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Impact of Somatic Mutations on Survival Outcomes in Patients With Anaplastic Thyroid Carcinoma

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PURPOSE Anaplastic thyroid carcinoma (ATC) uniformly present with aggressive disease, but the mutational landscape of tumors varies. We aimed to determine whether tumor mutations affect survival outcomes in ATC.

MATERIALS AND METHODS Patients who underwent mutation sequencing using targeted gene panels between 2005 and 2019 at a tertiary referral center were included. Associations between mutation status and survival outcomes were assessed using Cox proportional hazards models.

RESULTS A total of 202 patients were included, where 122 died of ATC (60%). The median follow-up was 31 months (interquartile range, 18-45 months). The most common mutations were in *TP53* (59%), *BRAF* (41%), *TERT* promoter (37%), and the *RAS* gene family (22%). Clinicopathologic characteristics and overall survival (OS) significantly correlated with mutations in *BRAF*V600E and *RAS*, which were mutually exclusive. The *BRAF*V600E mutation was associated with the presence of a papillary thyroid carcinoma precursor and significantly better OS (median OS: 24 months). *RAS*-mutated patients more commonly presented without cervical lymph node involvement but had the worst OS (median OS: 6 months). Tumors that were wild-type for both *BRAF* and *RAS* were enriched for *NF1 mutations* and harbored intermediate prognosis (median OS: 15 months). In multivariate analyses, *RAS* mutations were associated with a more than 2.5-fold higher risk of death (adjusted hazard ratio, 2.64; 95% CI, 1.66 to 4.20) compared with *BRAF*V600E. In patients treated with BRAF-directed therapy (n = 60), disease progression occurred in 48% of patients (n = 29). The median progression-free survival was 14 months. The presence of a *TP53* mutation was independently associated with reduced progression-free survival in *BRAF*V600E-mutated patients treated with BRAF-directed therapy (adjusted hazard ratio, 2.89; 95% CI, 1.35 to 6.21).

CONCLUSION Mutation analysis provides prognostic information in ATC and should be incorporated into routine clinical care.

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INTRODUCTION

Anaplastic thyroid carcinoma (ATC) is a rare malignancy with an age-adjusted incidence of 0.11 cases per 100,000 person-years in the United States.[1] It represents < 2% of all thyroid cancers but accounts for up to 50% of thyroid cancer–related mortality.[2] ATC is characterized by advanced disease at presentation and rapid progression within weeks. For decades, the median survival for ATC was < 5 months.[2-4] Multimodal therapy including cytotoxic chemotherapy, radiotherapy, and surgery improves outcomes, but most patients do not survive beyond 1 year.[5] Until recently, tumor mutation status has not been routinely used to inform clinical management in ATC. The US Food and Drug Administration's approval of dabrafenib and trametinib for *BRAF*V600E-mutated ATC in 2018 has shifted the treatment paradigm toward targeted therapy and increased utilization of mutation testing in clinical practice.[6] Although several studies have characterized the mutational landscape of ATC using targeted and whole-exome sequencing, the clinical significance of mutation profiles including associations with clinicopathologic characteristics, treatment outcomes, and survival has not been examined in detail.[7-11] The purpose of this study is to determine whether the tumor mutation profile assessed at diagnosis is associated with clinicopathologic characteristics, overall survival (OS), and progression-free survival (PFS).

MATERIALS AND METHODS

Patients with ATC treated at the University of Texas MD Anderson Cancer Center (Houston, TX) between 2005 and 2019 were identified via retrospective chart

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Recent sequencing efforts have elucidated the genomic landscape of anaplastic thyroid carcinoma (ATC), demonstrating that it is genetically heterogeneous. It remains unknown whether differences in clinical manifestations and prognosis exist among subtypes of ATC determined by tumor mutation status. To our knowledge, this is the largest report assessing the impact of tumor mutation status on ATC clinical outcomes.

Knowledge Generated

ATCs can be divided into three subtypes on the basis of driver mutations in *BRAF* and *RAS*, which are mutually exclusive: (1) *RAS*-mutated, (2) *BRAFV600E*-mutated, and (3) *BRAF* and *RAS* wild-type. *RAS*-driven tumors appear to be the most aggressive and harbor the worst prognosis. *BRAFV600E*-mutated tumors have the best prognosis, largely because of benefits from BRAF-directed therapy. *BRAF* and *RAS* wild-type tumors harbor intermediate prognosis.

Relevance

These findings support inclusion of tumor mutation testing during the clinical workup of ATC and treatment decision making guided by tumor driver mutation status.

review. Approval for the study was obtained from the MD Anderson Institutional Review Board. For this study, a waiver of informed consent was granted by the Institutional Review Board as the study was determined to be minimal risk using existing information derived from patient care. All included patients had mutation testing and confirmation of ATC diagnosis by a head and neck pathologist.

