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

IMPORTANCE *BRAF* V600E and *TERT* promoter mutations can coexist in papillary thyroid cancer (PTC). This genetic duet was indicated to be involved in the aggressiveness of PTC, but its prognostic value in PTC-related mortality remains to be specifically established.

OBJECTIVE To establish the prognostic power of this genetic duet in PTC-specific mortality.

DESIGN, SETTING, AND PARTICIPANTS This genetic-clinical correlation study examined *BRAF* V600E and *TERT* promoter mutations (chr5:1,295,228C>T and chr5:1,295,250C>T) and PTC-specific mortality in 1051 patients (764 women and 287 men) with a median (interquartile range [IQR]) age of 46 (36-57) years, with a median (IQR) follow-up time of 89 (48-142) months (7.4 years).

MAIN OUTCOMES AND MEASURES *BRAF* V600E and *TERT* promoter mutation patterns and associated patient deaths caused by PTC.

RESULTS Papillary thyroid cancer–specific mortality occurred in 4 of 629 patients (0.6%) with neither mutation; 7 of 292 (2.4%) with *BRAF* V600E alone; 4 of 64 (6.3%) with *TERT* promoter mutation alone; and 15 of 66 (22.7%) with the genetic duet; and deaths per 1000-person years in patients harboring neither mutation, *BRAF* V600E alone, *TERT* mutation alone, or both mutations were 0.80 (95% CI, 0.30-2.13), 3.08 (95% CI, 1.47-6.46), 6.62 (95% CI, 2.48-17.64), and 29.86 (95% CI, 18.00-49.52), respectively. Compared with patients harboring neither mutation, HRs (95% CIs) for PTC-specific mortality were 3.08 (0.87-10.84) for *BRAF* V600E alone; 8.18 (2.04-32.75) with *TERT* mutation alone; and 37.77 (12.50-114.09) with both mutations. Papillary thyroid cancer–specific mortality for cases with both mutations remained significant (HR, 9.34; 95% CI, 2.53-34.48) after adjustment for clinicopathological factors, and the genetic duet showed a strong incremental and synergistic impact over either mutation alone. Kaplan-Meier analyses revealed a flat PTC-specific patient survival curve with neither mutation, a modest decline in the curve with either mutation alone, and a sharp decline in the curve with coexisting mutations. Even more robust mortality associations of the genetic duet were seen when only conventional-variant PTC (CPTC) was analyzed (HR, 54.46; 95% CI, 12.26-241.82), which remained strongly significant (HR, 18.56; 95% CI, 2.97-116.18) after adjustment for clinicopathological factors.

CONCLUSIONS AND RELEVANCE These results demonstrate a simple 4-genotype classification of PTC, particularly CPTC, with a disease-specific mortality risk order of the genetic duet>>>>*BRAF* V600E alone = *TERT* promoter mutation alone > wild-type for both genes, representing a powerful molecular prognostic system that can help pinpoint patients with the highest mortality risk.

Invited Commentary
page 209

Supplemental content

Author Affiliations: Laboratory for Cellular and Molecular Thyroid Research, Division of Endocrinology, Diabetes, & Metabolism, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland (Liu, Zhu, Zhang, Ladenson, Xing); Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland (Bishop).

Corresponding Author: Michael Mingzhao Xing, MD, PhD, Professor of Medicine, Oncology and Pathology, Division of Endocrinology, Diabetes, & Metabolism, The Johns Hopkins University School of Medicine, 1830 E Monument St, Ste 333, Baltimore, MD 21287 (mxing1@jhmi.edu).

JAMA Oncol. 2017;3(2):202-208. doi:10.1001/jamaoncol.2016.3288
Published online September 1, 2016.

Papillary thyroid cancer (PTC) is a common endocrine malignancy with a rapidly rising incidence in recent decades.[1,2] This cancer consists of several histological variants, the majority of which is conventional-variant PTC (CPTC). Papillary thyroid cancer generally has an excellent prognosis, but about 5% to 10% of patients have a particularly aggressive disease course, accounting for virtually all PTC-related mortality.[3] The underlying genetic background for the unusually high aggressiveness and mortality of PTC in these patients is unknown. Concerns propelled by the poor clinical outcomes, particularly the high mortality, in this small group of patients commonly drive overtreatment of patients with PTC in general, even though the overall 5-year mortality rate was only 2% to 3% in large general cohorts of patients with thyroid cancer.[1] This creates a major clinical challenge in today's thyroid cancer medicine. The main reason is the lack of a risk stratification system that could accurately and effectively identify the small group of patients with the highest mortality risk for targeted aggressive treatments. The current risk stratification for PTC is solely based on conventional clinicopathological risk factors,[3] which are too common to be risk pinpointing and are therefore insufficiently accurate in identifying the small group of PTC patients with the highest mortality risk.

