Dissecting Anaplastic Thyroid Carcinoma: A Comprehensive Clinical, Histologic, Immunophenotypic, and Molecular Study of 360 Cases

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Background: Anaplastic thyroid carcinoma (ATC) is nearly always fatal. Large studies on ATC are exceedingly rare. We aimed to study the clinical, genotypic, and histologic characteristics of ATC in the largest retrospective cohort of ATC to date.

Methods: Three hundred sixty patients with ATC from two tertiary centers were studied. Molecular testing was performed in 126 cases including 107 using next-generation sequencing.

Results: The median patients' age was 68 years. Differentiated thyroid carcinoma (DTC) was present in 208 cases (58%), the most common being papillary carcinoma ($n=150$). The 1-, 2-, 3-, and 5-year overall survival (OS) was 36%, 17%, 13%, and 11%, respectively. On univariate analysis, age, resectability, chemotherapy, radiotherapy, margin status, encapsulation, gross residual disease, gross extrathyroidal extension, percentage, and size of ATC in the primary tumor predicted OS ($p<0.05$). Age, resectability, chemotherapy, and gross residual disease were independent prognostic factors in the entire cohort, while gross residual disease was the only independent predictor of OS in patients who had resection of their tumor. *BRAF*, *RAS*, *TERT* promoter, *TP53*, *PIK3CA*, *E1F1AX*, and *PTEN* mutations were detected in 45%, 24%, 75%, 63%, 18%, 14%, and 14% of ATC, respectively. Concomitant *BRAF*/*RAS* and *TERT* mutations were associated with worse outcome than mutation in only one of the genes. *BRAF*-mutated and *RAS*-mutated ATCs had similar frequency of nodal and distant metastasis. Twelve cases were pure squamous cell carcinoma, 60% of which carried *BRAF*^{V600E} mutation and showed a similar OS to other ATCs.

Conclusions: (i) Gross residual disease remains the most crucial indicator of outcome in ATC. (ii) Encapsulation, margin status, percentage, and size of ATC in the primary were prognostically relevant. (iii) Pure thyroid squamous cell carcinoma may be considered as ATC given a *BRAF*^{V600E} genotype and similar outcome. (iv) In contrast to DTC, *BRAF*-mutated and *RAS*-mutated ATCs have similar metastatic spread. (v) Concomitant mutations of *BRAF* or *RAS* with *TERT* confer a worse prognosis.

Keywords: anaplastic thyroid carcinoma, undifferentiated thyroid carcinoma, prognosis, *BRAF*, *RAS*, *TERT*

Introduction

ANAPLASTIC THYROID CARCINOMA (ATC) is a rare but lethal form of thyroid cancer with an incidence of 0.9 to 1.2 cases per 1,000,000 population and a median survival of 3–4 months based on the Surveillance, Epidemiology, and End Results (SEER) data (1,2).

Significant advances have been made in recent years in understanding the molecular pathogenesis of ATC. While *BRAF* and *RAS* mutations remain the main drivers in ATC

similar to differentiated thyroid carcinoma (DTC), ATCs commonly contain additional molecular events, including high frequency of *TERT* promoter and *TP53* mutations, as well as frequent alterations in gene encoding effectors in the PIK3CA-PTEN-AKT-mTOR pathway, SWI-SNF complex, histone methyltransferases, and mismatch repair (MMR) (3–6). The identification of these targetable molecular alterations in ATC led to incorporation of targeted therapies into the traditional multimodality treatment of ATC (7,8). For example, combination of dabrafenib (*BRAF* inhibitor) and

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trametinib (MEK inhibitor) treatment demonstrates robust responses in *BRAF*^{V600E}-mutated ATCs (9).

Little is known about the prognostic factors in this cohort of patients. Population/registration-based studies (1,2,10,11) have reported distant metastasis, lymph node metastasis, age, surgical resectability, surgical margin status, tumor size, radiation therapy, and/or chemotherapy as independent prognostic factors in ATC. However, a contemporary large-scale clinicopathologic and molecular study with meticulous histopathologic review to identify possible prognostic factors is lacking.

Aiming to understand the clinical–pathologic–molecular correlation and the prognostic factors of ATC, we herein present the largest comprehensive retrospective cohort of 360 ATC patients from 2 tertiary centers, including 178 primary thyroidal ATCs, which underwent surgical resection, and 126 analyzed at the molecular level.

Methods

Characteristics of the study cohort and clinicopathologic review

The study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY) and Royal North Shore Hospital (RNSH, Sydney, Australia). Pathology database at MSKCC was searched for “anaplastic carcinoma” or “dedifferentiated carcinoma.” All ATC cases with slides available for review diagnosed between 1985 and 2019 were included in this study. The slides of all patients were reviewed by at least one of four endocrine pathologists (B.X., A.J.G., T.F., and R.G.) to confirm the ATC diagnosis and to gather detailed pathologic features. A total of 360 patients with ATC were included in this retrospective study (MSKCC *n* = 289, RNSH *n* = 71).

The following clinical and pathologic characteristics were collected for each case: sex, age at first ATC diagnosis, site of ATC (thyroid primary, nodal metastasis, distant metastasis, and/or locoregional recurrence), type of procedure (cytology/core biopsy, incisional/excisional biopsy, and resection), the presence and type of prior or concurrent DTC, the predominant cytologic features of ATC, mitotic index (per 10 high-power fields within the most mitotically active area, field diameter 0.55 mm), atypical mitoses, true tumor necrosis (rather than infarct-type necrosis secondary to prior procedure), immunophenotype (PAX8 polyclonal, TTF-1, thyroglobulin, cytokeratin AE1/AE3, and CAM5.2), and treatment (attempted surgical resection, chemotherapy, radiation therapy, targeted therapy, and immunotherapy).

