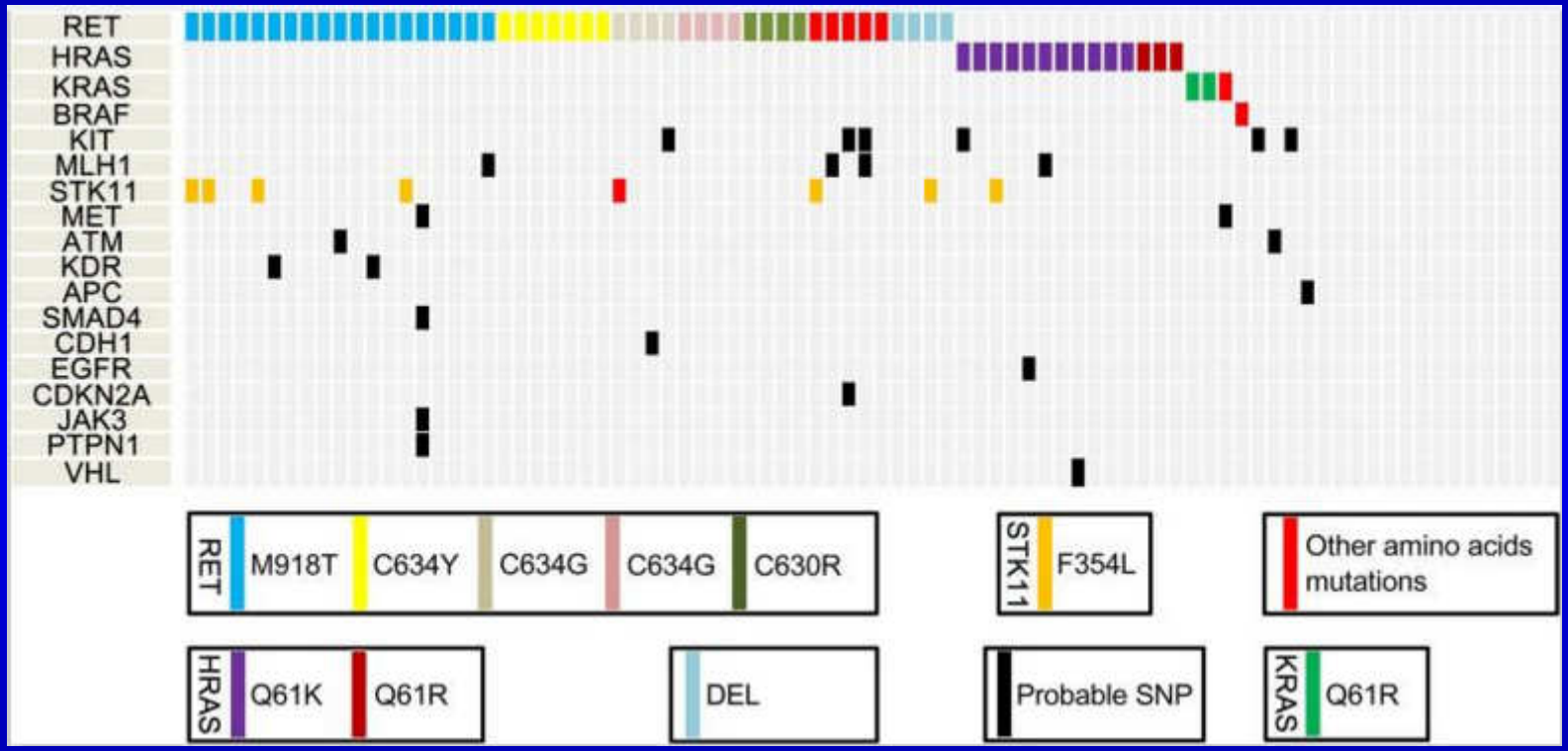
TERT point mutations in thyroid cancer



- **469 patients with FCDTC**
- **Mean follow-up 7.8 ± 5.8 y**

Melo M et al. JCEM 2014:99(5):E754-65

Identification of driving ALK fusion genes and genomic landscape of medullary thyroid cancer



JH et al. PLoS. Genet. 2015 Aug 21;11(8):e1005467. doi: 10.1371/journal.pgen.1005467. eCollection 2015.