Molecular-based approaches to risk stratification for thyroid cancer hold considerable promises.[4] In this context, the prognostic value of *BRAF* V600E (the V600E mutation results in an amino acid substitution at position 600, from a valine [V] to a glutamic acid [E]), a prominent oncogene in PTC,[5] has been widely studied.[6-8] Large multicenter studies have demonstrated an association between *BRAF* V600E and PTC recurrence,[9,10] as well as PTC-specific mortality.[11] However, given the high prevalence of *BRAF* V600E, it may not be practical to generally recommend aggressive treatment for *BRAF* mutation-positive PTC. A stratified use of *BRAF* V600E in combination with clinical or other molecular prognostic makers has been proposed to be a potentially effective strategy to improve the risk stratification of PTC,[4,9,11] but this has not been specifically established for mortality risk stratification of PTC.

The recently discovered 2 mutually exclusive *TERT* promoter mutations—chr5:1,295,228C>T and chr5:1 295 250 C>T (termed C228T and C250T, respectively, with the former being far more prevalent)—have been widely reported to be associated with *BRAF* V600E in PTC.[12,13] These mutations confer the *TERT* promoter increased transcriptional activities.[14,15] However, the clinical effect of *BRAF* mutation or *TERT* promoter mutation alone was modest. Interestingly, coexisting *BRAF* V600E and *TERT* promoter mutations were shown to be particularly associated with high-risk clinicopathological characteristics of PTC.[16-18] Coexistence of *BRAF* V600E and *TERT* promoter mutations was also found to be strongly associated with high-risk clinicopathological characteristics of melanoma.[19] These results suggest that the genetic duet of *BRAF* V600E and *TERT* promoter mutations is a unique genetic driving mechanism for the aggressiveness of PTC and, as such, may be prognostically valuable. However, independent prognostic power of this unique genetic duet in PTC-related mortality, the most concerned clinical outcome of this cancer, has not been established. Our initial study[20] published in a previous abstract showed a cooperative effect of the 2 mutations on PTC-related mortality. In the present study, we report the full analysis on the role of coexisting *BRAF* V600E and *TERT* promoter mutations in PTC-related mortality on an extensively expanded large cohort of patients with PTC with a long clinical follow-up time. With the large scale of the study, we were able to particularly investigate the synergistic interactions between the 2 mutations for the first time and establish a robust independent prognostic power of this genetic duet for PTC-related mortality far beyond the influence of either mutation alone and beyond conventional clinicopathological risk factors.

Key Points

Question What is the prognostic value of the genetic duet of coexisting *BRAF* V600E and *TERT* promoter mutations in papillary thyroid cancer (PTC)-related mortality?

Findings In this genetic-clinical correlation study of 1051 patients with PTC, a *BRAF* or *TERT* mutation alone was modestly associated with mortality, while the genetic duet strongly associated with mortality.

Meaning The genetic duet of coexisting *BRAF* and *TERT* promoter mutations identifies robustly the highest mortality risk for patients with PTC and represents a molecular prognostic profile for this disease.

Methods

Patients and Clinicopathological Data

The study included 1051 consecutive cases of PTC patients (764 women and 287 men), with a median (interquartile range [IQR]) age of 46 (36-57) years, who were treated and clinically followed for PTC at the Johns Hopkins Hospital, between 1990 and 2015, with an overall median (IQR) clinical follow-up time of 89 (48-142) months after the initial treatment. The study was approved by our institutional review board and informed patient consent was obtained where required. Patient demographic data in various genetic settings is shown in eTable 1 in the Supplement. All patients received total or near-total thyroidectomy. Cervical lymph node dissection and radioiodine therapy were pursued as clinically determined as previously described.[10,16] Iodine-131 doses used in various genetic groups of patients are summarized in eTable 1 in the Supplement. Clinicopathological data were collected from patient medical records. Pathological diagnoses, including the variant classification of PTC, were established following the World Health Organization criteria[21] as documented previously[10,16] and confirmed in the present study by an expert thyroid cancer pathologist (J.B.). The American Joint Committee on Cancer staging system was used to define the disease stages of PTC. Patient follow-up time was defined as the time from initial thyroidectomy to the most recent clinical contact or, in the case of deceased patients, the time of patient death. Papillary thyroid cancer–specific mortality was defined as patient death directly caused by incurable invasive and/or metastatic PTC. Genetic analyses, which were performed after the surgical and radioiodine treatments, had no influence on therapeutic decision-making.

