NATIONAL INSTITUTES OF HEALTH

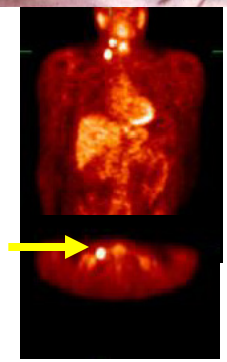
Genomics in Medicine Series

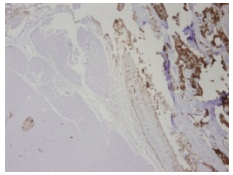
NCI ENDOCRINE ONCOLOGY BRANCH

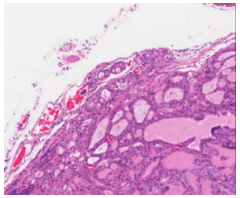
GENETICS AND GENOMICS OF THYROID NEOPLASMS

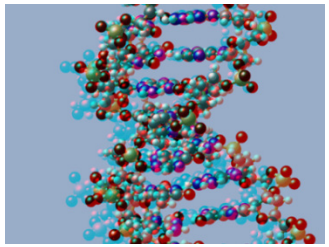
MOVING CLOSER TOWARDS PERSONALIZED PATIENT CARE

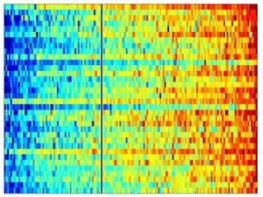
Electron Kebebew, MD, FACS





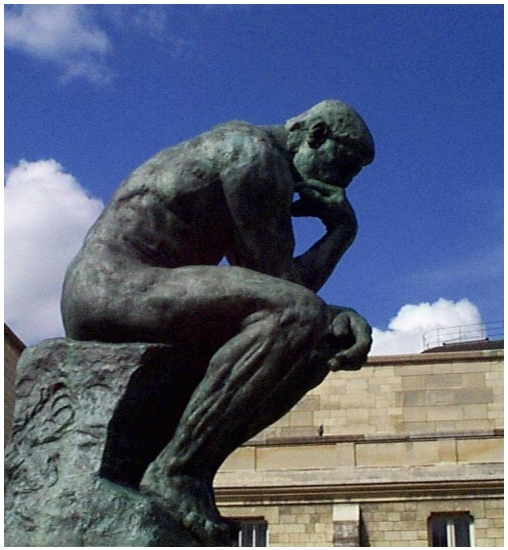






Outline

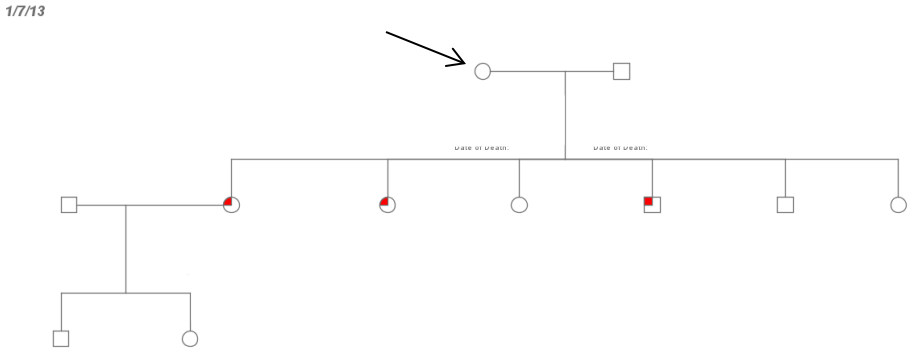
- To assess the change in thyroid cancer epidemiology
- To review our current knowledge on the genetic and genomic changes associated with thyroid cancer initiation and progression
- To consider the impact of genetic and genomic testing towards personalizing patient care



Illustrative Clinical Cases

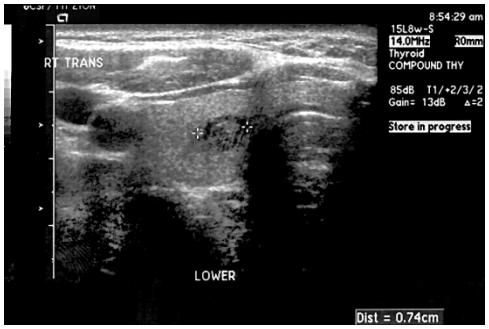
2013

63 year old woman with a left thyroid nodule & family history of thyroid cancer



2002

A 55-year-old woman with a long history of a neck mass.



- **Papillary thyroid cancer**

Illustrative Clinical Cases

63 year old woman with a left thyroid nodule & family history of thyroid cancer

↓

Biopsy showed atypical cells

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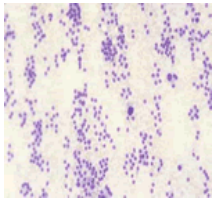
Biopsy 8 years ago which was inconclusive.



Now presents with an enlarging high right neck mass.

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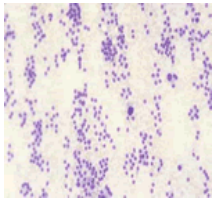

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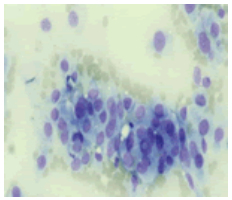

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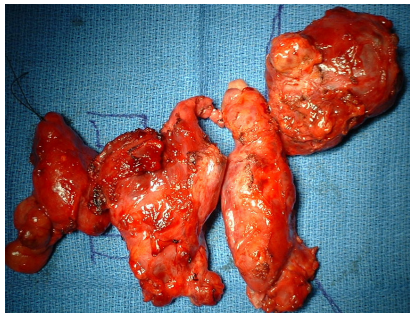

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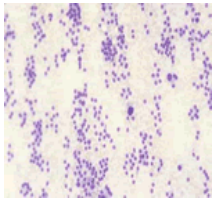

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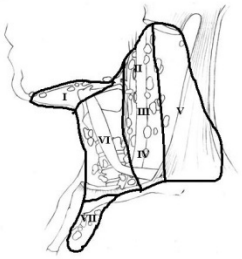

1. Total thyroidectomy and left and right central neck node dissection extending into level 7.
2. Right level 2, 3 and 4 lymph node dissection.
3. Left level 2, 3 and 4 lymph node dissection.

Illustrative Clinical Cases

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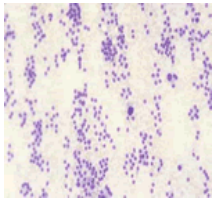

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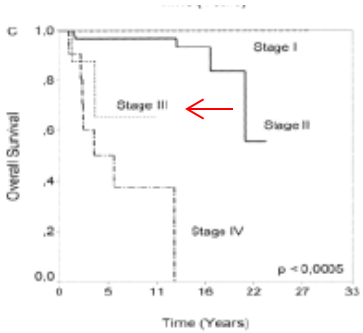


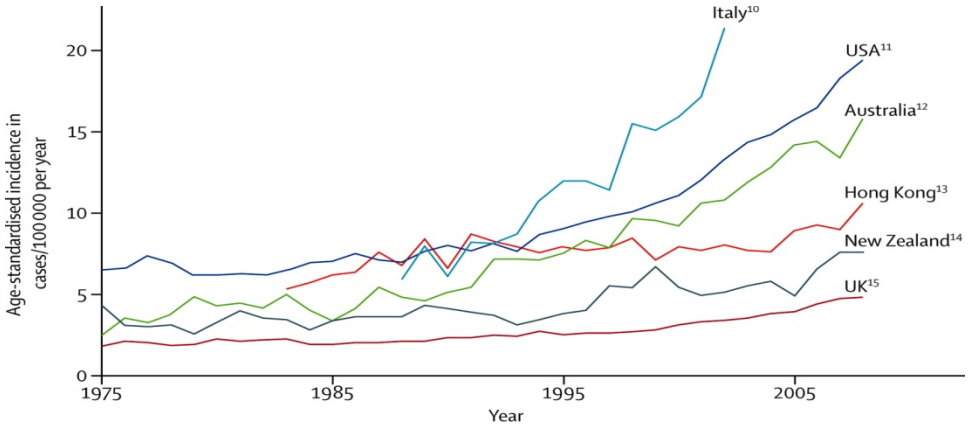
T1bN1bMx (15 of 75 nodes positive)
Stage III Papillary thyroid cancer

Common Cancers in the US

	Common Types of Cancer	Estimated New Cases 2013	Estimated Deaths 2013
1.	Prostate Cancer	238,590	29,720
2.	Breast Cancer	232,340	39,620
3.	Lung and Bronchus Cancer	228,190	159,480
4.	Colon and Rectum Cancer	142,820	50,830
5.	Melanoma of the Skin	76,690	9,480
6.	Bladder Cancer	72,570	15,210
7.	Non-Hodgkin Lymphoma	69,740	19,020
8.	Kidney and Renal Pelvis Cancer	65,150	13,680
9.	**Thyroid Cancer**	**60,220**	**1,850**
10.	Endometrial Cancer	49,560	8,190

Thyroid Cancer

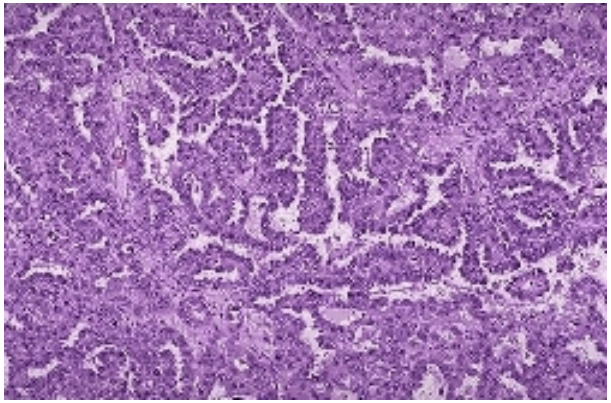
- **Over 60,220 new cases will occur in 2013 in US**
- **Prevalence of thyroid nodules has increased dramatically (10% → >50%)**



Incidence of thyroid cancer in women over

McLeod et al. Lancet 2013

Histologic type	Frequency
Papillary	85%
Follicular	7%
Hürthle cell carcinoma	3%
Medullary	4%
Anaplastic	<1%
Other	<1%



papillary thyroid cancer

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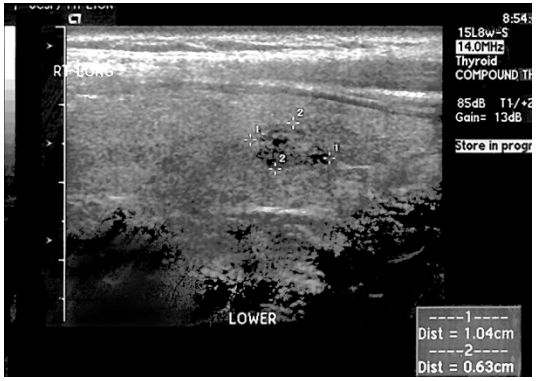

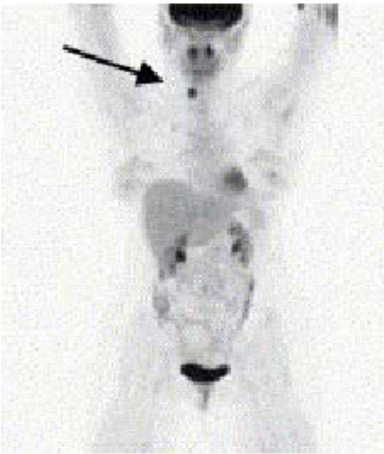

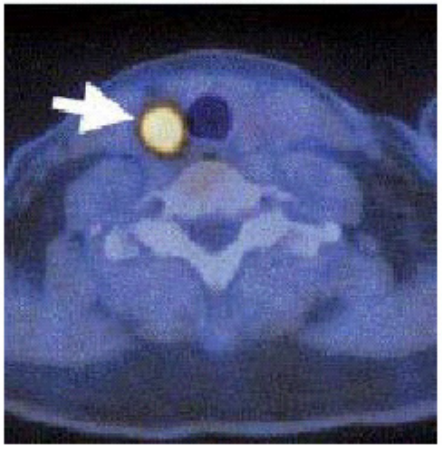

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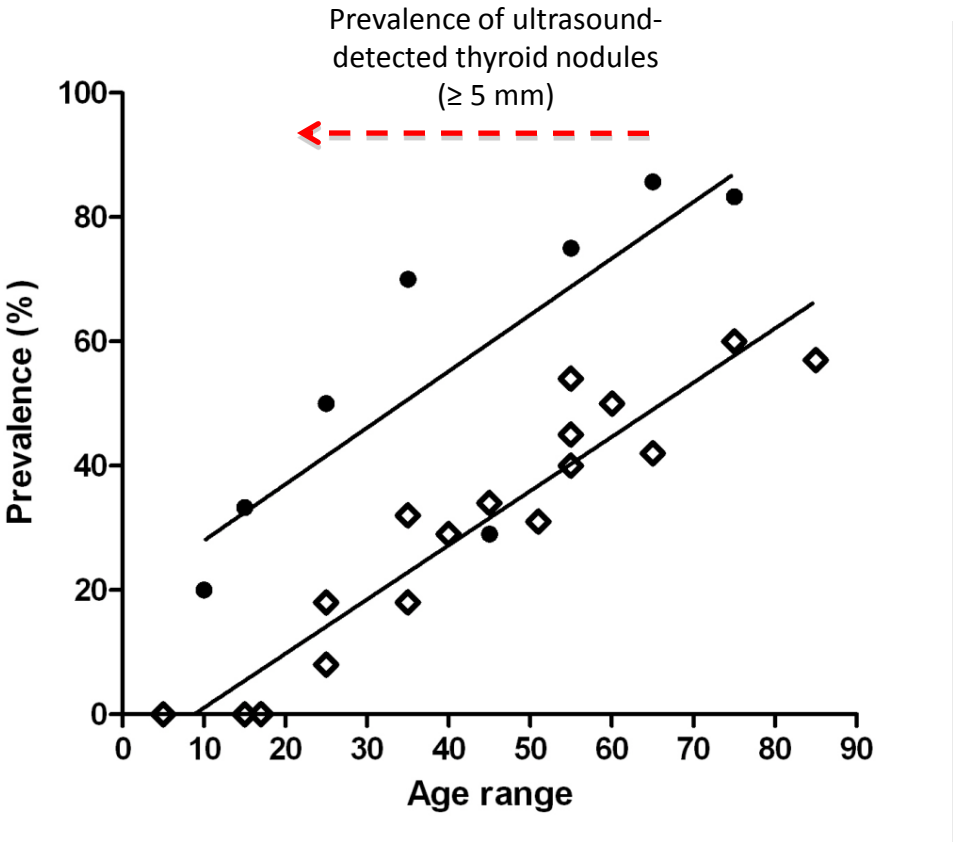

