Role for molecular testing in perioperative risk stratification

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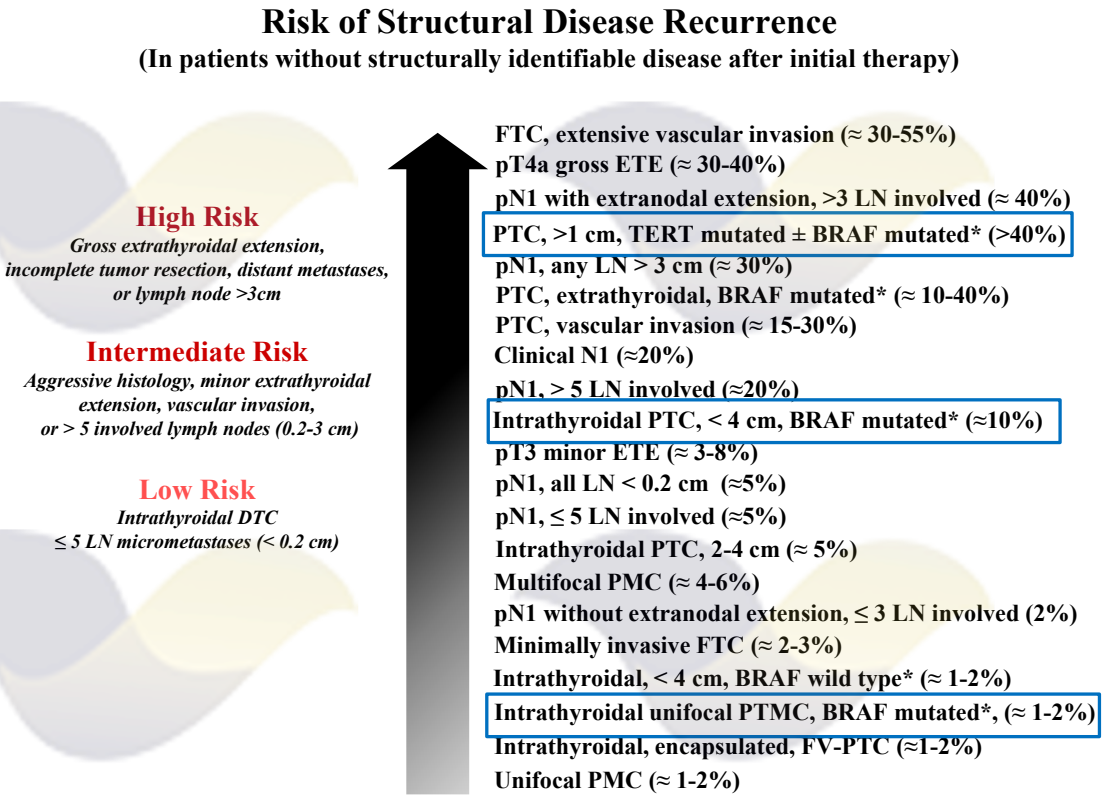
UPMC LIFE CHANGING MEDICINE

Disclosures

- Own IP related to ThyroSeq through University of Pittsburgh (royalties)
- Consultant to Sonic Healthcare USA (consultant fees)

Thyroid cancer risk stratification



Haugen BR et al. *Thyroid*. 2016, 26:1-133

Molecular Markers of Cancer Risk

- Can molecular markers provide **robust** risk stratification of thyroid cancer independent on pathology?
- Can this be achieved preoperatively?

TERT Promoter Mutation as an Early Genetic Event Activating Telomerase in Follicular Thyroid Adenoma (FTA) and Atypical FTA

Na Wang, MD[1]; Tiantian Liu, MD[2]; Anastasios Sofiadis, MD, PhD[1]; C. Christofer Juhlin, MD, PhD[1]; Jan Zedenius, MD, PhD[3,4]; Anders Höög, MD, PhD[1]; Catharina Larsson, MD, PhD[1]; and Dawei Xu, MD, PhD[2]

TABLE 1. Mutations and Follow-Up for the 58 Patients With a Primary FTA

	Mutation					Follow-Up			
Case No.	*TERT* Promoter	*RAS* Gene	Age at Diagnosis, y	Sex (M/F)	Primary Tumor	Disease Recurrence	Patient Outcome	Time, mo	Final Diagnosis
FTA-1	wt	–	55	F	FTA	no	DWOD	172	FTA
FTA-2	wt	–	40	F	FTA	no	AWOD	316	FTA
FTA-3	wt	–	52	M	FTA	no	DWOD	87	FTA
FTA-4	wt	–	32	F	FTA	no	AWOD	314	FTA
FTA-5	wt	–	46	F	FTA	no	AWOD	313	FTA
FTA-6	wt	–	40	M	FTA	no	DWOD	277	FTA
FTA-7	wt	–	46	M	FTA	no	AWOD	309	FTA
FTA-8	wt	–	50	F	FTA	no	AWOD	309	FTA
FTA-9	wt	–	25	M					
FTA-10	wt	–	61	F					
FTA-11	wt	–	55	F					
FTA-12	wt	–	50	F					
FTA-13	wt	–	32	M					
FTA-14	wt	–	64	F					
FTA-15	wt	–	37	F					
FTA-16	wt	–	62	F					
FTA-17	wt	–	43	M					
FTA-18	wt	–	54	F					
FTA-19	wt	–	49	M					
FTA-20	wt	–	78	F					
FTA-21	C228T	*NRAS* Q61R	69	F					

TABLE 1. Mutations and Follow-Up for the 58 Patients With a Primary FTA

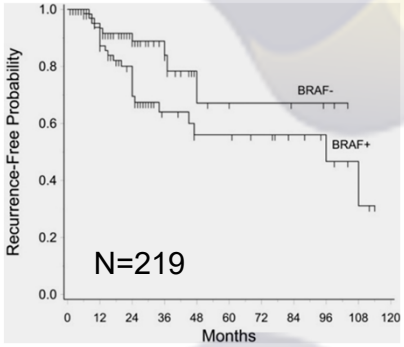
	Mutation					Follow-Up			
Case No.	*TERT* Promoter	*RAS* Gene	Age at Diagnosis, y	Sex (M/F)	Primary Tumor	Disease Recurrence	Patient Outcome	Time, mo	Final Diagnosis
FTA-21	C228T	*NRAS* Q61R	69	F	FTA	yes, FTC	DOD	250	FTC

Wang N, et al. *Cancer*. 2014;120:2965-79

Molecular Markers of Cancer Risk

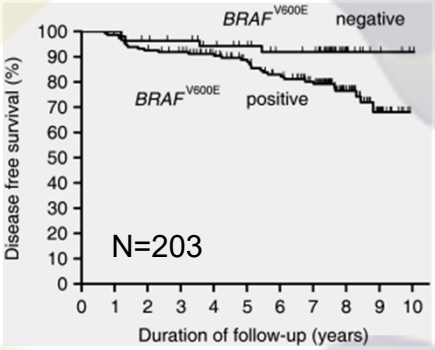
Early years - BRAF

All Variants of PTC included



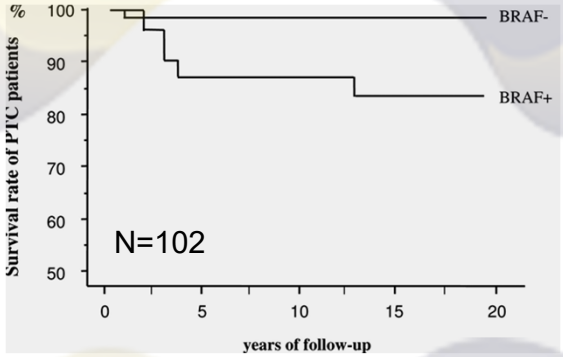
Xing M et al. *JCEM (*2005)

Conventional PTC Only



Kim TY et al. *Clin Endocrinol* (2006)

All Variants of PTC included



Elisei R et al. *JCEM* (2008)

Molecular Markers of Cancer Risk

Early years - BRAF

ORIGINAL CONTRIBUTION

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

Mingzhao Xing, MD, PhD
Ali S. Alzahrani, MD
Kathryn A. Carson, ScM
David Viola, MD
Rossella Elisei, MD
Bela Bendlova, PhD
Linwah Yip, MD

Importance *BRAF* V600E is a prominent oncogene in papillary thyroid cancer (PTC), but its role in PTC-related patient mortality has not been established.

Objective To investigate the relationship between *BRAF* V600E mutation and PTC-related mortality.

Design, Setting, and Participants Retrospective study of 1849 patients (1411 women and 438 men) with a median age of 46 years (interquartile range, 34-58 years) and an overall median follow-up time of 33 months (interquartile range, 13-67 months) after initial treatment at 13 centers in 7 countries between 1978 and 2011.

Main Outcomes and Measures Patient deaths specifically caused by PTC.