Table 1. Cooperative Impacts of *BRAF* V600E and *TERT* Promoter Mutations on PTC-Related Mortality

Types of PTC and Death	Overall	No Mutation	*BRAF* Mutation Only	*P* Value	*TERT* Mutation Only[a]	*P* Value	*BRAF* and *TERT* Mutations	*P* Value
All PTC								
Total cases, No.	1051	629	292		64		66	
PTC Deaths, No. (%)	30 (2.9)	4 (0.6)	7 (2.4)	.04	4 (6.3)[b]	.004	15 (22.7)	<.001
Deaths from other causes, No. (%)	30 (2.9)	16 (2.5)	8 (2.7)	.86	4 (6.3)	.10	2 (3.0)	.69
Deaths of all causes, No. (%)	60 (5.7)	20 (3.2)	15 (5.1)	.15	8 (12.5)	<.001	17 (25.8)	<.001
CPTC								
Total cases, No.	793	442	256		41		54	
PTC Deaths, No. (%)	22 (2.8)	2 (0.5)	6 (2.3)	.057	1 (2.4)	.23	13 (24.1)	<.001
Deaths from other causes, No. (%)	19 (2.4)	11 (2.5)	4 (1.6)	.59	2 (4.9)	.30	2 (3.7)	.64
Deaths of all causes, No. (%)	41 (5.2)	13 (2.9)	10 (3.9)	.51	3 (7.3)	.15	15 (27.8)	<.001

Abbreviations: CPTC, conventional PTC; PTC, papillary thyroid cancer.

[a] *TERT* promoter mutations included collectively both C228T and C250T.

[b] The 4 cases here included 1 CPTC, 1 follicular-variant PTC, 1 tall-cell PTC, and 1 columnar PTC.

Table 2. Synergy Test of Interactions Between *BRAF* V600E and *TERT* Promoter Mutations in PTC-Related Mortality

TERT Mutation	PTC Type	Synergy Index (95% CI)[a]	*P* Value
C228T and C250T	All PTC	3.67 (1.25-10.78)	.02
	CPTC	7.83 (1.73-35.34)	.01
C228T only	All PTC	3.50 (1.14-10.74)	.03
	CPTC	8.68 (1.67-45.07)	.01

Abbreviations: CPTC, conventional PTC; PTC, papillary thyroid cancer.

[a] A Synergy Index other than 1 is evidence of significant interaction; a Synergy Index greater than 1 is evidence of synergism; and a Synergy Index less than 1 is evidence of antagonism.

Mutational Analyses

Genomic DNA was isolated from primary PTC by standard procedures involving phenol-chloroform extraction and ethanol precipitation, as described previously.[10,13] For *BRAF* V600E, the polymerase chain reaction (PCR) primers and conditions described previously[10] were used to amplify exon 15 of the *BRAF* gene containing the mutation hot spot. For *TERT* promoter mutations, our previously established PCR primers and conditions were used to amplify the *TERT* promoter containing the 2 mutation hot spots—chr5:1,295,228C>T and chr5: 1 295 250 C>T (termed C228T and C250T, respectively).[13] The PCR products were subjected to Big Dye (Applied Biosystems) reaction for Sanger sequencing and mutations were recognized on sequencing electropherograms.

Statistical Analyses

To analyze the association between genetic variants and clinical parameters, we summarized continuous data using medians and IQRs and categorical data using frequencies and percentages. Categorical variables were compared using χ^2 tests or, in the case of small sample sizes, Fisher exact tests. The Wilcoxon-Mann-Whitney test was used for non-normally distributed continuous variables. Cox regression analyses were used to examine independent associations of mutations with patient mortality. Patient survival curves by mutation status were examined by Kaplan-Meier analyses with log-rank tests and Cox proportional hazards regression analyses, censoring patients at the time of patient death or, in the case of no death, at the time of last clinical contact. The HRs were used to show the magnitude of the effect of the mutations on mortality and 95% CIs were used to indicate the significance of the risk.[22] The synergy index was calculated to examine the additive interactions between *BRAF* V600E with *TERT* promoter mutations in affecting the mortality following the method of Hosmer.[23] All *P* values were 2-sided and *P* less than .05 was considered significant. Analyses were performed using Stata/SE version 12 (Stata Corp) and GraphPad Prism version 6 (GraphPad Software Inc).

Results

Synergistic Effects of *BRAF* V600E and *TERT* Promoter Mutations on PTC-Specific Mortality

BRAF V600E and *TERT* promoter mutations were significantly associated with each other whether the analysis was performed on all PTC or only CPTC (eTable 2 in the Supplement), with their coexistence being seen in 66 of 1051 (6.3%) of all cases of PTC and 54 of 793 (6.8%) of all cases of CPTC. The overall PTC-specific mortality was 30 of 1051 patients (2.9%), consistent with the general report.[1] Dividing the whole PTC cohort into 4 genotype groups revealed mortalities of 4 of 629 patients (0.6%) harboring neither mutation; 7 of 292 (2.4%) harboring *BRAF* V600E alone; 4 of 64 (6.3%) harboring *TERT* mutation alone; and 15 of 66 (22.7%) harboring both mutations (**Table 1**). The 2 mutations, either alone or in coexistence, had no effect on patient mortalities of other (nondisease-specific) causes, although they had effects on mortalities of all causes (including PTC) (Table 1).

Synergism analysis of *BRAF* and *TERT* promoter mutations revealed synergy indices of 3.67 (95% CI, 1.25-10.78; *P* = .02) in all PTC and 7.83 (95% CI, 1.73-35.34; *P* = .01) in CPTC (**Table 2**) that were both well above 1.0, thus demonstrating strong synergistic interactions between the 2 oncogenes in affecting the PTC-specific mortality.