Descriptive statistics were used to summarize demographic and clinicopathologic characteristics of included patients. Survival curves were constructed using the Kaplan-Meier method and compared using the log-rank test. Hazard ratios (HRs) and 95% CIs were calculated through univariate or multivariate Cox proportional hazard regression adjusting for age at diagnosis, surgery, and overall stage.

See the Data Supplement for additional information.

RESULTS

Patient Cohort

A total of 202 consecutive patients with ATC were included in the study. The clinicopathologic characteristics of the included patients are shown in Table 1. The median age at diagnosis was 66 years (range, 32-92 years). A majority of patients presented with distant metastases (stage IVC disease; n = 109, 54%). Locoregionally advanced disease was also prevalent, with 86% (n = 173) of patients presenting with T4 disease and 73% of patients (n = 147) presenting with lateral neck lymph node metastases (N1b). Ninety-six patients (47%) were noted to have a component of another coexisting thyroid carcinoma on histopathology. Papillary thyroid carcinoma (PTC) was the most common coexisting precursor and found in 72 patients. A history of well-differentiated thyroid cancer was reported in 21 patients (10%) where they had clinically documented transformation from PTC to ATC. The median duration of follow-up was 31 months. A majority of patients received multimodality treatment (Table 1). In terms of systemic therapy, 55% of patients received cytotoxic chemotherapy (n = 112), 40% received immunotherapy (n = 80), and 30% received BRAF-directed therapy (n = 60). Detailed treatment modality information is included in the Data Supplement.

Spectrum of Identified Somatic Mutations

One hundred eighty-three patients were profiled using tissue-based assays. The majority had NGS performed on thyroid tissues (n = 133), whereas cervical lymph nodes and distant metastases were used in 39 and 11 patients, respectively. In 19 patients where tumor tissues were not available, testing was performed on blood using the LB70 liquid biopsy assay. Overall, for patients who had tests of both solid tissue biopsy and liquid biopsy (n = 57), we observed an average gene-wise concordance of 96% and an average within-patient concordance of 95%.

In patients who had both liquid-based and tissue-based assays, the mutation profile from tissue-based assay was used for subsequent analyses. Identified somatic mutations are summarized in Figure 1.

No mutations were identified in 21 patients. These patients did not differ in terms of clinicopathologic characteristics from the rest of the cohort. Missense mutations in the mitogen-activated protein kinase (MAPK) pathway genes, namely, *BRAF*, *NRAS*, *KRAS*, and *HRAS*, were prevalent (Fig 1). All identified mutations in *BRAF* were V600E with the exception of a D594N mutation, a G469E mutation, and two in-frame deletions. In the *RAS* gene family, *NRAS* mutations (13%) were the most common followed by *KRAS* (4.4%) and *HRAS* (3.0%). The *BRAF*V600E and *RAS* mutations were mutually exclusive. Three groups of patients emerged on the basis of MAPK driver mutation status: (1) *BRAF*V600E-mutated and *RAS* wild-type, (2) *RAS*-mutated and *BRAF* wild-type, and (3) *BRAF* wild-type and *RAS* wild-type, which each accounted for 40%, 22%, and 38% of patients, respectively.

TABLE 1. Summary of Patient Demographic and Clinicopathologic Characteristics (N = 202)

Patient Characteristics	
Age at diagnosis, years, median (range)	66 (32-92)
Sex, No. (%)	
Male	107 (53.0)
Female	95 (47.0)
Overall stage, No. (%)	
IVA	4 (2.0)
IVB	88 (43.8)
IVC	109 (54.3)
Tumor stage, No. (%)	
T3	13 (6.4)
T4	173 (85.6)
TX	16 (7.9)
Nodal stage, No. (%)	
N0	33 (16.4)
N1a	13 (6.4)
N1b	147 (72.8)
NX	9 (4.5)
Metastasis stage, No. (%)	
M0	93 (46.0)
M1	109 (54.0)
Concomitant histopathology, No. (%)	
PTC	72 (35.6)
Non-PTC	24 (11.9)
None	106 (52.5)
Mutation testing assay, No. (%)	
46	6 (3.0)
50	70 (34.6)
Solid tumor V1	47 (23.3)
Solid tumor 2018	57 (28.2)
MDA-409	3 (1.5)
LB 70	19 (9.4)
Treatment modality, No. (%)	
No treatment	9 (4.4)
Surgery only	12 (5.9)
Radiotherapy only	8 (4.0)
Systemic therapy only[a]	23 (11.4)
Bimodal therapy	87 (43.1)
Trimodal therapy	63 (31.2)
Follow-up, months, median (IQR)	31 (18-45)

Abbreviations: IQR, interquartile range; M, metastasis; N, node; PTC, papillary thyroid carcinoma; T, tumor.