Tumor necrosis was composed of degenerating cytoplasm and punctate karyorrhectic nuclear debris. The presence of fibroblastic stromal reaction, hemorrhage, or an identifiable needle track in the necrotic area was attributable to reaction induced by prior fine-needle aspiration and was therefore not regarded as tumor necrosis.

The cytologic features of ATC were defined as follows: (i) spindle cells with prominent nuclear spindling; (ii) pleomorphic with significant variation in nuclear size; (iii) epithelioid ATC with cohesive nests/sheets of epithelioid cells exhibiting round nuclei and no definite line of differentiation; (iv) squamous cell carcinoma with keratinization and/or intercellular bridges; (v) rhabdoid in which tumor cells contain prominent round cytoplasmic inclusion and eccentrically located nuclei;

(vi) osteoclast giant cell-rich; and (vii) tumor giant cells defined as ATC tumor cells with multinucleation (Fig. 1).

The antibodies utilized for immunohistochemistry included PAX8 (polyclonal, dilution 1:100; Proteintech), TTF-1 (8G7G3/1, monoclonal, ready-to-use [RTU]; Ventana), thyroglobulin (2H11+6E1, RTU; Cell Marque), cytokeratin AE1/AE3 (1:1600; Dako), CAM5.2 (1:75; Becton & Dickinson), CK 5/6 (D5/16B4, RTU; Ventana), and *BRAF*^{V600E} (VE1, 1:400; Abcam).

Additionally, for the resected primary thyroidal ATC with or without gross residual disease, the following pathologic features were collected: the size of the entire carcinoma, the size and percentage of the ATC component, infiltration versus encapsulation, capsular invasion, vascular invasion, the presence and extent of microscopic extrathyroidal extension (ETE), gross ETE observed intraoperatively or radiologically, margin status, gross residual disease documented in the operative reports by the surgeons, nodal status, size and histotype of nodal metastasis, size and percentage of ATC within the lymph node, and extranodal extension.

Three hundred twenty-seven patients had clinical follow-up data available. The primary outcome was overall survival (OS). Disease-specific survival (DSS) was also calculated.

Molecular analysis

Molecular analysis using various platforms was conducted in a subset of 126 cases on formalin-fixed paraffin-embedded tissue blocks, including Sanger sequencing for *BRAF* mutation and/or *NRAS* mutations (*BRAF*: *n* = 9, *NRAS*: *n* = 5) (12), Sequenom (*n* = 10) (12,13), and next-generation sequencing (NGS, *n* = 107). Among the 107 cases that were subjected to NGS, 101 were tested using MSK-IMPACT™ (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets) platform (14,15), 3 using FoundationOne™ (16), and 3 using a customized panel covering mutation hot spots in 14 genes (*AKT1*, *BRAF*, *CTNNB1*, *GNAS*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PTEN*, *RET*, *TP53*, *TSHR*, *TERT*, and *EIF1AX*) and 76 fusions (including the most common fusions in thyroid carcinoma, *RET*, *NTRK*, *PAX8-PPARG*, and *ALK*; full list provided in Supplementary Table S1).

Twenty-nine cases that were subjected to MSK-IMPACT platform were previously reported (3,4). Among the 101 cases tested using MSK-IMPACT platform, 35 (35%) were biopsies or cytology cell blocks, 9 (9%) were incision or excision, and 57 (56%) were resection specimens.

The details of all molecular platforms were previously described. In brief, MSK-IMPACT is a Food and Drug Administration (FDA)-approved deep-coverage, targeted NGS assay detecting single nucleotide variants (SNVs), small insertions/deletion (indels), copy number variants, and fusion/structural variants in 468 oncogenes, using custom DNA probes designed for targeted sequencing of all exons and selected introns, including canonical and selected noncanonical transcripts (14,15). FoundationOne is a commercially available FDA-approved NGS platform detecting SNV, indels, copy number alterations, and rearrangement in 324 genes (16).

Sequenom is a MassARRAY system based on matrix-assisted laser desorption/ionization time-to-flight mass spectrometry that was used to interrogate the presence of single nucleotide variation in 91 hot spots of 8 oncogenes: *BRAF*, *NRAS*, *KRAS*, *EGFR*, *PIK3CA*, *AKT1*, *MEK1*, and *ERBB2*

FIG. 1. Histologic features of ATC. ATC may show spindle (A), pleomorphic (B), epithelioid (C), squamous (D), rhabdoid (E), osteoclast giant cell-rich (F) features. Inset in (D) PAX8 immunohistochemistry is positive in the anaplastic squamous cell carcinoma component. Inset in (E) BRAF^{V600E} immunostain is positive in the rhabdoid ATC cells. (G–I) Immunoprofile of the osteoclast cell-rich ATC (F). (G) PAX8, (H) TTF-1, and (I) BRAF^{V600E}. The papillary thyroid carcinoma component (right) is positive for PAX8, TTF-1, and BRAF^{V600E}. The ATC component (left) is focally and weakly positive for PAX8 and TTF-1, whereas diffusely positive for BRAF^{V600E}. Note that BRAF immunostain is negative in osteoclast giant cells, suggesting that they are reactive rather than neoplastic in nature. (J–L) Unusual features of ATC, showing myxoid stroma (J), osteosarcomatous differentiation (K), and chondrosarcomatous differentiation (L). ATC, anaplastic thyroid carcinoma.

(13). Only the 101 cases with MSK-IMPACT were subsequently plotted using cBioPortal Oncoprint (17). For the remaining 25 cases, alterations in *BRAF*, *RAS*, *TERT* promoter, and *TP53* mutations were documented when available and were incorporated into our statistical analysis.

Statistical analyses

All statistical analyses were performed using the SPSS software 24.0 (IBM Corporation, New York, NY). The prognostic significance of each parameter on OS was calculated using log-rank test or univariate Cox proportional hazard model. All clinical and pathologic parameters collected were subjected to univariate survival analysis. Factors significant on univariate analysis were subsequently subjected to multivariate analysis using Cox proportional haz-

ards model. Additionally, the clinicopathologic features of ATC subgroups, *BRAF*-mutated versus *RAS*-mutated ATC, and ATC with pure squamous cell carcinoma phenotype versus other ATCs were compared using chi-square test or Fisher's exact test. *p*-Values less than 0.05 were considered to be statistically significant.