Results Overall, mortality was 5.3% (45/845; 95% CI, 3.9%-7.1%) vs 1.1% (11/1004; 95% CI, 0.5%-2.0%) ($P < .001$) in *BRAF* V600E–positive vs mutation-negative patients. Deaths per 1000 person-years in the analysis of all PTC were 12.87 (95% CI, 9.61-17.24) vs 2.52 (95% CI, 1.40-4.55) in *BRAF* V600E–positive vs mutation-negative patients; the hazard ratio (HR) was 2.66 (95% CI, 1.30-5.43) after adjustment for age at diagnosis, sex, and medical center. Deaths per 1000 person-years in the analysis of the conventional variant of PTC were 11.80 (95% CI, 8.39-16.60) vs 2.25 (95% CI, 1.01-5.00) in *BRAF* V600E–positive vs mutation-negative patients; the adjusted HR was 3.53 (95% CI, 1.25-9.98). 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 (HR, 1.21; 95% CI, 0.53-2.76). A higher *BRAF* V600E–associated patient mortality was also observed in several clinicopathological subcategories, but statistical significance was lost with adjustment for patient age, sex, and medical center. For example, in patients with lymph node metastasis, the deaths per 1000 person-years were 26.26 (95% CI, 19.18-35.94) vs 5.93 (95% CI, 2.96-11.86) in *BRAF* V600E–positive vs mutation-negative patients (unadjusted HR, 4.43 [95% CI, 2.06-9.51]; adjusted HR, 1.46 [95% CI, 0.62-3.47]). In patients with distant tumor metastasis, deaths per 1000 person-years were 87.72 (95% CI, 62.68-122.77) vs 32.28 (95% CI, 16.14-64.55) in *BRAF* V600E–positive vs mutation-negative patients (unadjusted HR, 2.63 [95% CI, 1.21-5.72]; adjusted HR, 0.84 [95% CI, 0.27-2.62]).

Conclusions and Relevance In this retrospective multicenter study, the presence of the *BRAF* V600E mutation was significantly associated with increased cancer-related mortality among patients with PTC. Because overall mortality in PTC is low and the association was not independent of tumor features, how to use *BRAF* V600E to manage mortality risk in patients with PTC is unclear. These findings support further investigation of the prognostic and therapeutic implications of *BRAF* V600E status in PTC.

JAMA. 2013;309(14):1493-1501 www.jama.com

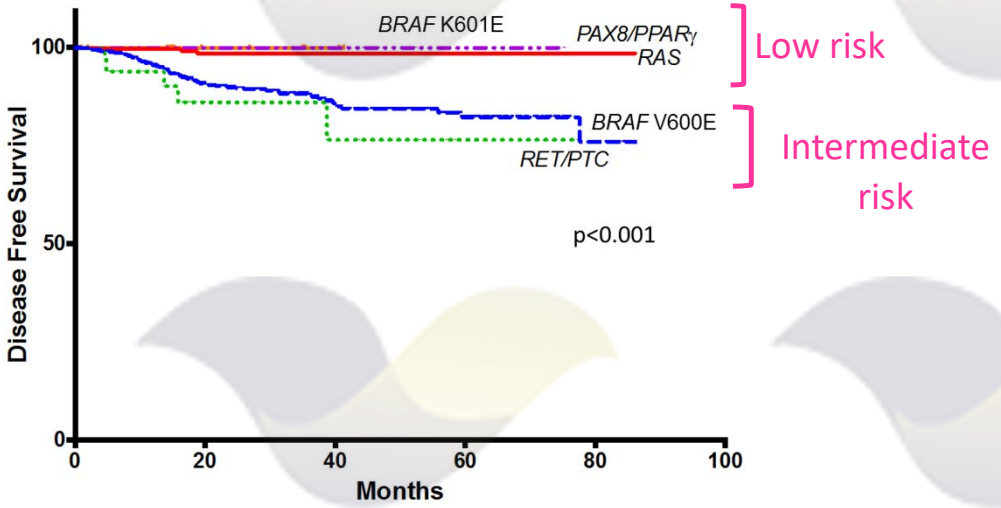
JAMA, April 10, 2013—Vol 309, No. 14

Molecular Markers of Cancer Risk

More knowledge

Tumor Genotype Determines Phenotype and Disease-related Outcomes in Thyroid Cancer: A Study of 1510 Patients

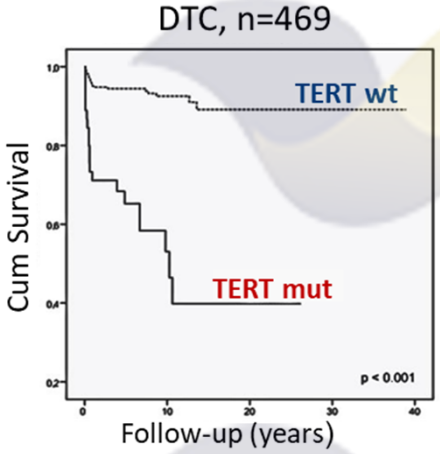
- 1510 patients, 97% with PTC
- Excised tumors tested for 7 common mutations
- 70% of tumors found mutation-positive
- Mean follow-up 33 ± 21.2 months with PTC



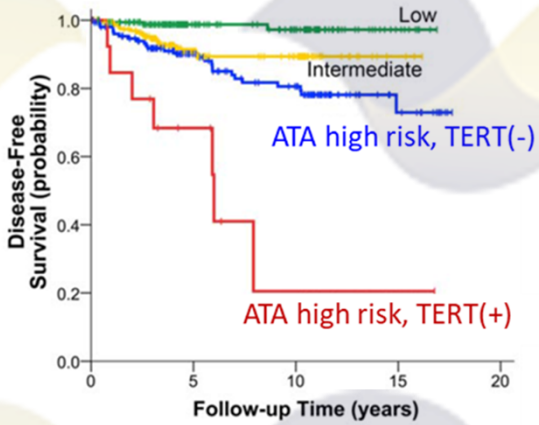
Yip et al. *Ann Surg* 262:519-25 (2015)

Molecular Markers of Cancer Risk in DTC

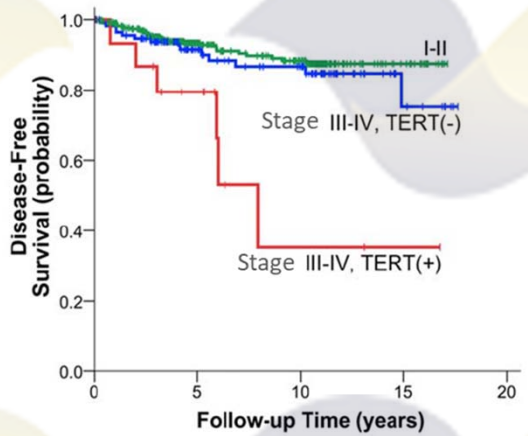
TERT mutations



Melo M et al. *JCEM* (2014)

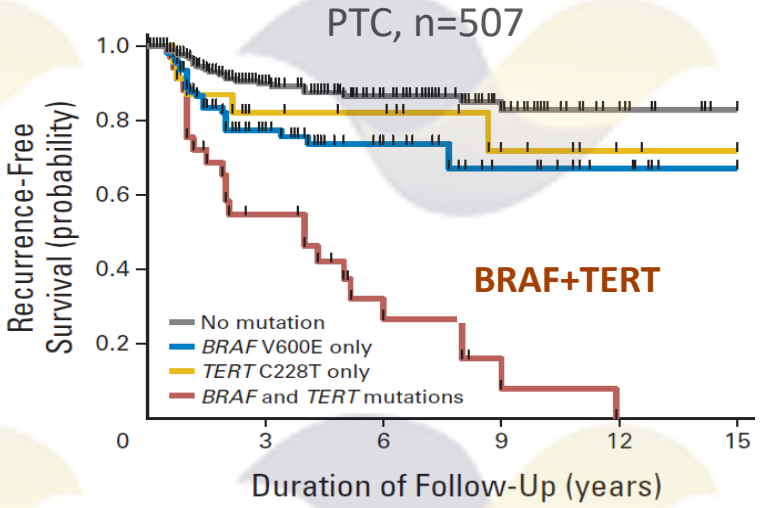


DTC, n=551

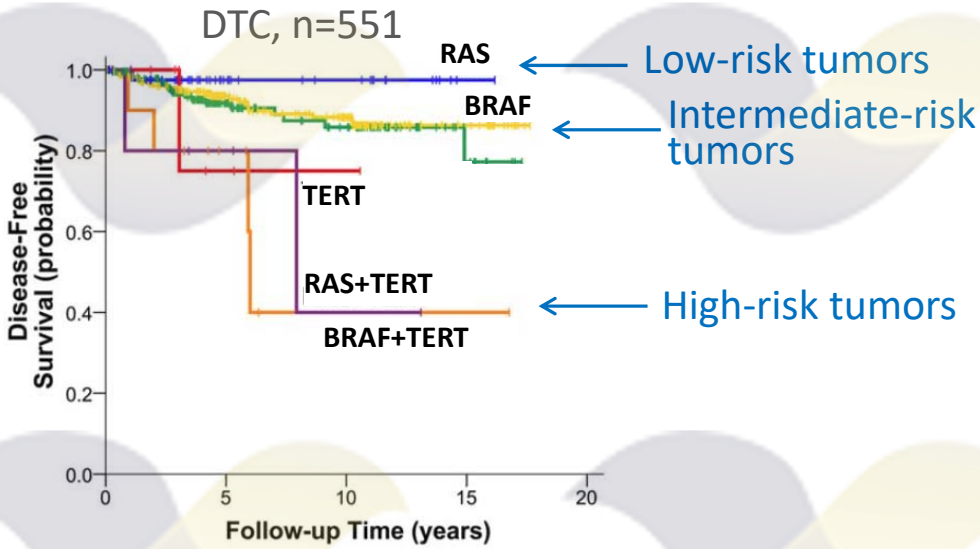


Song YS et al. *Cancer* (2016)