[a]Systemic therapy included any treatment with BRAF/MEK, inhibitors, immunotherapy, or cytotoxic chemotherapy.

Mutations in *TP53* were the most common, identified in 54% of patients (n = 110 of 202). These mutations commonly co-occurred with *BRAF* and *RAS* mutations, 46% (n = 38 of 84) in *BRAF*-mutated and 51% (n = 23 of 45) in *RAS*-mutated. A higher prevalence of *TP53* mutations (64%, n = 49 of 77) was identified in patients who were *BRAF* and *RAS* wild-type. The majority of identified *TP53* mutations were missense (70%). Frameshift (6%), nonsense (17%), and splice site (6%) accounted for the remaining mutations. Multiple *TP53* mutations were identified in 10 patients, where five patients had mutations of different classes. Mutually exclusive *PIK3CA and PTEN* mutations were also identified in a significant proportion of patients, 12% and 7%, respectively. Although *PIK3CA* mutations were all missense, *PTEN* mutations included missense (33%), nonsense (33%), frameshift deletions (20%), and splice site (13%). In patients where the *TERT* promoter, *NF1*, and *NF2* genes were assessed, 37% (46 of 123), 10% (13 of 126), and 8% (9 of 107) harbored mutations, respectively. *TERT* promoter mutations were equally distributed across driver mutation groups in tested patients (38%, 41%, and 33% in *BRAF*-mutated, *RAS*-mutated, and *BRAF* and *RAS* wild-type, respectively). *NF1* mutations were only detected in patients who were *BRAF* wild-type and *RAS* wild-type. Other notable but less frequent mutations were identified in *RB1*, *CDKN2A* (n = 8 of 202, 4%), *ATM* (n = 6 of 202, 3%), and *ARID1A* (n = 4 of 79, 5%).

Clinicopathologic Characteristics and Mutation Status

Driver mutation status was associated with histopathology and disease presentation (Table 2). ATCs with PTC precursors were enriched for the *BRAF*V600E mutation. Of 72 ATCs with coexisting PTC on histopathology, 48 (67%) harbored the *BRAF*V600E mutation. Similarly, the majority of patients (n = 17 of 21, 81%) with clinically documented transformation from PTC to ATC were *BRAF*V600E-mutated. *RAS* mutations were infrequently detected in these cases and found in four tumors with coexisting PTC on histopathology and two tumors with documented PTC to ATC transformation. *BRAF*V600E was less prevalent in ATCs with non-PTC precursors (n = 7, 29%). A higher prevalence of *RAS* mutations was identified in these patients (n = 8, 33%).

In terms of disease presentation, *RAS*-mutated patients more commonly presented with T4b disease and without metastases to neck lymph nodes (N0 disease) compared with patients who were *BRAF*V600E-mutated or *BRAF* and *RAS* wild-type. Although not statistically significant, a higher proportion of M1 disease was also observed in *RAS*-mutated patients (Table 2).

OS and Mutation Status

The total number of deaths within the study was 122. All deaths were due to ATC. Survival rates and estimates by driver mutation status are shown in Figure 2. The median survival time was 24 months in *BRAF*V600E-mutated patients, 15 months in *BRAF* and *RAS* wild-type patients, and 6 months in *RAS*-mutated patients. In multivariate