Table 3. Papillary Thyroid Cancer–Specific Deaths per 1000 Person-Years and Hazard Ratios in Various Genetic Groups

Tumor Type and Mutation Status	Deaths per 1000 Person-Years (95% CI)	Unadjusted		Adjustment 1[a]		Adjustment 2[b]	
		Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value
All PTC							
No mutation	0.80 (0.30-2.13)	1 [Reference]		1 [Reference]		1 [Reference]	
BRAF mutation only	3.08 (1.47-6.46)	3.08 (0.87-10.84)	.08	1.18 (0.25-5.54)	.84	1.16 (0.24-5.55)	.85
TERT mutation only[c]	6.62 (2.48-17.64)	8.18 (2.04-32.75)	.003	5.73 (0.96-34.25)	.056	6.00 (0.73-49.48)	.096
BRAF and *TERT* mutations	29.86 (18.00-49.52)	37.77 (12.50-114.09)	<.001	9.34 (2.53-34.48)	.001	8.71 (2.34-32.46)	.001
CPTC							
No mutation	0.57 (0.14-2.27)	1 [Reference]		1 [Reference]		1 [Reference]	
BRAF mutation only	3.05 (1.37-6.79)	4.17 (0.82-21.29)	.09	2.01 (0.23-17.38)	.53	1.90 (0.26-13.91)	.53
TERT mutation only[c]	2.40 (0.34-17.01)	4.32 (0.39-47.68)	.23	209.80 (0.01-6209035)	.31	NA	
BRAF and *TERT* mutations	30.78 (17.87-53.00)	54.46 (12.26-241.82)	<.001	18.56 (2.97-116.18)	.002	18.08 (2.92-112.10)	.002

Abbreviations: CPTC, conventional PTC; NA, not available; PTC, papillary thyroid cancer.

[a] Adjusted for patient age and sex, tumor size, tumor multifocality, extrathyroidal invasion, vascular invasion, and cervical lymph node metastasis.

[b] Adjusted for patient age and sex, tumor size, tumor multifocality, extrathyroidal invasion, vascular invasion, cervical lymph node metastasis, and radioiodine treatment.

[c] *TERT* promoter mutations included collectively both C228T and C250T.

Deaths per 1000-person years in patients harboring neither mutation, *BRAF* V600E alone, *TERT* mutation alone, or both mutations were 0.80 (95% CI, 0.30-2.13), 3.08 (95% CI, 1.47-6.46), 6.62 (95% CI, 2.48-17.64), and 29.86 (95% CI, 18.00-49.52), respectively (**Table 3**). Compared with patients harboring neither mutation, HRs for PTC-specific mortality were 3.08 (95% CI, 0.87-10.84) with *BRAF* V600E alone, 8.18 (95% CI, 2.04-32.75) with *TERT* mutation alone, and 37.77 (95% CI, 12.50-114.09) with both mutations. After adjustment for patient age and sex, tumor size, tumor multifocality, extrathyroidal invasion, vascular invasion, and cervical lymph node metastasis, these HRs became 1.18 (95% CI, 0.25-5.54), 5.73 (95% CI, 0.96-34.25), and 9.34 (95% CI, 2.53-34.48), respectively. Thus, while each mutation alone had a modest effect, coexistence of the 2 mutations exhibited a robustly synergistic effect on PTC-specific mortality that remained strongly significant after multivariate adjustment. This effect remained even after additional adjustment for radioiodine treatment (Table 3).

All the above genetic-clinical correlations in the analyses of all PTC were also observed when only CPTC was analyzed (Tables 1, 2, and 3). In fact, the synergistic effect of the 2 mutations was even more robust in CPTC, which is the most common PTC variant; while the HR was not significant in patients harboring the *BRAF* V600E or *TERT* mutation alone, it was robustly significant (HR, 54.46; 95% CI, 12.26-241.82) with the mutation duet and remained strongly significant (HR, 18.56; 95% CI, 2.97-116.18) after adjustment for all the conventional clinicopathological risk factors (Table 3).

Incremental Effects of Coexisting *BRAF* V600E and *TERT* Promoter Mutations Over Either Mutation Alone on PTC-Specific Mortality

There was no difference in PTC-specific mortality between patients harboring *BRAF* V600E alone or *TERT* mutation alone ($P = .11$) (**Table 4**). There was a much higher mortality in patients harboring both mutations than *BRAF* V600E alone (HR, 14.01; 95% CI, 5.08-38.64; $P < .001$), or *TERT* mutation alone (HR, 4.83; 95% CI, 1.59-14.62, $P = .01$), demonstrating a strong incremental effect of the 2 mutations over either alone. Similar results were obtained when only CPTC patients were analyzed (Table 4).

