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ORIGINAL REPORT

Patient Age–Associated Mortality Risk Is Differentiated by *BRAF* V600E Status in Papillary Thyroid Cancer

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ABSTRACT

Purpose
For the past 65 years, patient age at diagnosis has been widely used as a major mortality risk factor in the risk stratification of papillary thyroid cancer (PTC), but whether this is generally applicable, particularly in patients with different *BRAF* genetic backgrounds, is unclear. The current study was designed to test whether patient age at diagnosis is a major mortality risk factor.

Patients and Methods
We conducted a comparative study of the relationship between patient age at diagnosis and PTC-specific mortality with respect to *BRAF* status in 2,638 patients (623 men and 2,015 women) with a median age of 46 years (interquartile range, 35 to 58 years) at diagnosis and a median follow-up time of 58 months (interquartile range, 26 to 107 months). Eleven medical centers from six countries participated in this study.

Results
There was a linear association between patient age and mortality in patients with *BRAF* V600E mutation, but not in patients with wild-type *BRAF*, in whom the mortality rate remained low and flat with increasing age. Kaplan-Meier survival curves rapidly declined with increasing age in patients with *BRAF* V600E mutation but did not decline in patients with wild-type *BRAF*, even beyond age 75 years. The association between mortality and age in patients with *BRAF* V600E was independent of clinicopathologic risk factors. Similar results were observed when only patients with the conventional variant of PTC were analyzed.

Conclusion
The long-observed age-associated mortality risk in PTC is dependent on *BRAF* status; age is a strong, continuous, and independent mortality risk factor in patients with *BRAF* V600E mutation but not in patients with wild-type *BRAF*. These results question the conventional general use of patient age as a high-risk factor in PTC and call for differentiation between patients with *BRAF* V600E and wild-type *BRAF* when applying age to risk stratification and management of PTC.

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INTRODUCTION

Thyroid cancer is a common endocrine malignancy, and its incidence has rapidly increased in recent decades.[1-4] The most common histologic type is papillary thyroid cancer (PTC), accounting for > 85% of all thyroid malignancies, with conventional PTC (CPTC) being the dominant variant.[5,6] Risk stratification is a critical component of standard management of thyroid cancer and is currently based mainly on clinicopathologic risk factors, among which patient age at diagnosis is a major factor. In 1953, Crile and Hazard[7] described in detail the association between advanced patient age and unfavorable prognosis of thyroid cancer. Since then, numerous studies have confirmed this relationship. Thus, patient age has long been routinely applied as a major risk factor in risk stratification of thyroid cancer, which has profoundly impacted clinical practice in the management of thyroid cancer.[8-10]

The most important prognostic significance of patient age in thyroid cancer is its effect on patient mortality; older patient age is strongly associated with thyroid cancer–specific mortality.[11,12] In fact, thyroid cancer is the only type of cancer for which patient age is a metric for disease staging in the American Joint Committee on Cancer (AJCC) and

ASSOCIATED CONTENT

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several other staging systems, reflecting the unique importance of patient age as a risk factor in thyroid cancer. The age of 45 years has been conventionally treated as a cutoff point demarcating the age-associated risk in thyroid cancer[13]; however, this has been recently changed to 55 years in the revised eighth edition of AJCC.[14] Yet, some studies have suggested that the mortality risk of thyroid cancer continuously increases as patient age increases.[15-18] A recent analysis by Adam et al[19] of 31,802 patients with PTC in the SEER database demonstrated that age was associated with PTC-specific mortality in a continuous linear manner without an age cutoff point. However, critical questions remain unanswered as to why older patient age has such a remarkable adverse effect on PTC-specific mortality and whether age is a risk factor universally applicable to all patients with PTC.

The *BRAF* V600E mutation has been well known to be a main oncogenic driver of PTC, occurring in approximately 45% of patients.[20-22] Many studies have demonstrated an association between *BRAF* V600E and older patient age as well as poor clinical outcomes, including recurrence of PTC[23,24] and PTC-specific mortality.[25,26] Given these data, we hypothesized that *BRAF* V600E might play an important role in the effect of patient age on PTC-specific mortality, and that, in the absence of *BRAF* V600E, patient age might not be a risk factor. We conducted this multicenter study to test this hypothesis.

PATIENTS AND METHODS

Study Medical Centers, Countries, and Patients

With the approval of the institutional review boards of the participating institutions and, where required, informed written patient consent, data from 2,638 patients with PTC on clinicopathologic characteristics and PTC-specific patient death were collected from 11 medical centers in six countries (Appendix Table A1, online only). These patients included 623 men (23.6%) and 2,015 women (76.4%) and had a median age of 46 years (interquartile range, 35 to 58 years) at diagnosis of PTC and a median clinical follow-up time of 58 months (interquartile range, 26 to 107 months) after the initial surgery. *BRAF* genetic testing failed in 20 patients, whereas 1,524 patients had wild-type *BRAF* and 1,094 patients had *BRAF* V600E mutation. Mortality analysis was focused on PTC-specific patient death, as previously defined (ie, death that occurred as a result of incurable PTC disease that invaded and compromised vital organs, causing the patient to die).[25] Patient clinicopathologic characteristics that are well-known risk factors for PTC-specific mortality are listed in Table 1. For a separate analysis of patients with CPTC, a subset of 1,893 patients with CPTC was identified, and exclusion of 14 patients without *BRAF* information left 996 and 883 patients who had wild-type *BRAF* and *BRAF* V600E. All of these patients were consecutively selected and were treated with total or near-total thyroidectomy for PTC; other treatments, such as radioiodine ablation, were pursued as clinically indicated. Histopathologic diagnoses of thyroid cancer were established according to the WHO criteria.[27] *BRAF* V600E mutation in primary PTC was examined and documented as previously described.[23,25] *BRAF* V600E mutation status was determined after surgical and medical treatments in all patients and did not affect decision making regarding treatments.