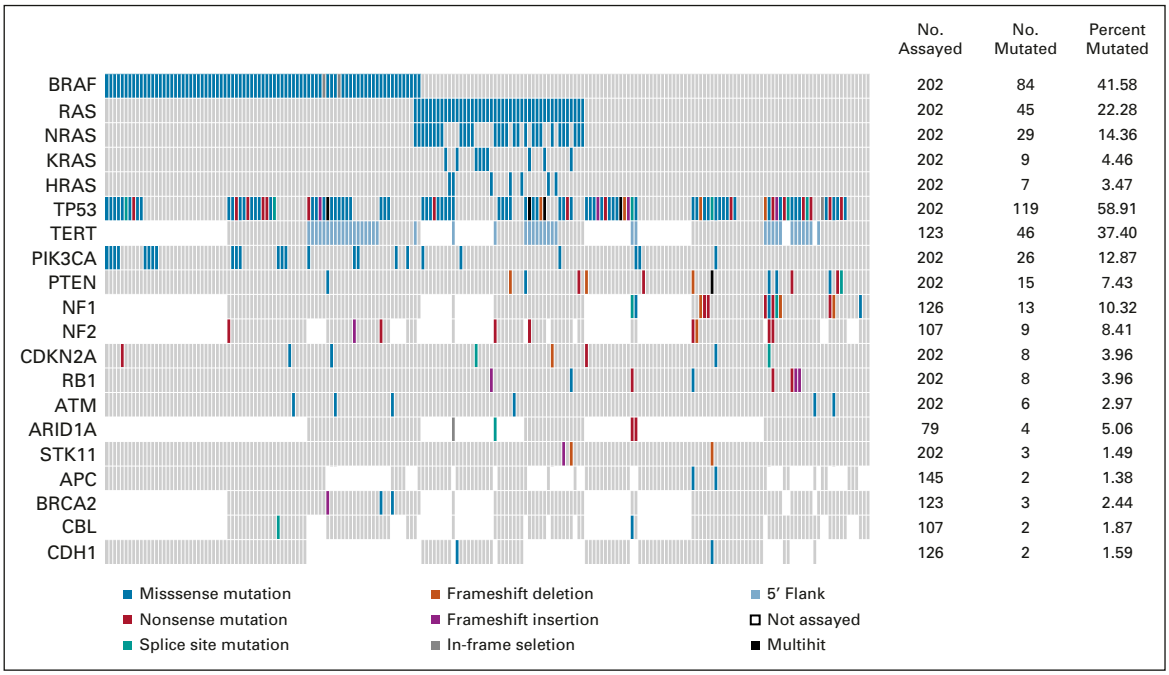

FIG 1. Somatic Mutations in 202 patients with ATC. OncoPrint showing recurrently mutated genes identified within the overall ATC cohort. *RAS* gene family (*NRAS*, *KRAS*, and *HRAS*) mutations are shown together and individually. Of 19 genes that are recurrently mutated, 11 were covered across panels. In the eight genes that were covered in selected panels only, the number assayed indicates the number of patients who were tested with panels covering the specific gene(s). The number and percent mutated indicate the proportion of patients who had mutation(s) detected among those who were assayed. Color key for the types of genetic alterations identified is shown on the bottom legend. ATC, anaplastic thyroid carcinoma.

analyses, after adjustment for age at diagnosis, overall stage, and treatment with surgery, driver mutation status was significantly associated with OS ($P < .001$; Table 3). Compared with *BRAF*V600E-mutated patients, *RAS*-mutated patients had a more than two-and-a-half-fold higher risk of death (adjusted HR, 2.64; 95% CI, 1.66 to 4.20; Table 3). No differences in OS were observed comparing patients with *NRAS*, *KRAS*, and *HRAS* mutations. Although *BRAF* and *RAS* wild-type patients had shorter OS compared with those who were *BRAF*V600E-mutated, this difference was not statistically significant (HR, 1.45; 95%, 0.95 to 2.22) in multivariate analyses (Table 3).

We also evaluated recurrently mutated genes with identified frequencies > 5% in terms of survival outcomes. This included mutations in *TP53*, *TERT* promoter, *PIK3CA*, *PTEN*, and *NF1*. No significant associations were identified for these mutations. Furthermore, panel-derived tumor mutational burden (TMB) did not significantly affect survival outcomes, including in the context of immunotherapy (Data Supplement).

OS in Patients with *BRAF* Wild-Type ATC

Survival outcomes for RAS-mutated and BRAF and RAS wild-type patients by treatment are shown in the Data Supplement. In *BRAF* and *RAS* wild-type patients, surgery was associated with improved survival in multivariate analyses (adjusted HR, 0.49; 95% CI, 0.24 to 0.99; Data Supplement). Although the 13 patients who harbored a *NF1* mutation had marginally better OS compared with wild type, this difference was not significant. Surgery in *RAS*-mutated patients did not significantly alter OS in multivariate analyses (Data Supplement). However, treatment with immune checkpoint blockade in this population trended towards improved survival (adjusted HR, 0.47; 95% CI, 0.22 to 1.01; Data Supplement). Co-occurrence of *RAS* mutations with other mutations did not significantly alter OS.

OS *in Patients With BRAF*V600E-*Mutated ATC*

Of the 80 patients who had *BRAF*V600E-mutated ATC, 60 (75%) received BRAF-directed therapy. All 20 patients who did not receive BRAF-directed therapy were diagnosed before the US Food and Drug Administration approval of dabrafenib/trametinib in April 2018. These patients had poorer survival outcomes similar to those who were *BRAF* wild-type with a median OS of 6 months (Fig 3A). Most patients treated with BRAF-directed therapy received both a BRAF inhibitor and a MEK inhibitor (n = 54 of 60, 90%). Compared with patients who did not receive BRAF-directed therapy, treated *BRAF*V600E-mutated patients had significantly better OS (Fig 3B). After adjusting for age, stage, and surgery, BRAF-directed therapy was associated with a 76% reduction (HR, 0.24; 95% CI, 0.11 to 0.54) in the risk of death from any cause.