Molecular Markers of Cancer Risk in DTC
Current stage



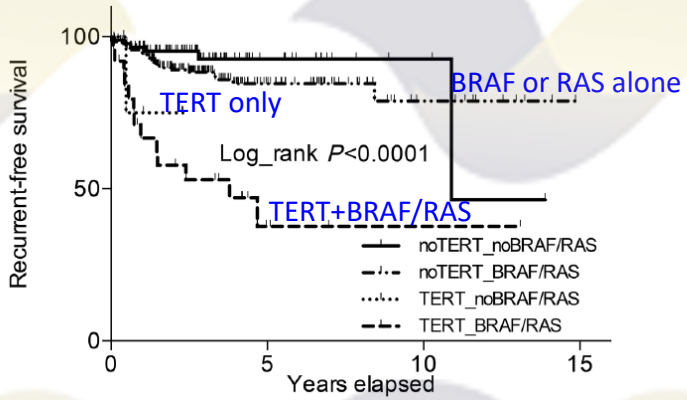
Xing M et al. *JCO* (2014)



Song YS et al. *Cancer* (2016)

Molecular Markers of Cancer Risk in PTC
Current stage

PTC, n=388, 19 sites (TCGA study)



TERT+BRAF/RAS
Recurrence rate=52%
HR=8.17 (95% CI 3-22)
HR adjusted=14.71 (95% CI 3-78)

Shen X et al. *ERC* (2017)

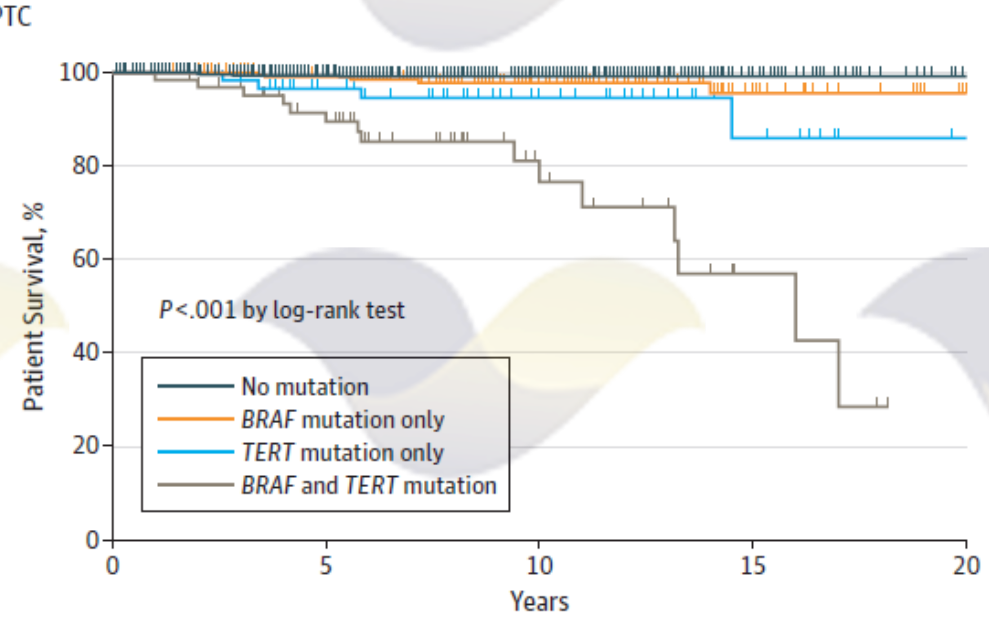
Molecular Markers of Cancer Risk in PTC
Survival

JAMA Oncology | Original Investigation

Mortality Risk Stratification by Combining *BRAF* V600E and *TERT* Promoter Mutations in Papillary Thyroid Cancer
Genetic Duet of *BRAF* and *TERT* Promoter Mutations in Thyroid Cancer Mortality

Rengyun Liu, PhD; Justin Bishop, MD; Guangwu Zhu, BS; Tao Zhang, PhD; Paul W. Ladenson, MD; Mingzhao Xing, MD, PhD

Adjusted RR 9.34 (2.53-34.48)



Molecular Markers of Cancer Risk

SYSTEMATIC REVIEW
published: 03 November 2020
doi: 10.3389/fonc.2020.540238

The Coexistence of Genetic Mutations in Thyroid Carcinoma Predicts Histopathological Factors Associated With a Poor Prognosis: A Systematic Review and Network Meta-Analysis

Ling Zhao[1,2†], Lin Wang[1†], Xiaomeng Jia[1], Xiaodong Hu[1], Ping Pang[3], Sitong Zhao[1], Yajing Wang[1], Jing Wang[4], Yingshi Zhang[5] and Zhaohui Lyu[1*]*

Front Oncol. 2020

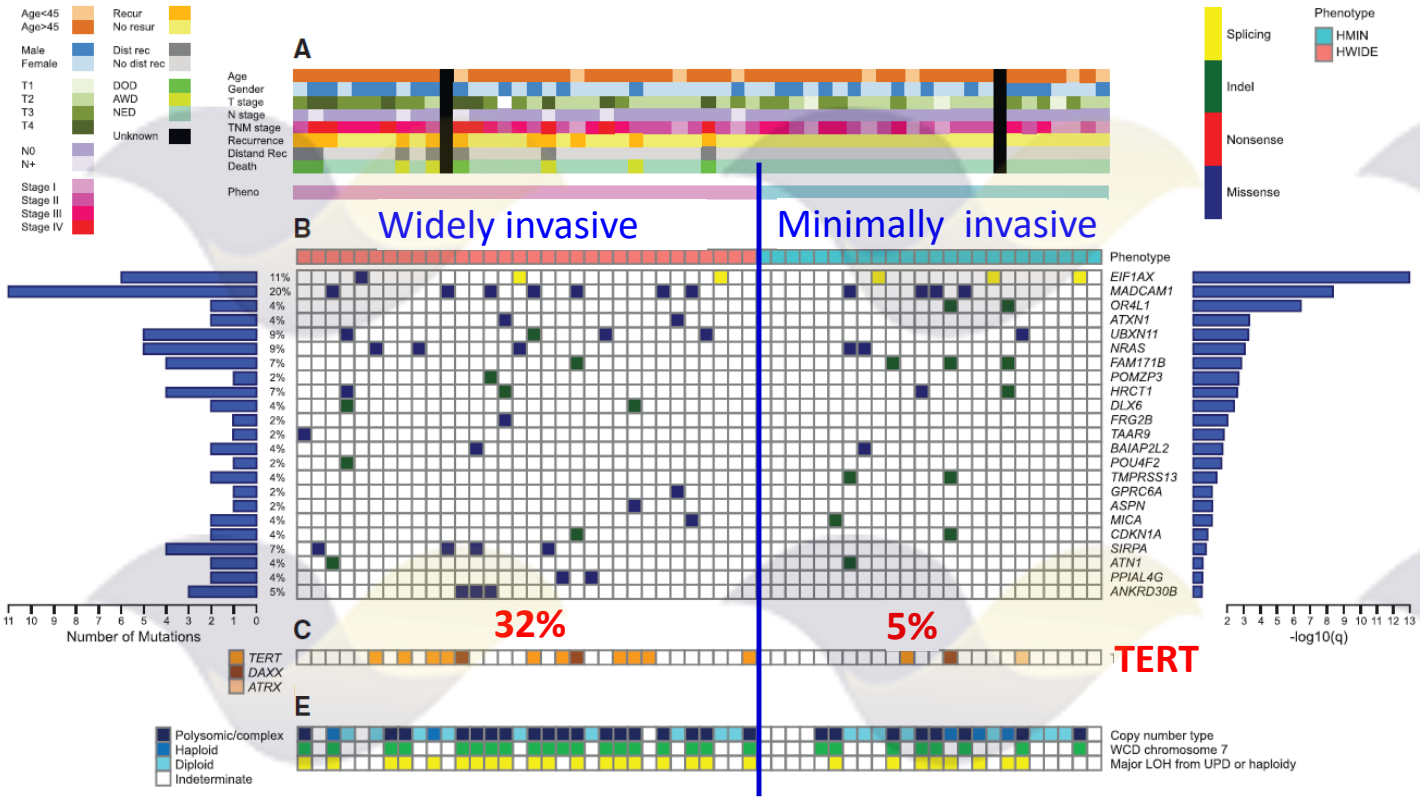
- Meta-analysis of 27 studies reporting 8,388 TC patients

TABLE 2 | Network meta-analysis results for the outcomes in thyroid carcinoma.