Sadowski et al Surgery 2013
• Familial population
◊ Mazzaferri, 1993

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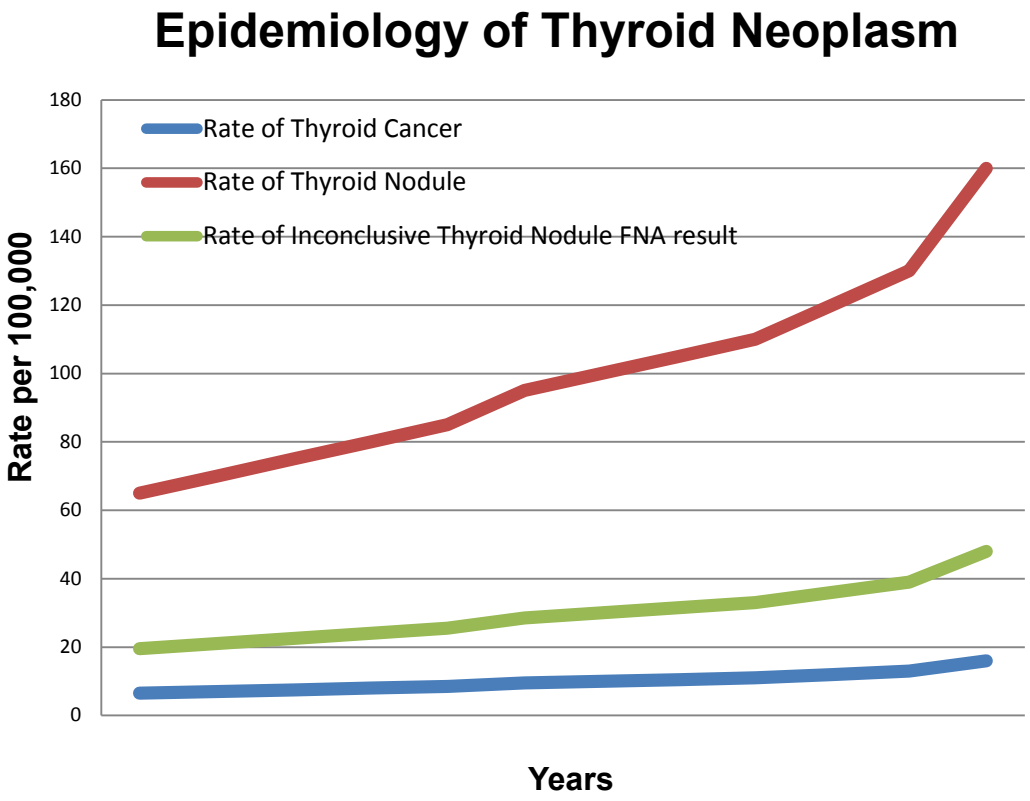

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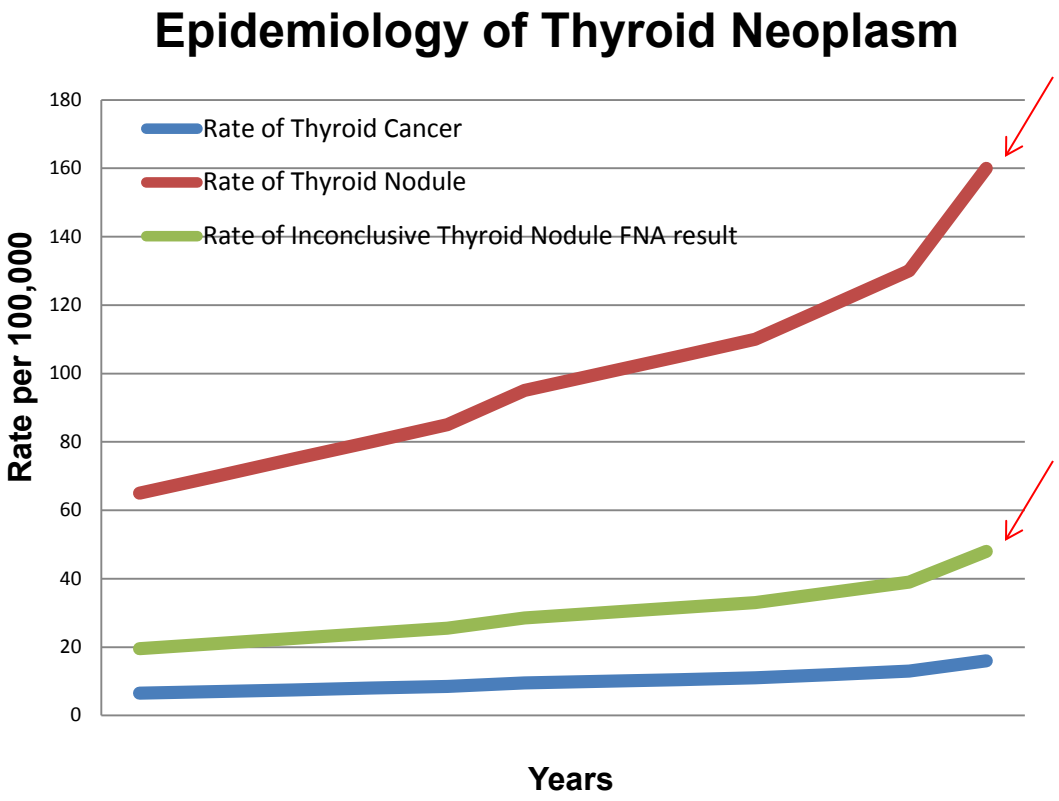
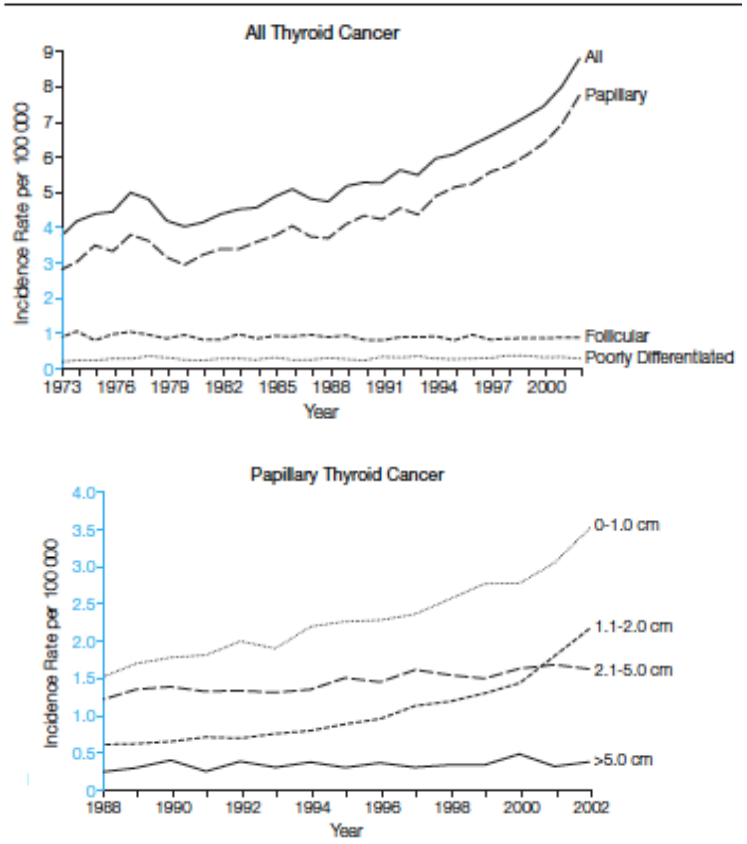


Thyroid Cancer

Why has the incidence increased?

- **Overdiagnosis**

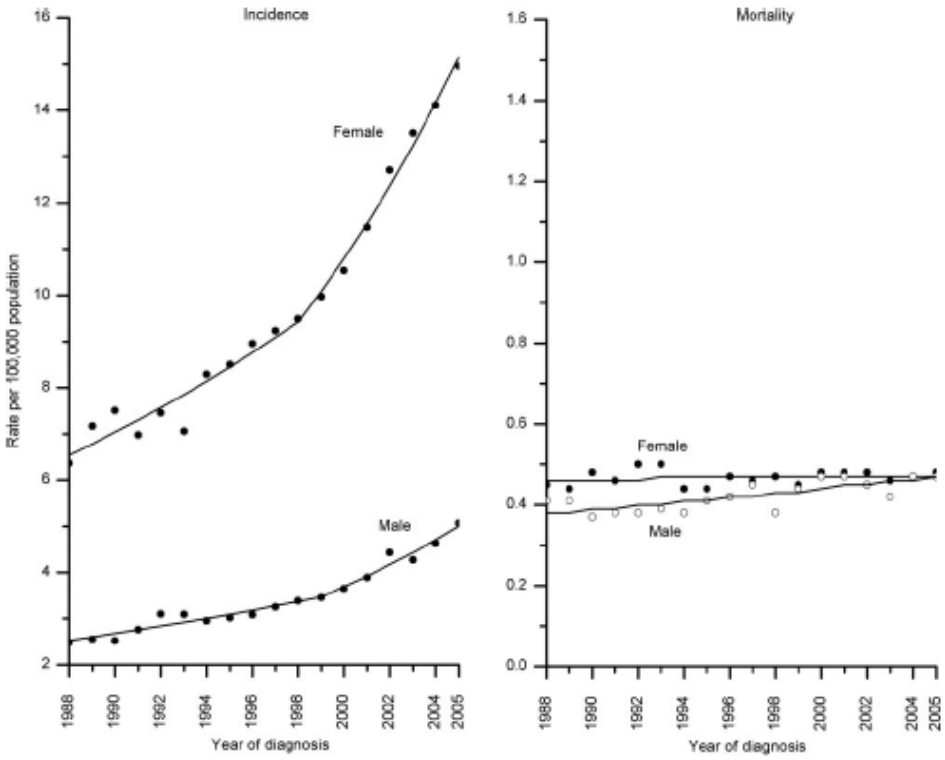


Davies & Welch. JAMA 2006

Thyroid Cancer

Why has the incidence increased?

- **Overdiagnosis**
- **Real increase in disease burden**



Chen et al. Cancer 2009

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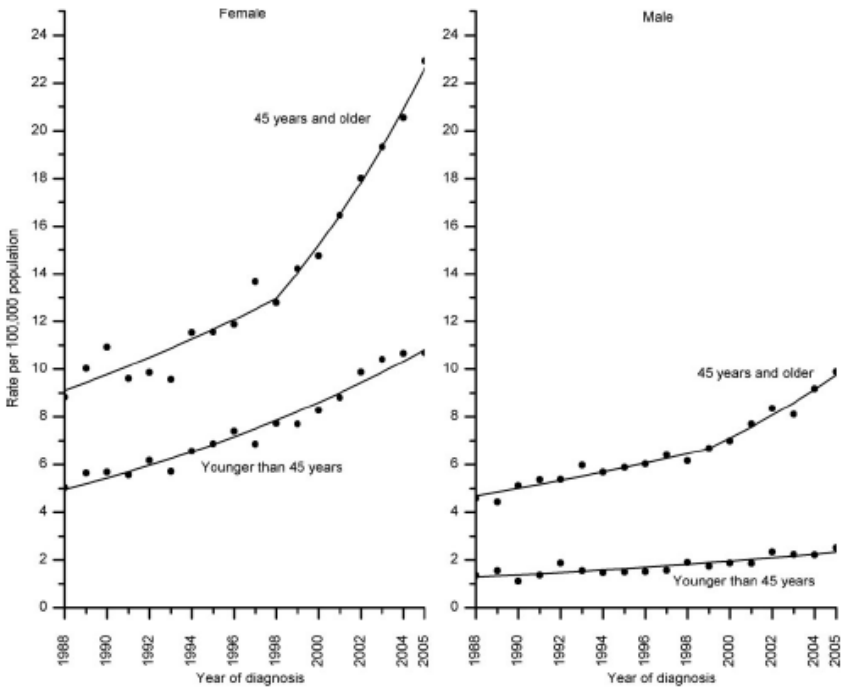


Dots represent observed values and solid lines fitted values.

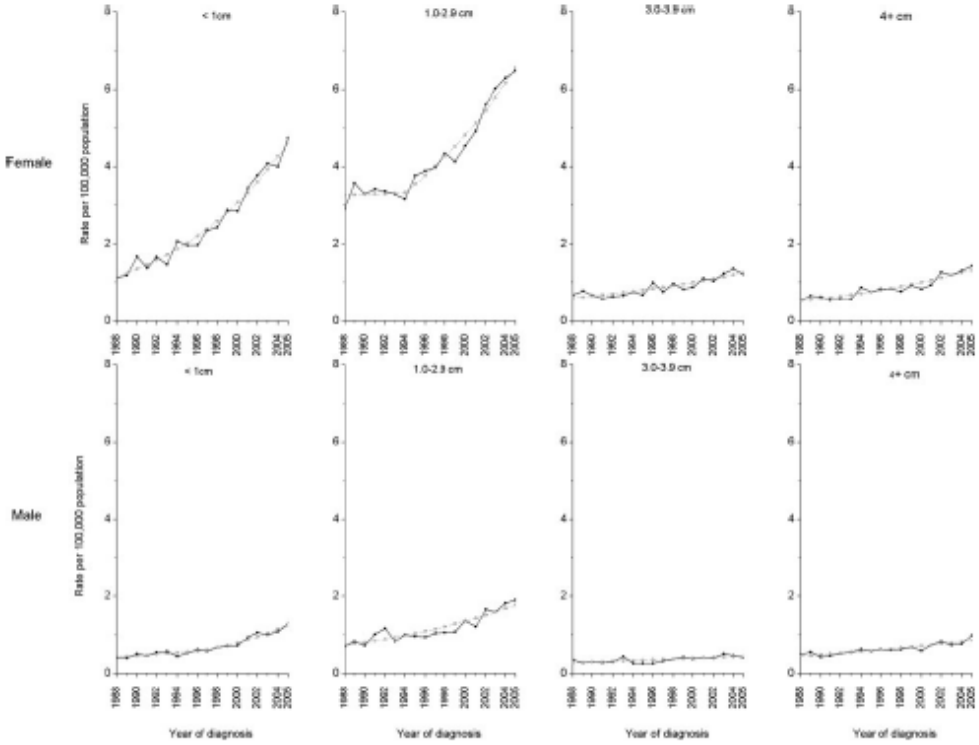
Chen et al. Cancer 2009

Thyroid Cancer

Why has the incidence increased?

- **Overdiagnosis**
- **Real increase in disease burden**

All cases

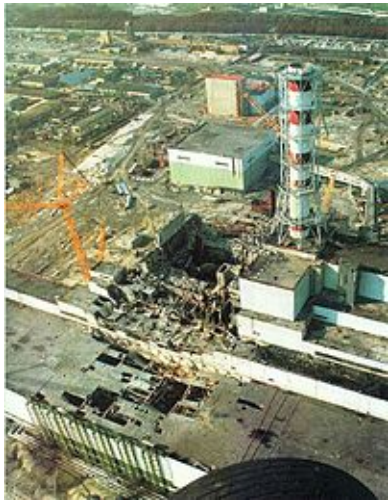


Chen et al. Cancer 2009

Thyroid Cancer

Why has the incidence increased?

- Overdiagnosis
- Real increase in disease burden
- Environmental
  - Chernobyl
  - Fukushima



Thyroid Cancer

Why has the incidence increased?

- **Overdiagnosis**
- **Real increase in disease burden**
- **Environmental**
- **Genetic landscape of the disease**

Table 4. Molecular Testing Results for 228 Cases of Conventional Papillary Thyroid Cancer

Mutation	Group I (n=74), 1991-1995	Group II (n=103), 1996-2000	Group III (n=51), 2001-2005	*P*
Somatic mutation	68% (51/74)	64% (66/103)	92% (47/51)	≤.002[a]
BRAF mutation	51% (38/74)	43% (44/103)	88% (45/51)	<.001[a]
RET/PTC1	0%	3% (3/103)	0%	.15
RET/PTC3	14% (10/74)	16% (16/103)	4% (2/51)	.18
NRAS	1% (1/74)	1% (1/103)	0%	.44
KRAS	3% (2/74)	2% (2/103)	0%	.52

[a] For comparison of group I and/or II to group III.