Statistical Analyses

Spearman correlation coefficient was calculated to evaluate the association between patient age and PTC-specific mortality. Variance inflation factor to test multicollinearity was calculated for each clinicopathologic characteristic in the Cox hazards regression model; all variance inflation factors were low (ie, < 1.58), ensuring that multicollinearity was not a problem in the regression models. Multivariate Cox proportional hazards regression models with restricted cubic splines (RCS) and adaptive splines were used to demonstrate the continuous relationship between patient age and PTC-specific mortality.[19] Hazard ratios (HRs) were natural logarithm-transformed and adjusted for multivariate clinicopathologic characteristics. The RCS model (knot number, 3) was used to estimate the HR and 95% CI of different ages compared with age 45 years. Comparing the statistical fitness of different

Table 1. Clinicopathologic Characteristics of Patients With PTC

Characteristic	All Patients (N = 2,638)*	Patients With Wild-Type *BRAF* (n = 1,524)	Patients With *BRAF* V600E (n = 1,094)
Median age at diagnosis, years (IQR)	46 (35-58)	44 (34-56)	48 (36-59)
Female sex, No. (%)	2,015 (76.4)	1,175 (77.1)	822 (75.1)
Median tumor size, cm (IQR)	1.5 (1.0-2.5)	1.5 (0.9-2.5)	1.6 (1.1-2.5)
Subtype, No. (%)			
CPTC	1,893 (71.8)	996 (65.4)	883 (80.7)
FVPTC	525 (19.9)	413 (27.1)	107 (9.8)
TCPTC	100 (3.8)	26 (1.7)	74 (6.8)
Other	120 (4.5)	89 (5.8)	30 (2.7)
AJCC stage, No./total No. (%)			
I	1,819/2,618 (69.5)	1,138/1,512 (75.3)	667/1,086 (61.4)
II	185/2,618 (7.1)	118/1,512 (7.8)	66/1,086 (6.1)
III	414/2,618 (15.8)	174/1,512 (11.5)	235/1,086 (21.6)
IV	200/2,618 (7.6)	82/1,512 (5.4)	118/1,086 (10.9)
Extrathyroidal extension, No./total No. (%)	668/2,634 (25.4)	274/1,522 (18.0)	387/1,092 (35.4)
Lymph node metastasis, No./total No. (%)	896/2,613 (34.3)	449/1,505 (29.8)	437/1,088 (40.2)
Vascular invasion, No./total No. (%)	158/1,051 (15.0)	83/693 (12.0)	75/358 (20.9)
Distant metastasis, No./total No. (%)	118/2,615 (4.5)	64/1,508 (4.2)	54/1,087 (5.0)
^{131}I treatment, No./total No. (%)	1,984/2,559 (77.5)	1,067/1,481 (72.0)	897/1,058 (84.8)
Median administered activities of ^{131}I, mCi (IQR)	100 (30-100)	78 (0-100)	100 (50-104)
Recurrence, No. (%)	423 (16.0)	183 (12.0)	239 (21.4)
PTC-specific mortality, No. (%)	58 (2.2)	16 (1.0)	42 (3.8)
Median follow-up time, months (IQR)	58 (26-107)	62 (28-118)	51 (24-96)

Abbreviations: AJCC, American Joint Committee on Cancer; CPTC, conventional papillary thyroid cancer; FVPTC, follicular-variant papillary thyroid cancer; IQR, interquartile range; PTC, papillary thyroid cancer; TCPTC, tall-cell papillary thyroid cancer.
*Including 20 patients with no *BRAF* information.

number knots showed that the model with 3 knots had the lowest Akaike information criterion estimate, thus providing the best fit to the data. Adaptive splines are knot-free and do not rely on knot number. Statistical analyses were performed using the mgcv[28] and rms[29] packages in R (version 3.2.4; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Association Between Patient Age and PTC-Specific Mortality in Patients With BRAF *V600E But Not Wild-Type* BRAF

As shown in Figure 1, before the age of 45 years, the mortality rates (percentages of deaths in the cohort) were low in all of the patient groups. After the age of 45 years, mortality rates increased as patient age increased in all patients, and mortality rates increased even more rapidly in patients with *BRAF* V600E mutation. However, in striking contrast, there was no increase in mortality overall in patients with wild-type *BRAF* (Fig 1A). Accumulated mortality rates also increased continuously after age 45 years in all patients and increased even more rapidly and steeply in patients with *BRAF* V600E mutation, whereas there was only a marginal increase in accumulated mortality in patients with wild-type *BRAF* at age 45 to 64 years (Fig 1B). After age 65 years, the mortality rate began to decrease (Fig 1A) and the accumulated mortality rate stayed flat (Fig 1B) in patients with wild-type *BRAF*, whereas both the mortality rate and the accumulated mortality rate continuously and sharply increased as