Outcomes		Molecular markers	Mutations type	OR(95% CrI)
Primary outcomes	Lymph node metastasis	Coexistent mutations	$BRAF^{V600E}$+*TERT*	1.62 (0.97,2.70)
			TERT+*RAS*	1.38 (0.14,13.61)
			$BRAF^{V600E}$+*RET/PTC*	3.91 (0.37,41.10)
			$BRAF^{V600E}$+*CHEK2*	1.08 (0.18,6.33)
		Single mutation	$BRAF^{V600E}$	1.24 (0.80,1.93)
			TERT	0.88 (0.46,1.68)
			RAS	0.37 (0.08,1.79)
			RET/PTC	18.21 (0.44,748.49)
			CHEK2	1.77 (0.27,11.50)
	Distant metastasis	Coexistent mutations	$BRAF^{V600E}$+*TERT*	7.86 (3.46,17.84)*
			TERT+*RAS*	39.84 (5.23,303.73)*
			$BRAF^{V600E}$+*RET/PTC*	54.02 (1.37,2124.33)*
			$BRAF^{V600E}$+*CHEK2*	86.43 (0.09,78676.88)
		Single mutation	$BRAF^{V600E}$	0.67 (0.29,1.58)
			TERT	6.56 (2.24,19.23)*
			RAS	3.54 (0.60,21.00)
			RET/PTC	36.16 (0.25,5177.90)
			CHEK2	6.34 (0.06,680.43)
Secondary outcomes	Tumor recurrence	Coexistent mutations	$BRAF^{V600E}$+*TERT*	7.21 (3.59,14.47)*
			TERT+*RAS*	92.47 (0.08,106876.03)
			$BRAF^{V600E}$+*CHEK2*	48.02 (0.03,67332.82)
		Single mutation	$BRAF^{V600E}$	1.58 (0.91,2.77)
			TERT	2.67 (1.00,7.15)*
			RAS	43.64 (0.04,47930.52)
			CHEK2	44.51 (0.03,70519.05)
	Mortality	Coexistent mutations	$BRAF^{V600E}$+*TERT*	9.00 (3.03,26.74)*
			TERT+*RAS*	29.85 (2.36,378.42)*
			$BRAF^{V600E}$+*CHEK2*	95.18 (0.04,225987.43)
		Single mutation	$BRAF^{V600E}$	0.85 (0.29,2.46)
			TERT	3.54 (0.87,14.36)
			RAS	3.69 (0.02,610.95)
			CHEK2	88.08 (0.04,209091.33)

*significant difference.