Results

Clinical features of the study cohort

The clinicopathologic characteristics of the study cohort are summarized in Table 1. The median age of patients during ATC diagnosis was 68 years (range 29–99 years). Only seven patients (2%) were diagnosed with ATC when they were younger 40 years. There was a slight female predominance

TABLE 1. CLINICAL AND PATHOLOGIC CHARACTERISTICS OF ANAPLASTIC THYROID CARCINOMA IN THE ENTIRE COHORT ($N=360$) AND IN PRIMARILY RESECTED SPECIMENS ($N=178$)

Entire cohort ($n=360$)	
Sex	
Female	196 (54)
Male	164 (46)
Age, years	68 (29–99)
Specimen type	
Cytology/core biopsy	102 (28)
Incisional/excisional biopsy	31 (9)
Resection	227 (63)
Pathologically confirmed ATC: site of origin	
Primary thyroid	271 (75)
Thyroid only	199
Thyroid/LN	53
Thyroid/DM	12
Thyroid/LN/DM	3
Thyroid/LN/LRR	3
Thyroid/LRR	1
Other	89 (25)
LN only	35
DM only	31
LRR only	22
LRR/DM	1
DTC (history or concurrent)	
No DTC	152 (42)
With DTC	208 (58)
Papillary thyroid carcinoma	150
Tall cell variant	68
Classic variant	37
Follicular variant	24
Poorly differentiated carcinoma	68
Hürthle cell carcinoma	21
Follicular carcinoma	10
ATC % (ATC area/total tumor area) ($n=331$)	
$\leq 10\%$	15 (5)
11–89%	95 (29)
$\geq 90\%$	221 (67)
Predominant cytologic features of ATC ($n=354$) ^a	
Spindle	92 (26)
Pleomorphic	80 (23)
Squamous	76 (21)
Epithelioid	66 (19)
Rhabdoid	28 (8)
Osteoclast cell rich	12 (3)
Mitotic index/10 HPFs ($n=296$)	
0–5/10 HPFs	91 (31)
6–10/10 HPFs	94 (32)
11–20/10 HPFs	66 (22)
$>20/10$ HPFs	45 (15)
Atypical mitosis ($n=291$)	
Absent	45 (15)
Present	246 (85)
Tumor necrosis ($n=332$)	
Absent	76 (23)
Present	256 (77)
Neutrophilic infiltrate ($n=314$)	
Absent	92 (29)
Present	222 (71)
Thyroid primary resection specimen ($n=178$)	
Size of primary tumor, cm	5.0 (2.1–15.0)
Size of ATC in the primary tumor, cm	3.0 (0.4–14.0)
Size of primary ATC, cm ($n=160$)	

(continued)

TABLE 1. (CONTINUED)

≤ 1	10 (6)
1.1–2	25 (16)
2.1–4	63 (39)
>4	62 (39)
Mitotic index/10 HPFs ($n=152$)	
0–5/10 HPFs	33 (22)
6–10/10 HPFs	47 (31)
11–20/10 HPFs	40 (26)
$>20/10$ HPFs	32 (21)
Atypical mitosis ($n=146$)	
Absent	13 (9)
Present	133 (91)
Tumor necrosis ($n=177$)	
Absent	25 (14)
Present	152 (86)
Infiltration/encapsulation ($n=176$)	
Infiltrative	162 (92)
Encapsulated	14 (8)
Without capsular invasion	5
With capsular invasion	9
Lymphovascular invasion ($n=159$)	
Absent	36 (21)
Present	123 (79)
Extensive	56
Focal	35
With vessel wall invasion	12
Microscopic ETE ($n=167$)	
Absent	21 (13)
Present	146 (87)
Fibroadipose tissue	63
Muscle	68
Other organ(s)	15
Gross ETE ($n=71$)	
Absent	6 (8)
Present	65 (92)
Margin status ($n=160$)	
Negative	36 (22)
Positive	124 (78)
Gross residual disease ($n=71$)	
No gross residual disease	47 (66)
Gross residual disease	24 (34)
Nodal status ($n=167$)	
pN0/pNx	88 (53)
pN1	79 (47)
Largest nodal metastatic focus, cm	1.5 (0.1–7.5)
Size of ATC in nodal metastasis, cm	1.3 (0–7.5)
Extranodal extension ($n=74$)	
Absent	29 (39)
Present	45 (61)

Data are expressed as n (%) for categorical variables and median (range) for continuous variables.

^aPredominant cytologic feature is defined as the cytologic feature that accounts for the largest percentage within an ATC.

ATC, anaplastic thyroid carcinoma; DM, distant metastasis; DTC, differentiated thyroid carcinoma; ETE, extrathyroidal extension; HPFs, high power fields; LN, lymph node; LRR, locoregional recurrence.

with a female:male ratio of 1.2:1. The diagnosis of ATC was most frequently rendered on surgical resection specimen ($n=227$, 63%), including 178 resections of the primary ATC in the thyroid gland proper. A pathologic diagnosis of ATC was pathologically confirmed in the thyroid gland ($n=271$, 75%), lymph node metastasis ($n=94$, 26%), distant metastasis ($n=41$, 11%), and/or locoregional recurrence ($n=27$, 8%).

A history of DTC and/or concurrent DTC was noted in 208 patients (58%), including papillary thyroid carcinoma ($n=150$), poorly DTC defined using MSKCC criteria ($n=68$) (18), Hürthle cell carcinoma ($n=21$), and follicular thyroid carcinoma ($n=10$). In 41 cases, the DTC contained multiple histotypes, a poorly differentiated and a well-differentiated component (being papillary carcinoma, follicular carcinoma, or Hürthle cell carcinoma). Among papillary thyroid carcinoma, tall cell variant was the most common subtype ($n=68$), followed by classic variant ($n=37$) and follicular variant ($n=24$). Other variants of papillary thyroid carcinoma documented in our cohort included solid ($n=2$), hobnail ($n=1$), and diffuse sclerosing variant ($n=1$). In 17 cases, the variants of papillary carcinoma were not available.