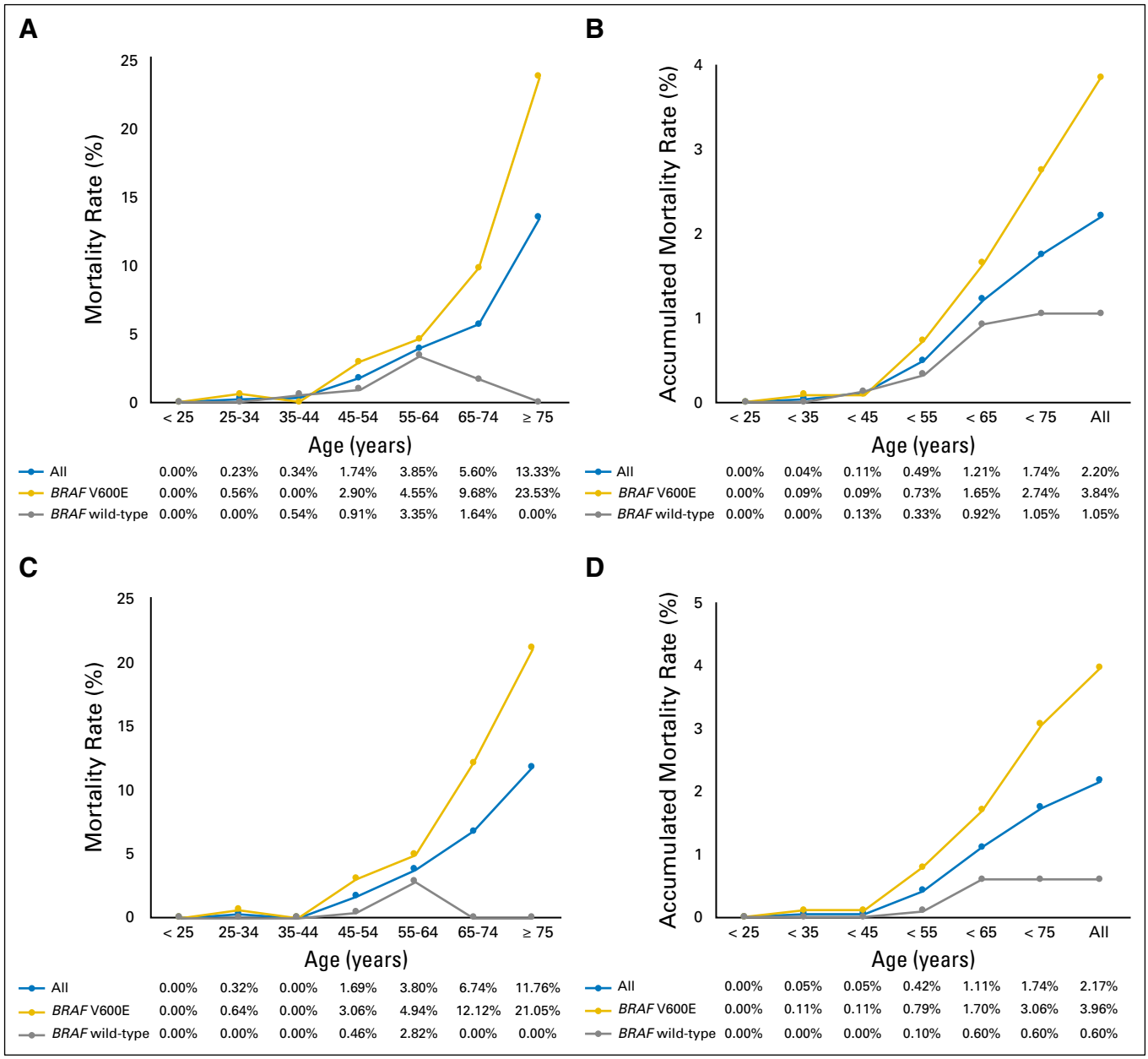


Fig 1. Relationship between patient age and papillary thyroid cancer (PTC)–specific mortality in all patients, patients with *BRAF* V600E mutation, and patients with wild-type *BRAF*. (A) Mortality rates and (B) accumulated mortality rates by patient age in all patients with PTC. (C) Mortality rates and (D) accumulated mortality rates of patients with conventional PTC.

patient age increased in patients with *BRAF* V600E (Figs 1A and 1B). Spearman correlation analysis showed a strongly positive correlation between patient age and mortality rate in *BRAF* V600E patients ($P = .002$, $r = 0.94$), but the correlation was not significant in wild-type *BRAF* patients ($P = .36$, $r = 0.41$). Virtually identical results were obtained when only patients with CPTC were analyzed (Figs 1C and 1D). Spearman correlation analysis also showed a strongly positive correlation between patient age and mortality in patients with CPTC harboring *BRAF* V600E ($P < .002$, $r = 0.94$), but not in patients with CPTC harboring wild-type *BRAF* ($P = .70$, $r = 0.18$). These results suggest that the association between patient age and PTC-specific mortality depends on *BRAF* V600E status.

Rapidly Progressive Decline in Kaplan-Meier Survival Curve With Increasing Age in Patients With BRAF *V600E But Not Wild-Type* BRAF

In the analysis of all patients, Kaplan-Meier survival curves progressively declined as patient age increased, particularly after age 45 years; decline was sharpest in patients ≥ 75 years old (Fig 2A). An even more rapidly progressive decline in survival curve was seen in patients with *BRAF* V600E as patient age increased (Fig 2B). In striking contrast, there was no progressive decline in survival curve in patients with wild-type *BRAF* as patient age increased (Fig 2C). Specifically, in patients with *BRAF* V600E, survival curves in patients younger than 45 years old were largely flat, and only one death occurred in the 25- to 34-year age group at a follow-up time of 300 months. Starting at age 45 years, the older the patients were, the more rapidly the survival curve declined and the most rapid decline occurred in patients ≥ 75 years old (Fig 2B). Similar results were observed when only patients with CPTC were analyzed (Fig 3). These results demonstrate a *BRAF* V600E–dependent association between decreasing PTC-specific patient survival and increasing patient age.

Independent Linear Association Between Mortality Risk and Increasing Age in Patients With BRAF *V600E But Not Wild-Type* BRAF

We used multivariate Cox proportional hazards regression models with RCS to further analyze the relationship between patient age and PTC-specific mortality with adjustment for the classic clinicopathologic characteristics of patient sex, tumor size, extrathyroidal extension, lymph node metastasis, distant metastasis, and administered activities of radioactive iodine (mCi), which are factors known to affect clinical outcomes of patients with PTC, as well as study center (Fig 4). To be comparable, for all RCS plots, patient age of 45 years, which was close to the median age of our cohort, was chosen as the reference for HR calculation. In all patients combined, RCS analysis demonstrated a nearly linear association between patient age and PTC-specific mortality risk, with the adjusted log HR continuously increasing as patient age increased (Fig 4A). In patients with *BRAF* V600E, an even stronger and steeper linear relationship between patient age and adjusted log HR of PTC-specific mortality risk was observed (Fig 4B). In contrast, in patients with wild-type *BRAF*, no significant relationship was observed between patient age and mortality risk; the mortality risk at various age segments generally did not show significant difference, and the line stayed flat as the patient age increased, even after age 75 years (Fig 4C). The increasing line before age 45 years is a result of the large variance from the low mortality rate in this young patient age range, which displayed insignificant HRs in reference to patient age of 45 years.