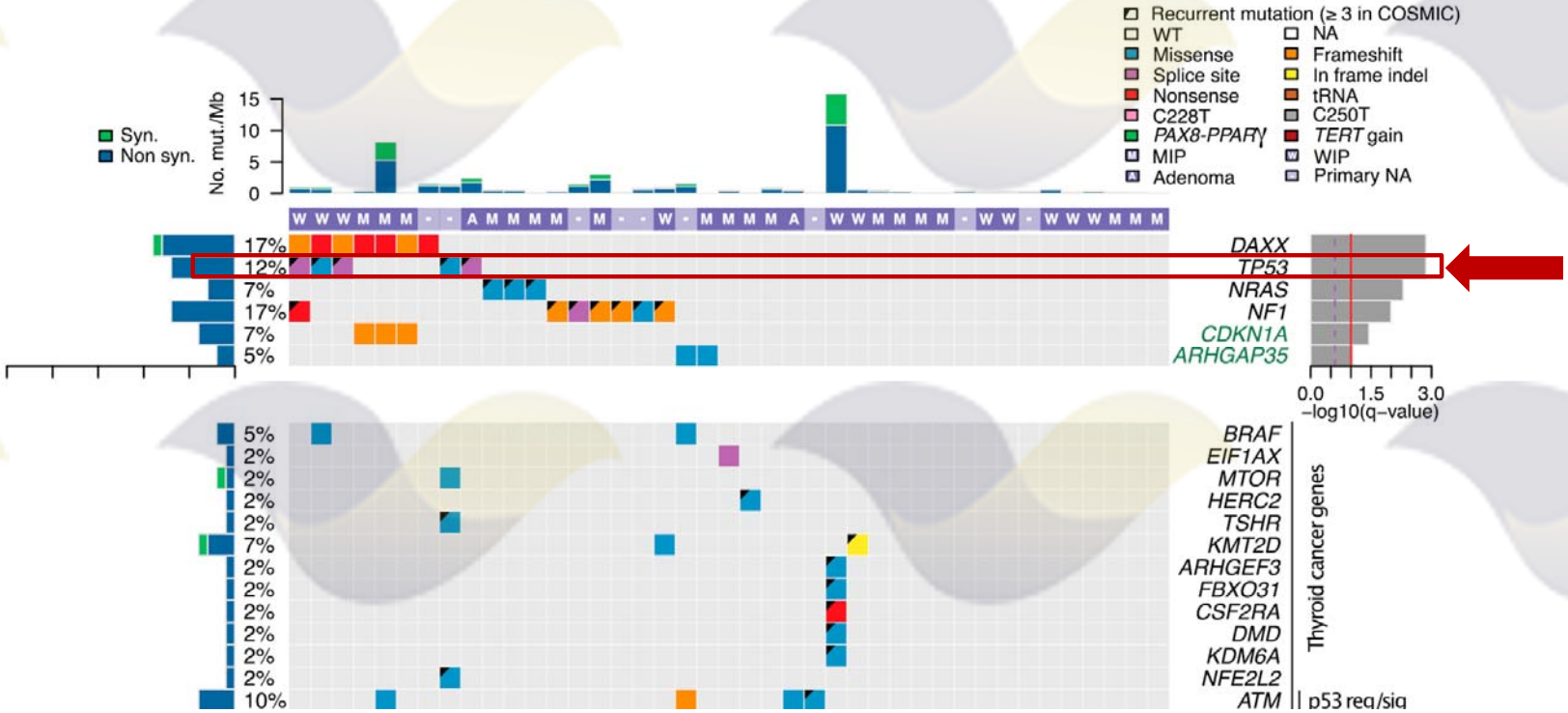
Hurthle cell carcinomas



Ganly et al. *Cancer Cell* 2018

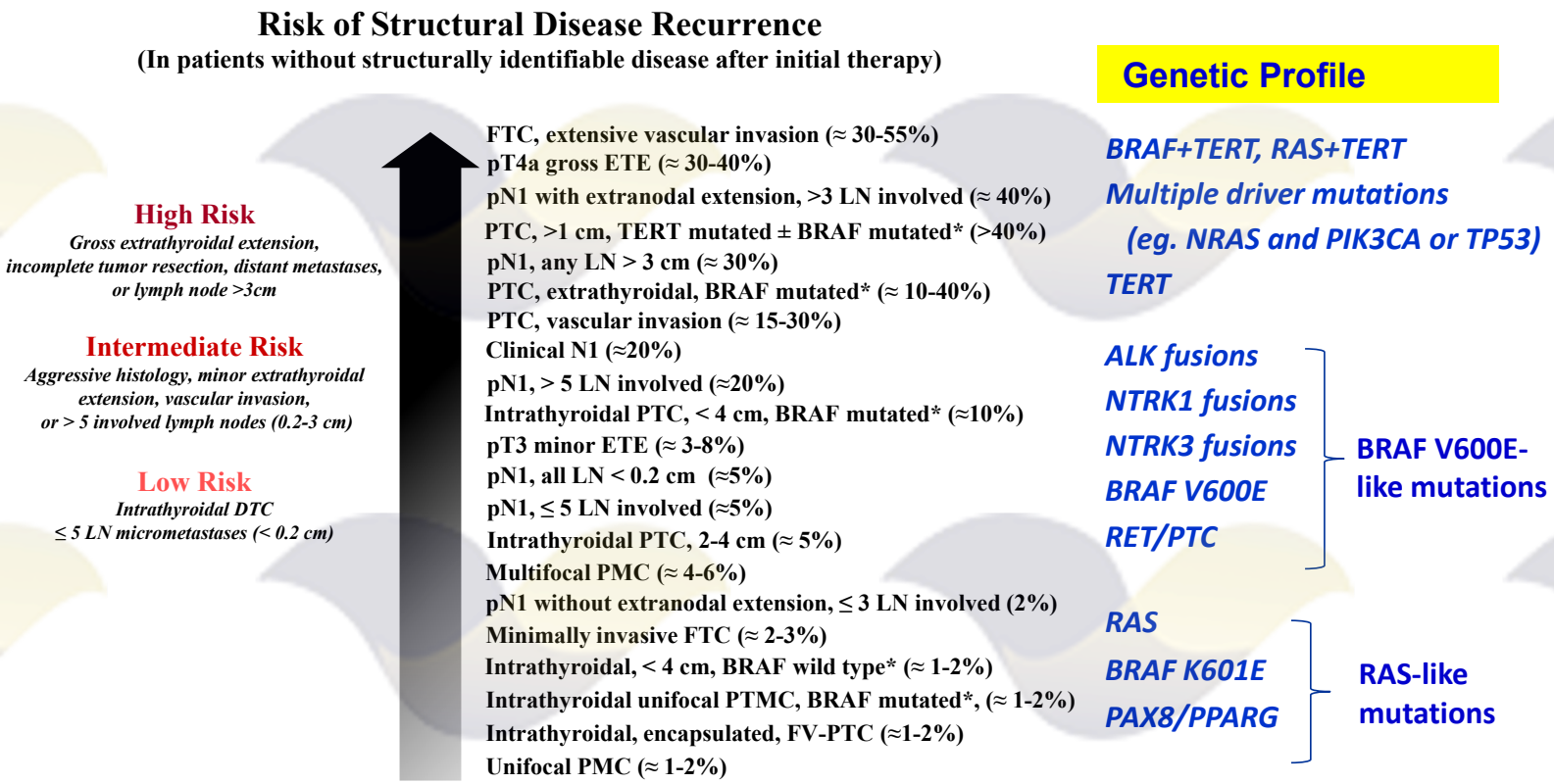
High-risk Hurthle cell carcinomas

_TP53_ mutations observed more often in widely-invasive vs minimally-invasive HCC (p=0.04)



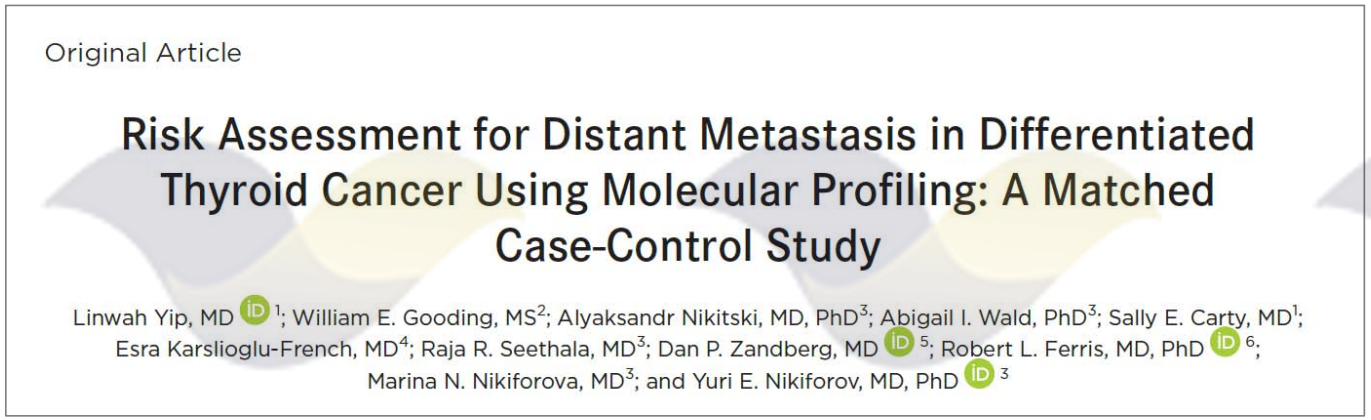
Gopal et al. *Cancer Cell* 2018

Cancer risk stratification using molecular markers



Haugen BR et al. *Thyroid*. 2016, 26:1-133

Cancer risk stratification using molecular markers



Original Article

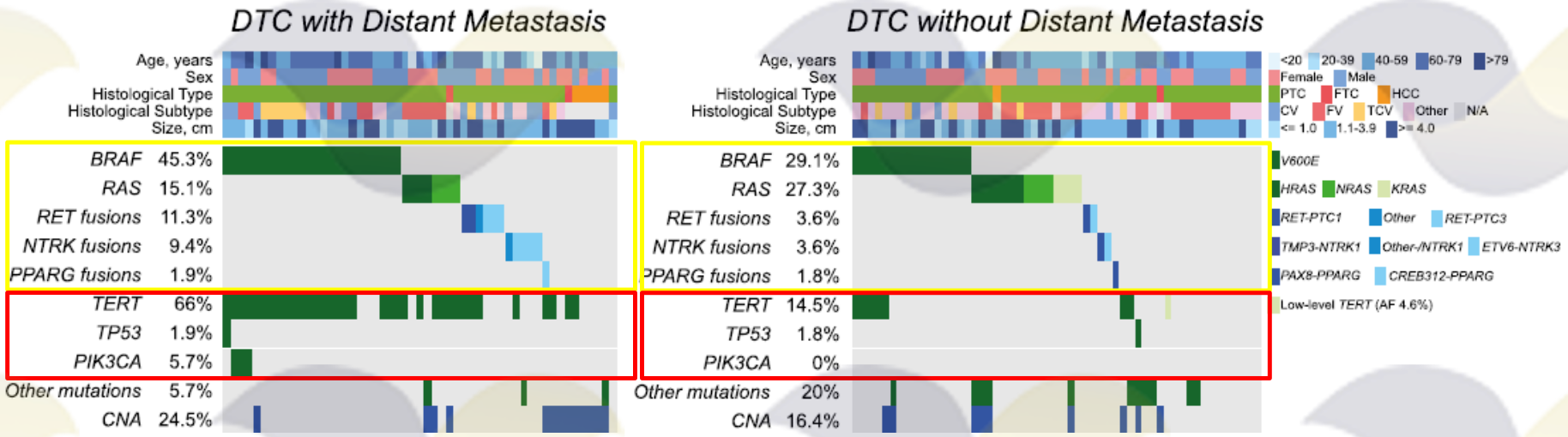
Risk Assessment for Distant Metastasis in Differentiated Thyroid Cancer Using Molecular Profiling: A Matched Case-Control Study

Linwah Yip, MD [1]; William E. Gooding, MS[2]; Alyaksandr Nikitski, MD, PhD[3]; Abigail I. Wald, PhD[3]; Sally E. Carty, MD[1]; Esra Karslioglu-French, MD[4]; Raja R. Seethala, MD[3]; Dan P. Zandberg, MD [5]; Robert L. Ferris, MD, PhD [6]; Marina N. Nikiforova, MD[3]; and Yuri E. Nikiforov, MD, PhD [3]

- Case-control study
  - 62 patients with DTC with distant mets
  - Propensity matched cohort with DTC without distant mets
- At least 5 yrs follow-up
- ThyroSeq v3 targeted NGS panel (112 genes) to classify as high, intermediate, and low risk

Yip L, et al. *Cancer.* 2021. doi: 10.1002/cncr.33421.

Cancer risk stratification using molecular markers



Yip L, et al. *Cancer.* 2021. doi: 10.1002/cncr.33421.

Cancer risk stratification using molecular markers

TABLE 3. Distribution of Molecular Alterations in Patients With Distant Metastasis (Cases) Compared With Propensity-Matched Controls