On Kaplan-Meier analyses of 4 groups divided from the entire PTC cohort (**Figure**, A), the disease-specific survival curve of patients harboring neither mutation stayed flat, and a *BRAF* V600E mutation alone or *TERT* mutation alone was associated with a modest decline in the survival curve. In striking contrast, the mutation duet was associated with a sharp decline in the survival curve. Similar results were obtained when only CPTC cases were analyzed (Figure, B). Thus, the data demonstrated a robust synergistic effect of the genetic duet of *BRAF* V600E and *TERT* promoter mutations but only a modest effect of either mutation alone on PTC-related mortalities. The data are overall consistent with existence of 4 genotypes in PTC, particularly CPTC, with a PTC-specific mortality risk order of coexisting mutations>>>>*BRAF* V600E alone = *TERT* promoter mutation alone > the wild-type for both genes.

BRAF V600E and *TERT* promoter mutations, either alone or in coexistence, did not have any effect on nondisease-specific patient survival, either in the analysis of all patients with PTC (eFigure 1A-C in the Supplement) or patients with CPTC (eFigure 1D-F in the Supplement). The 2 mutations were associated with a decline in all-cause survival both in all patients with PTC (eFigure 2A-C in the Supplement) and patients with CPTC (eFigure 2D-F in the Supplement), with coexisting mutations again displaying a sharp effect. When only *TERT* C228T was examined, whether alone or in coexistence with *BRAF* V600E and whether on all PTC or CPTC, similar results as above on the collective *TERT* C228T and *TERT* C250T were observed, including a similar robust synergistic effect with *BRAF* V600E (Table 2) (eTable 3, eTable 4, and eFigure 3 in the Supplement).

Table 4. Incremental Effect of Coexisting *BRAF* V600E and *TERT* Promoter Mutations Over Either Mutation Alone on PTC-Related Patient Mortality

Tumor Type and Comparison	HR (95% CI)	*P* Value
All PTC		
TERT[a] mutation only vs *BRAF* V600E only	2.95 (0.79-10.98)	.11
BRAF and *TERT* mutations vs *BRAF* V600E only	14.01 (5.08-38.64)	<.001
BRAF and *TERT* mutations vs *TERT* mutation only	4.83 (1.59-14.62)	.01
CPTC		
TERT mutation only vs *BRAF* V600E only	1.15 (0.13-10.28)	.90
BRAF and *TERT* mutations vs *BRAF* V600E only	14.97 (4.88-45.98)	<.001
BRAF and *TERT* mutations vs *TERT* mutation only	13.68 (1.78-105.18)	.01

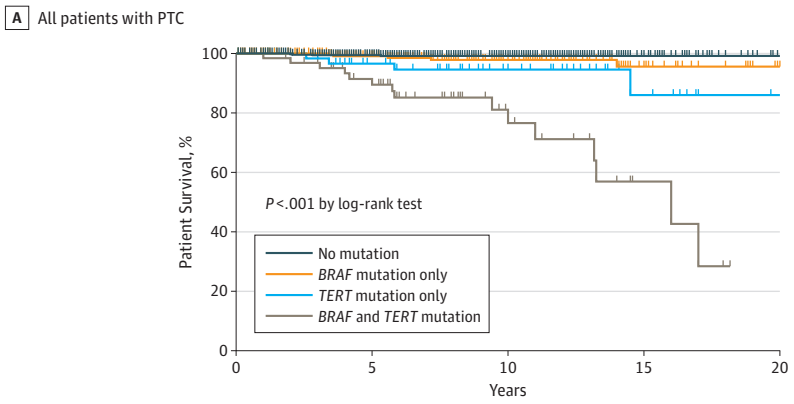
Abbreviations: CPTC, conventional PTC; HR, hazard ratio; PTC, papillary thyroid cancer.

[a] *TERT* promoter mutations included both C228T and C250T collectively.

Discussion

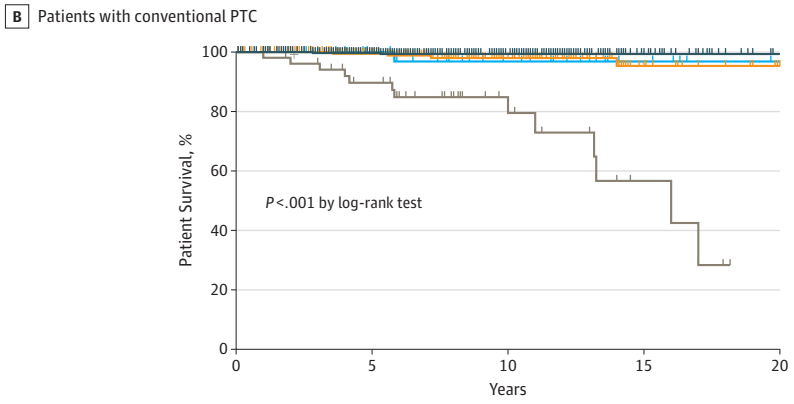
The association between *BRAF* V600E and *TERT* promoter mutations in PTC has been widely confirmed[12,16,17,24-29] since its initial report[13] and was demonstrated again in the present study. This association of the 2 mutations has been also reported in melanoma in several studies.[14,19,30] From an evolutionary perspective, such a consistently close relationship of 2 prominent oncogenes in cancers has strong functional implications—it likely confers oncogenic and tumor survival advantages. Indeed, this unique genetic duet was found to be associated with high-risk clinicopathological characteristics and recurrence of PTC.[12,16-18] It was also found to be associated with aggressive clinicopathological characteristics of melanoma.[19] Our initial results,[20] previously reported in an abstract, showed a cooperative role of *BRAF* V600E and *TERT* promoter mutations in PTC-related mortality. This finding was confirmed in a recent Korean study,[18] which, for certain study limita-