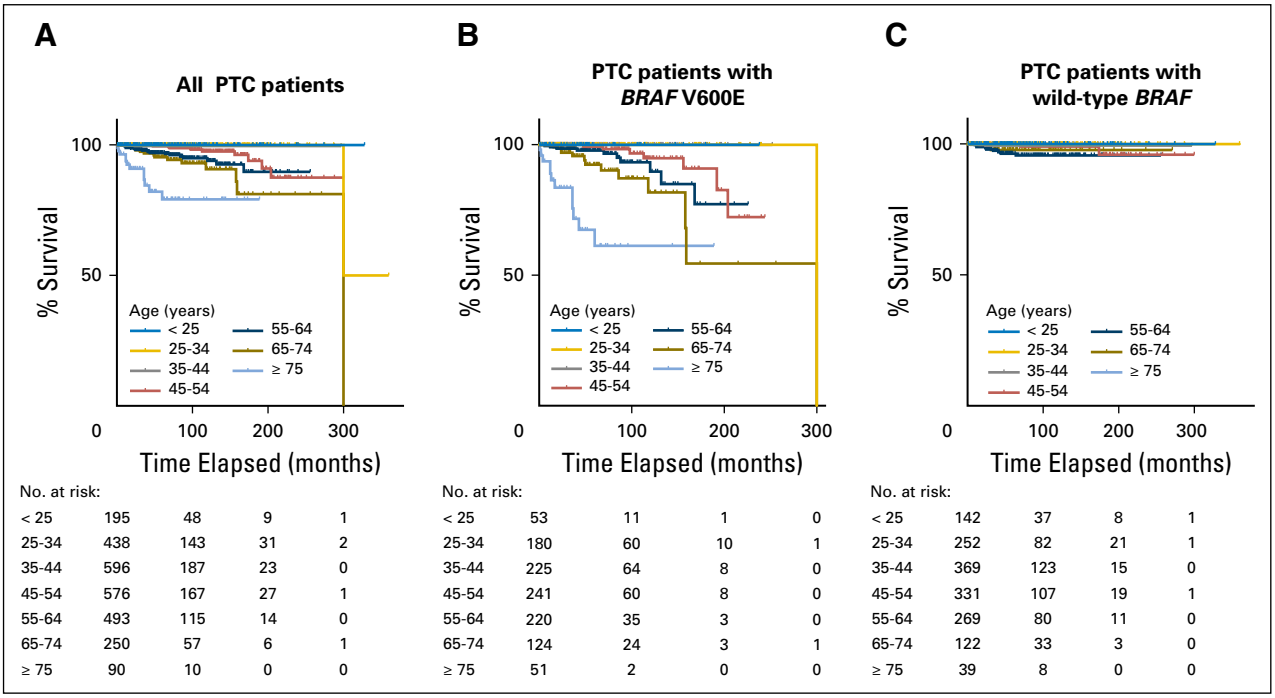


Fig 2. Kaplan-Meier analysis of disease-specific survival curves of patients with papillary thyroid cancer (PTC) in various patient age groups: (A) all patients; (B) patients with *BRAF* V600E mutation; and (C) patients with wild-type *BRAF*.

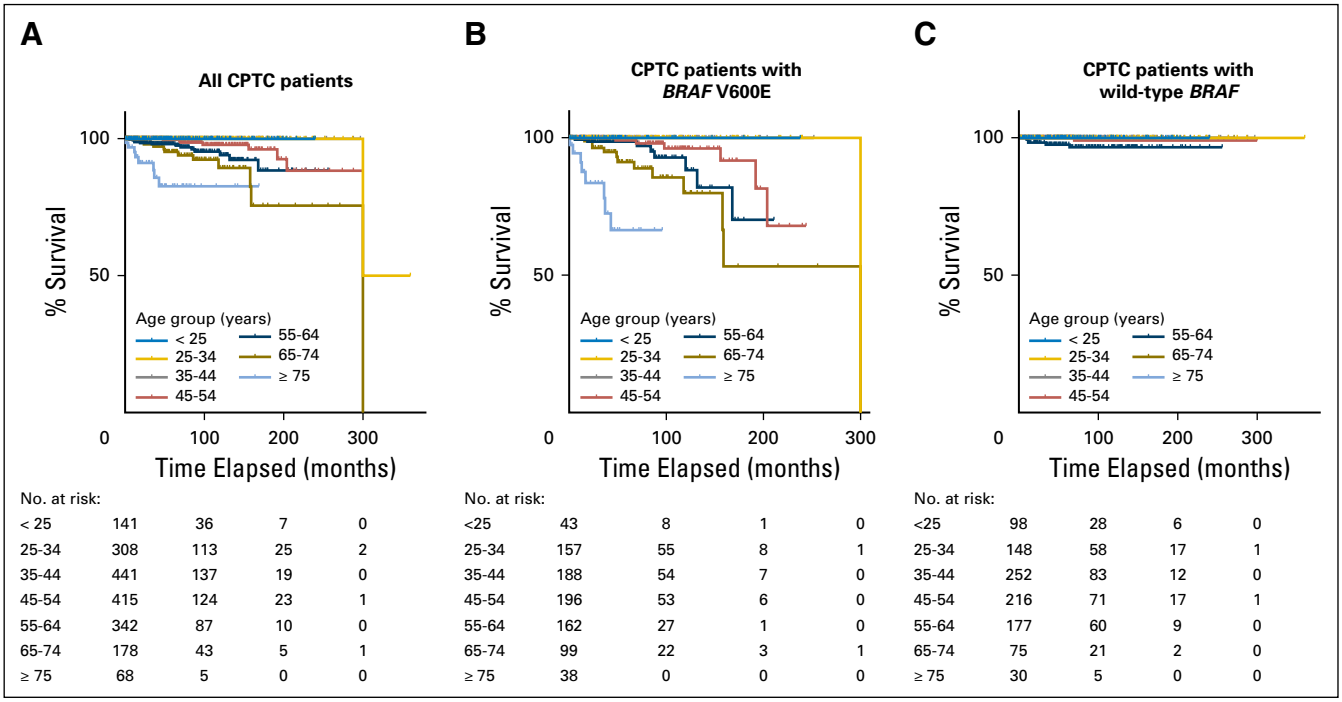


Fig 3. Kaplan-Meier analysis of disease-specific survival curves of patients with conventional papillary thyroid cancer (CPTC) in various patient age groups: (A) all patients; (B) patients with *BRAF* V600E; and (C) patients with wild-type *BRAF*.