Typical genotype-phenotype relationships in differentiated thyroid cancer

Genotype	Histological Phenotype
Ret/PTC Family	Classic PTC
Ret/PTC 1	Classic PTC Often radiation related
Ret/PTC 3	Solid variant PTC Often radiation related
TRK	PTC Often radiation related
ALK Fusions	Poorly differentiated thyroid cancer Anaplastic thyroid cancer
PAX8-PPARγ	Follicular thyroid cancer Follicular variant, papillary thyroid cancer Follicular adenoma
RAS	Follicular thyroid cancer Follicular variant, papillary thyroid cancer Poorly differentiated thyroid cancer Anaplastic thyroid cancer Follicular adenoma
BRAF V600E	Tall cell variant PTC Classic PTC
PI3K/AKT	Follicular thyroid cancer Poorly differentiated thyroid cancer Anaplastic thyroid cancer
PTEN	Poorly differentiated thyroid cancer Anaplastic thyroid cancer
TERT	PTC Poorly differentiated thyroid cancer Anaplastic thyroid cancer Hürthle cell carcinoma

Schlumberger M, Pacini F, Tuttle RM, Thyroid tumors. (4th ed), Institut Médico-Educatif, Paris, 2015.

Genetic alterations in thyroid cancers in Hungary

n=177	Age (year)
men (n=52)	52.9 ± 15.9
women (n=125)	50.3 ± 14.7

	Number of DNA samples	BRAF	HRAS	KRAS	NRAS
papillary cc.	154	59 (38,3%)	3 (1,95%)	1 (0.9%)	2 (1,3%)
follicular cc.	16	2 (16.7%)	1 (8.3%)	0	4 (25.0%)
other cc.	7	0	0	0	1 (14.3%)
normal tissue	163	0	0	0	0
total	340				

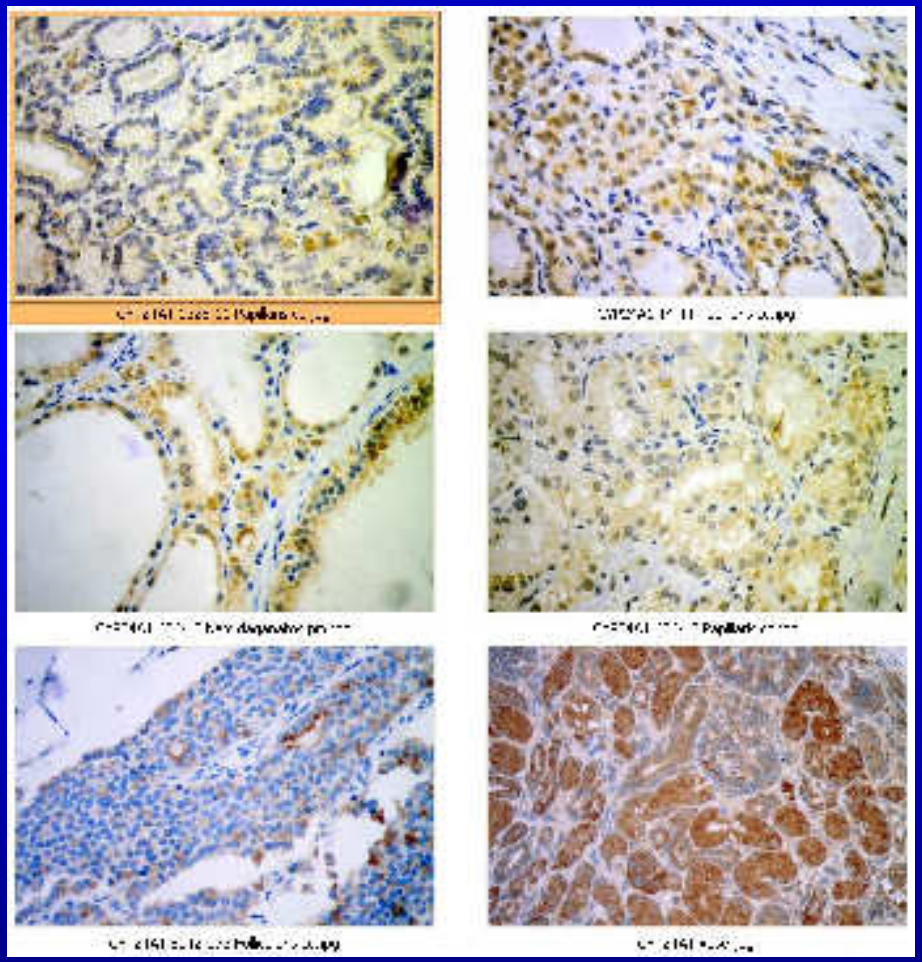
No PAX8/PPARγ!