Histologic and immunophenotypic characteristics of ATC

Histologically, ATC demonstrated various cytomorphologic features, including pleomorphic ($n=184$), spindle cell ($n=167$), epithelioid ($n=127$), squamous cell carcinoma ($n=109$), rhabdoid ($n=71$), osteoclast giant cell-rich ($n=45$), and tumor giant cells ($n=17$). It was not uncommon for an ATC to exhibit multiple cytomorphologic features (Supplementary Table S2).

The predominant feature of each tumor defined as the cytologic features comprising the highest percentage of tumor volume within the ATC component was available in 354 cases, and it was: spindle cell ($n=92$, 26%), pleomorphic ($n=80$, 23%), squamous cell carcinoma ($n=76$, 21%), epithelioid ($n=66$, 19%), rhabdoid ($n=28$, 8%), and osteoclast giant cell-rich ($n=12$, 3%, Fig. 1).

Other uncommon histologic features that may be noted focally within the ATC included heterologous differentiation ($n=5$, including 3 osteosarcomatous and 2 chondrosarcomatous differentiation); myxoid stroma ($n=4$), focal glandular differentiation ($n=3$), and focal clear cell changes ($n=2$). Two cases in our cohort met the description of paucicellular variant of ATC (19).

The mitotic activity noted within the ATC component varied with a median mitotic index of 8 per 10 high-power fields (range 0–78). Notably, 91 (31%) cases of ATC had a

mitotic index of 5 or less per 10 high-power fields, including 5 cases with no mitotic activity. In the absence of prominent mitotic activity, especially in small (biopsy) material, other histologic features, such as marked nuclear pleomorphism and loss of thyroid differentiation demonstrated by histology and immunohistochemistry, were required to render the ATC diagnosis. Atypical mitoses, tumor necrosis, and prominent neutrophil infiltrate within the tumors were common features of ATC, being observed in 85%, 77%, and 71% of tumors, respectively.

Our cohort contained 178 resected primary thyroid ATC, and their features are summarized in Table 1. The median size of the entire primary carcinoma and the ATC component was 5.0 cm (range 2.1–15.0 cm) and 3.0 cm (range 0.4–14.0 cm), respectively. Although the majority (92%) of the cohort was composed of infiltrative tumors, 14 ATCs (8%) were entirely encapsulated. The frequency of various parameters was: vascular invasion 79%, microscopic ETE 87%, gross ETE 92%, microscopic positive resection margin 78%, gross residual disease documented intraoperatively 34%, and nodal metastasis 47%.

The immunohistochemical profile of ATC is shown in Table 2. ATCs were frequently positive for PAX8 polyclonal (126/179, 70%) and cytokeratins, cytokeratin AE1/AE3 (130/195, 67%), CAM5.2 (90/133, 68%), 34 β E12 (35/75, 68%), CK18 (34/52, 65%), and CK5/6 (18/33, 55%), occasionally positive for TTF-1 (78/256, 30%), whereas nearly always negative for thyroglobulin (238/248, 96%). Abnormal p53 immunorexpression was observed in seven of nine cases tested (78%), being overexpressed in five and absence of any nuclear staining (null type) in two.

Molecular profile of ATC

Among the 126 cases tested, the frequency of *BRAF*, *RAS*, *TP53*, and *TERT* promoter mutations was 45% (57/126, *BRAF*^{V600E} 56 cases and *BRAF* deletion 1 case), 24% (29/122, including *NRAS* $n=21$, *KRAS* $n=3$, and *HRAS* $n=5$), 63% (66/105), and 75% (78/104), respectively. The most common *RAS* mutations were *NRAS* Q61R ($n=13$), *NRAS* Q61K ($n=7$), and *HRAS* G13R ($n=3$). Other alterations in the MAPK pathway included *CCDC6-RET* fusion in two

TABLE 2. IMMUNOHISTOCHEMICAL PROFILE OF ANAPLASTIC THYROID CARCINOMA

Immunohistochemistry	Total stained	Negative, n (%)	+, n (%)	++, n (%)	+++, n (%)
PAX8 (polyclonal)	179	53 (30)	3 (2)	26 (15)	97 (54)
TTF-1	256	178 (70)	9 (4)	39 (15)	30 (12)
Thyroglobulin	248	238 (96)	4 (2)	4 (2)	2 (1)
Any cytokeratins	225	57 (25)	168 (75)		
Cytokeratin AE1/AE3	195	65 (33)	2 (1)	32 (16)	96 (49)
CAM5.2	133	43 (32)	5 (4)	21 (16)	64 (48)
34 β E12	75	40 (53)		5 (7)	30 (40)
CK18	52	18 (35)		6 (12)	28 (54)
CK5/6	33	15 (45)		5 (15)	13 (39)
p63	36	13 (36)		6 (17)	17 (47)
p40	18	12 (67)		1 (6)	5 (28)
p53 ^a	9	2 (22)		7 (78)	

^aFor p53 immunostain, the absence of abnormal expression is considered as negative, whereas abnormal expression defined as overexpression ($n=5$) or absence of any nuclear stain ($n=2$) is regarded as positive.

+, immunopositivity in rare cells; ++, focal and/or weak immunopositivity; +++, multifocal or diffuse immunopositivity.

cases, *NTRK1-IRF2BP2* fusion in one case, *NTRK2-CRNDE* fusion in one case, and *NTRK3* R630W mutation in one case. The significance of *NTRK3* R630W in ATC is unclear at present.