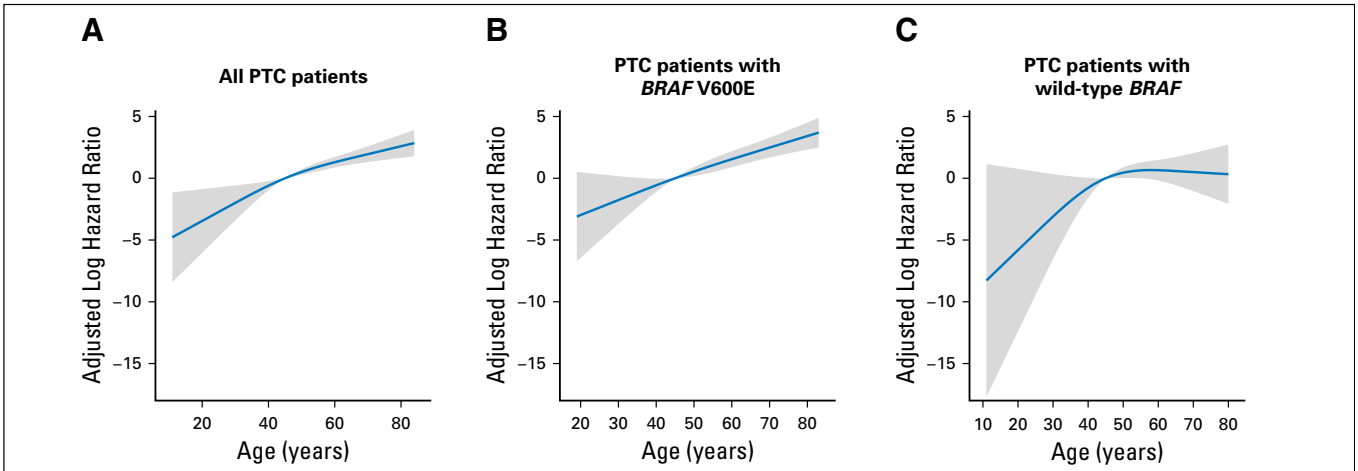
The adjusted specific HRs at different age points are presented in Fig 4D. HRs increased from age 20 to 80 years in the analysis of all patients. An even stronger upward trend in HRs was observed from age 20 to 80 years in patients with *BRAF* V600E, particularly after age 50 years. In contrast, in patients with wild-type *BRAF*, the HR was marginally significant only at age 50 years and was insignificant at all other age points (Fig 4D). Similar results were observed when only patients with CPTC were analyzed using RCS (Appendix Fig A1, online only).

We also used adaptive smoother spline (Fig 5), used previously by Adam et al,[19] to analyze the relationship between patient age and PTC-specific mortality and obtained similar results. Specifically, in analyses of all patients, a near-linear association between patient age and mortality risk was seen (Fig 5A). An even steeper linear association between patient age and mortality risk was seen in patients with *BRAF* V600E (Fig 5B). In contrast, no significant association between patient age and mortality risk was seen in patients with wild-type *BRAF* (Fig 5C). Similar results were obtained when only patients with CPTC were analyzed using the adaptive smoother spline (Appendix Fig A2, online only).

DISCUSSION

Since Crile and Hazard described the association between advanced patient age and aggressiveness of thyroid cancer almost 65 years ago,[7] numerous studies have confirmed this phenomenon. Today, patient age is a well-established mortality risk factor in the prognostication of thyroid cancer; various clinical guidelines and risk assessment models uniformly incorporate patient age as a major risk factor in the management of thyroid cancer.[8-10,30,31] To further support the prognostic importance of patient age, a linear relationship between patient age and PTC-specific mortality was recently demonstrated, suggesting a continuous adverse impact on PTC prognosis as patient age increases.[19] For thyroid cancer, the previous and recent editions of the AJCC staging system heavily emphasize the general risk of patient age.[13,14] Thus, patient age has profoundly influenced the risk stratification and management of PTC. However, it remains to be determined whether patient age is a major risk factor for all patients with PTC.

This study explored the effect of *BRAF* V600E on age-associated mortality risk in patients with PTC. We reproduced the findings of Adam et al[19] by demonstrating a similar linear association between patient age and PTC-specific mortality in the analysis of all patients combined. However, this linear relationship was even steeper in patients with *BRAF* V600E, particularly in patients older than age 45 years. In contrast, this association was lost in patients with wild-type *BRAF*, in whom the PTC-specific mortality risk remained flat with increasing patient age, even after age 45 years. Thus, the long-observed age-associated mortality risk in PTC is *BRAF* V600E dependent; patient age itself, in the absence of *BRAF* V600E, is not a significant risk factor. These findings challenge the conventional belief that older patient age is uniformly a mortality risk factor in PTC and question its universal application in risk stratification of PTC. Instead, the utility of patient age as a prognostic risk factor depends on *BRAF* V600E status. Specifically, in patients with *BRAF* V600E, age has a strong and continuous adverse effect on the prognosis of patients with PTC throughout the entire age spectrum examined, and in fact, the effect intensifies as patient age increases. Thus, in patients with *BRAF* V600E mutation, age is an important factor in risk stratification and management of PTC as conventionally applied. In contrast, in patients with wild-type *BRAF*, age is not a risk



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Patient Group	Hazard Ratio (95% CI) for Age 20-80 Years						
	20 Years	30 Years	40 Years	50 Years	60 Years	70 Years	80 Years
All PTC patients	0.03 (0.002 to 0.42)*	0.14 (0.03 to 0.56)*	0.55 (0.38 to 0.79)*	1.66 (1.32 to 2.09)*	3.66 (2.38 to 5.64)*	7.03 (3.70 to 13.37)*	13.35 (5.20 to 34.25)*
PTC patients with *BRAF* V600E	0.30 (0.03 to 3.50)	0.45 (0.12 to 1.79)	0.73 (0.51 to 1.07)	1.47 (1.15 to 1.88)*	4.03 (2.52 to 6.44)*	12.92 (6.17 to 27.03)*	42.35 (13.69 to 131.06)*
PTC patients with wild-type *BRAF*	0.002 (0 to 2.14)	0.05 (0.001 to 1.40)	0.46 (0.20 to 1.04)	1.56 (1.01 to 2.41)*	1.92 (0.83 to 4.43)	1.64 (0.35 to 7.67)	1.39 (0.12 to 15.45)