	Number of RNA samples	RET/PTC1	RET/PTC3
papillariy cc.	97	7 (7,2%)	2 (2,1%)
follicular cc.	16	0	0
other cc.	7	0	0
normal tissue	120	0	0
total	240		

Tobias B et al, Pathol Oncol Res. 2015 Aug 11. Epub ahead of print

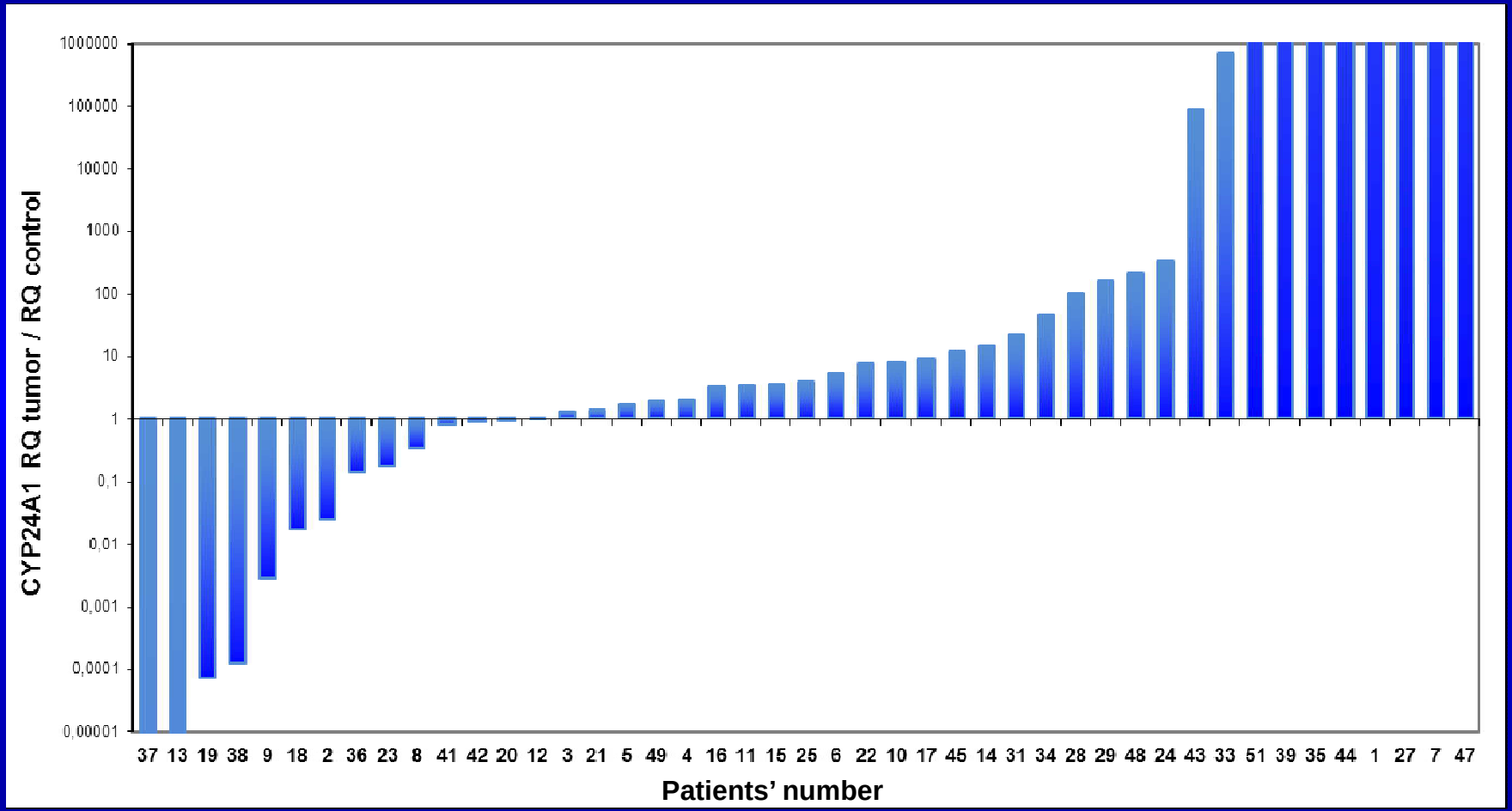
CYP24A1 gene expression in PTC

- **CYP24A1 is a calcitriol-neutralizing enzyme**
- **Increased expression in colon and liver cc**