BRAF-mutated ATC was associated with a high frequency of pure squamous cell carcinoma histotype and a high rate of associated DTC, in particular papillary thyroid carcinoma (Fisher's exact test, $p=0.002$ and 0.001 , respectively, Table 3). Other pathologic features, such as predominant cytomorphic features of ATC, vascular invasion, and risk of nodal and distant metastasis, did not differ between *BRAF*-mutated and *RAS*-mutated ATCs (Fisher's exact test, $p>0.05$). Additionally, *BRAF*-mutated and *RAS*-mutated ATCs had similar OS (log rank test, $p=0.945$).

The Oncoprint of the molecular profile of 101 ATCs tested using MSK-IMPACT platform is shown in Figure 2A. The median mutation count per tumor was 5 (range 0–34). Common alterations detected included *CDKN2A/2B* alteration in 25%, *E1F1AX* in 14%, *PTEN* in 14%, *NF2* in 12%,

NF1 in 12%, *KMT2D* in 9%, *RB1* in 9%, *ARID2* in 9%, MMR genes (*MLH1*, *PMS2*, *MSH2*, and *MSH6*) in 8%, *ATM* in 7%, *KMT2A* in 6%, *MTOR* in 5%, *PBRM1* in 5%, and *IL7R* in 5%.

The presence of *TERT* promoter mutations in ATC was associated with shorter OS (log-rank test, $p=0.016$, 1-year OS was 36% and 46% in ATC with and without *TERT* promoter mutation, respectively, Fig. 2B). Furthermore, ATC with both *BRAF/RAS* mutation and *TERT* promoter mutation (*BRAF* and *TERT* or *RAS* and *TERT*) was associated with worse clinical outcome compared with ATC without any of these three mutations or with *BRAF*, *RAS* mutation, or *TERT* promoter mutation alone (log-rank pairwise comparison, $p<0.05$, 1-year OS was 25%, 45%, 44%, and 47% in *BRAF/RAS* and *TERT* double mutant, *TERT*, *BRAF/RAS*, and wild-type group, respectively, Fig. 2C). Mutation count and other molecular alterations, *TP53*, *E1F1AX*, *PTEN*, and *PIK3CA* mutations, did not impact OS (log-rank test, $p>0.05$).

Mutations in MMR genes were detected in eight cases: including four with *MSH6*, two with *MSH2*, one with *MLH1*, and one with *MSH6* and *MSH2* mutations. Among them, four tumors also contained alteration in genes involved in the MAPK pathway, including three *BRAF*^{V600E} and one *NTRK3* R630W mutation. The OS did not differ significantly from those without MMR gene defects (log-rank test, $p=0.182$, 1-year OS was 47%).

BRAF^{V600E} immunohistochemistry was a sensitive and specific screening tool for *BRAF*^{V600E} mutations in ATC

In our cohort, 32 cases were tested for both *BRAF*^{V600E} immunostain and *BRAF* mutation molecular testing. Among them, 19 ATCs were positive for *BRAF*^{V600E} mutation, 1 showed *BRAF* deletion, whereas the remaining 12 did not have *BRAF* alteration. *BRAF*^{V600E} immunostain was positive in 18 of 19 *BRAF*^{V600E}-mutated ATC, whereas negative in all 13 ATCs without *BRAF*^{V600E} mutations. The case with *BRAF* deletion was negative for *BRAF*^{V600E} by immunostain. The sensitivity and specificity of *BRAF*^{V600E} immunohistochemistry in detecting the underlying *BRAF*^{V600E} mutation in ATC were 95% and 100%, respectively.

Clinical outcome and prognostic factors of ATC

Follow-up data were available in 327 patients, including 163 patients who underwent resection of their primary thyroid ATC. The 1-, 2-, 3-, and 5-year OS and DSS were 36%, 17%, 13%, and 11%; and 36%, 17%, 14%, and 14%, respectively. The median OS was nine months in the entire cohort. Sixty-one percent (189/310) of our cohort had distant metastasis, including 83 patients with distant metastasis at first ATC diagnosis.

On univariate analysis, younger age at ATC diagnosis, the presence of DTC, resection of ATC, chemotherapy, and radiation therapy were associated with longer OS in the entire cohort (Cox proportional model, $p<0.05$, Table 4 and Fig. 3). Other features, such as site of ATC, cytomorphic features, tumor necrosis, atypical mitoses, mitotic index and distant metastasis, were not associated with outcome (Supplementary Table S3). Multivariate analysis using Cox proportional model showed that age at ATC diagnosis, resection, and chemotherapy were independent prognostic factors in

TABLE 3. GENOTYPE-PHENOTYPE CORRELATION IN ANAPLASTIC THYROID CARCINOMA

	BRAF (n=57), n (%)	RAS (n=29), n (%)	No BRAF or RAS mutations (n=40), n (%)	p ^a
Pure squamous versus nonsquamous ATC				
Nonsquamous	43 (75)	29 (100)	38 (95)	0.002
Pure squamous	14 (25)	0 (0)	2 (5)	
Predominant cytologic features of ATC				
Squamous	15 (26)	3 (10)	5 (12.5)	0.535
Spindle cell	11 (19)	8 (28)	10 (25)	
Epithelioid	13 (23)	7 (24)	13 (32.5)	
Osteoclast cell rich	4 (7)	1 (3)	1 (2.5)	
Pleomorphic	9 (16)	6 (21)	9 (22.5)	
Rhabdoid	5 (9)	4 (14)	2 (5)	
DTC				
Absent	14 (25)	18 (62)	24 (60)	0.001
Present	43 (75)	11 (38)	16 (40)	
PDTC	12	5	7	
Papillary carcinoma	38	5	7	
Follicular carcinoma	0	2	0	
Hürthle cell carcinoma	0	1	4	
Vascular invasion				
Absent	4 (16)	2 (12.5)	1 (5)	1.000
Present	21 (84)	14 (87.5)	19 (95)	
Distant metastasis				
Absent	14 (25)	6 (20)	13 (32.5)	0.790
Present	42 (75)	23 (79)	27 (67.5)	
Nodal status				
N0/Nx	12 (35)	6 (43)	9 (45)	0.746
N1	22 (65)	8 (57)	11 (55)	

Bold values indicate significant p -values.