Fig 4. Multivariate Cox proportional hazards regression analysis of papillary thyroid cancer (PTC)–specific mortality risk with restricted cubic splines (RCS). Continuous linear association between patient age and PTC-specific mortality was observed (A) in the analysis of all patients and (B) even more significantly in patients with *BRAF* V600E, but (C) not in patients with wild-type *BRAF*. The blue line represents the fitted line of the association between patient age and the estimated hazard ratio (HR) of mortality after adjustment; the shaded region represents the 95% CI. The models were adjusted for the following clinicopathologic characteristics: patient sex, tumor size, extrathyroidal extension, lymph node metastasis, distant metastases, administered activities of radioactive iodine, and study center. The RCS plots were performed with the age of 45 years as the reference for HR calculation. (D) Specific HRs and 95% CIs are presented for the indicated patient age points. (*) Significantly different HRs in reference to patient age of 45 years.

factor for poor prognosis; in these patients, both younger and older patients have a similar PTC-specific mortality risk and may be managed similarly. This new concept will likely have a major impact on the clinical management of PTC because the prevalence of *BRAF* V600E mutation in PTC is, on average, 45%.[20] Thus, the majority of patients with PTC have wild-type *BRAF*, and in these patients, conventional use of patient age as a major risk factor is not valid. As such, many older patients will be able to avoid more aggressive treatment that would otherwise be administered as a result of the conventional concept of older patient

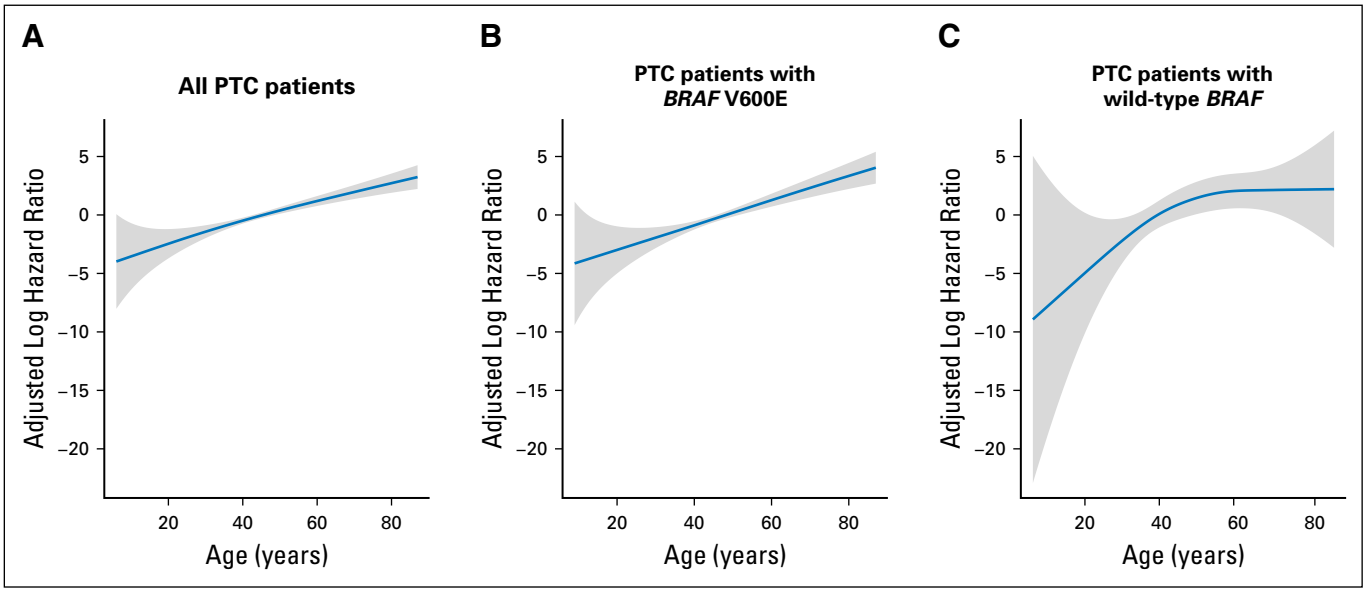

Fig 5. Multivariate Cox proportional hazards regression analysis of papillary thyroid cancer (PTC)–specific mortality risk with adaptive smoother splines in (A) all patients with PTC, (B) patients with *BRAF* V600E mutation, and (C) patients with wild-type *BRAF*. The blue line represents the fitted line of the association between patient age and the estimated hazard ratio of mortality after adjustment; the shaded region represents the 95% CI. The models were adjusted for the following clinicopathologic characteristics: patient sex, tumor size, extrathyroidal extension, lymph node metastasis, distant metastases, administered activities of radioactive iodine, and study center.

age being a general high-risk factor. Our study calls for a *BRAF* genotype–based modification of the conventionally used risk assessment systems,[8-10,30,31] as well as the recently developed quantitative risk assessment nomogram,[32] which all incorporate patient age as a general risk factor for thyroid cancer. In addition, given this differentiating role of *BRAF* V600E status in patient age-related mortality risk of PTC, use of the conventional cutoff age of 45 years[13] or the new cutoff age of 55 years[14] in the risk stratification of PTC is inaccurate. Our study addressed the role of *BRAF* V600E mutation in PTC-specific mortality risk related to patient age at diagnosis. It would be interesting for future studies to investigate the role of the mutation in the dynamic effect, if any, of patient age on the prognosis of PTC as the age of the same patient increases after the diagnosis.

The large multicenter cohort of patients is a major strength of this study and is one of the largest cohorts of patients in *BRAF* mutation–related studies in thyroid cancer. The multicenter nature, however, is inherently associated with the potential limitation of data heterogeneity, as seen in population data such as the SEER data.[19] Nevertheless, our study only looked at the single outcome parameter of PTC-specific patient death, which has a universally straightforward definition, and the binary data of *BRAF* mutation–positive and –negative status from each participating center were similarly included in the analysis. The participating centers are well-known thyroid cancer centers that actively follow contemporary standard practice guidelines in the management of thyroid cancer, minimizing the heterogeneity in the management of thyroid cancer. The fact that the overall analysis of all patients in the current study fully reproduced the findings of the linear effect of patient age on PTC-specific mortality in the study by Adam et al[19] is consistent with the good generalizability of the current study. Another limitation is that *TERT* promoter mutation, which is also a prognostic genetic event in PTC, was not included in this study. However, *TERT* promoter mutations are relatively uncommon and mostly coexist with *BRAF* mutation in PTC.[33,34] Moreover, *TERT* promoter mutation alone has limited or virtually no effect on PTC-specific mortality.[35,36] Therefore, lack of information on *TERT* promoter mutation should not affect the clinical implications of this study on the use of *BRAF* V600E status in differentiating patient age–related mortality risk in PTC.