Mathur et al. Cancer 2011

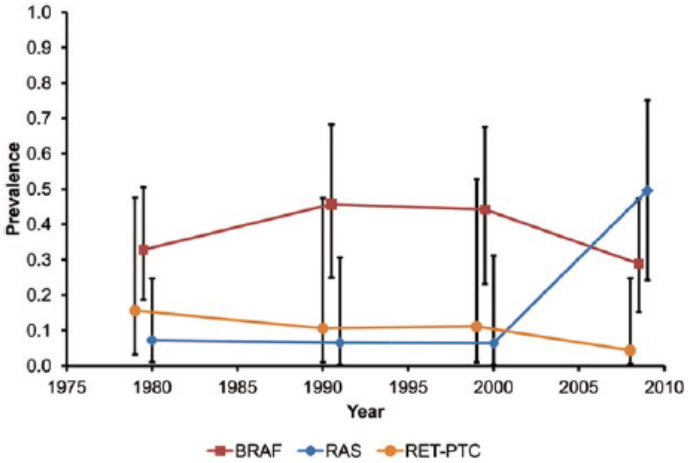
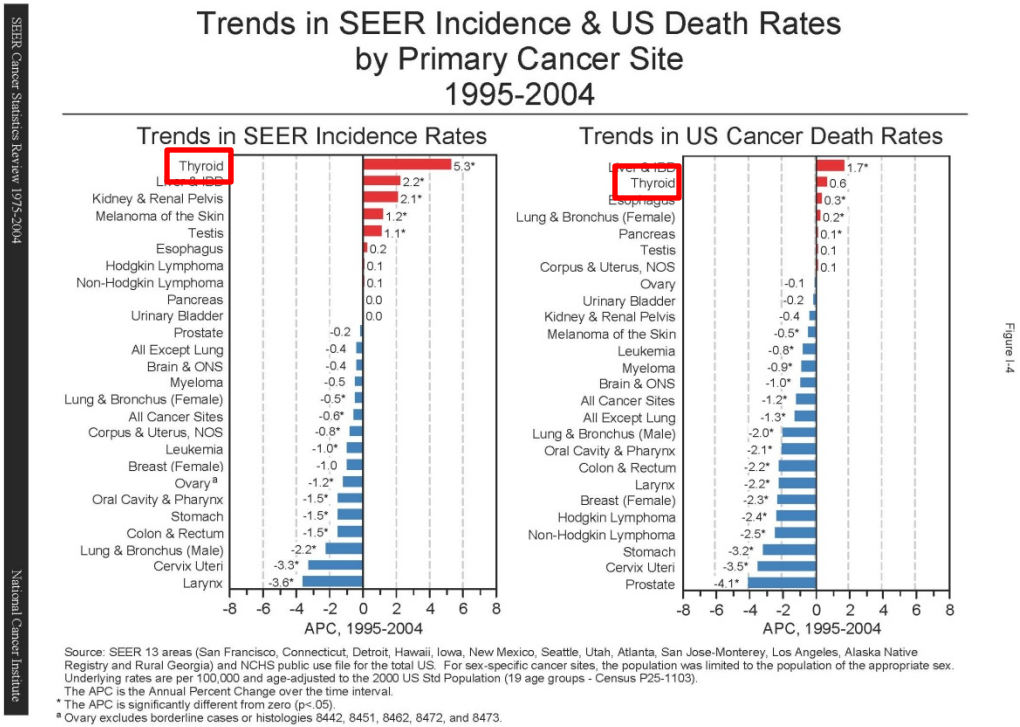


Figure 2. Time trends in the age- and gender-adjusted prevalence (with 95% CI) of the three mutation types in papillary thyroid carcinoma. University of Pittsburgh Medical Center, 1974–2009.

Jung et al. J Clin Endocrinol Metab. 2013

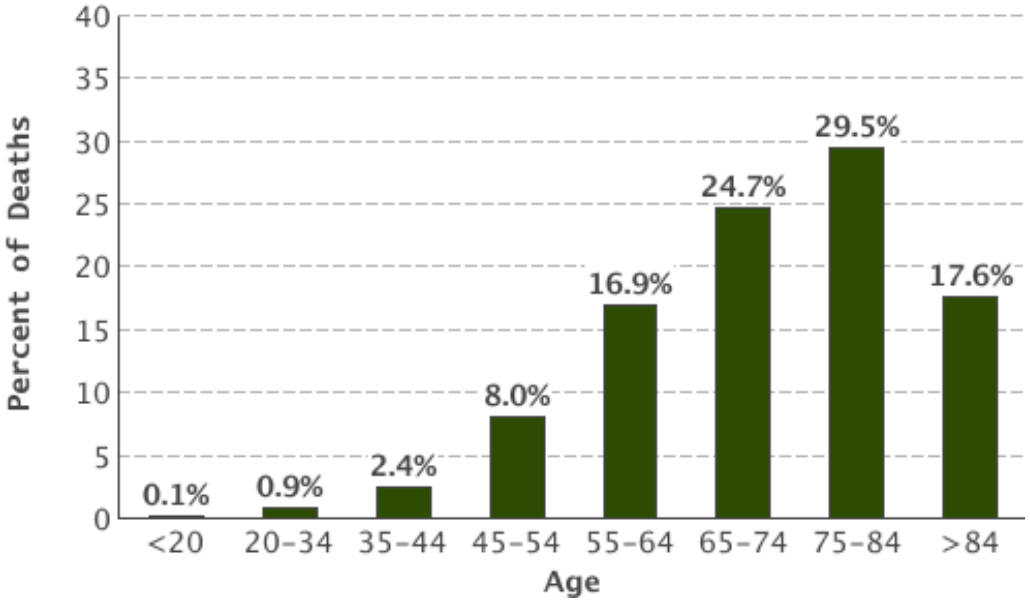
Thyroid Cancer

- **Over 60,220 new cases will occur in 2013 in US**
- **Multifactorial increase in the incidence of thyroid cancer across all histologies**



http://seer.cancer.gov/cgi-bin/csr/1975_2004/search.pl#results

Thyroid Cancer
Percent of Deaths by Age Group



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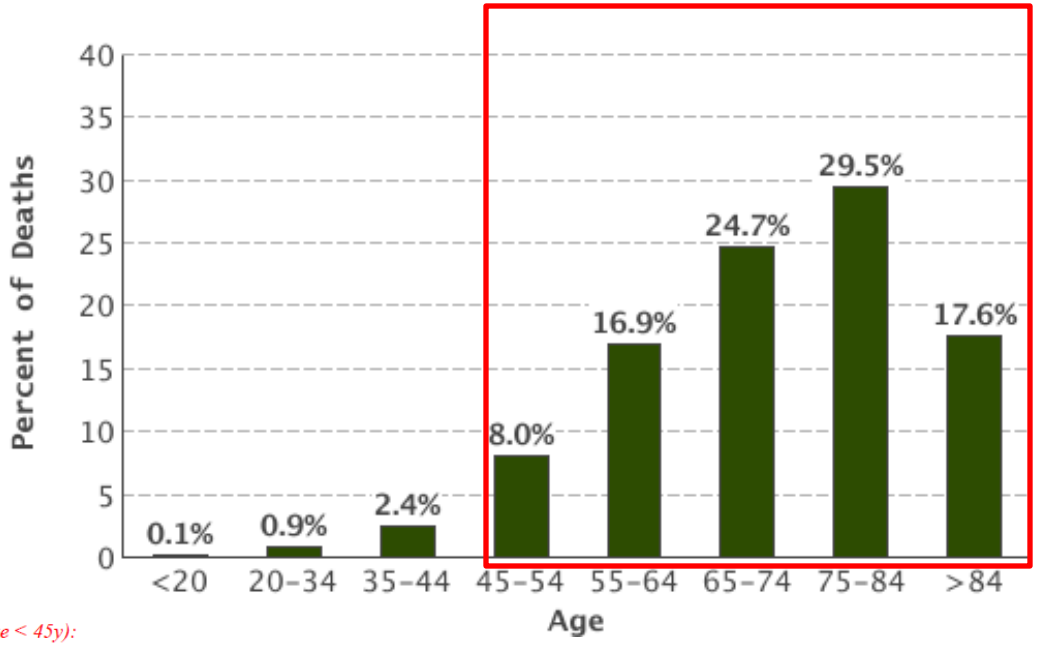


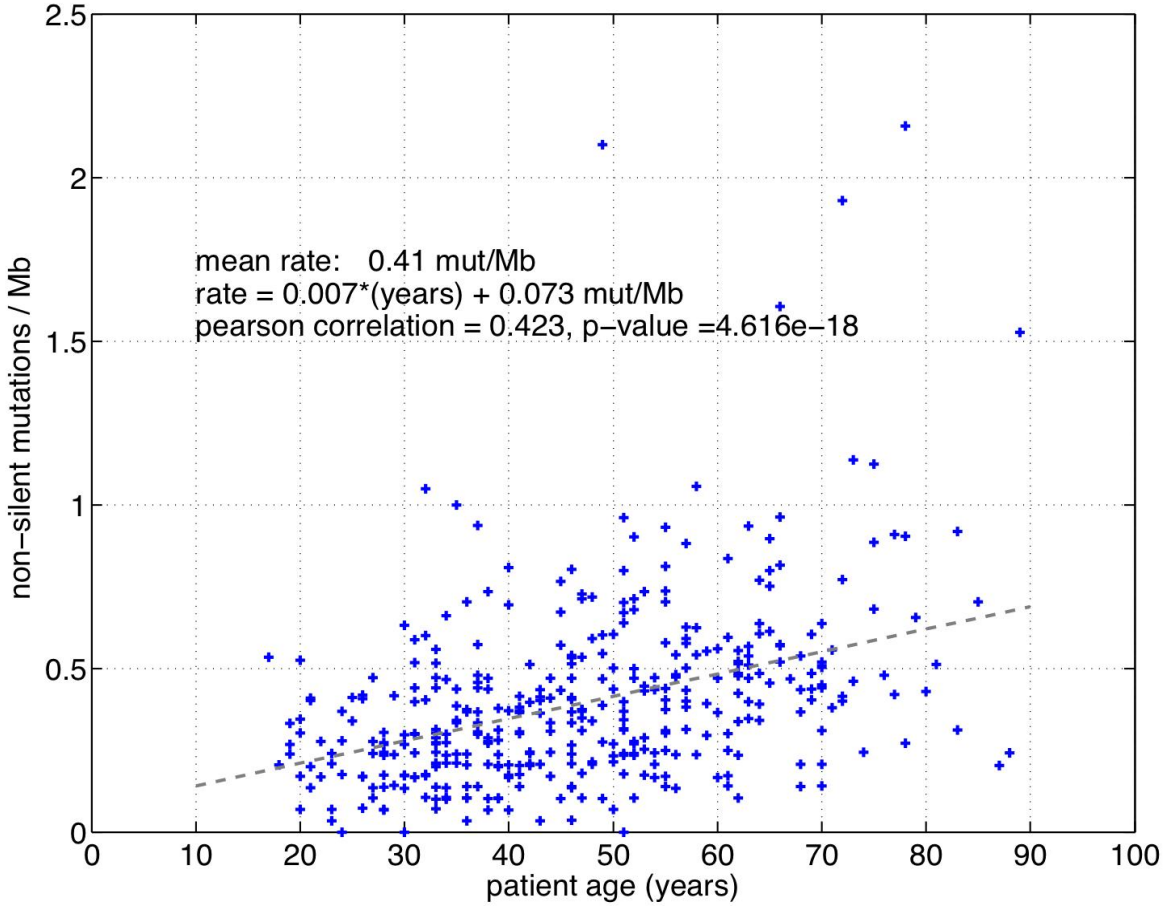
Papillary and follicular thyroid cancer (age < 45y):

Stage	T	N	M
I	Any T	Any N	M0
II	Any T	Any N	M1

Papillary and follicular; differentiated (age ≥ 45y):

Stage	T	N	M
I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
IVA	T1-3	N1a	M0
	T4a	N1b	M0
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1

The Cancer Genome Atlas (TCGA)
Analysis of Papillary Thyroid Carcinomas



Unpublished data

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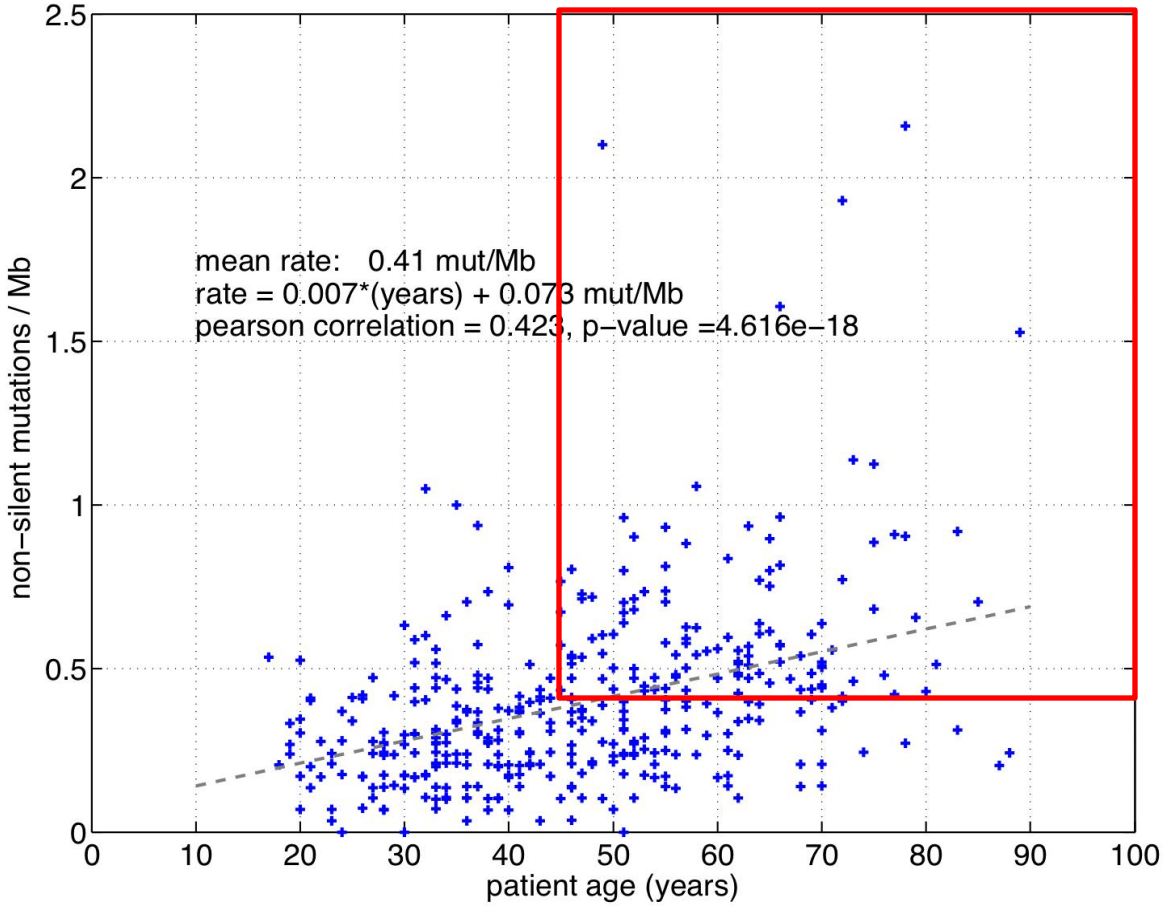