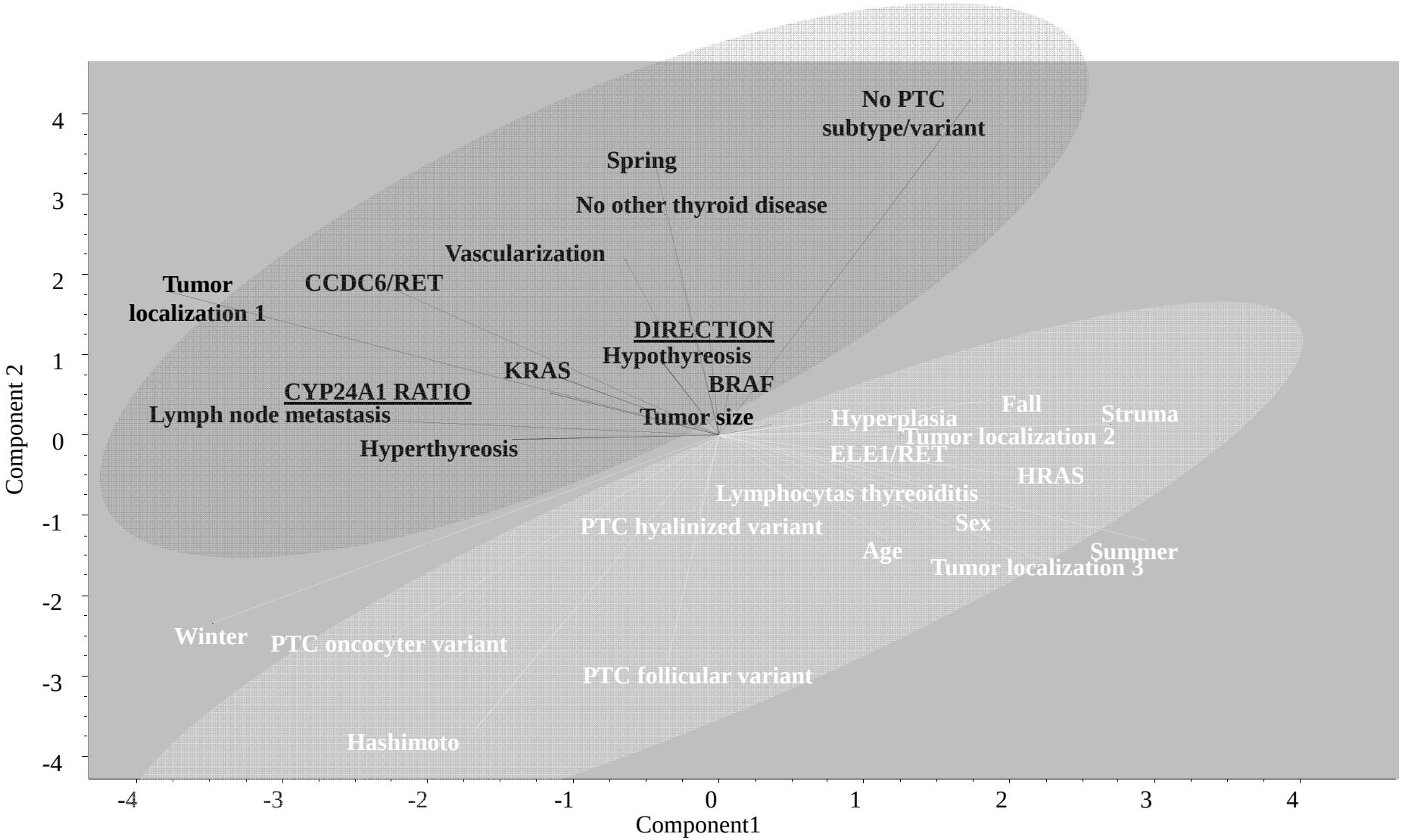
Balla B et al, Thyroid 2011. 21(4):459-460.