Figure. Effects of *BRAF* V600E and *TERT* Promoter Mutations on Disease-Specific Survival of Patients With PTC



No. at risk

No mutation	629	415	225	66	17
BRAF mutation only	292	188	98	29	8
TERT mutation only	64	50	27	10	2
BRAF and *TERT* mutation	66	48	18	4	0



No. at risk

No mutation	442	288	155	50	15
BRAF mutation only	256	165	82	22	8
TERT mutation only	41	33	19	8	2
BRAF and *TERT* mutation	54	39	16	4	0

Kaplan-Meier curves show (A) the results of the analyses of patients with papillary thyroid cancer (PTC) of all types, and (B) the analyses of patients with conventional-variant PTC only. The patients were divided into 4 genotype groups—no mutation (dark blue line), *BRAF* V600E only (orange line), *TERT* promoter mutation only (bright blue line), and coexistence of the 2 mutations (brown line). *TERT* promoter mutations here included collectively *TERT* C228T and *TERT* C250T. The curves are truncated at 20 years of follow-up.

tions (eg, relatively small cohort), could not address the important prognostic properties of this genetic duet in affecting PTC-specific mortalities. These include the synergistic interactions between the 2 oncogenes, their power independent of conventional clinicopathological risk factors, their role within conventional PTC variant (the most common PTC variant), and the role of individual *TERT* C228T (the most common *TERT* promoter mutation). Two other studies[24,26] also examined coexisting *BRAF* V600E and *TERT* promoter mutations in PTC-related mortality but did not reveal a significant effect, likely owing to the small number of cases studied. The present study on an extensively expanded cohort of PTC patients with a long-term clinical follow-up took a major step forward by addressing these issues and firmly established a critical prognostic role of this genetic duet in PTC-related mortality. This study, for the first time demonstrated a robustly synergistic and independent role of coexisting *BRAF* V600E and *TERT* promoter mutations in PTC-related mortality far beyond the influence of either mutation alone and conventional clinicopathological risk factors. The prevalence of this genetic duet at 6% to 8% corresponds numerically surprisingly well to the classically known 5% to 10% of PTC patients that account virtually for all PTC-related mortality.[3] This prompted our hypothesis that coexisting *BRAF V600E* and *TERT* promoter mutations constituted a genetic background driving the highest mortality risk for PTC, which was proven here to be true.

As an example of the robustness of the prognostic role of the genetic duet, our data showed that *BRAF* V600E and *TERT* promoter mutation each alone had a similarly modest effect while coexisting mutations exhibited a strongly synergistic effect on PTC-related mortality with a high HR of 37.77 in all PTC and 54.46 in CPTC. Remarkably, virtually no death occurred in patients harboring neither mutation while, in contrast, PTC-related deaths virtually exclusively occurred with the coexisting *BRAF* V600E and *TERT* promoter mutations, suggesting that this genetic duet is a primary genetic mechanism for PTC-related mortality. The effect of this mutation duet remained strongly significant after multivariate adjustment for all the conventional clinicopathological characteristics, demonstrating its strong independent and incremental role in PTC-related mortality. The mutations had no effect on nondisease-specific mortality, confirming the specificity of their role in PTC-related mortality. These results are consistent with a genotype classification of PTC patients into 4 groups with a mortality risk order of the genetic duet of coexisting mutations>>>>*BRAF* mutation alone = *TERT* mutation alone > the wild-type for both genes. This is particularly evident for CPTC, which, unlike the follicular variant of PTC, harbored virtually no *RAS* mutations.[31,32] As recently proposed,[12] coexisting *RAS* and *TERT* promoter mutations may also confer increased aggressiveness in thyroid cancer. The genetic duet of *BRAF* and *TERT* promoter mutations represents the first molecular marker system that most effectively identifies the small group of patients with PTC who have the highest mortality risk. Overtreatment of PTC is currently a major challenge widely encountered owing to the concern of mortality risk, albeit generally low, and the lack of a prognostic system that could accurately pinpoint the patients with the highest mortality risk. Prognostic use of this genetic duet of *BRAF* V600E and *TERT* promoter mutations will most likely be helpful in improving the mortality risk stratification of PTC.