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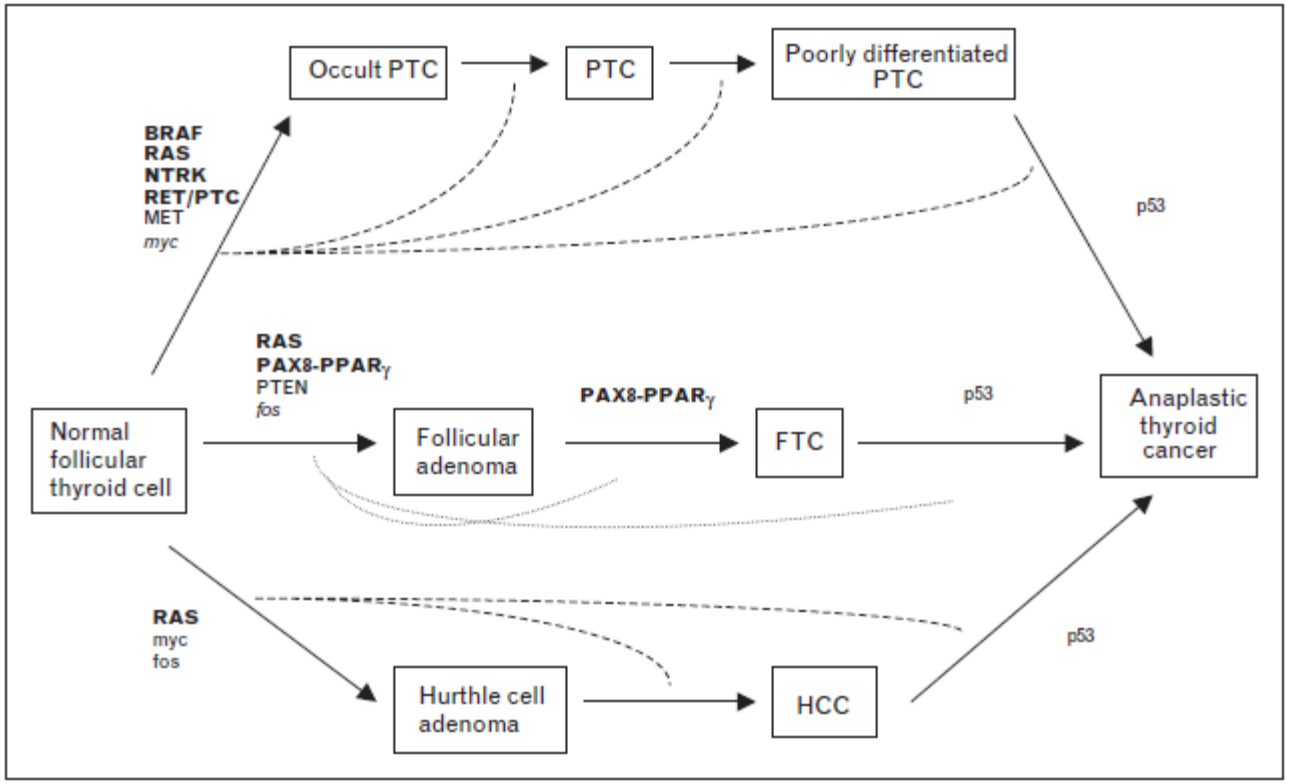
Pathogenesis of thyroid cancer of follicular cell origin



Shibru, et al. Curr Opin Oncol 2008

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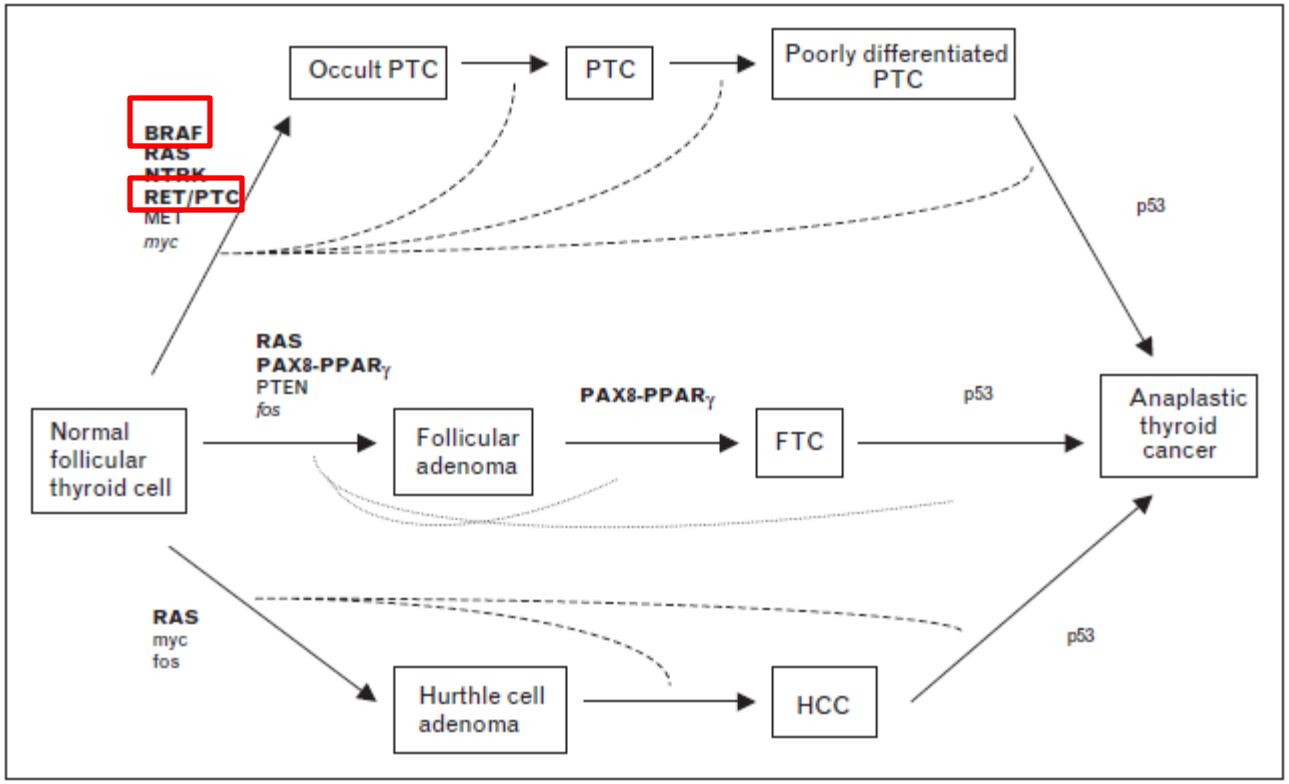

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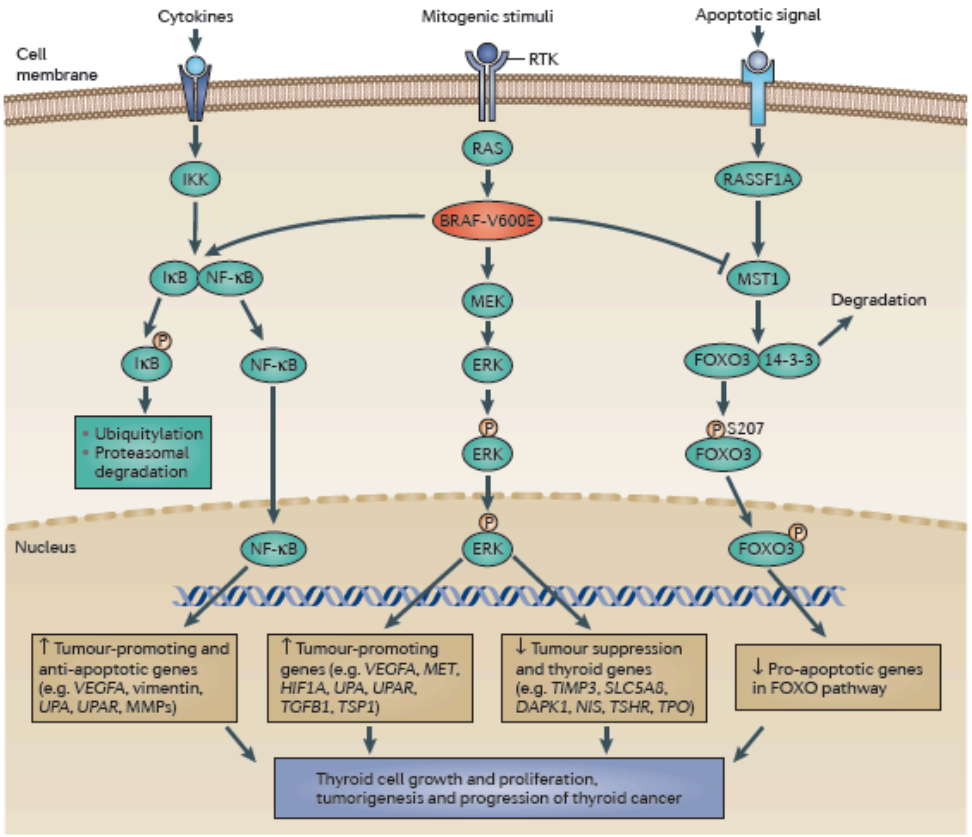

Xing M. Nat Rev. Cancer 2013

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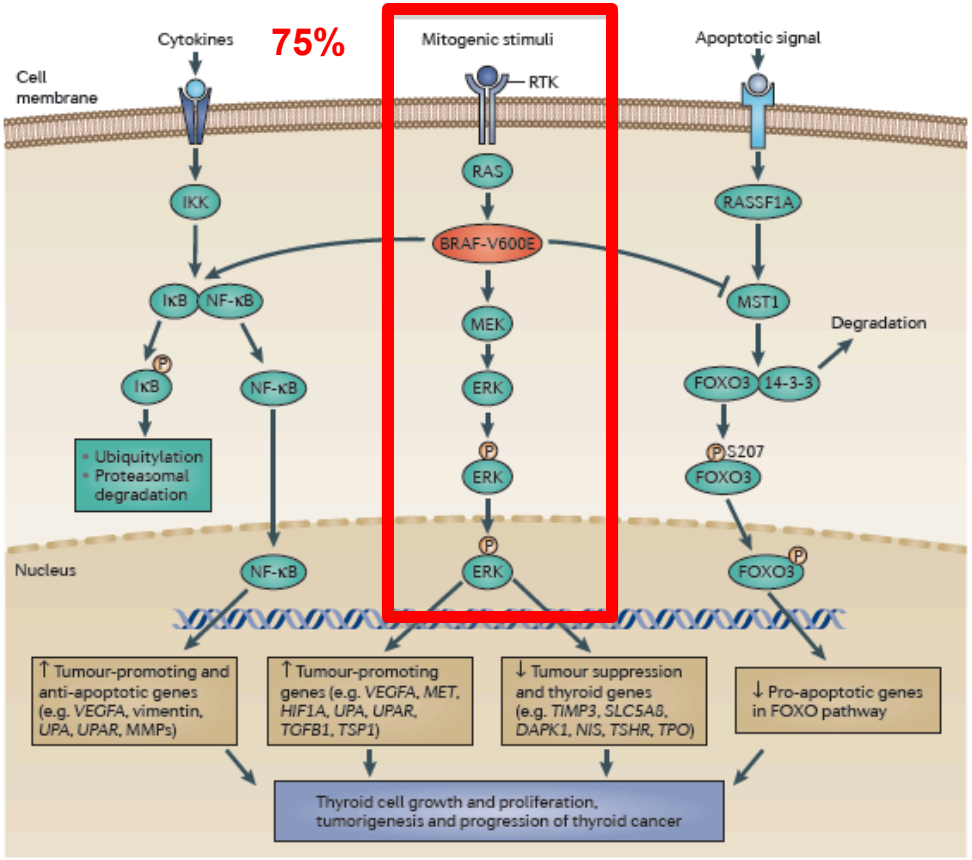

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Illustrative Clinical Cases

63 year old woman with a left thyroid nodule & family history of thyroid cancer

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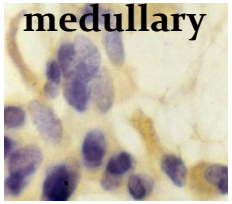
Biopsy showed atypical cells

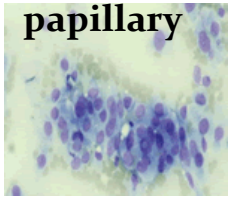
Thyroid nodules FNA biopsy results

Benign (80%)

≥ 95% accurate

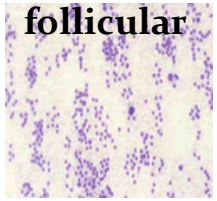
Malignant (≤10%)





Therapeutic thyroidectomy

Suspicious/Indeterminate (5-20%)



Diagnostic thyroidectomy

Nondiagnostic (<10%)

5 – 50% risk of cancer

Thyroid nodules FNA biopsy results

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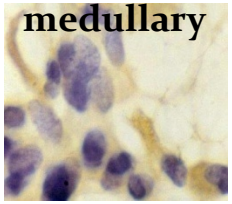
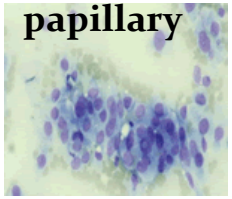
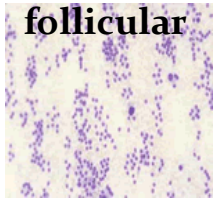