TABLE 2. Driver Mutation Status and Clinicopathologic Characteristics

Clinicopathologic Characteristic	No.	*BRAFV600E*-Mutated (n = 80), No. (%)	*RAS*-Mutated (n = 45), No. (%)	*BRAF* and *RAS* Wild-Type (n = 77), No. (%)	*P*[a]
Tumor stage					.017
T3	13	3 (3.8)	3 (6.7)	7 (9.1)	
T4a	110	43 (53.8)	22 (48.9)	45 (58.4)	
T4b	63	21 (26.2)	19 (42.2)	23 (29.9)	
TX	16	13 (16.2)	1 (2.2)	2 (2.6)	
Nodal stage					.009
N0	33	7 (8.8)	12 (26.7)	14 (18.2)	
N1a	13	2 (2.5)	1 (2.2)	10 (13.0)	
N1b	147	67 (83.8)	31 (68.9)	49 (63.6)	
NX	9	4 (5.0)	1 (2.2)	4 (5.2)	
Metastasis stage					.14
M0	92	37 (46.2)	15 (33.3)	40 (52.0)	
M1	110	43 (53.8)	30 (66.7)	37 (48.0)	
Documented transformation					< .001
Yes	21	17 (21.2)	2 (4.4)	2 (2.6)	
No	181	63 (78.8)	43 (95.6)	75 (97.4)	
Concomitant histopathology					< .001
PTC	72	48 (60.0)	4 (8.9)	20 (26.0)	
Non-PTC	24	7 (8.8)	8 (17.8)	9 (11.7)	
None	106	25 (31.2)	33 (73.3)	48 (62.3)	

Abbreviations: M, metastasis; N, node; PTC, papillary thyroid carcinoma; T, tumor.
[a]*P* values for the chi-square test or Fisher's exact test.

OS and PFS in *BRAFV600E*-Mutated Patients Treated With BRAF-Directed Therapy

Forty-eight percent of patients who received BRAF-directed therapy (n = 29 of 60) developed disease progression. The majority progressed in their distant metastatic disease with or without locoregional progression. Distribution of mutations for progressed versus disease-free patients is shown in the Data Supplement. The median OS and PFS in patients who received BRAF-directed therapy were 26 months and 14 months, respectively. The presence of *TP53* mutations was independently associated with both reduced OS and PFS after BRAF-directed treatment (Fig 3C and Data Supplement). This association was further stratified by *TP53* mutation type, where protein-truncating mutations (frameshift and nonsense) were associated with worse OS compared with missense mutations (HR, 2.61; 95% CI, 1.01 to 6.73). *BRAF* allele frequency and co-occurrence with other mutations besides *TP53* did not affect OS or PFS in patients receiving BRAF-directed therapy (Data Supplement).

Surgery significantly improved PFS and OS, whereas radiotherapy did not affect survival outcomes in patients treated with BRAF-directed therapy (Data Supplement). Moreover, 36 patients received both BRAF-directed therapy and immunotherapy. These patients had better PFS and OS compared with those who received BRAF-directed therapy alone (Data Supplement). Multivariable Cox regression analysis also demonstrated a significant benefit in survival outcomes with a combination of BRAF-directed and immune checkpoint inhibitor therapies (Data Supplement), after adjusting for age, stage, and surgery.

DISCUSSION

ATC is one of the most lethal human malignancies. For decades, the rarity of ATC, its short survival times, and the lack of sufficient tumor tissues for molecular studies impeded progress in the field. Recent characterization of the ATC mutational landscape has paved the way for more effective therapies and improved outcomes for patients with ATC, particularly for those with *BRAF*V600E-mutated tumors.[7-11,15] As in differentiated thyroid carcinomas, mutually exclusive mutations in the *BRAF*V600E and *RAS* gene family are the main driver mutations in ATC. In this study, we found that although clinically documented transformation from PTC to ATC occurred in only 10% of patients, evidence of a differentiated thyroid carcinoma precursor was prevalent on histopathology. Confirming prior observations, the *BRAFV600E* mutation was associated with the presence of a PTC precursor and documented

A

Driver Mutation Groups	1-Year OS, % (95% CI)	2-Year OS, % (95% CI)	3-Year OS, % (95% CI)	5-Year OS, % (95% CI)
All patients	54.9 (47.7-62.1)	35.1 (27.8-42.4)	33.4 (26.1-40.7)	25.7 (17.0-34.4)
*BRAF*V600E-mutated	64.3 (53.2-75.3)	47.0 (35.1-59.0)	45.1 (33.0-57.2)	30.1 (14.0-46.1)
BRAF and RAS wild-type	58.4 (46.9-69.8)	33.4 (21.8-45.1)	33.4 (21.8-45.1)	28.7 (15.4-41.9)
RAS-mutated	31.6 (17.0-46.1)	16.7 (4.4-29.1)	11.2 (0-23.3)	11.2 (0-23.3)