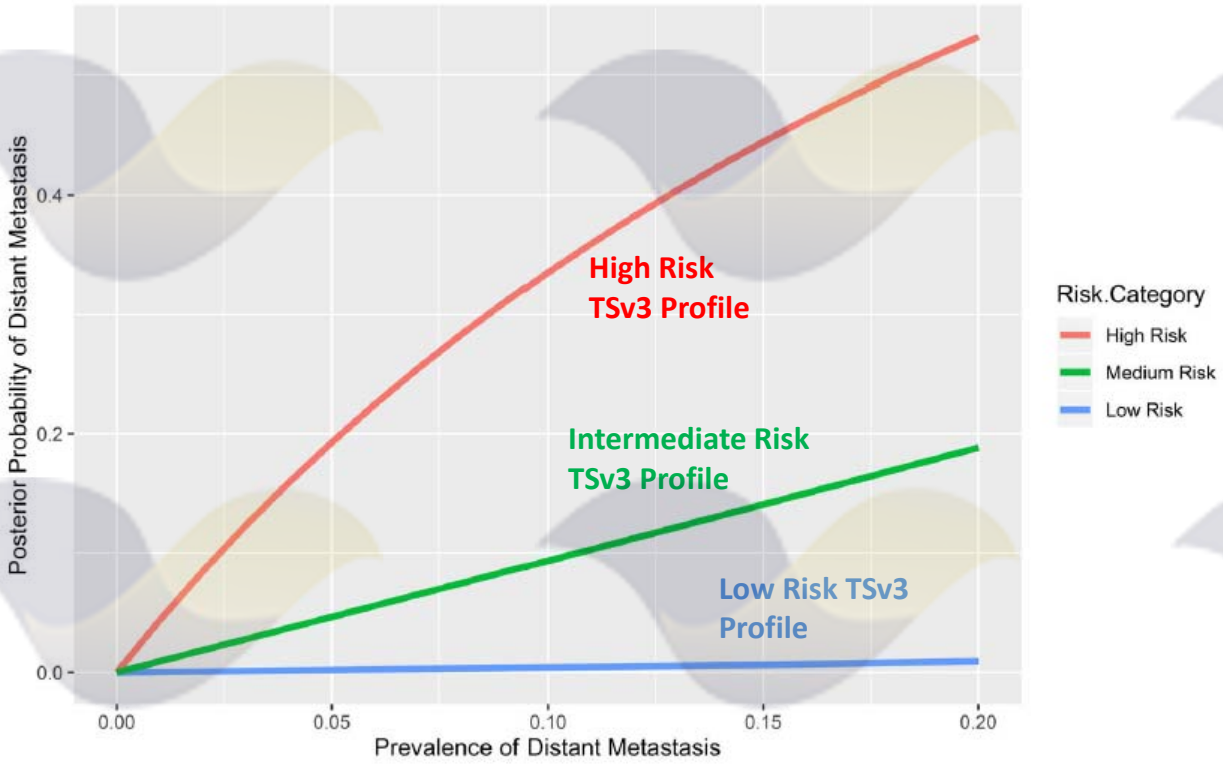
Molecular Alteration	No. (%)		OR	95% CI	Adjusted *P*
	Cases, N = 53	Controls, N = 55			
Molecular risk group					
Low	1 (2)	28 (51)	—[a]		
Intermediate	17 (32)	19 (35)	—[b]		
High	35 (66)	8 (15)			
TERT	35 (66)	8 (15)	11.42	4.46-29.27	<.0001
Late secondary hits: *TERT*, *TP53*, *PIK3CA*	35 (66)	8 (15)	11.42	4.46-29.27	<.0001
Gene expression analysis	29 (55)	20 (36)	2.11	0.98-4.57	.12
BRAF V600E	24 (45)	16 (29)	2.02	0.91-4.46	.1454
RAS	8 (15)	15 (27)	0.47	0.18-1.24	.1795
RET fusions	6 (11)	2 (4)	3.38	0.65-17.58	.1795
NTRK fusions	5 (9)	2 (4)	2.76	0.51-14.90	.2416
Copy number alterations	13 (25)	9 (16)	1.66	0.64-4.29	.2914

Abbreviation: OR, odds ratio.
[a]The OR for the high-risk group relative to the intermediate-risk group was 25.1 (95% CI, 3.07-204.4; $P < .001$).
[b]The OR for the high-risk group relative to the low-risk group was 122.5 (95% CI, 14.5-1038.4; $P < .001$).

Yip L, et al. *Cancer.* 2021. doi: 10.1002/cncr.33421.

Cancer risk stratification using molecular markers

Prevalence-adjusted predicted probability of DM associated with molecular risk groups detected by ThyroSeq



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Cancer risk stratification using molecular markers

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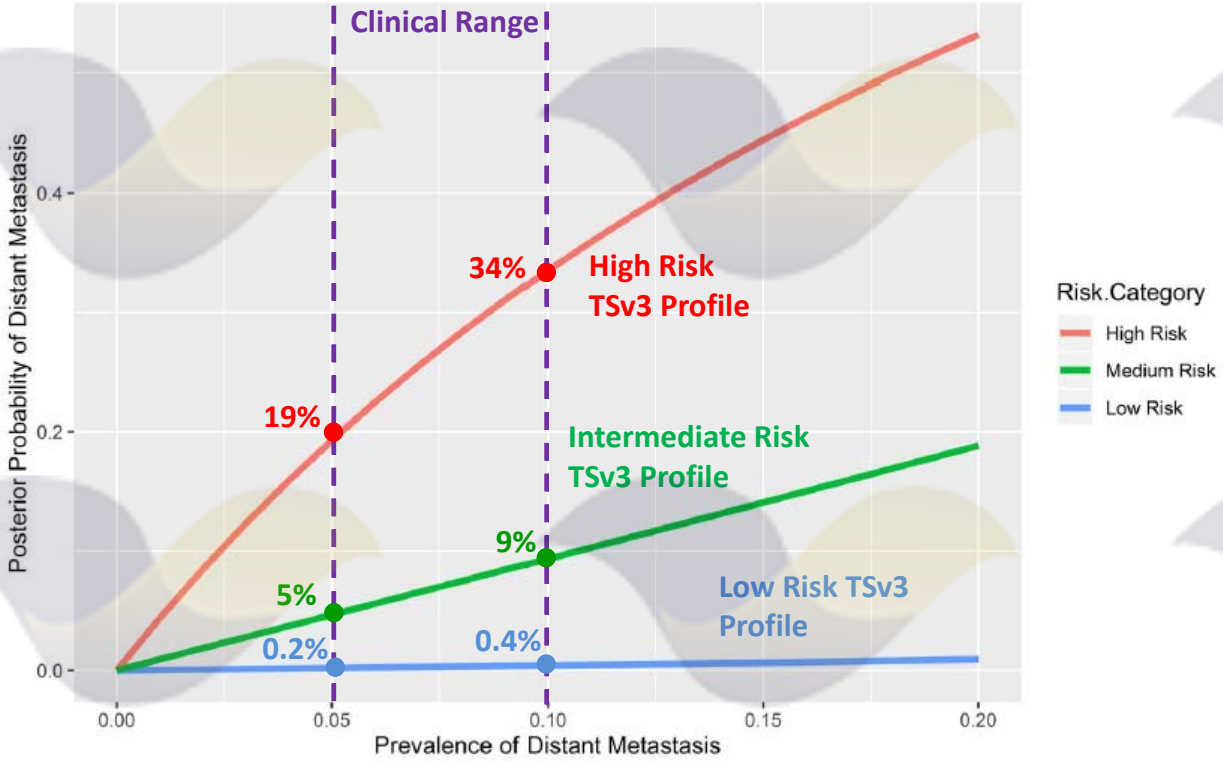
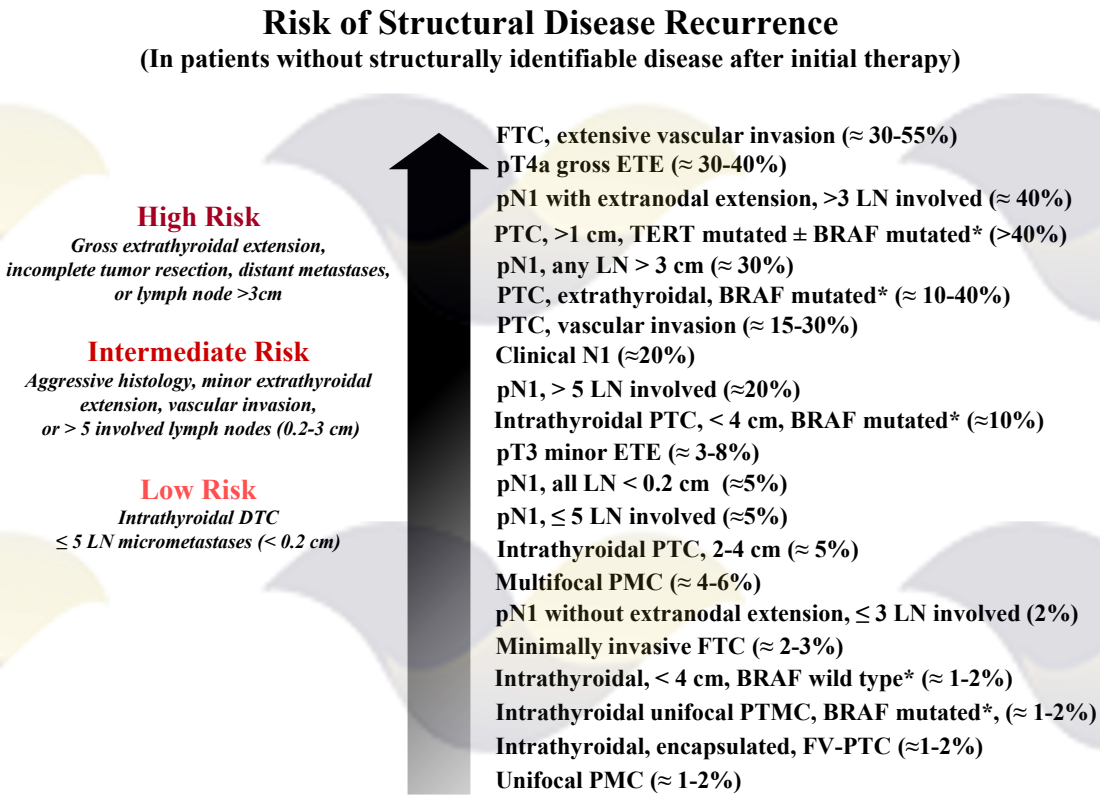


Yip L, et al. *Cancer.* 2021. doi: 10.1002/cncr.33421.