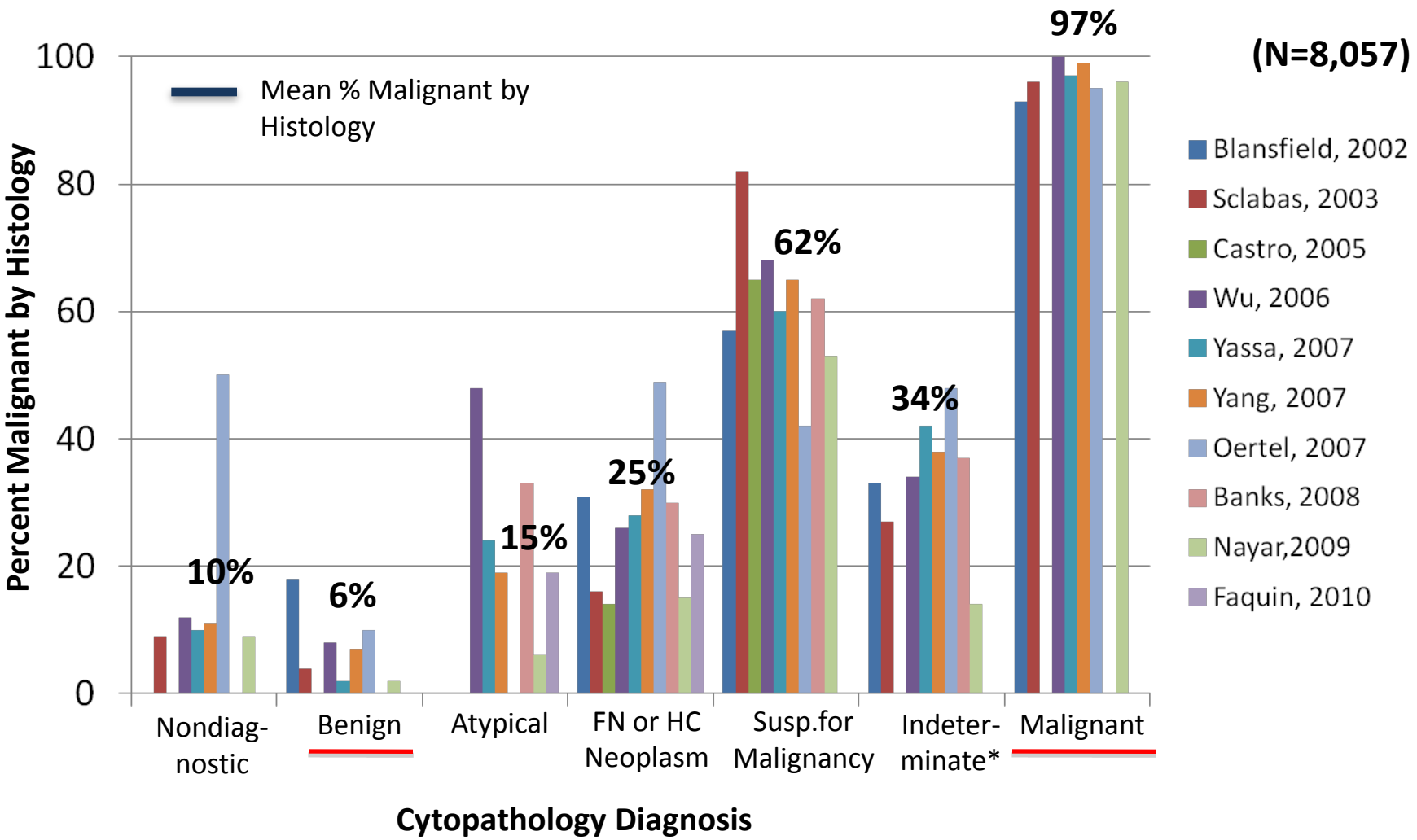


5 – 50% risk of cancer

Therapeutic thyroidectomy

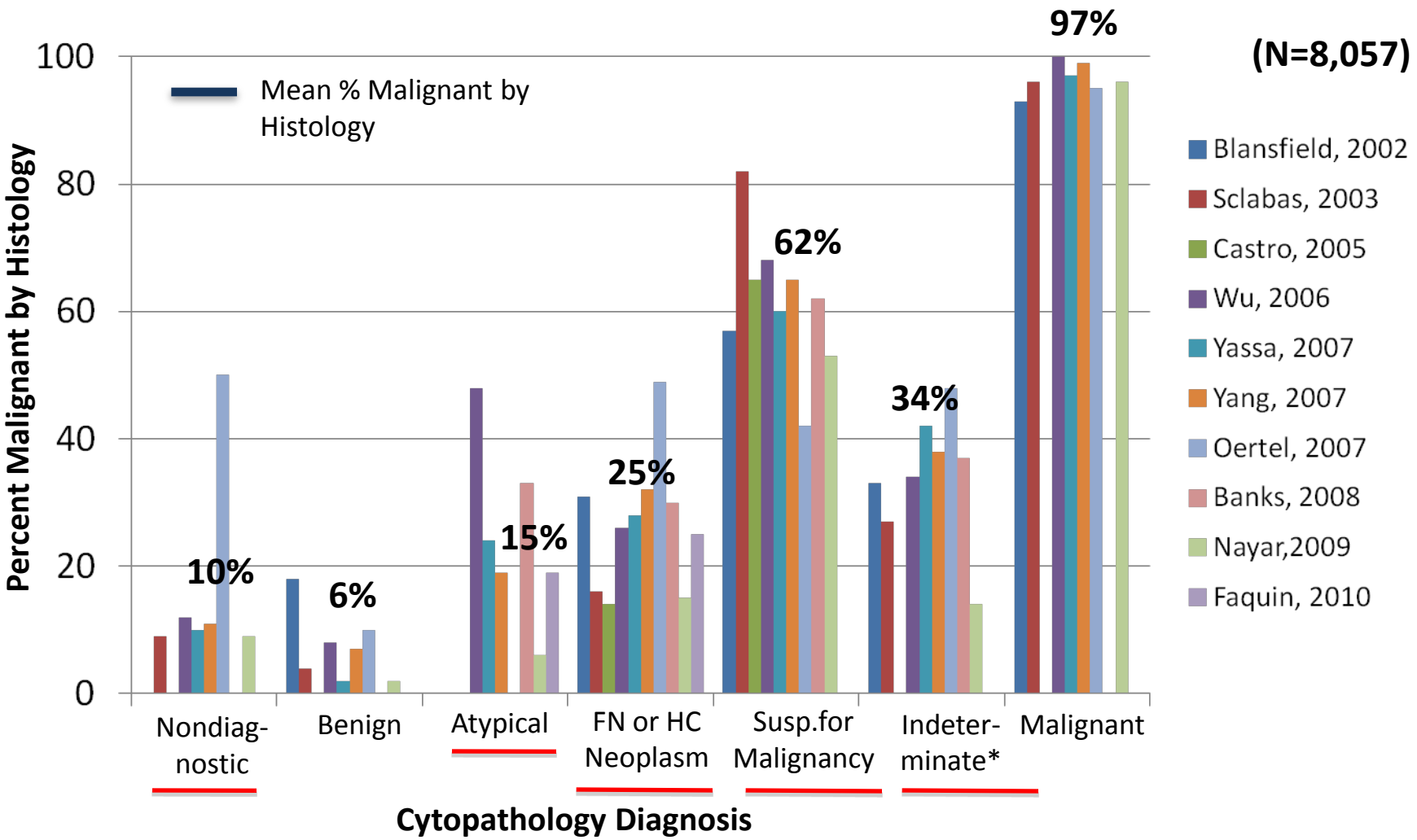
Diagnostic thyroidectomy

Thyroid Nodule FNA Biopsy Studies



*Indeterminate: atypical/FLUS, follicular or Hürthle cell neoplasm, and suspicious for malignancy.

Thyroid Nodule FNA Biopsy Studies



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Thyroid FNA Classification & Risk of Malignancy

The Bethesda System for Reporting Thyroid Cytopathology: Implied risk of malignancy and recommended clinical management			
	Diagnostic Category	Risk of Malignancy	Usual Management
I.	Non diagnostic or unsatisfactory	NA	Repeat FNAB with US guidance
II.	Benign	0% - 3%	Clinical follow-up
III.	Atypia of undetermined significance or follicular lesion of undetermined significance	~5% - 15%	Repeat FNAB
IV.	Follicular neoplasm or suspicious for a follicular neoplasm	15% - 30%	Lobectomy
V.	Suspicious for malignancy	60% - 75%	Near-total thyroidectomy or Lobectomy
VI.	Malignant	97% - 99%	Near-total thyroidectomy

Marker development for thyroid cancer – where are we?

Patient sample

↓

Marker identification

↓

Marker development for thyroid cancer – where are we?

Patient sample

↓

Marker identification

↓

Analytical and clinical validation

↓

FDA approval 'safe and effective'

↓

Cost-benefit analysis by CMS/Insurance Co. 'reasonable and necessary'

↓

Routine use of biomarker for patient care

Molecular Testing for somatic mutations

TABLE 1. Summary of four studies with a panel of mutation analyses

	First author, year (Ref.)			
	Nikiforov *et al.*, 2009 (40)	**Moses** *et al.*, 2010 (20)	**Ohori** *et al.*, 2010 (41)	**Cantara** *et al.*, 2010 (39)
Cytology categories investigated: sample number	Indeterminate: 52; cancer positive: 22; cancer negative: 12	Indeterminate: 110; malignant: 57; suspicious: 27; nondiagnostic: 2; benign: 257	Follicular lesion of indeterminate significance/atypia of indeterminate significance: 117	Indeterminate: 41; suspicious: 54; inadequate: 53; benign: 87
Material	FNAB (4 needle passes)—a small portion-Roche nucleic acids preservative solution for cytology and remaining material from needle washing for molecular analysis	FNAB—cytological analysis and remaining material for molecular analysis	FNAB (3 or 4 needle passes)—direct smear and monolayered slides by ThinPrep processing. Residual FNA material—nucleic acid preservative solution for molecular analysis	FNAB (1 or 2 needle passes) 2/3 of the material for cytology and 1/3 for molecular analysis. Tissue collected at surgery with 1.5 ml microcentrifuge tube containing Allprotect tissue reagent
Mutation identified (indeterminate samples)	Indeterminate: *N-*, *H-*, *KRAS*: 5; *BRAF*: 7; *RET/PTC1*-3: 2; *PAX8/PPARγ*: 1	Indeterminate: *BRAF*: 3; *RET/PTC1,3*: 1; *NRAS*: 8; *KRAS*: 0	Follicular lesion of indeterminate significance/atypia of indeterminate significance: *BRAF*: 3; *NRAS*: 7; *HRAS*: 1; *PAX8/PPARγ*: 1	Indeterminate: *N-*, *H-*, *KRAS*: 3; *BRAF*: 2; *RET/PTC1,3*: 2; *PAX8/PPARγ*: 0
Histology for indeterminate samples	PTC: 17; FTC: 4; FA: 4; HN: 27	PTC: 19; FA: 29; FTC: 8; Hurthle cell adenoma: 6; hyperplastic nodule: 40; Hurthle cell carcinoma: 2; lymphocytic thyroiditis: 6	PTC: 20; nonneoplastic: 79; adenoma: 18	PTC: 7; FA: 26; HN: 8
	Indeterminate	Indeterminate	Follicular lesion of indeterminate significance	Indeterminate; suspicious
FP/FN (n)	0/6	4/21[a]	0/8	1/1; 0/9
Sensitivity/specificity (%)	71/100	38/95[a]	60/100	85.7/97; 80.4/100

Salvatore *et al.* (37) study was not included in this table because they used just PTC as final histology. This would lead to a false specificity of 0% by the absence of TN samples. HN, Hyperplastic nodule.

[a] It was not possible to calculate the suspicious samples because the data correlating histology and mutation were not given.

Ferraz et al. J Clin Endocrinol Metab. 2011

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Ferraz et al. J Clin Endocrinol Metab. 2011

Molecular Testing for somatic mutations

TABLE 3. Means from studies classified according to the type and number of mutations investigated and FNAB category

	Indeterminate	
	Several mutations analysis[a]	**RET/PTC rearrangements analysis[b]**
FP	1.25 (0–4)	0
FN	9 (1–21)	3.5 (1–6)
Sensitivity	63.7% (38–85.7%)	55% (50–60%)
Specificity	98% (95–100%)	100%

Data are expressed as means (range). The means for suspicious samples were not calculated because only data from Cantara *et al.* (39) were available. The suspicious samples from Moses *et al.* (20) were not calculated because data for a correlation of histology with mutation status were not given.

[a] Means from four studies (20, 39–41) that analyzed a panel of mutations of indeterminate samples.

[b] RET/PTC rearrangements analysis (23, 34) of indeterminate samples.

Ferraz et al. J Clin Endocrinol Metab. 2011

Impact of Mutational Testing on the Diagnosis and Management of Patients with Cytologically Indeterminate Thyroid Nodules: A Prospective Analysis of 1056 FNA Samples

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- Cytology category according to Bethesda Reporting System
- 967 of 1056 (91.6%) adequate epithelial cells and nucleic acid
  - 50 insufficient nucleic acid
  - 39 insufficient epithelial cells

jcem.endojournals.org J Clin Endocrinol Metab, November 2011, 96(11):3390–3397

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TABLE 1. Cancer probability in thyroid nodules with indeterminate cytology based on specific cytological diagnosis and results of molecular testing performed in FNA samples

	Cancer risk			
	AUS/FLUS cytology (n = 247)	**FN/SFN cytology (n = 214)**	**SMC cytology (n = 52)**	**All indeterminate cytology (n = 513)**
Cytology only	14%	27%	54%	24%
Any mutation identified	88%	87%	95%	89%
RAS	84%	85%	88%	85%
BRAF	100%	100%	100%	100%
PAX8/PPARγ	100%	100%	100%	100%
RET/PTC	na	na	100%	100%
No mutation identified	5.9%	14%	28%	11%

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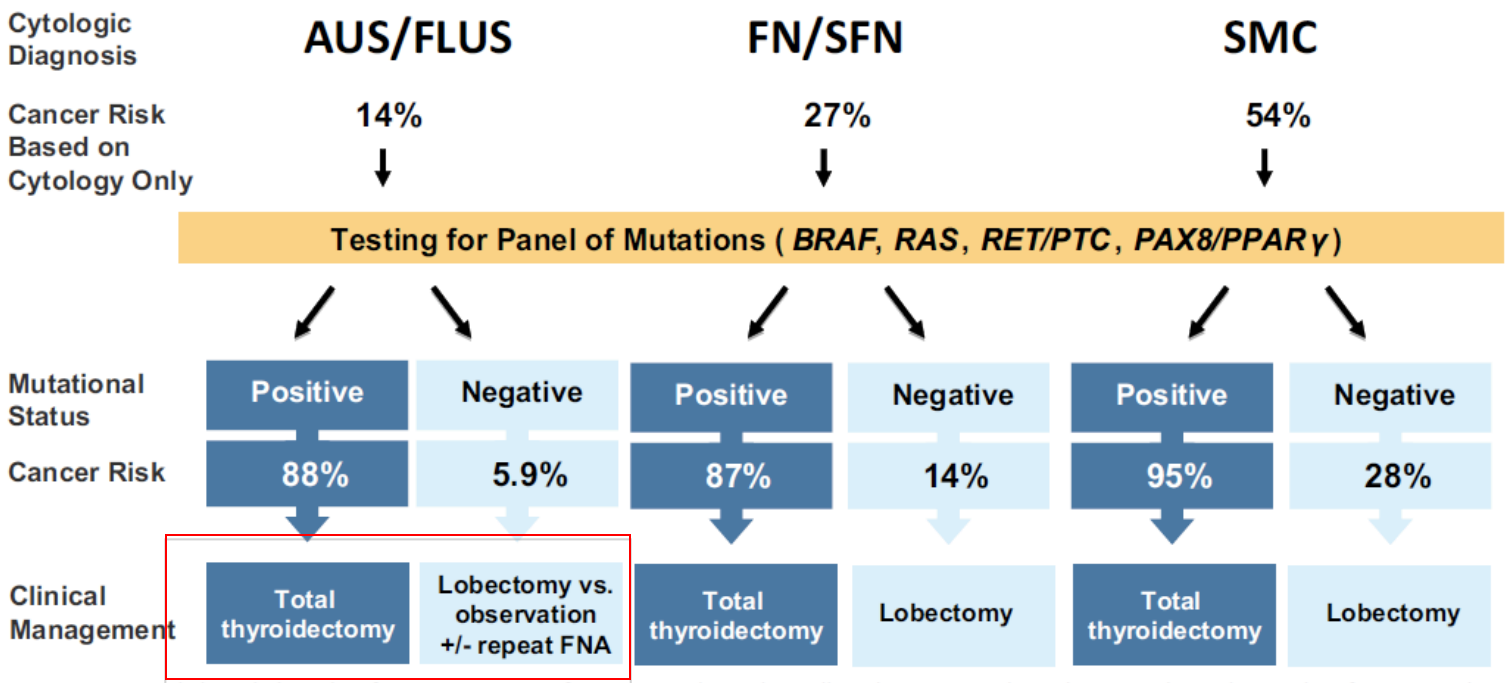

FIG. 3. Proposed clinical algorithm for management of patients with cytologically indeterminate thyroid FNA applying the results of mutational analysis.