CYP24A1 gene expression in PTC compared to normal thyroid tissues

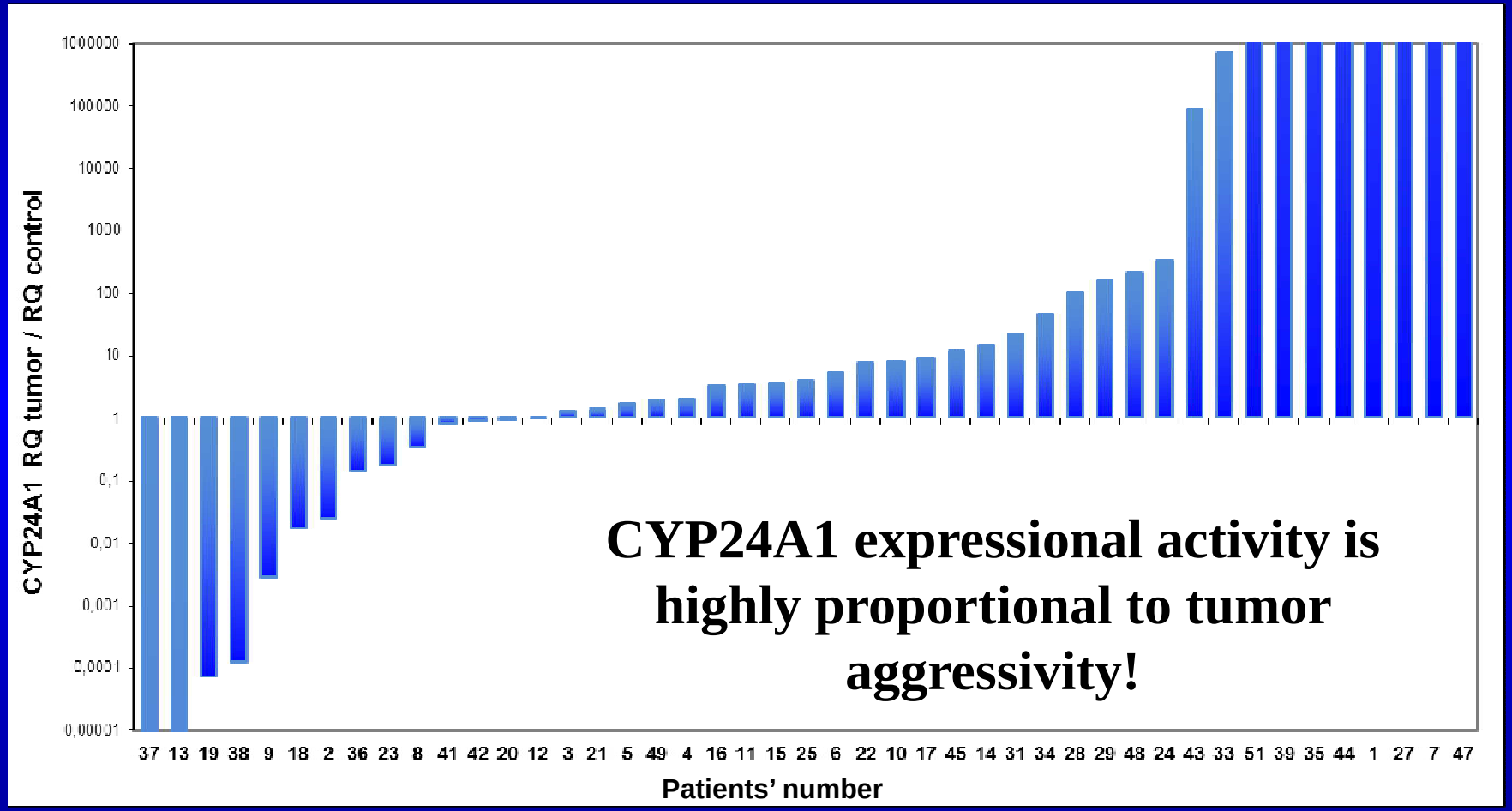


Balla B et al, J Endocrinol Invest. 2015. 38(3):313-321.

Principal component analysis



CYP24A1 gene expression in PTC compared to normal thyroid tissues



Balla B et al, J Endocrinol Invest. 2015. 38(3):313-321.

Follow-up study

- FNAB samples collected
- 779 samples collected (cytologically benign)
- Genetic alteration examined
- At least 3 yrs of follow-up planned
- Frequency of genetic alterations, incidence of malignancies recorded
- Can genetic alterations predict malignancy?

Follow-up of 779 FNAB samples

Genetic alterations	Frequency
BRAF	39
NRAS	23
HRAS	9
KRAS	1
RET/PTC3	1
	73

year 1.

Specificity: 93,3%, sensitivity 46.2%
negative predictive value is 96%

Follow-up of 504 FNAB samples

Genetic alterations	Frequency
BRAF	12
NRAS	5
HRAS	7
KRAS	1
RET/PTC3	1
	26

year 2.

Specificity: 96,4%, sensitivity 30%
negative predictive value is 95.6%

Follow-up of 250 FNAB samples

Genetic alterations	Frequency
BRAF	4
NRAS	1
HRAS	7
KRAS	1
RET/PTC3	1
	14

year 3.

Specificity: 96,2%, sensitivity 38.5%
negative predictive value is 96.6%

Single gene/limited gene mutation panel conventional sequencing

Gene mutations (DNA)
BRAF
NRAS
HRAS
KRAS

Gene mutations (DNA)
RET/PTC1
RET/PTC3
PAX8/PPARG

BRAF only

Sensitivity 45-50%; Specificity >99%

Meta-analysis of 16170 pts/9924 FNA samples
Fnais et al. Hum. Pathol. 2015:48:1443

7-8 gene panels

Sensitivity 60-70%; Specificity 60-95%

Nikiforov et al. JCEM 2009;94:2092
Cantara et al. JCEM 2010;95:1365
Nikiforov et al. JCEM 2011;96:3390
Giordano et al. Hum. Pathol. 2014;45:1339

Molecular testing from FNAB

- **Cleveland Clinic TSHR mRNA assay**
- **Veracyte Afirma Gene Classifier (167-gene panel)**
- **Asuragen miRInform (7-gene panel)**
- **ThyroSeq v.2 (56-gene panel)**
- **PentaCore Thyreon (7-gene panel)**