The MAPK pathway plays a fundamental role in PTC tumorigenesis.[32] The present study firmly established a robust link of this pathway through *BRAF* V600E, with TERT at a patient mortality level. It was proposed that the MAPK pathway could promote the expression of *TERT* through up-regulating the ETS factors that bind the consensus binding site in DNA created by the *TERT* promoter mutations.[14,15] Indeed, coexistence of *BRAF* V600E and *TERT* promoter mutations was shown to be associated with increased expression of *TERT* in thyroid cancer.[28] Recent studies have demonstrated a robust role of TERT in tumor growth and aggressiveness of several cancers in animal models.[33-35] This provides a molecular mechanism explaining the strong synergism between *BRAF* V600E and *TERT* promoter mutations in promoting the mortality of PTC. Because *BRAF* V600E and *TERT* promoter mutations are among the most common and prominent oncogenes in human cancers[36-38] and can coexist in other human cancers such as melanoma, our results have general prognostic implications for other human cancers.

The patients with coexisting *BRAF* V600E and *TERT* promoter mutations likely received more aggressive treatments because of their more aggressive initial disease (eTable 1 in the Supplement). Because such treatments generally improve clinical outcomes of patients with aggressive thyroid cancer,[3] they likely caused an underestimate of the effect of the genetic duet on PTC-related mortality in the present study. Also, it would be ideal to have a multicenter study to consolidate the findings from the present study even though it was a large patient cohort.

Conclusions

This large study demonstrates for the first time a strong independent association of coexisting *BRAF* V600E and *TERT* promoter mutations with PTC-specific mortality, establishing a robust role of this oncogenic genetic duet in driving PTC-specific mortality far beyond the influence of either mutation alone and the conventional clinicopathological risk factors. A mortality risk stratification of PTC based on the 4 genotype system identified here has a strong potential to improve the clinical management of PTC by helping pinpoint the small group of PTC patients that have the highest mortality risk. Particularly fitting the concept of precision medicine, testing for this genetic duet can help optimize individualized treatment of patients with PTC. The data also have strong implications for the development of novel therapeutic strategies targeting *BRAF* V600E and *TERT* in PTC harboring both *BRAF* V600E and *TERT* promoter mutations. The results may similarly have general prognostic and therapeutic implications for other human cancers harboring both mutations.

ARTICLE INFORMATION

Accepted for Publication: June 29, 2016.

Published Online: September 1, 2016. doi:10.1001/jamaoncol.2016.3288

Author Contributions: Dr Xing had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data. Drs Liu and Bishop are equal first authors.
Concept and design: Xing.
Acquisition, analysis, or interpretation of data: All Authors.
Drafting of the manuscript: Xing.
Critical revision of the manuscript for important intellectual content: All Authors.
Statistical analysis: Liu, Xing.
Obtaining funding: Xing.
Administrative, technical, or material support: Bishop, Ladenson, Xing.
Study supervision: Xing.

Conflict of Interest Disclosures: Dr Xing reported receiving royalties as coholder of a licensed USA patent related to *BRAF* V600E mutation in thyroid cancer. No other disclosures are reported.

Funding/Support: This project was supported by National Institutes of Health (NIH) (grants R01CA113507 and R01CA189224 to Dr Xing).

Role of the Funder/Sponsor: The NIH had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Disclaimer: The content of this article is solely the responsibility of the authors and does not necessarily reflect the official views of the NIH.

Previous Presentation: This article was presented at the 17th International Congress of Endocrinology in collaboration with the 15th Annual Meeting of the Chinese Society of Endocrinology; September 1, 2016; Beijing, China.

REFERENCES

1. Howlader N, Noone AM, Krapcho M, et al, eds. SEER Cancer Statistics Review, 1975-2013, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2013/, based on November 2015 SEER data submission, posted to the SEER web site, April 2016. Accessed on May 21, 2016.

2. Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. *J Cancer Epidemiol*. 2013;2013:965212.

3. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association Guidelines Task Force on thyroid nodules and differentiated thyroid cancer. *Thyroid*. 2016;26(1):1-133.

4. Xing M, Haugen BR, Schlumberger M. Progress in molecular-based management of differentiated thyroid cancer. *Lancet*. 2013;381(9871):1058-1069.

5. Xing M. BRAF mutation in thyroid cancer. *Endocr Relat Cancer*. 2005;12(2):245-262.

6. Tufano RP, Teixeira GV, Bishop J, Carson KA, Xing M. BRAF mutation in papillary thyroid cancer and its value in tailoring initial treatment: a systematic review and meta-analysis. *Medicine (Baltimore)*. 2012;91(5):274-286.

7. Xing M. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. *Endocr Rev*. 2007;28(7):742-762.

8. Xing M. Prognostic utility of BRAF mutation in papillary thyroid cancer. *Mol Cell Endocrinol*. 2010;321(1):86-93.

9. Xing M, Alzahrani AS, Carson KA, et al. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. *J Clin Oncol*. 2015;33(1):42-50.