B

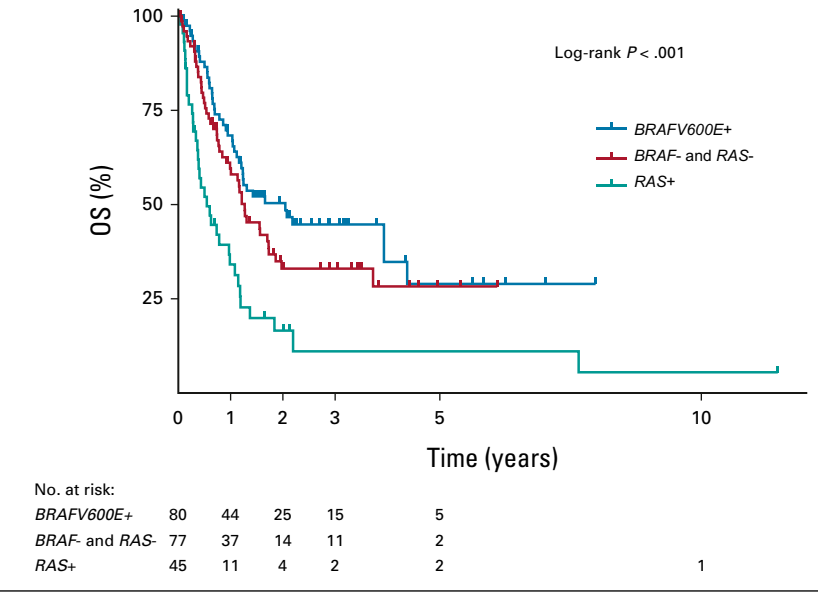

FIG 2. OS of patients with ATC by driver mutation status: (A) OS rates at 1, 2, 3, and 5 years and (B) survival analysis. ATC, anaplastic thyroid carcinoma; OS, overall survival.

transformation from PTC to ATC.[10,16-18] *RAS* mutations, on the other hand, were associated with other differentiated thyroid carcinoma precursors including follicular thyroid carcinoma. In patients where a precursor was not reported, fine-needle aspiration biopsy instead of a core biopsy likely contributed to lower identification rates and underestimation

TABLE 3. Univariate and Multivariate Analyses for Overall Survival in All Patients

	Univariate		Multivariate	
Variable	**HR (95% CI)**	***P***	**HR (95% CI)[a]**	***P***
Overall stage				
IVA/B	1.00		1.00	
IVC	1.78 (1.22-2.58)	.003	1.40 (0.94-2.07)	.10
Surgery				
No	1.00		1.00	
Yes	0.39 (0.27-0.56)	< .001	0.43 (0.29-0.64)	< .001
Driver mutation status		< .001		< .001
BRAFV600E-mutated	1.00		1.00	
BRAF and *RAS* wild-type	1.35 (0.88-2.05)	.17	1.45 (0.95-2.22)	.088
RAS-mutated	2.66 (1.68-4.20)	< .001	2.64 (1.66-4.20)	< .001