Hodak SP, Rosenthal DS, Thyroid 2013. 23(2):131.
Milas M et al, Ann Surg 2010. 252:253.
Chudova D et al, JCEM 2010. 95:5296.
Alexander EK et al, NEJM 2012. 367:705.
Li H et al, JCEM 2011. 96:E1719.
Cooper DS et al, Thyroid 2009. 19:1167.
Nikiforov YE et al, JCEM 2011. 96:3390.
Cantara S et al, JCEM 2010. 95:1365.
Nikiforov YE et al, JCEM 2009. 94:2092.
Nikiforov et al. Thyroid 2015. Sept 10. [Epub]

ThyroSeq v.2 (56-gene panel)

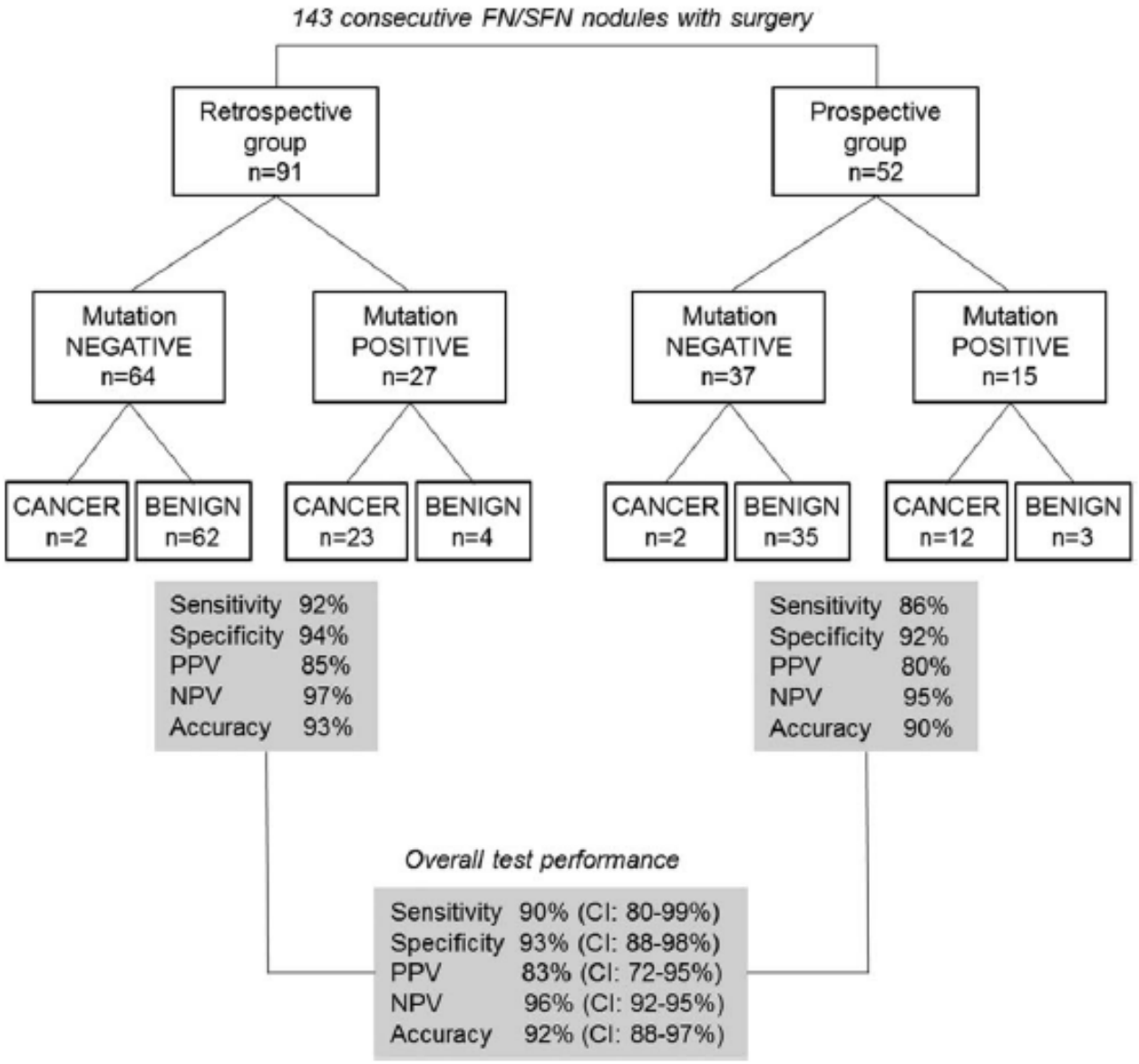
Gene mutations (DNA)		Gene Fusions (RNA)
BRAF	RET	RET
NRAS	TSHR	PPARG
HRAS	AKT1	NTRK1
KRAS	TP53	NTRK3
PIK3CA	GNAS	BRAF
PTEN	CTNNB1	ALK
TERT	EIF1AX	Other