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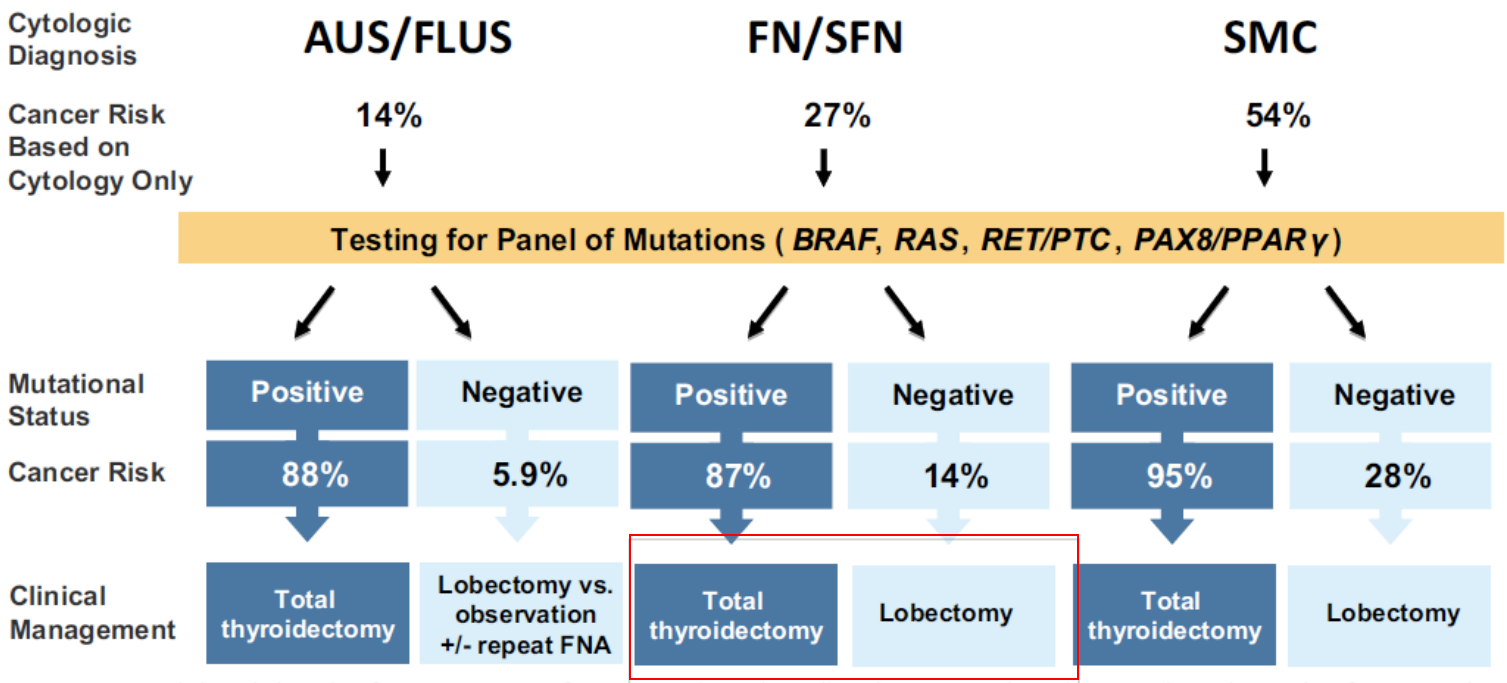

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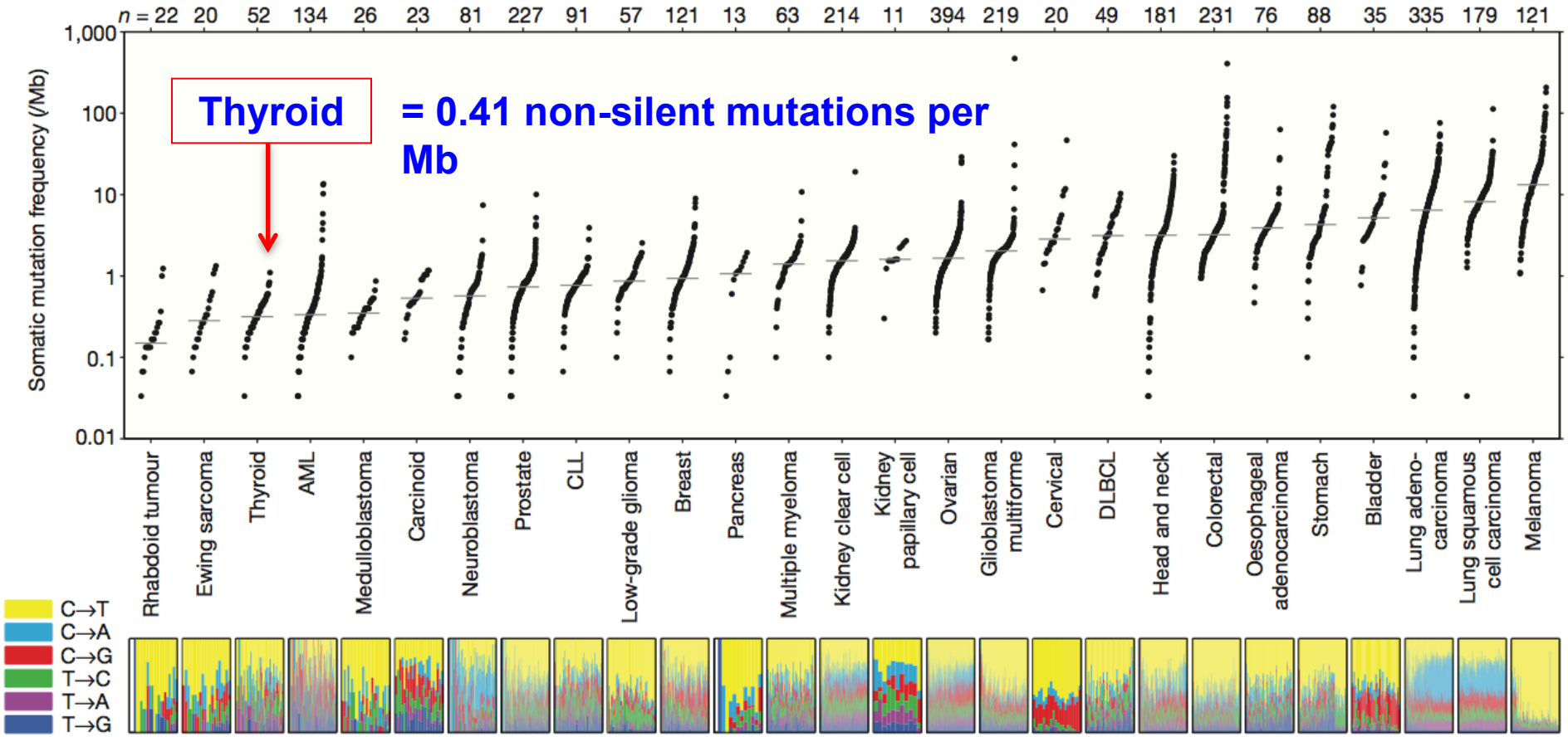
Relative Mutation Rate



Somatic mutation frequencies observed in exomes from 3,083 tumor-normal pairs.

Lawrence et al. Nature 2013:499;214-218

The Cancer Genome Atlas (TCGA)
Analysis of Papillary Thyroid Carcinomas

Number of Cancer Specific Genetic Events

Driver Mutation	# driver mutations	% driver mutation
BRAF – all	248	61.7
RAS – all	52	12.9
EIF1AX	6	1.5
Fusions – all non-BRAF	42	10.5
Arm level SCNAs (non-mutated, non-fusion)	28	7.0
Focal PTEN del	2	0.05
TERT promoter mutations (non-mutated, non-fusion)	2	0.05
All	380	94.5

Unpublished data

Genome Wide Gene expression analysis

Author	Year	Study cohort size	Differentially expressed genes
Barden *et al.*	2003	17	105
Finley *et al.*	2004	59	627
Mazzanti *et al.*	2004	73	10 and 6 gene model
Weber *et al.*	2005	24 (12 FTC 12FA)	80 genes 3 gene model

Shibru, et al. Curr Opin Oncol 2008

Genome Wide Gene expression analysis

Author	Year	Study cohort size	Differentially expressed genes	Accuracy of markers
Barden *et al.*	2003	17	105	Overall 100%
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Shibru, et al. Curr Opin Oncol 2008

The new england journal of medicine
original article

Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology

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- Additional FNA pass
- Thyroid FNA samples collected from 49 clinical sites
- 3,789 patients with 4,812 thyroid nodules (> 1 cm) enrolled
- 414 indeterminate on FNA and histologic diagnosis
  - 51 inadequate/degraded RNA
  - 36 “excessive study site storage time”
  - 16 “unavailable reference standard”
  - 265 had thyroidectomy

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- 85 of 265 indeterminate nodules were malignant (32%)

- The gene expression classifier
  - 78 of 85 as ‘suspicious’
  - 92% Sensitivity, [84%-97%] 95% CI
  - 52% Specificity, [44%-59%] 95% CI

  - Overall NPV from 93%
  - Atypia/FLUS NPV = 95%
  - Follicular neoplasm NPV = 94%
  - Suspicious for malignancy NPV = 85%

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Table 2. Performance of Gene-Expression Classifier, According to Histopathological Subtype.

Histopathological Subtype	No. of Nodules (%)	Result with Gene-Expression Classifier *no. benign/no. suspicious*
Benign		
Total	180 (100)	
Benign follicular nodule*	71 (39.4)	41/30
Follicular adenoma	64 (35.6)	37/27
Follicular tumor of uncertain malignant potential	11 (6.1)	5/6
Well-differentiated tumor of uncertain malignant potential	9 (5.0)	4/5
Hürthle-cell adenoma	21 (11.7)	4/17
Chronic lymphocytic thyroiditis	2 (1.1)	0/2
Hyalinizing trabecular adenoma	2 (1.1)	2/0
Malignant		
Total	85 (100)	
Papillary thyroid carcinoma†	42 (49.4)	4/38
Papillary thyroid carcinoma, follicular variant	19 (22.4)	2/17
Hürthle-cell carcinoma‡	10 (11.8)	1/9
Follicular carcinoma §	10 (11.8)	0/10
Medullary thyroid cancer	2 (2.4)	0/2
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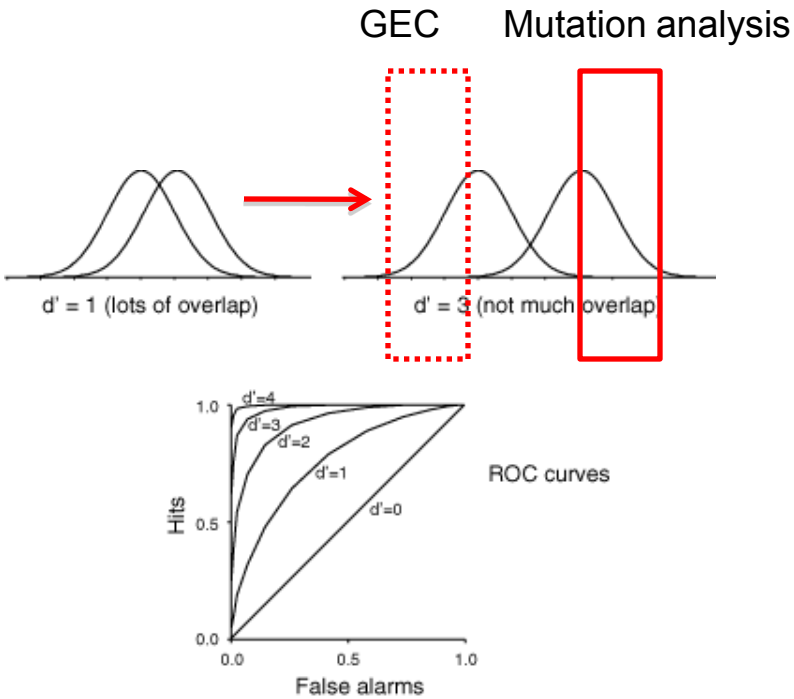
Thyroid cancer marker application: The future?

- **Diagnostic molecular markers:**
  - **Some markers beginning to be applied clinically**
  - **Limitations in FNA and early detections of small cancers are likely to increase and result in significant healthcare expenditure.**



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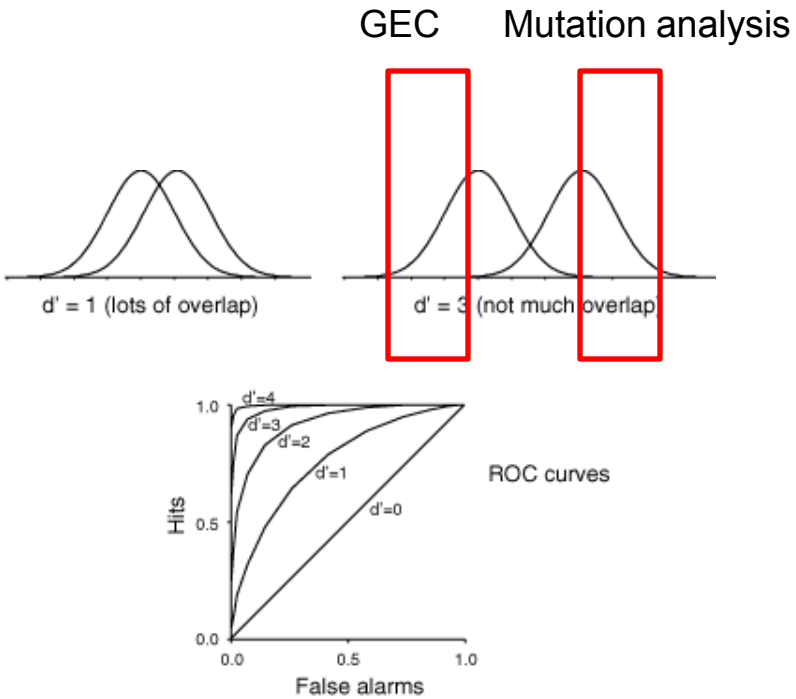

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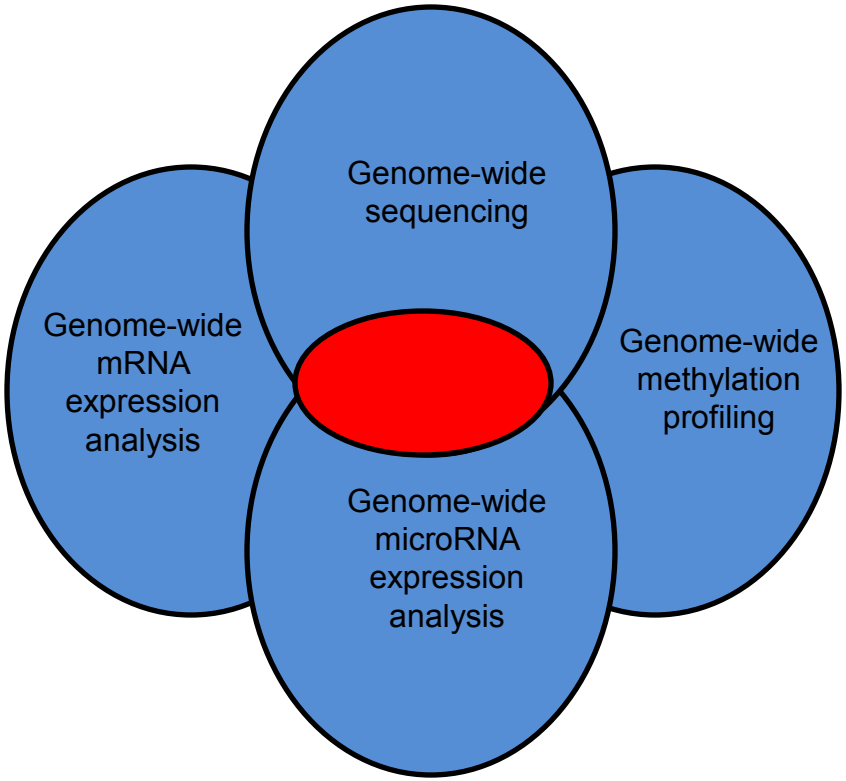