Molecular Markers for Cancer Risk Stratification



Genetic Profile		5-year risk of distant metastasis
BRAF+TERT, RAS+TERT *Multiple driver mutations (eg. NRAS and PIK3CA or TP53)* *TERT*		*High-risk profile (20-35%)*
ALK fusions *NTRK1 fusions* *NTRK3 fusions* *BRAF V600E* *RET/PTC*	BRAF V600E-like mutations	*Intermediate-risk profile (5-10%)*
RAS *BRAF K601E* *PAX8/PPARG*	RAS-like mutations	*Low-risk profile (<1%)*

Yip L et al. *Cancer* (2021)

Haugen BR et al. *Thyroid*. 2016, 26:1-133

Does this work in FNA samples?

Endocrine Pathology
https://doi.org/10.1007/s12022-020-09641-2

Check for updates

Correlation of ThyroSeq Results with Surgical Histopathology in Cytologically Indeterminate Thyroid Nodules

Patrick D. Chin[1] · Catherine Y. Zhu[1] · Dipti P. Sajed[2] · Gregory A. Fishbein[2] · Michael W. Yeh[1] · Angela M. Leung[3,4] · Masha J. Livhits[1]

- **Design:**
  - 78 patients with Bethesda III/IV nodules, positive TSv2/TSv3 result and surgical outcome
- **Findings:**
  - TERT/TP53 and BRAF-like ThyroSeq mutations were associated with increased cancer probability and risk of recurrence defined by histopathologic features
  - RAS-like mutations were associated with lower cancer probability and indolent disease

Molecular Alteration (No.)	Cancer Type (No.)	Histopathology				ATA Risk Stratification
		ETE	LVI	LN	DM	
***TERT/TP53* Combination Mutation Group**						
TERT + NRAS	Poorly differentiated CA					intermediate
TERT + HRAS	Follicular carcinoma					low
TERT + BRAF V600E + PI3CA	PTC - classic	G	EV	>5		high
TP53 + EIF1AX	PTC - follicular					low
***BRAF*- like Group**						
BRAF V600E (6)	PTC - follicular					low
	PTC - tall cell (2)	G				high
						intermediate
	PTC - classic (3)	M	L			intermediate
				≤5		low
						low
RET/CCDC6 fusion	PTC - classic			≤5		low
BRAF/AGK fusion	PTC - classic					low
***RAS*- like Group**						
NRAS (9)	Follicular carcinoma					low
	PTC - follicular (2)					low
						low
	PTC - classic (6)			≤5		low
						low
						low
						low
						low
						low
HRAS (3)	PTC - classic					low
	PTC – follicular (2)					low
						low
KRAS	PTC - classic					low
EIF1AX	Follicular carcinoma					low
NRAS + EIF1AX	PTC - follicular					low
PPARG/PAX8 fusion	Follicular carcinoma		EV			low
Other						
Gene Expression Alt (2)	PTC - classic					low
	Follicular carcinoma		FV			low
Copy Number Alt	PTC - classic			>5		intermediate
TERT promoter	Poorly differentiated CA		FV			intermediate

Does this work in FNA samples?

Endocrine Pathology
https://doi.org/10.1007/s12022-020-09641-2

Check for updates

Correlation of ThyroSeq Results with Surgical Histopathology in Cytologically Indeterminate Thyroid Nodules

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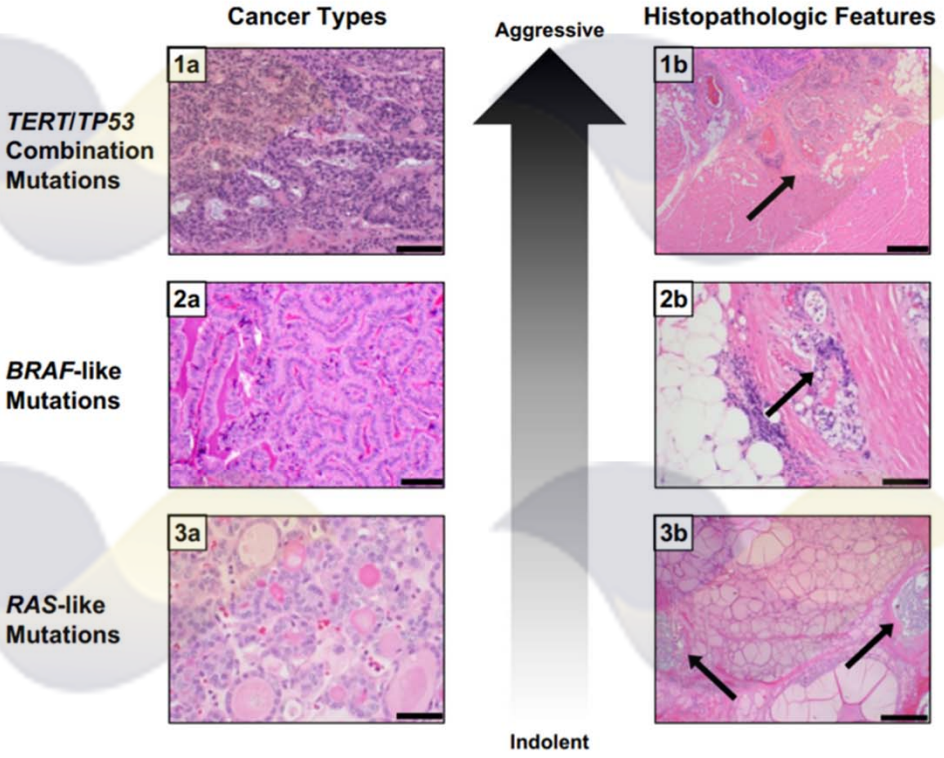
- **Conclusions:**
  - *Individualized management, including extent of surgery, should be considered based on specific genetic alterations found in cytologically indeterminate thyroid nodules*



Chin PD et al. *Endocr Pathol.* 2020 Dec;31(4):377-384.

Are high-risk thyroid cancers large and clinically apparent?

MOLECULAR PROFILE AND CLINICAL OUTCOMES IN DIFFERENTIATED THYROID CANCER PATIENTS PRESENTING WITH BONE METASTASIS

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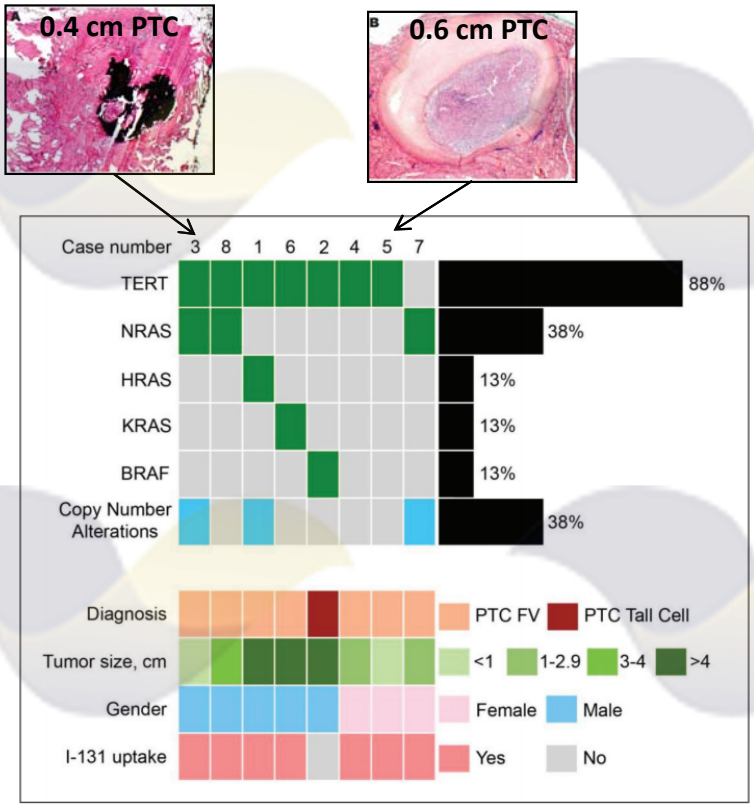
Malik N et al. *Endocr Pract*. 2019

- 8 patients presented with symptomatic bone metastasis from unknown primary
- Bone biopsy – thyroid cancer
- Thyroid surgery: 7 - follicular variant PTC; 1 - tall cell variant PTC
- Primary tumor size 0.4-7.5 cm