Gene expression (RNS)
PGK1
KRT7
TG
TTF1
NIS
Calcitonin
PTH
KRT20
Other

- **14 genes for mutations, > 1000 hotspots**
- **42 fusion types**
- **16 genes for expression**

Nikiforov et al. Thyroid 2015. Sept 10. [Epub]

ThyroSeq v2 performance in AUS/FLUS cytology nodules



Nikiforov et al. Cancer 2014; 120:3627-34

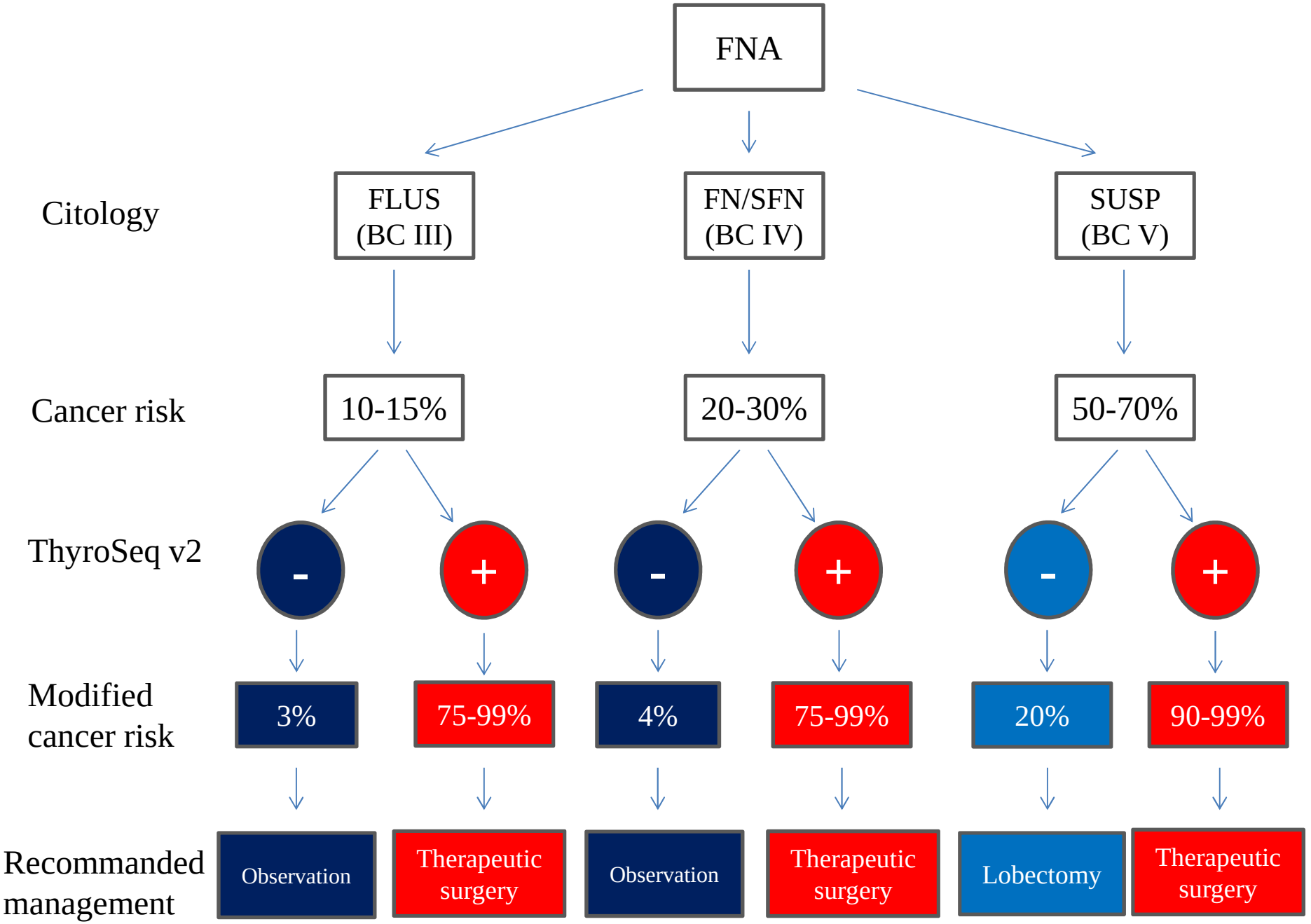

FNA
Citology
FLUS (BC III)
FN/SFN (BC IV)
SUSP (BC V)
Cancer risk
10-15%
20-30%
50-70%
ThyroSeq v2
-
+
-
+
-
+
Modified cancer risk
3%
75-99%
4%
75-99%
20%
90-99%
Recommanded management
Observation
Therapeutic surgery
Observation
Therapeutic surgery
Lobectomy
Therapeutic surgery