Abbreviations: HR, hazard ratio.
[a]Adjusted for age, stage, and surgery

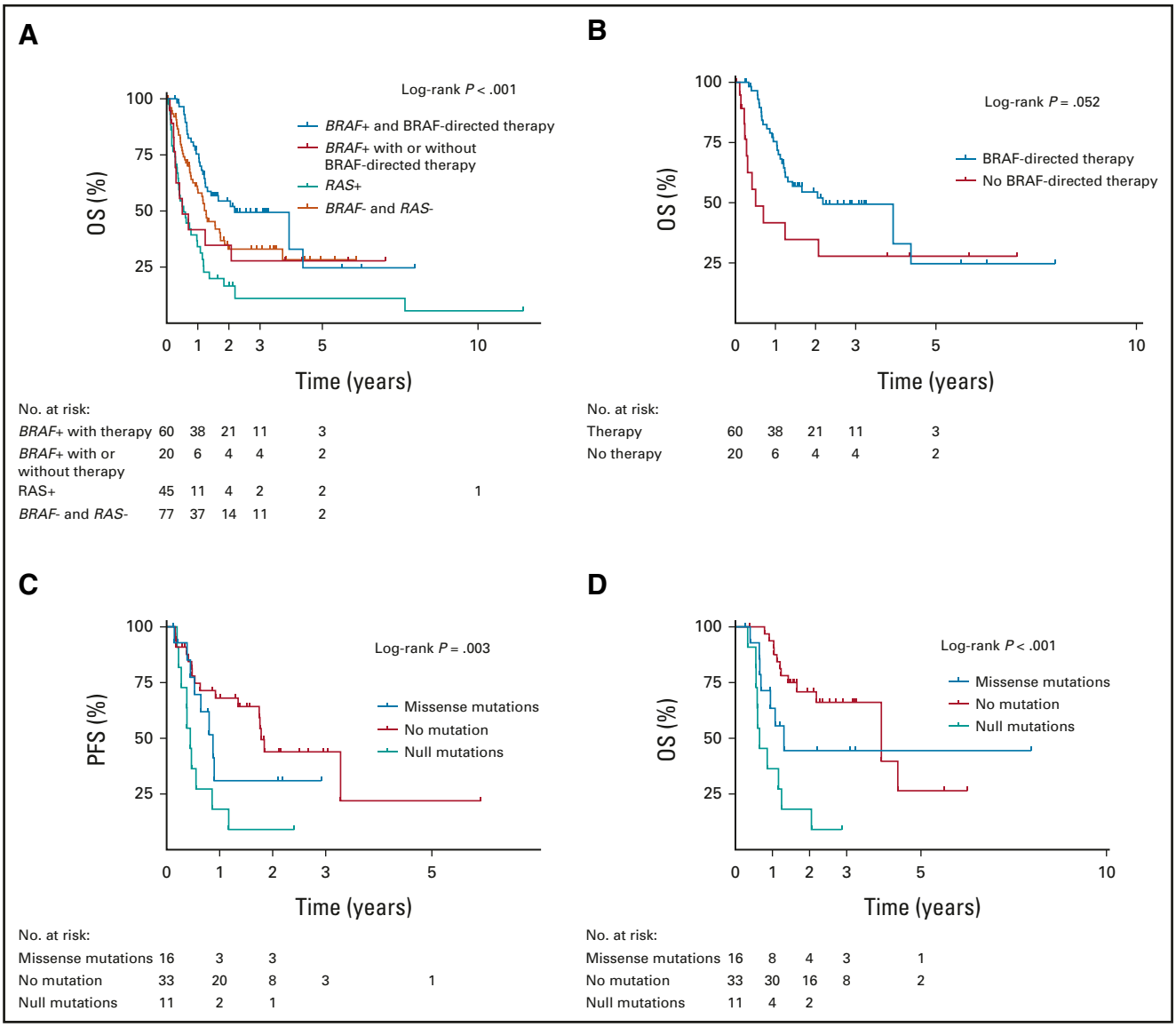

FIG 3. Survival outcomes analyses in *BRAFV600E*-mutated ATC: (A) OS in all patients by driver mutation status and *BRAF*-directed therapy, (B) OS in *BRAFV600E*-mutated patients by *BRAF*-directed therapy status, (C) PFS by *TP53* mutation status in patients treated with *BRAF*-directed therapy, and (D) OS by *TP53* mutation status in patients treated with *BRAF*-directed therapy. ATC, anaplastic thyroid carcinoma; OS, overall survival; PFS, progression-free survival.

of precursor prevalence. These findings support the current theory that although ATC may develop de novo, the majority of ATCs arise from differentiated thyroid carcinomas.[17,19,20] The reported prevalence of the *BRAF*V600E mutation in ATC varies, ranging from 25% to 45%.[7-11,21] In this study, which examined the largest cohort of sequenced patients with ATC with clinical outcomes data reported to date, the *BRAF*V600E mutation was identified in 41% of patients, which aligns with previous reports.[7,8,10] The rates of *RAS* mutations (25%) in this cohort were also similar to those in previous studies, yet we observe a lower prevalence of *TP53* mutations.[7,8,10] This difference may be due to a higher variant allele frequency used for filtering in our tissue-based next generation sequencing panels (≥ 5%) compared with MSK-IMPACT and Foundation Medicine.

We observed that survival outcomes in ATC were primarily determined by MAPK driver mutation status. *BRAF*V600E-mutated patients had the best prognosis with a median OS of 31 months followed by patients who were *BRAF* and *RAS* wild-type. The improved outcomes in *BRAF*V600E patients were attributed to the utilization of BRAF-directed therapy in our cohort. Without treatment with BRAF/MEK inhibitors, *BRAF*V600E-mutated patients had a median OS of 11 months, which was 4 months shorter than patients who were *BRAF* and *RAS* wild-type. Strikingly, the presence of *RAS* gene family mutations (*NRAS*, *KRAS*, and *HRAS*) was associated with significantly reduced survival, with a median OS of 6 months. Although *RAS*-mutated tumors showed less propensity for cervical lymph node