The molecular mechanism for the *BRAF* mutation–dependent effect of patient age on the prognosis of PTC remains to be defined. It is possible that certain age-associated genes, such as immune response–related genes,[37] may cooperate with mutant *BRAF* in conferring poor prognosis because *BRAF* V600E was shown to be linked to abnormal immune responses in human cancers, including PTC.[38-40] Another potential and more likely mechanism is the coexistence of *BRAF* V600E and *TERT* promoter mutations, which are synergistically associated with poor clinical outcomes in PTC, including disease recurrence and patient mortality.[35,36] Both *BRAF* V600E[20-22] and *TERT* promoter mutations[34] occur in PTC more commonly in older patients. The present results are also consistent with a previous finding that *BRAF* V600E and older patient age had a synergistic effect on PTC-related mortality.[25]

In summary, in contrast to the long-held practice of treating patient age as a general risk factor for PTC, this large multicenter study demonstrates that age is a strong and continuous mortality risk factor only in patients with *BRAF* V600E mutation, and not in the more commonly seen patients with wild-type *BRAF*. These results call for differentiation between patients with wild-type *BRAF* and *BRAF* V600E when applying age to risk stratification and management of patients with PTC. This study has broad clinical implications.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Administrative support: Mingzhao Xing
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Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Patient Age–Associated Mortality Risk Is Differentiated by *BRAF* V600E Status in Papillary Thyroid Cancer

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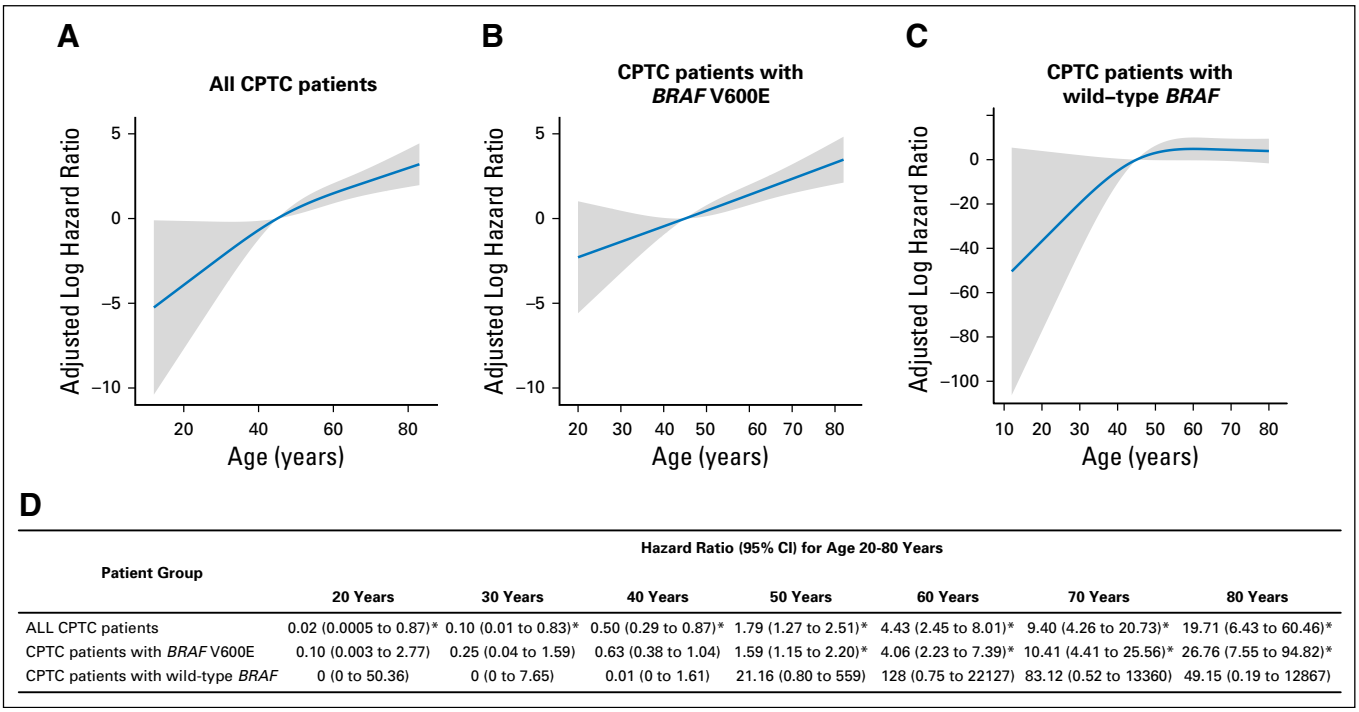
Appendix



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Patient Group	Hazard Ratio (95% CI) for Age 20-80 Years						
	20 Years	30 Years	40 Years	50 Years	60 Years	70 Years	80 Years
ALL CPTC patients	0.02 (0.0005 to 0.87)*	0.10 (0.01 to 0.83)*	0.50 (0.29 to 0.87)*	1.79 (1.27 to 2.51)*	4.43 (2.45 to 8.01)*	9.40 (4.26 to 20.73)*	19.71 (6.43 to 60.46)*
CPTC patients with *BRAF* V600E	0.10 (0.003 to 2.77)	0.25 (0.04 to 1.59)	0.63 (0.38 to 1.04)	1.59 (1.15 to 2.20)*	4.06 (2.23 to 7.39)*	10.41 (4.41 to 25.56)*	26.76 (7.55 to 94.82)*
CPTC patients with wild-type *BRAF*	0 (0 to 50.36)	0 (0 to 7.65)	0.01 (0 to 1.61)	21.16 (0.80 to 559)	128 (0.75 to 22127)	83.12 (0.52 to 13360)	49.15 (0.19 to 12867)