^a p -Values were obtained using Fisher's exact test comparing *BRAF*-mutated and *RAS*-mutated tumors.

PDTC, poorly differentiated thyroid carcinoma.

FIG. 2. Molecular profile of ATC. (A) Oncoprint of 102 ATCs that are sequenced using MSK-IMPACT next-generation sequencing panel. (B) *TERT* promoter mutation is associated with worse overall survival in ATC (log-rank test, $p=0.016$). (C) Prognostic significance of *BRAF*, *RAS*, and *TERT* promoter mutations in ATC: ATC carrying both *BRAF/RAS* and *TERT* promoter mutations is associated with significantly shorter overall survival compared with those without either mutations (wild type), *BRAF/RAS* mutation alone, or *TERT* promoter mutation alone (log-rank pairwise comparison, $p<0.05$). MMR, mismatch repair.

TABLE 4. CLINICAL AND PATHOLOGIC PROGNOSTIC PARAMETERS IN ANAPLASTIC THYROID CARCINOMA

	Univariate			Multivariate		
	HR	CI	p	HR	CI	p
All patients						
Age at ATC diagnosis	1.871	1.453–2.410	<0.001	1.671	1.276–2.188	<0.001
Resectability	0.680	0.529–0.875	0.003	0.510	0.389–0.670	<0.001
Chemotherapy	0.587	0.453–0.761	<0.001	0.697	0.493–0.985	0.041
Radiation therapy	0.650	0.497–0.850	0.002	0.863	0.607–1.228	0.863
Primary resection						
Encapsulation	0.312	0.115–0.851	0.023	0.699	0.238–2.052	0.514
Percentage of ATC	1.847	1.321–2.583	<0.001	0.768	0.238–2.476	0.659
Size of ATC	1.596	1.255–2.031	<0.001	1.451	0.620–3.398	0.391
Margin status	2.359	1.398–3.982	0.001	1.391	0.516–3.748	0.514
Gross residual disease	2.718	1.515–4.874	0.001	2.391	1.141–5.010	0.021
Gross ETE	4.678	1.133–19.319	0.033	2.109	0.479–9.286	0.324

Bold values indicate significant *p*-values.

CI, 95% confidence interval; HR, hazard ratio.

ATC (hazard ratio 1.671 [CI 1.276–2.188]; 0.510 [CI 0.389–0.670]; and 0.697 [CI 0.493–0.985], respectively).

Among the 163 patients who underwent thyroid resection with follow-up data, factors that were associated with OS on univariate analysis included encapsulation, percentage and size of the ATC component within the primary tumor, microscopic margin status, gross residual disease, and gross ETE (Table 4). Other parameters, such as the presence and extent of microscopic ETE, the presence and extent of vascular invasion, nodal status, and extranodal extension, were not significantly associated with OS. On multivariate analysis, the only factor that predicted OS was gross residual disease (hazard ratio 2.391 [CI 1.141–5.010], *p* = 0.021).

ATC with squamous cell carcinoma phenotype

In the study cohort, 51 cases demonstrated a pure squamous cell carcinoma phenotype. Compared with other ATCs, tumors with pure squamous cell carcinoma phenotype were associated with a higher frequency of previous/concurrent DTC (76% vs. 55%, *p* = 0.004), previous/concurrent papillary thyroid carcinoma (95% vs. 67% *p* < 0.001), and *BRAF*^{V600E} mutation (87.5% vs. 39%, *p* < 0.001, Table 5). ATC with a pure squamous phenotype carried a similar dismal outcome compared with all other ATCs (log-rank test, *p* = 0.190), with a median OS of 12 and 9 months, respectively.

Immunophenotypically, ATC with squamous cell carcinoma phenotype was commonly positive for PAX8 (20/22, 91%, focal in 2 and multifocal/diffuse in 18) and *BRAF*^{V600E} (7/9, 78%), occasionally positive for TTF-1 (13/34, 38%, focal in 9 and multifocal/diffuse in 4), and generally negative for thyroglobulin (negative in 31/32 cases tested, 97%; focally positive in 1 case). Not surprisingly, these tumors showed a squamous immunophenotype, demonstrated by immunopositivity for high-molecular weight cytokeratins 34βE12 (8/8, 100%) and CK5/6 (4/4, 100%), p63 (6/6, 100%), and p40 (4/5, 80%).

Additionally, there were 12 tumors with a pure squamous cell carcinoma phenotype and without any previous/concurrent DTC. In theory, these tumors could be classified as primary thyroid squamous cell carcinoma, an entity recognized by the 2017 World Health Organization (WHO) clas-

sification (20). These 12 patients had a similar outcome to all other ATC patients, with a median OS of 14 months (log-rank test, *p* = 0.784, Table 5). Among them, the molecular signatures of five tumors were examined using MSK-IMPACT platform. Three carried *BRAF*^{V600E} mutations, and one harbored an *NTRK2-CRNDE* fusion.

There was no significant difference between squamous cell carcinoma with and without associated DTC in term of genotype and outcome (*p* > 0.05, data not shown).

Discussion

In concordance with previous reports (1,2,10,11,21), we show that ATC commonly affect elderly patients and is exceedingly rare in patients younger than 40 years. In our cohort, the median survival was 9 months and the 1-year OS was 36%. Although dismal, the survival of ATC patients in our cohort appears to be better than the 3–4 months median survival and ~20% 1-year survival reported from the SEER database (1,2) and/or cancer registry studies (10,11,21). This improved survival may be in part due to a referral bias and/or treatment differences as all our cases were collected from tertiary referral centers that regularly treat ATC with multimodality therapies. Indeed, a retrospective cohort study from the Mayo clinic, another tertiary center, also reported a median survival of 9 months and a 1-year survival of 42% in ATC patients treated with multimodality therapy (22).