Molecular profiles of test-positive nodules

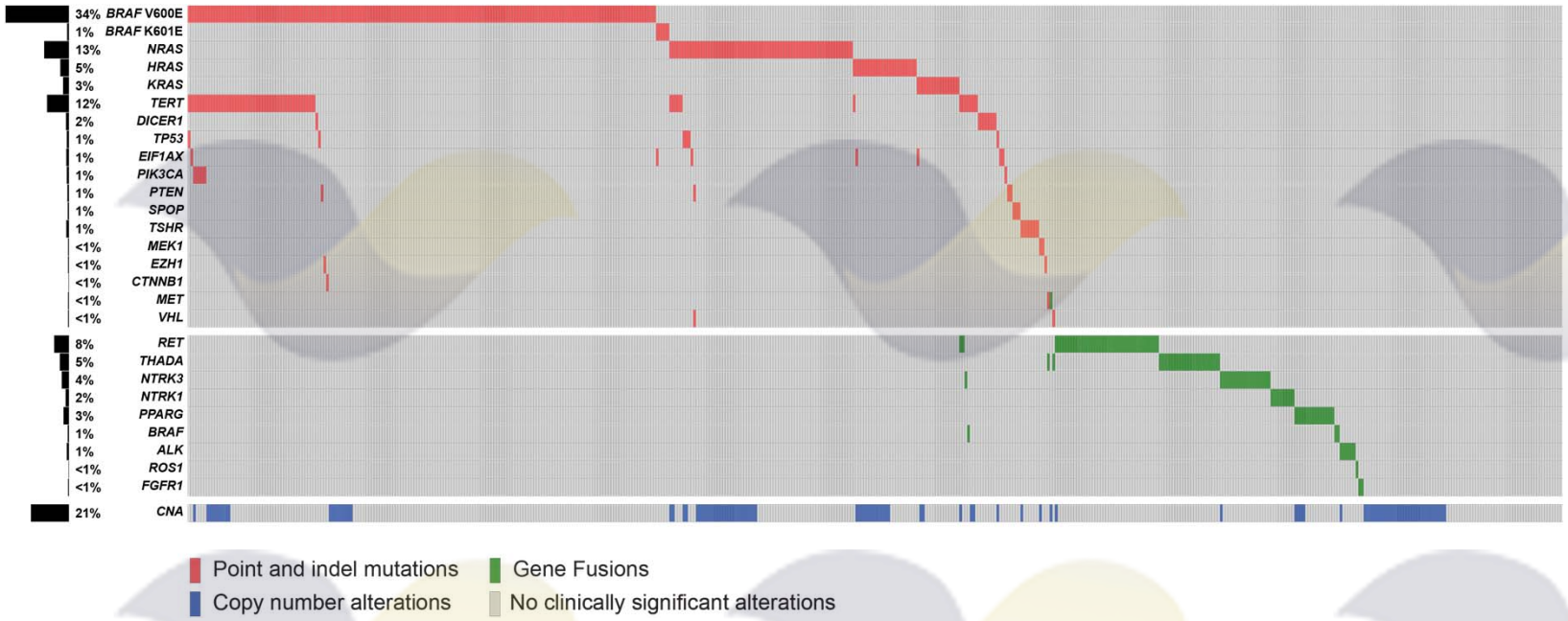
- Prospective double-blind multicenter study
- Bethesda III-V cytology with surgical outcome
- Primary outcome: accuracy of detection of cancer+NIFTP
- 257 nodules
- 68 cancers/NIFTP

Table 3. Probability of Cancer/NIFTP in Specific Molecular Alteration Groups

Group	Molecular Alterations, No.	Prevalence in Test-Positive Samples, No. (%)	Histopathologic Diagnosis, %		Cancer Type/ NIFTP (%)
			Cancer/ NIFTP	Benign	
High-risk group	*TERT* (and *HRAS*) (1) *TP53* (and *MEN1*) (1)	2 (2)	100	0	Papillary carcinoma (50) Follicular carcinoma (50)
BRAF-like group	*BRAF V600E* (9) *NTRK3* fusions (2) *RET* fusions (1) *BRAF* fusions (1)	13 (12)	100	0	Classical papillary carcinoma (92) Follicular variant papillary carcinoma (8)
RAS-like group	*NRAS* (21) *HRAS* (18) *KRAS* (5) *EIF1AX* (5) *BRAF K601E* (3) *PTEN* (1) *IDH2* (1) *DICER1* (1) *PPARG* fusions (4) *THADA* fusions (4)	60 (57)	62	38	Follicular variant papillary carcinoma (22) Papillary carcinoma, other variants (17) NIFTP (15) Follicular carcinoma (3) Hürthle cell carcinoma (5)
Copy number alterations group	Copy number alterations	22 (21)	59	41	Hürthle cell carcinoma (32) Follicular variant papillary carcinoma (14) Papillary carcinoma, other variants (9) NIFTP (5)
Gene expression alterations group	Gene expression alterations	8 (8)	75	25	Classical papillary carcinoma (37) NIFTP (13) Other cancers (MTC, mRCC) (25)

Steward DL et al. JAMA Oncology (2018)

Spectrum of genetic alterations in PTC (n=512)

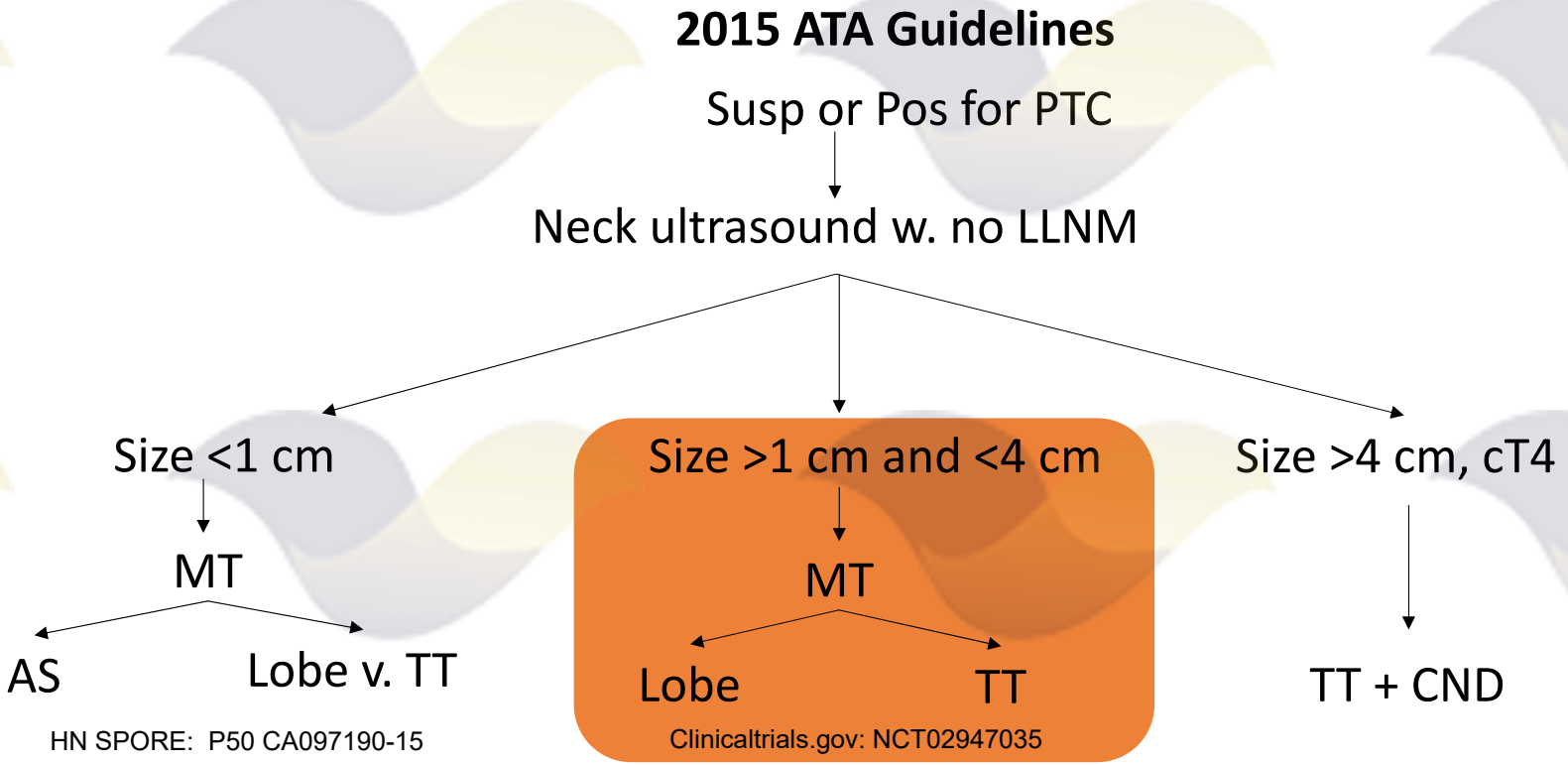


Tumor with TERT as a late event ~10% of PTC

Nikitski A, Condello V, Wald A, Nikiforova M, Chiosea S, Nikiforov Y. ATA Abstract. Thyroid, Volume: 31 Issue S1: 2021

Molecular Markers to Direct Extent of Thyroidectomy

Trial NCT02947035 PI: Lin Yip



Summary

- Abundant retrospective data on molecular markers association with recurrence, distant mets, and survival
- Rapidly increasing number of studies on cancer risk stratification in FNA samples
- Results of prospective trials pending

Thank you!