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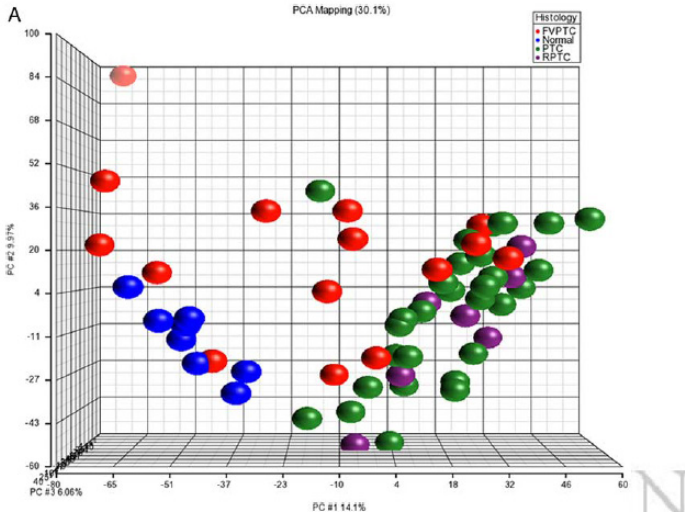

Ellis et al. J Clin Endocrinol Metab. 2013

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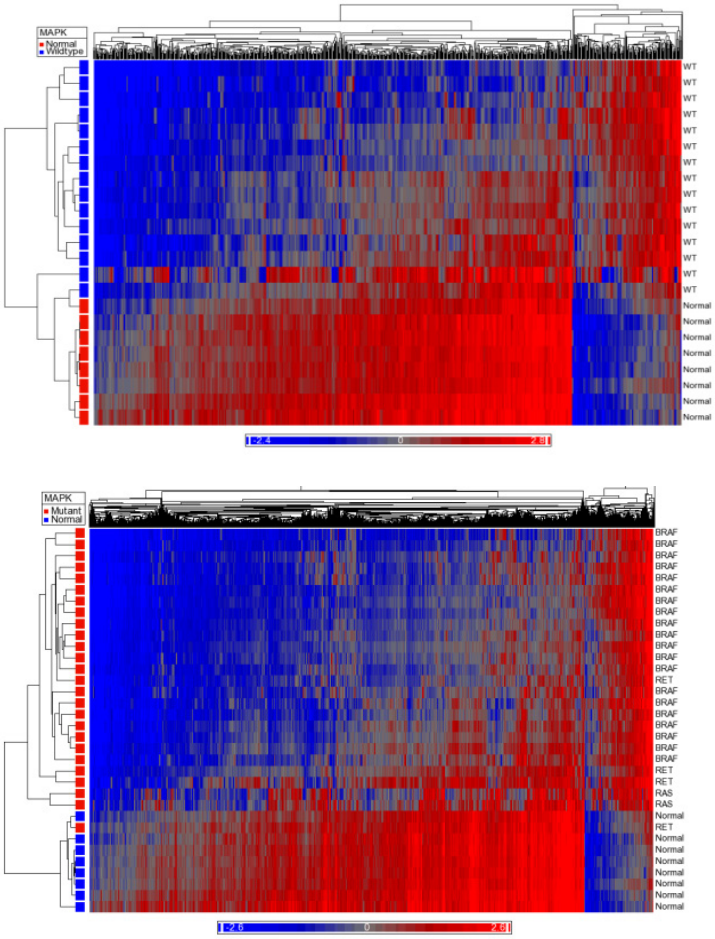
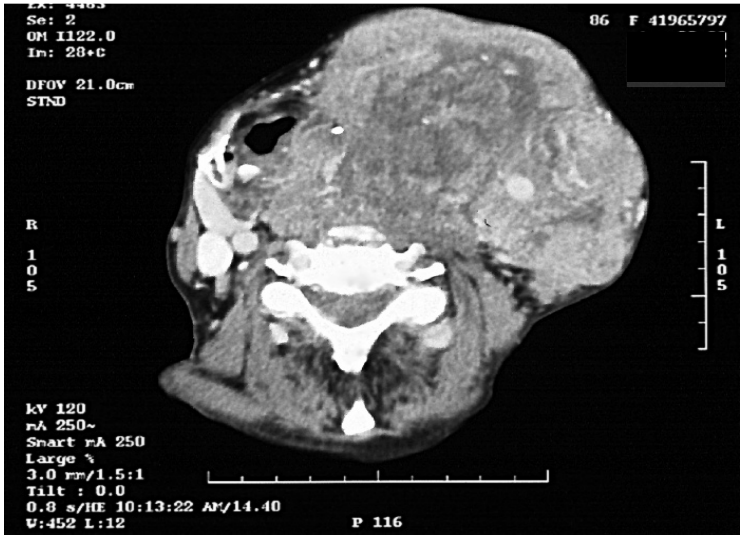


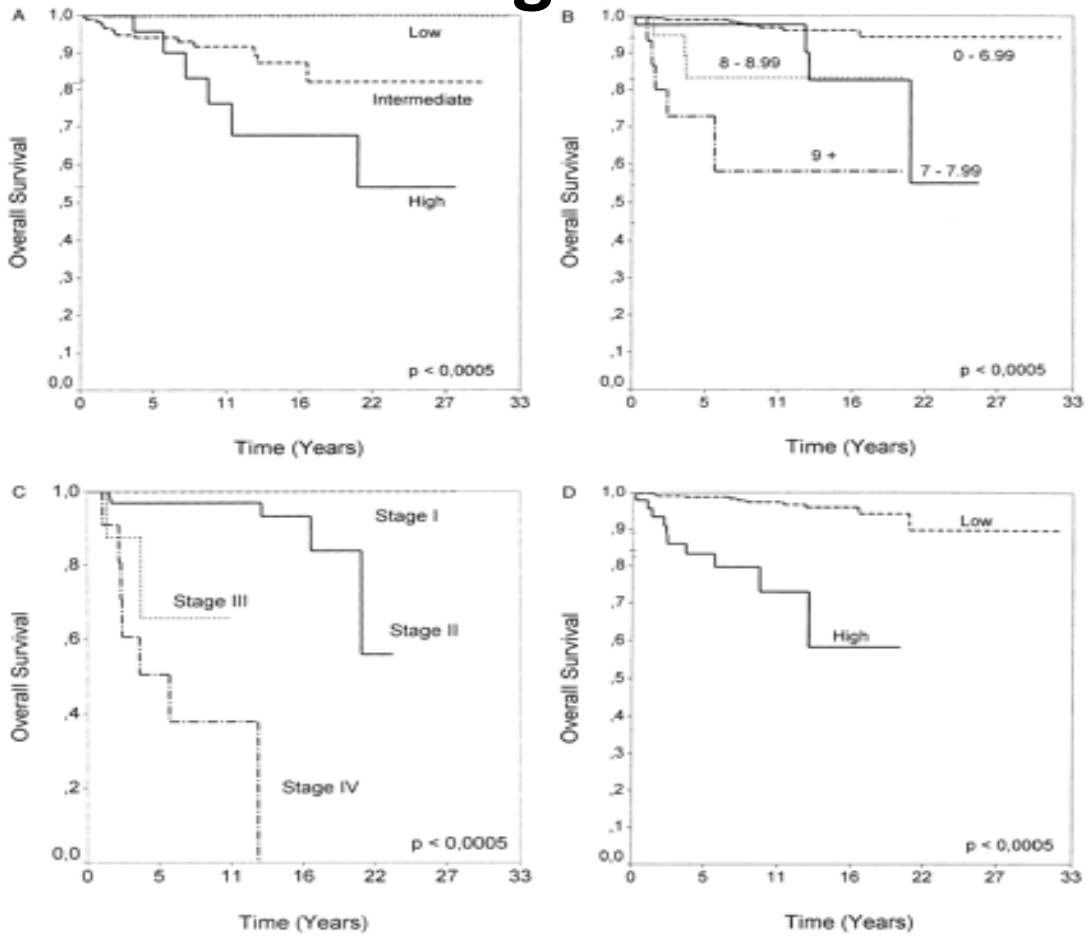
Ellis et al. J Clin Endocrinol Metab. 2013

Prognostic classification systems for thyroid cancer of follicular cell origin

System	Prognostic Factors Used
EORTC	Gender, tumor histology type, extrathyroidal invasion, distant metastases
AGES	Age, tumor grade, extrathyroidal invasion, distant metastases, tumor size
MACIS	Age, extrathyroidal invasion, distant metastases, completeness of resection, tumor size
AMES	Age, extrathyroidal invasion, distant metastases, tumor size
TNM	Primary tumor size, lymph node status, distant metastases
DeGroot class*	Cervical node metastases, distant metastases, extrathyroidal invasion
SAG*	Sex, age, grade of tumor (vascular invasion, nuclear atypia, or tumor necrosis)
Mazzaferri staging system	Tumor size, cervical node status, multiple tumors (>3), extrathyroidal invasion, distant metastases



Prognostic classification systems for differentiated thyroid cancer of follicular cell origin



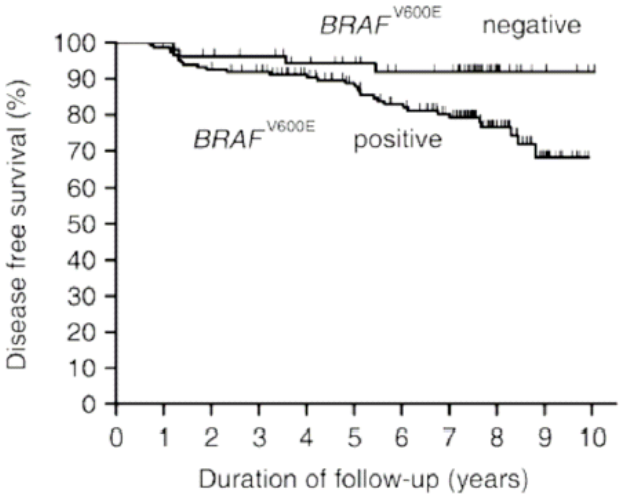
Jukkola A, et al. Endocrine-Related Cancer, 2004

Is BRAF V600E a marker of thyroid cancer aggressiveness?

Is BRAF V600E mutation associated with aggressive tumor phenotype?

Controversial because of

- lack of multivariate analysis,
- small study cohorts,
- inclusion of different histologic subtypes of thyroid cancer,
- lack of both follow up data,
- different treatment approaches



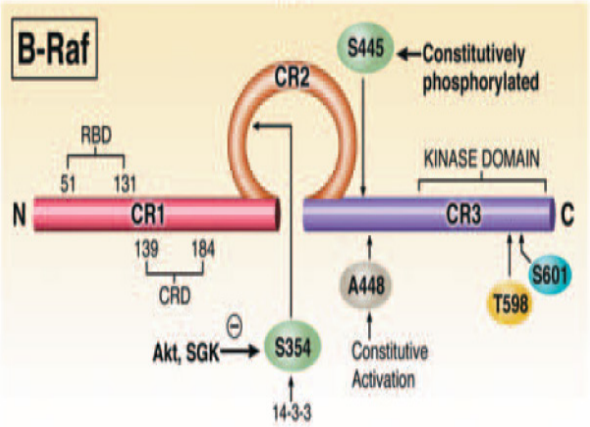
Xing. J Clin Endocrinol Metab, 2005

BRAF in thyroid cancer

BRAF V600E Genotype-Phenotype association

BRAF V600E mutations associated with

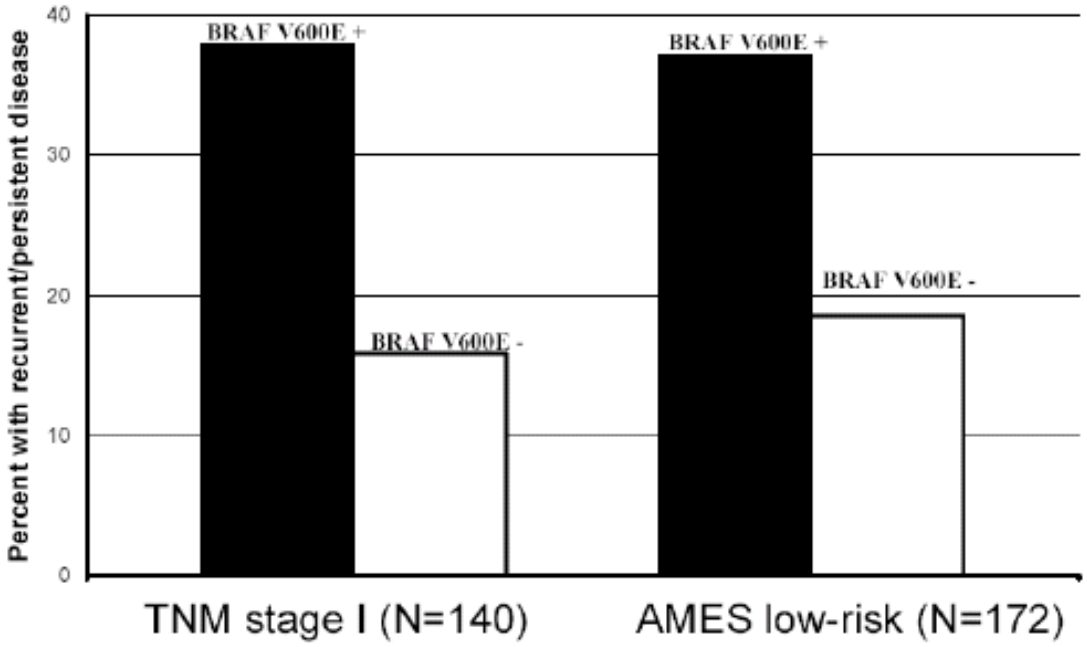
- Older age
- Lymph node & distant metastasis
- Higher TNM stage
- Higher AMES risk group
- Higher recurrence rate



Kebebew et al. Ann Surg 2007

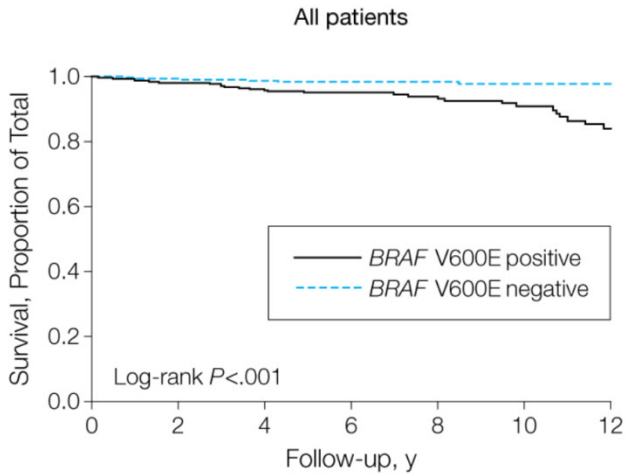
BRAF in thyroid cancer

BRAF V600E is a risk factor for recurrence even for AMES low-risk or TNM Stage I conventional papillary thyroid cancer