The prognostic factors in ATC, especially the pathologic ones, have not been comprehensively studied. To date, only several registry-based studies have identified lack of distant metastasis (at presentation), lack of lymph node metastasis, smaller tumor size, younger age, surgical resection, a negative margin status, radiation therapy, and chemotherapy as independent prognostic factors that are associated with improved survival (1,2,10,11,21). For example, Sugitani *et al.* studied 677 ATCs using a Japanese ATC Research Consortium with careful annotation of clinicopathologic parameters and reported age, primary tumor size, T4b stage, presence of acute symptoms, leukocytosis, and distant metastasis as independent prognostic factors for ATC (21).

In this study, we have performed a detailed clinicopathologic review to investigate the prognostic significance of

FIG. 3. Kaplan-Meier plots of overall survival. OCGCR, osteoclast giant cell rich.

multiple pathologic parameters. In addition to confirming the prognostic role of patient age, surgical resection, chemotherapy, radiation therapy, margin status, and gross residual disease, we have found that several pathologic parameters, such as pre-existing/co-existing DTC, encapsulation, percentage of ATC within the primary tumor, and the actual size of ATC (rather than size of entire tumor), are prognostically significant on univariate survival analysis. Therefore, in primary resection of ATC, assessing the actual amount of ATC (percentage and size) as well as the encapsulation status may provide additional prognostic information for patients' follow-up and treatment.

Interestingly, Sugitani *et al.* reported that incidentally detected ATC was associated with improved survival on univariate analysis. It is plausible that small ATC detected incidentally are associated with improved outcome (21). Other histopathologic features, such as cytomorphic features, mitotic index, and tumor necrosis, which play a role in the prognosis of DTC (18,23), do not affect the outcome of ATC. As the current study and registry-based studies were retrospective in nature, it is unclear whether the survival advantage observed in patients having surgical resection, radiation therapy, and/or chemotherapy is related to a true benefit of such therapies or to confounding factors, such as selection bias.

TABLE 5. COMPARISON BETWEEN ANAPLASTIC THYROID CARCINOMA WITH SQUAMOUS CELL CARCINOMA PHENOTYPE AND OTHER ANAPLASTIC THYROID CARCINOMAS

	Pure squamous ATC (n=51)	Other ATCs (n=309)	p	Pure squamous ATC without DTC (n=12)	Other ATCs (n=348)	p
DTC	39/51 (76%)	169/309 (55%)	0.004	NA	NA	NA
PTC	37/39 (95%)	113/169 (67%)	<0.001	NA	NA	NA
<i>BRAF</i> ^{V600E} mutation	14/16 (87.5%)	43/110 (39%)	<0.001	3/5 (60%)	54/121 (45%)	0.658
1-year overall survival	50%	38%	0.190	63%	39%	0.784
2-year overall survival	20%	22%		0%	22%	
Median overall survival	12 months	10 months		14 months	10 months	

Bold values indicate significant *p*-values.

NA, not available; PTC, papillary thyroid carcinoma.

In this study, we also provide a comprehensive histopathologic description of ATC. Similar to what have been previously described (24,25), we demonstrate that ATC may exhibit various histologic features including spindle (tumor), giant cells, pleomorphic, osteoclast giant cell-rich, epithelioid and squamous morphology, with spindle cells being the most common histotype. Heterologous sarcomatoid component, although rare, may be present in <2% of cases. Immunophenotypically, ATCs are frequently positive for various cytokeratins (75%) and PAX8 (70%), whereas close to a third of cases may show TTF-1 immunopositivity. In contrast, thyroglobulin is nearly always negative in ATC. This immunohistochemical profile is consistent with what has been previously reported (26–28). A combination of cytokeratins, PAX8, TTF-1, thyroglobulin, and mutation-specific markers (such as *BRAF*^{V600E} as described below) may serve as a useful ancillary tool to distinguish ATC from its mimickers.

Interestingly, we did not detect any association between certain histologic characteristics (e.g., mitotic index, atypical mitoses, necrosis, and cytologic features) and genotype or prognosis. Even vascular invasion, a key prognostic factor and key component of risk stratification in DTC (29), was not associated with outcome of ATC. Understanding these pathologic features may be important in rendering a correct diagnosis of ATC, but it is the ATC diagnosis by itself rather than these histologic features that drives outcome. Of note, a proportion (31%) of ATC may have a mitotic index of ≤5/10 high-power fields. Therefore, a low mitotic count does not necessarily exclude a diagnosis of ATC.

In the current WHO classification (fourth edition, 2017), primary squamous cell carcinoma of the thyroid gland (defined as a tumor purely composed of squamous cells) is considered as a separate tumor entity from ATC (20). Herein, we demonstrate that the squamous phenotype is a common histotype of ATC and may coexist with other cytohistologic features. ATC with pure squamous cell carcinoma phenotype is often associated with *BRAF*^{V600E} mutations and DTC, particularly papillary thyroid carcinoma. Additionally, *de novo* squamous cell carcinoma of the thyroid gland, that is squamous cell carcinoma without any associated DTC, frequently harbors gene alterations in the MAPK pathway resembling thyroid follicular cell-derived carcinomas.

More importantly, we confirm that squamous cell carcinoma, with or without an associated DTC component, has a comparable dismal outcome as ATC. Similarly, an epide-

miologic study based on the National Cancer Database also reported a poor prognosis of thyroid squamous cell carcinoma akin to ATC with a 5-year OS of 18% (30). Together, these findings suggest that squamous cell carcinoma of the thyroid gland may be regarded as ATC, rather than a separate pathologic entity. Immunophenotypically, these tumors are commonly positive for PAX8 (31) and *BRAF*^{V600E}, which may be used as ancillary tools to differentiate them from squamous cell carcinoma of adjacent mucosal sites (e.g., larynx) and metastases from other organs.