Fig A1. Multivariate Cox proportional hazards regression analysis of mortality risk with restricted cubic splines (RCS) in patients with conventional papillary thyroid cancer (CPTC). (A) A continuous and nearly linear association between patient age and CPTC-specific mortality was observed in all patients. (B) The association was linear and even steeper in patients with *BRAF* V600E mutation. (C) A linear association was not seen in patients with wild-type *BRAF*. The blue line represents the fitted line of the association between patient age and the estimated hazard ratio (HR) of mortality risk after adjustment; the shaded region represents the 95% CI. The models were adjusted for the following clinicopathologic characteristics: patient sex, tumor size, extrathyroidal extension, lymph node metastasis, distant metastases, administered activities of radioactive iodine, and study center. The RCS plots were performed with the age of 45 years as the reference for HR calculation. (D) Specific HRs and 95% CIs were calculated for the indicated age points. (*) Significantly different HRs in reference to patient age of 45 years. Because of the small number of deaths in patients younger than age 45 years, there were large variations in log HRs in patients with CPTC harboring only wild-type *BRAF* in the young age ranges. Consequently, different *y*-axis scales are used for log HR for panels A, B, and C.

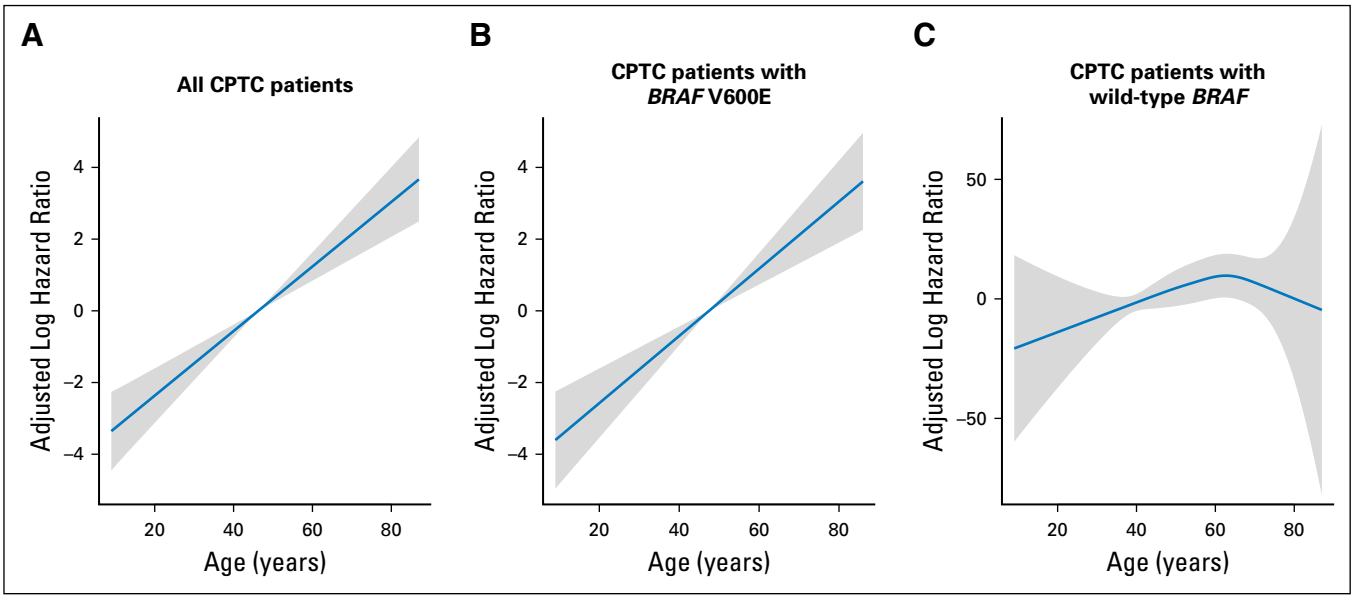

Fig A2. Multivariate Cox proportional hazards regression analysis of conventional papillary thyroid cancer (CPTC)–specific mortality risk with adaptive smoother splines: (A) all CPTC patients; (B) CPTC patients with *BRAF* V600E mutation; and (C) CPTC patients with wild-type *BRAF*. The blue line represents the fitted line of the association between patient age and the estimated hazard ratio (HR) of mortality risk after adjustment; the shaded region represents the 95% CI. The models were adjusted for the following clinicopathologic characteristics: patient sex, tumor size, extrathyroidal extension, lymph node metastasis, distant metastases, administered activities of radioactive iodine, and study center. Because of the small number of deaths in patients younger than age 45 years, there were large variations in log HRs in patients with CPTC harboring only wild-type *BRAF* in the young age ranges. Consequently, different *y*-axis scales are used for log HR for panels A, B, and C.

Table A1. Demographic Characteristics of Patients by Medical Center and Country

Center and Country	No. of Patients	Median (IQR) Age at Diagnosis (years)	No. of Male Patients (%)
By medical center			
Johns Hopkins Hospital (United States)	1,051	46 (36-57)	287 (27.3)
University of Pisa (Italy)	189	38 (28-51)	47 (24.9)
University of Perugia (Italy)	117	49 (37-59)	32 (27.4)
University of Milan (Italy)	265	45 (36-58)	63 (23.8)
Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology (Poland)	253	47 (35-59)	30 (11.9)
Griffith University (Australia)	76	40 (34-56)	20 (26.3)
University of Padua (Italy)	135	48 (39-57)	32 (23.7)
University of Pittsburgh (United States)	169	52 (38-63)	42 (24.9)
Hospital La Paz Health Research Institute, Madrid (Spain)	66	42 (32-54)	11 (16.7)
University of Sydney (Australia)	95	44 (34-59)	20 (21.1)
Institute of Endocrinology, Prague (Czech Republic)	222	47 (31-60)	39 (17.6)
By country			
United States	1,220	47 (37-58)	329 (27.0)
Italy	706	45 (34-56)	174 (24.6)
Poland	253	47 (35-59)	30 (11.9)
Australia	171	43 (34-57)	40 (23.4)
Spain	66	42 (32-54)	11 (16.7)
Czech Republic	222	47 (31-60)	39 (17.6)
Overall	2,638	46 (35-58)	623 (23.6)

Abbreviation: IQR, interquartile range.