involvement, there was a nonsignificant trend toward higher prevalence of distant metastatic disease at presentation, demonstrating their aggressive behavior and the usual pattern of metastasis seen in follicular and Hurthle cell thyroid cancers. Although other studies have implicated co-occurrence of *TERT* promoter mutations with *BRAF* and *RAS* as poor prognostic factors in ATC, the presence of *RAS* mutations alone as a negative prognostic factor in ATC was recently reported in a small cohort of 27 patients where 11 were *RAS*-mutated.[7,10,22] Similar to the study by Lai et al,[22] we did not identify significant associations between *TERT* promoter mutations and survival outcomes, including co-occurrences with *BRAF*V600E and *RAS*. Since the *TERT* promoter region was tested in 123 of 202 patients, missing cases might have contributed to a lower mutation prevalence in this cohort than previous reports. However, distribution of *TERT* promoter mutations was similar across driver mutation groups, which suggests that the poor survival outcome seen in *RAS*-mutated patients cannot be entirely attributed to co-occurrence of *RAS* with *TERT* promoter mutations. In *BRAF* and *RAS* wild-type patients, *NF1* mutations appear to act as driver mutations but were incompletely assessed across patients in this study, which is a limitation. A larger sample size of patients with *NF1* testing is needed to robustly investigate whether *NF1* mutations affect survival outcomes in ATC. Moreover, additional genomic alterations, which may include mutations in genes not captured in targeted panels (ie, *E1F1AX*, *SWI/SNF* genes, and mismatch repair pathway genes), oncogenic fusions, copy number alterations, and/or epigenetic alterations, may also affect ATC phenotypes and survival outcomes, which require further investigation in future studies.

Previous studies examining the genomic landscape of ATC have not evaluated survival outcomes in the context of BRAF-directed therapy.[7,10] In this large cohort of patients treated with a specialized multidisciplinary program for ATC at a tertiary referral center, we found that disease progression occurred in approximately half of treated patients. Comparable with observations in melanoma, the duration of response was limited with a PFS of approximately 1 year.[23] These findings suggest that despite high initial response rates to BRAF/MEK inhibition, treatment resistance remains a challenge precluding long-term survival in *BRAF*V600E-mutated ATC. Our group previously showed that the emergence of a *RAS* mutation after BRAF/MEK inhibition leads to treatment resistance and progression.[24] Here, we found that the presence of *TP53* mutations was an independent predictor of disease progression. Indeed, murine models of *BRAF*V600E-mutated ATC with p53 loss demonstrate evidence of intrinsic resistance to BRAF inhibition.[25,26] The combination of *BRAF*V600E and missense *TP53* mutations, on the other hand, remains less studied in vivo. Additional studies are required to further investigate the impact of specific *TP53* mutations on BRAF/MEK inhibition and to elucidate possible mechanisms of therapeutic resistance.

Immunotherapy with a checkpoint blockade is being actively investigated for ATC. In a recently published clinical trial evaluating the efficacy of programmed cell death protein (PD-1) blockade, the overall response rate to the checkpoint blockade was modest at 19%.[27] In this trial, patients were treated with single-agent spartalizumab and *BRAF*V600E-mutated patients (n = 12) were found to have lower response rates than BRAF wild-type patients (8% *v* 23%).[27] However, in this study, we found a survival benefit for 36 *BRAF*V600E-mutated patients treated with a checkpoint blockade and BRAF-directed therapy. These findings suggest that combination therapy with BRAF/MEK inhibition and checkpoint inhibition may be of benefit in *BRAF*V600E-mutated ATC. A potential benefit was also observed in *RAS*-mutated ATC, where effective therapies are urgently needed. Indeed, multiple ongoing clinical trials (ClinicalTrials.gov identifier: NCT03181100, NCT04675710, NCT04171622, and NCT04238624) are evaluating treatment strategies that include combination therapy with tyrosine kinase inhibitors and checkpoint inhibitors. Although TMB was not significantly associated with survival in patients treated with immunotherapy in this study, it has been demonstrated that TMB accuracy suffers when calculated from panel tests with low genome coverage.[28] Given the small size of the targeted panels used in this patient cohort, the accuracy of panel TMB estimates is likely low, which limits conclusions that can be drawn from these sensitivity analyses.

In conclusion, driver mutations in ATC are associated with distinct clinicopathologic features and survival outcomes, reflecting heterogeneity in tumor biology. Although treatment with BRAF/MEK inhibitors can be initiated on the basis of knowledge of *BRAF*V600E status alone (ie, using immunohistochemistry), knowledge of *RAS* and *TP53* mutation status allows for additional risk stratification and can guide further therapeutic decision making. As such, comprehensive tumor mutation profiling should be obtained for patients with ATC as a part of the routine clinical workup at diagnosis, which has been incorporated into the current American Thyroid Association treatment guidelines.[29]

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DATA SHARING STATEMENT

The data supporting findings from this study are available from the corresponding authors upon request.

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