Within the past few years, several groups have published genomic profiles of ATC using NGS techniques (3,4,6,32–37). Collectively (including the current study), close to 500 ATCs have been sequenced and the most frequent alterations are summarized in Supplementary Table S4. The findings of the current study confirm several findings that have been previously reported, including: (i) *BRAF* and *RAS* alterations are mutually exclusive and remain the key oncogenic drivers in ATC, together accounting for 65% of cases (*BRAF* 38%, *RAS* 27%) (4,6,32–36); (ii) *TP53* and *TERT* promoter mutations are highly recurrent events, being present in 62% (range 25–73%) and 50% (range 36–78%) of ATC, respectively; (iii) *EIF1AX* occurs in ~10–15% of ATC. It frequently coexists with *RAS* mutation and is absent in *BRAF*-altered ATC (3,4,32); (iv) cumulatively, *PIK3CA* and *PTEN* mutations occur in 13% (range 0–18%) and 11% (range 7–18%) of ATC, respectively; (v) *PTEN* and *NF1* mutations often coexist and are commonly seen in ATCs lacking *BRAF* or *RAS* mutations (3,4); and (vi) the frequent co-existence of *BRAF* and *PIK3CA* mutations. Similar to prior studies, we have reported rare cases of ATC with *CCDC6-RET* fusion (3,4,32).

Additionally, we are the first to report ATCs with *NTRK* fusions: one being *NTRK1-IRF2BP2*, the other with *NTRK2-CRNDE*, which may render the patients eligible for TRK-inhibitor targeted therapy. The finding that a large proportion of ATCs have a concurrent DTC component (58% of cases) and harbor the same MAPK pathway driver mutations that are found in DTC further support a stepwise oncogenic process of thyroid carcinoma from DTC to ATC (4,5).

More importantly, our study is the first to report a strong genotype–pathologic phenotype correlation in ATC. *BRAF*-mutated ATC is associated with a high frequency of DTC (particularly papillary thyroid carcinoma) and a significant higher percentage of squamous cell carcinoma histotype. Although previous studies have demonstrated a correlation between *BRAF*/*RAS* mutation status with route of spread in

DTC, namely BRAF-mutated tumors tend to spread to regional lymph nodes, whereas RAS-related tumors travel to distant sites (4,13), such difference in tumor spread according to genotype appears to be lost in ATC. Indeed, tumors with either mutations are capable to spread lymphatically (*BRAF*-mutant: 65%, *RAS*-mutant: 57%) and hematogenously (*BRAF*-mutant: 75%, *RAS*-mutant: 79%). The difference between *RAS*-mutated and *BRAF*-mutated ATCs seem to be also blurred at the mRNA expression level. Indeed, Landa *et al.* did not find difference in the BRAF-RAS-like score (a score based on mRNA expression) between *RAS*-mutated and *BRAF*-mutated ATCs (4).

In this study, we demonstrate that BRAF^{V600E} immunohistochemistry is a highly sensitive and specific marker for underlying BRAF^{V600E} mutations in ATC. Two previous smaller studies have reported similar findings, supporting the utility of BRAF^{V600E} immunostain as a rapid screening tool for underlying BRAF^{V600E} mutations in ATC patients (38,39). Recently, clinical trials have shown a dramatic response of *BRAF*-mutated ATC to combined MEK and BRAF-inhibitor therapy (9). Therefore, recognizing a link between squamous phenotype and *BRAF*-mutation status and the utility of BRAF^{V600E} immunostain in ATC may expedite the turnaround time for molecular testing and the selection of appropriate patients for FDA-approved dabrafenib and trametinib therapy.

Recently, it was reported that MMR protein deficiency can be seen in up to 14% of ATC and is associated with improved survival (40). In our cohort, eight cases (8%) contain somatic mutations in MMR machinery, including four affecting *MSH6*, two *MSH2*, one *MLH1*, and one *MSH6* and *MSH2*. All 8 tumors appear to be hypermutated with a mutation count of 9–34 (median 16). MMR deficiency does not correlate with any specific ATC phenotype and does not convey a survival advantage: the median survival was 10 months in this group ($p=0.182$ comparing ATC with and without MMR gene mutations).

Landa *et al.* have previously reported that co-occurrence of *BRAF*/*RAS* mutation and *TERT* promoter mutations is associated with a nonsignificant trend toward worse outcome compared with ATCs with *TERT* mutation alone, *BRAF*/*RAS* mutation alone, or neither mutation in 33 ATCs (4). Here, we demonstrate that ATCs with *TERT* mutations, in particular the *BRAF*/*RAS* and *TERT* double-mutants, are associated with a significantly worse outcome. It is postulated that *TERT* mutation generates binding motif of ETS families of transcription factors, a downstream effector of BRAF/*RAS* MAPK pathway, which in turn enhances *TERT* expression and results in the synergistic effects of *TERT* and *BRAF*/*RAS* mutations in ATC tumorigenesis (4).

In conclusion, based on the data from this large retrospective cohort of ATC with comprehensive clinical, pathologic, and molecular data, resectability and gross residual disease remain the most crucial indicators of outcome in ATC. It is important for pathologists to report encapsulation, margin, percentage, and size of ATC in a primary resection as they seem to provide prognostic information. The co-existence of *BRAF*/*RAS* and *TERT* promoter mutations is indicative of worse prognosis in ATC. The metastatic spread of *BRAF*-mutated and *RAS*-mutated ATCs is similar in contrast to its DTC counterparts. BRAF^{V600E} immunostain is a reliable and rapid tool to stratify ATC patient for BRAF^{V600E}-

targeted therapy. Finally, pure squamous cell carcinoma of thyroid may be considered as ATC given its frequent association with DTC, its underlying mutations affecting MAPK pathway, and its similar dismal outcome.

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Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4

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