TABLE 1. COMPARISON OF GENE CLASSIFIER, *BRAF*, *RAS*, *RET/PTC*, AND *PAX8/PPARγ* MOLECULAR PANEL, AND TSHR mRNA DIAGNOSTIC METHODS

	Gene classifier (%)	*Molecular panel (%)*	*TSHR mRNA (%)*	***ThyroSec v2***	***PentaCore Thyreon***
Sensitivity	92	60	61	***90***	***46***
Specificity	52	98	83	***93***	***96***
Cancers missed by indeterminate cytology category					
AUS/FLUS	10	37	n/a		***4***
FN/SFN	10	43	24		***2***
SMC	6	32	n/a		***2***

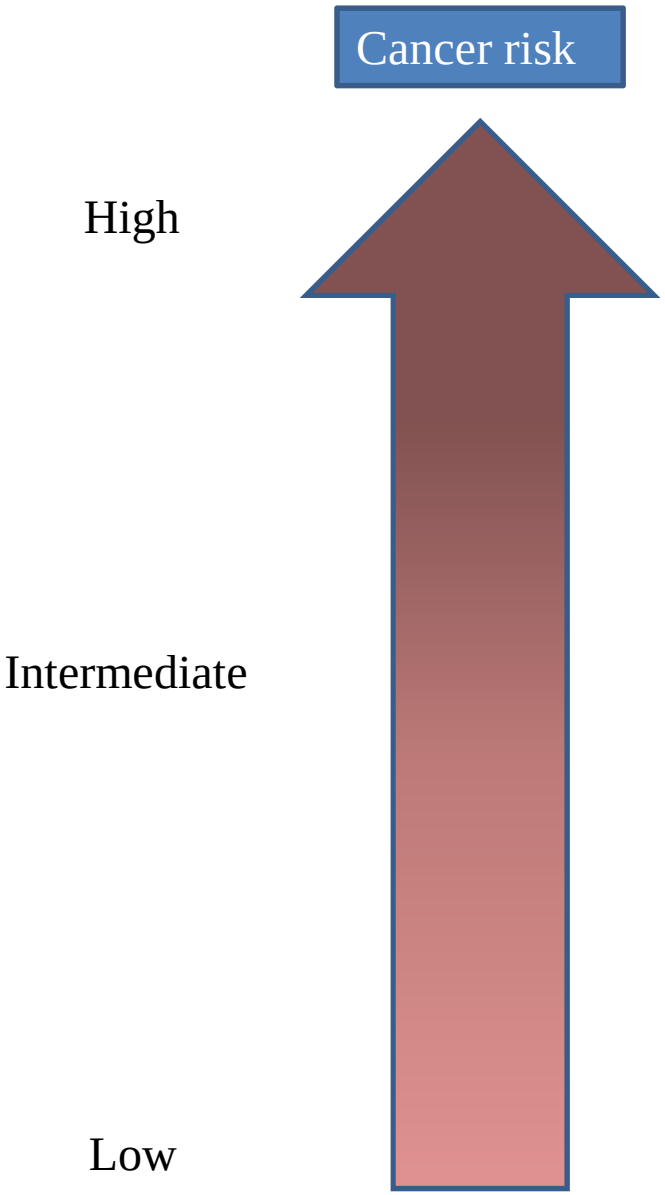
Sensitivity and specificity are reported for each method. The percentage of cancers missed based on indeterminate cytology category is shown.

Sources: All data from Refs. 4, 7, and 11.

TSHR mRNA, thyrotropin receptor messenger RNA; AUS/FLUS, atypia/follicular lesion of undetermined significance; FN/SFN, follicular or Hürthle cell neoplasm/suspicious for follicular neoplasm; SMC, suspicious for malignant cells; n/a, not applicable.

Hodak SP, Rosenthal DS, Thyroid 2013. 23(2):131.

Cancer risk stratification based on molecular profiling



Molecular structure

- Multiple dirver mutations (eg. BRAF and PIK3CA)
- TP53
- TERT

- ALK fusions
- NTRK1 fusions
- NTRK3 fusions
- BRAF V600E

- RET/PTC
- RAS
- PTEN
- BRAF K601E
- PAX8/PPARG

Contribution of molecular information to thyroid diagnostics and therapy

- Additional information to FNAB or histology
- Prediction of malignancy in thyroid nodules
- Individual treatment options (e.g. thyrosine kinase inhibition)

Thank you for your attention!