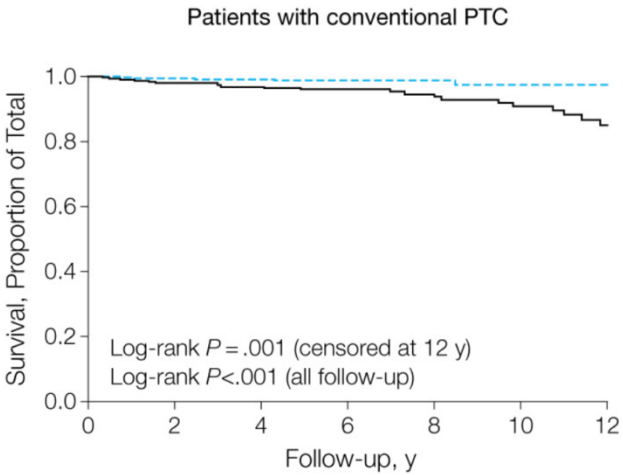
Kebebew et al. Ann Surg 2007

BRAF in thyroid cancer



No. at risk

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



No. at risk

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

Xing M, et al. JAMA. 2013

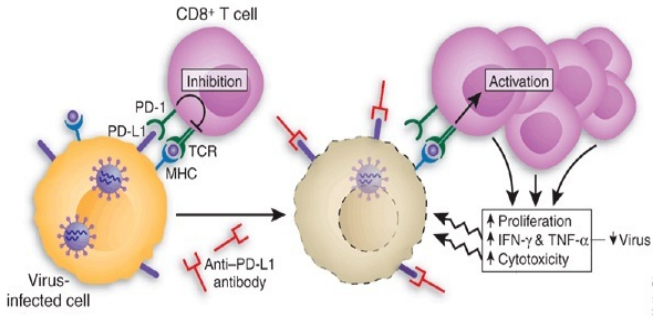
BRAF in thyroid cancer

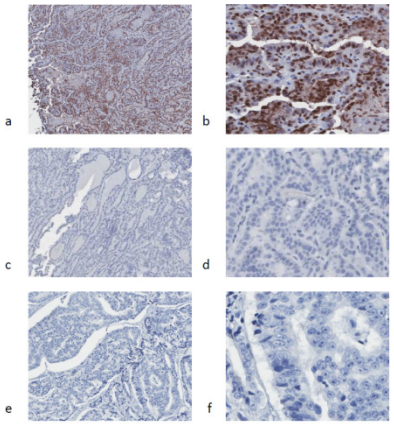
	No. of Patients	Age at Diagnosis, Median (IQR), y	Male, No. (%)	*BRAF* V600E Mutation, No. (%)	PTC-Related Deaths, No. (%)			Follow-up, Median (IQR), mo	
					All	*BRAF* V600E–Positive	*BRAF* V600E–Negative	All Patients	Survivors
By medical center									
Johns Hopkins Hospital	387	45 (35-57)	101 (26.1)	151 (39.0)	8 (2.1)	8 (5.3)	0	12 (1-30)	12 (1-28)
University of Pittsburgh	169	52 (38-63)	42 (24.8)	101 (59.8)	1 (0.6)	1 (1.0)	0	19 (11-26)	19 (11-26)
Memorial Sloan-Kettering Cancer Center	135	50 (35-63)	44 (32.6)	64 (47.4)	11 (8.2)	10 (15.6)	1 (1.4)	96 (1-144)	90 (1-144)
University of Pisa	189	38 (28-51)	47 (24.9)	65 (34.4)	9 (4.8)	6 (9.2)	3 (2.4)	72 (24-180)	84 (24-180)
University of Perugia	117	49 (37-59)	32 (27.4)	76 (65.0)	5 (4.3)	2 (2.6)	3 (7.3)	22 (6-39)	22 (6-40)
University of Milan	110	42 (34-55)	24 (21.8)	38 (34.6)	1 (0.9)	0	1 (1.4)	48 (24-64)	48 (24-64)
University of Padua	135	48 (39-57)	32 (23.7)	87 (64.4)	1 (0.7)	1 (1.2)	0	26 (22-30)	26 (22-30)
Kanagawa Cancer Center	49	55 (41-65)	16 (32.6)	33 (67.4)	9 (18.4)	7 (21.2)	2 (12.5)	68 (31-78)	65 (33-76)
Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology	99	49 (33-59)	10 (10.1)	42 (42.4)	1 (1.0)	1 (2.4)	0	48 (42-53)	48 (43-53)
Griffith Medical School	76	40 (34-56)	20 (26.3)	34 (44.7)	0	0	0	42 (4-82)	42 (4-82)
University of Sydney	95	44 (34-59)	20 (21.0)	55 (57.9)	5 (5.3)	5 (9.1)	0	103 (63-135)	104 (64-137)
Hospital La Paz, Health Research Institute	66	42 (32-54)	11 (16.7)	28 (42.4)	2 (3.0)	1 (3.6)	1 (2.6)	41 (30-57)	42 (30-57)
Institute of Endocrinology, Prague	222	47 (31-60)	39 (17.6)	71 (32.0)	3 (1.4)	3 (4.2)	0	50 (30-85)	50 (30-85)
By country									
United States	691	47 (36-59)	187 (27.1)	316 (45.7)	20 (2.9)	19 (6.0)	1 (0.3)	17 (2-36)	16 (2-32)
Italy	551	44 (34-56)	135 (24.5)	266 (48.3)	16 (2.9)	9 (3.4)	7 (2.5)	33 (20-70)	34 (20-72)
Japan	49	55 (41-65)	16 (32.6)	33 (67.4)	9 (18.4)	7 (21.2)	2 (12.5)	68 (31-78)	65 (33-76)
Poland	99	49 (33-59)	10 (10.1)	42 (42.4)	1 (1.0)	1 (2.4)	0	48 (42-53)	48 (43-53)
Australia	171	43 (34-57)	40 (23.4)	89 (52.0)	5 (2.9)	5 (5.6)	0	75 (32-118)	76 (33-118)
Spain	66	42 (32-54)	11 (16.7)	28 (42.4)	2 (3.0)	1 (3.6)	1 (2.6)	41 (30-57)	42 (30-57)
Czech Republic	222	47 (31-60)	39 (17.6)	71 (32.0)	3 (1.4)	3 (4.2)	0	50 (30-85)	50 (30-85)
Overall	1849	46 (34-58)	438 (23.7)	845 (45.7)	56 (3.0)	45 (5.3)	11 (1.1)	33 (13-67)	33 (13-65)

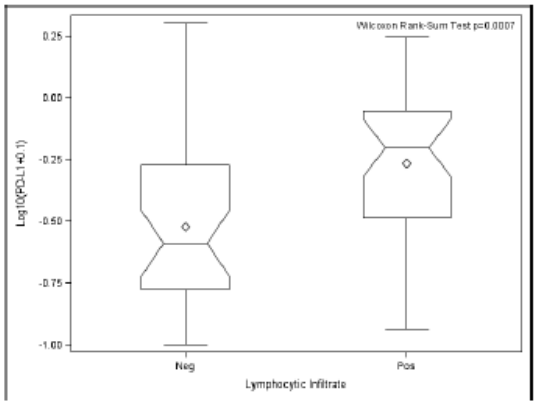
Abbreviation: IQR, interquartile range.

Xing M, et al. JAMA. 2013

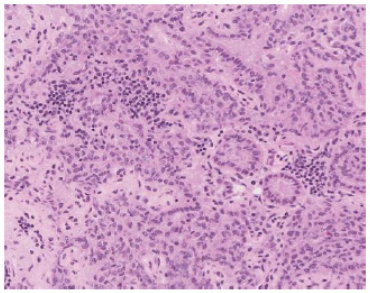
Beyond BRAF!







- Chronic lymphocytic thyroiditis has been linked to development of thyroid cancer
- PD-L1 is upregulated in papillary thyroid cancer



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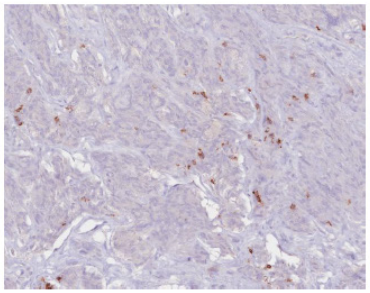

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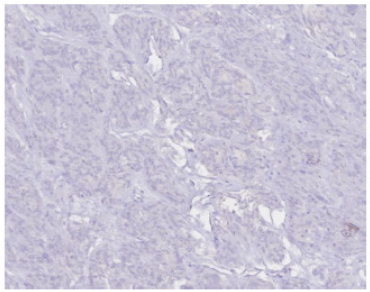
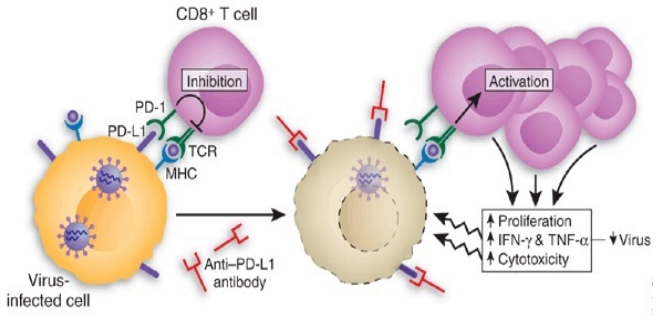
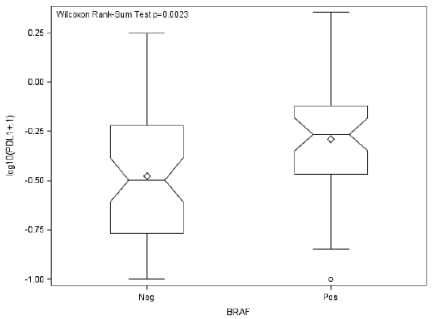
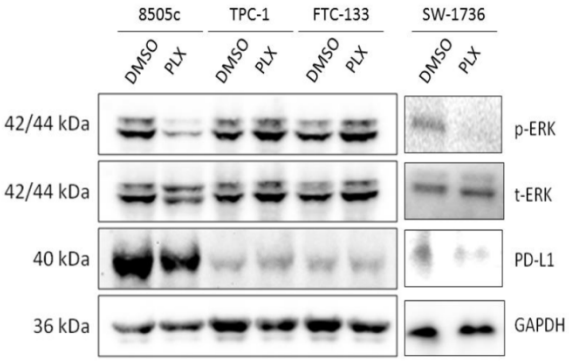


Unpublished data

Beyond BRAF!







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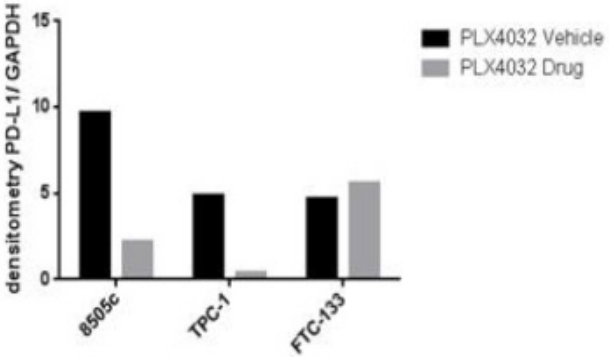

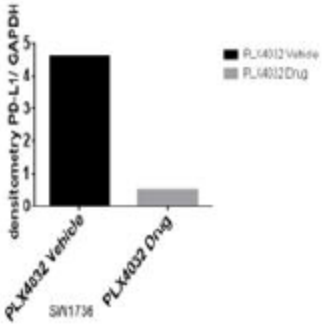

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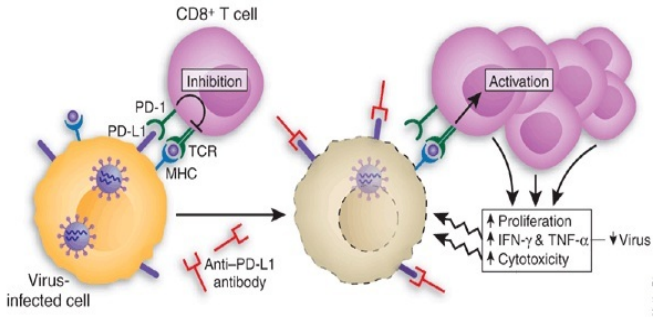

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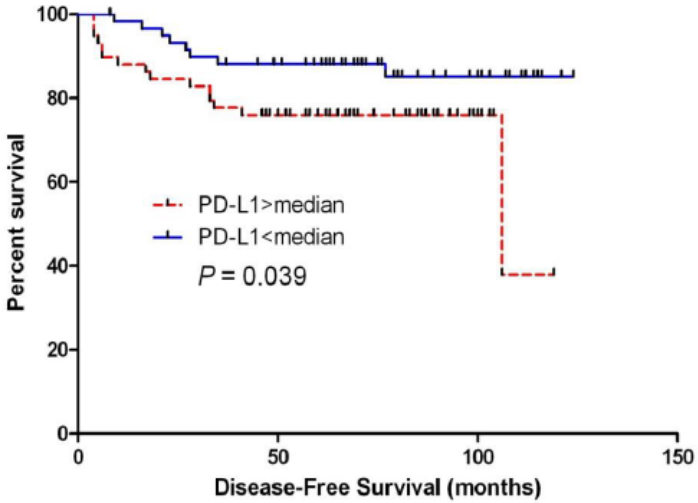

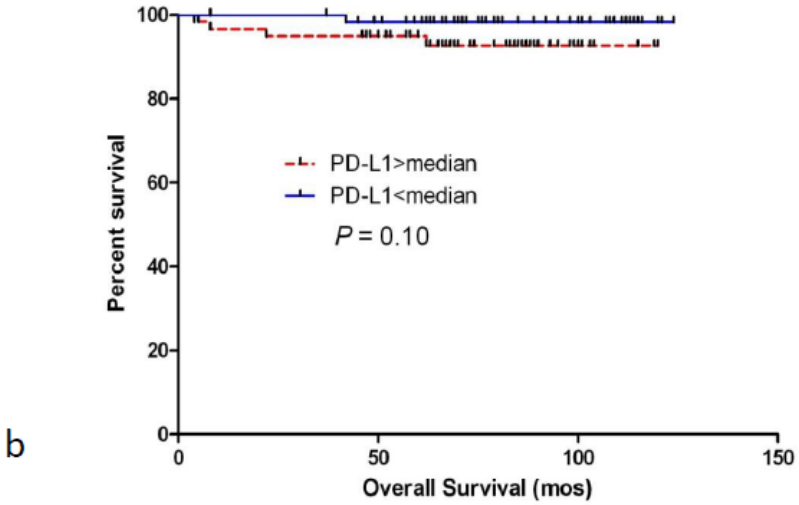

Unpublished data

Illustrative Clinical Cases

63 year old woman with a left thyroid nodule & family history of thyroid cancer

↓

Biopsy showed atypical cells

↓

Mutation testing for BRAF + for V600E mutation

↓

More aggressive treatment warranted

Summary

- Molecular testing
  - Somatic mutation and gene expression profiling are being applied with improvement in more accurate diagnosis
  - Room for improvement
- What is the natural history of benign neoplasm by molecular analysis?
- Is it cost effective?
- Prognostic markers of BRAF testing
  - Follow up
  - Adjuvant therapy

Thank You