10. Xing M, Westra WH, Tufano RP, et al. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *J Clin Endocrinol Metab*. 2005;90(12):6373-6379.

11. Xing M, Alzahrani AS, Carson KA, et al. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. *JAMA*. 2013;309(14):1493-1501.

12. Liu R, Xing M. TERT promoter mutations in thyroid cancer. *Endocr Relat Cancer*. 2016;23(3):R143-R155.

13. Liu X, Bishop J, Shan Y, et al. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. *Endocr Relat Cancer*. 2013;20(4):603-610.

14. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. *Science*. 2013;339(6122):959-961.

15. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. *Science*. 2013;339(6122):957-959.

16. Xing M, Liu R, Liu X, et al. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. *J Clin Oncol*. 2014;32(25):2718-2726.

17. Jin L, Chen E, Dong S, et al. BRAF and TERT promoter mutations in the aggressiveness of papillary thyroid carcinoma: a study of 653 patients. *Oncotarget*. 2016;7(14):18346-18355.

18. Song YS, Lim JA, Choi H, et al. Prognostic effects of TERT promoter mutations are enhanced by coexistence with BRAF or RAS mutations and strengthen the risk prediction by the ATA or TNM staging system in differentiated thyroid cancer patients. *Cancer*. 2016;122(9):1370-1379.

19. Macerola E, Loggini B, Giannini R, et al. Coexistence of TERT promoter and BRAF mutations in cutaneous melanoma is associated with more clinicopathological features of aggressiveness. *Virchows Arch*. 2015;467(2):177-184.

20. Xing M, Liu R, Bishop J. TERT promoter and BRAF mutations cooperatively promote papillary thyroid cancer-related mortality. *Thyroid*. 2014;24(S1):A-131.

21. DeLellis R, Lloyd R, Heitz P, Eng C, eds. *WHO Classification of Tumours—Pathology and Genetics of Tumours of Endocrine Organs*. Lyon, France: IARC Press; 2004.

22. Spruance SL, Reid JE, Grace M, Samore M. Hazard ratio in clinical trials. *Antimicrob Agents Chemother*. 2004;48(8):2787-2792.

23. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. *Epidemiology*. 1992;3(5):452-456.

24. Gandolfi G, Ragazzi M, Frasoldati A, Piana S, Ciarrocchi A, Sancisi V. TERT promoter mutations are associated with distant metastases in papillary thyroid carcinoma. *Eur J Endocrinol*. 2015;172(4):403-413.

25. Liu X, Qu S, Liu R, et al. TERT promoter mutations and their association with BRAF V600E mutation and aggressive clinicopathological characteristics of thyroid cancer. *J Clin Endocrinol Metab*. 2014;99(6):E1130-E1136.

26. Melo M, da Rocha AG, Vinagre J, et al. TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. *J Clin Endocrinol Metab*. 2014;99(5):E754-E765.

27. Shi X, Liu R, Qu S, et al. Association of TERT promoter mutation 1,295,228 C>T with BRAF V600E mutation, older patient age, and distant metastasis in anaplastic thyroid cancer. *J Clin Endocrinol Metab*. 2015;100(4):E632-E637.

28. Vinagre J, Almeida A, Pópulo H, et al. Frequency of TERT promoter mutations in human cancers. *Nat Commun*. 2013;4:2185.

29. Crescenzi A, Trimboli P, Modica DC, et al. Preoperative Assessment of TERT Promoter Mutation on Thyroid Core Needle Biopsies Supports Diagnosis of Malignancy and Addresses Surgical Strategy. *Horm Metab Res*. 2016;48(3):157-162.

30. Griewank KG, Murali R, Puig-Butille JA, et al. TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma. *J Natl Cancer Inst*. 2014;106(9):dju246. doi:10.1093/jnci/dju246.

31. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nat Rev Endocrinol*. 2011;7(10):569-580.

32. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. *Nat Rev Cancer*. 2013;13(3):184-199.

33. Ghosh A, Saginc G, Leow SC, et al. Telomerase directly regulates NF-κB-dependent transcription. *Nat Cell Biol*. 2012;14(12):1270-1281.

34. Koh CM, Khattar E, Leow SC, et al. Telomerase regulates MYC-driven oncogenesis independent of its reverse transcriptase activity. *J Clin Invest*. 2015;125(5):2109-2122.

35. Park YJ, Kim EK, Moon S, Hong DP, Bae JY, Kim J. Human telomerase reverse transcriptase is a promising target for cancer inhibition in squamous cell carcinomas. *Anticancer Res*. 2014;34(11):6389-6395.

36. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417(6892):949-954.

37. Fredriksson NJ, Ny L, Nilsson JA, Larsson E. Systematic analysis of noncoding somatic mutations and gene expression alterations across 14 tumor types. *Nat Genet*. 2014;46(12):1258-1263.

38. Killela PJ, Reitman ZJ, Jiao Y, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci U S A*. 2013;110(15):6021-6026.