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Primary High Grade Non-Anaplastic Thyroid Carcinoma: A Retrospective Study of 364 Cases

Bin Xu[1], **Julia David**[2], **Snjezana Dogan**[1], **Iñigo Landa**[3], **Nora Katabi**[1], **Maelle Saliba**[1], **Anjanie Khimraj**[1], **Eric J. Sherman**[4], **R Michael Tuttle**[2], **Giovanni Tallini**[5], **Ian Ganly**[6], **James A. Fagin**[2], **Ronald A Ghossein**[1]

[1]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[2]Division of Subspecialty Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[3]Department of Medicine, Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital, Boston, MD, USA

[4]Department of Medical Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[5]Pathology Unit, University of Bologna Medical Center, Bologna, Italy

[6]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract

Background: High grade non-anaplastic thyroid carcinomas (HGTC) are carcinomas of follicular cells with prognosis intermediate between well-differentiated and anaplastic carcinoma.

Methods: This study includes 364 HGTC patients: 200 patients (54.9%) were diagnosed as poorly differentiated thyroid carcinoma based on Turin consensus (HGTC-PDTC) and 164 with high grade features that did not meet Turin criteria (HGTC-nonPDTC).

Results: HGTC are aggressive: 3-year, 5-year, 10-year, and 20-year disease specific survival (DSS) were 89%, 76%, 60% and 35% respectively. Although DSS was similar between HGTC-PDTC and HGTC-nonPDTC, HGTC-PDTC was associated with higher rate of RAI avidity, higher frequency of *RAS* mutations, lower rate of *BRAF* V600E mutations, and higher propensity for distant metastasis (DM) compared with HGTC-nonPDTC. Independent clinicopathologic markers of worse outcome were older age, male sex, extensive necrosis, lack of encapsulation for DSS; older age, male sex, vascular invasion for DM free survival; older age, necrosis, positive margin, lymph node metastasis for locoregional recurrence free survival. The frequency of *BRAF*, *RAS*, *TERT*, *TP53*, and *PTEN* alterations was 28%, 40%, 55%, 11%, and 10%, respectively. *TP53*, *PTEN*, and *TERT* were independent molecular markers associated with unfavorable outcome independent of clinicopathologic parameters. Coexistence of *BRAF* V600E and *TERT* promoter mutation increased the risk of DM.

Corresponding authors: Ronald Ghossein, MD, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York, 10065, ghosseir@mskcc.org; Bin Xu, MD, PhD, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York, 10065, xub@mskcc.org.

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Conclusions: The above data supports the classification of high grade non-anaplastic thyroid carcinoma as a single group with two distinct subtypes based on tumor differentiation: HGTC-PDTC and HGTC-nonPDTC.

Keywords

High grade thyroid carcinoma; poorly differentiated thyroid carcinoma; *BRAF*; *RAS*; prognosis

Introduction

The prognosis of thyroid carcinomas of follicular cell derivation depends on histologic typing. At one end there are tumors that are well-differentiated with an overall favorable prognosis, at the other are tumors that are completely undifferentiated and almost universally fatal, i.e. anaplastic carcinoma. In between these extremes there are tumors with intermediate prognosis. How to recognize these tumors of intermediate prognosis has long been debated [1]. Consensus has been reached over the years that poor histologic differentiation is one reliable paradigm to identify such tumors [1]. Precise criteria, the Turin criteria, have been validated, and are endorsed by the World Health Organization (WHO) [2]. The Turin consensus defines a malignant follicular cell derived thyroid tumor as poorly differentiated thyroid carcinoma (PDTC) following three requirements: 1) solid, trabecular or insular growth pattern, 2) Absence of nuclear features of papillary thyroid carcinoma (PTC), and 3) at least one of the following features: convoluted nuclei, mitotic index ≥3/10 HPFs, and/or tumor necrosis.

In 2006, Hiltzik et al. from Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY, US) demonstrated that non-anaplastic thyroid carcinomas of follicular cells derivation with at least one of the following two pathologic features, the presence of elevated mitotic index ≥5/10 high power fields (HPFs) and/or tumor necrosis, have aggressive behavior with prognosis intermediate between that of conventional PTC and anaplastic carcinoma, regardless of the presence of poorly differentiated histology [3]. The prognostic relevance of these criteria (HGTC MSKCC criteria) has been repeatedly validated [1–7].

Both approaches identify thyroid tumors with intermediate prognosis [1–7]. Both take into consideration tumor necrosis and mitotic index, and therefore are best classified as HGTC. The Turin consensus has two mandatory components: solid, trabecular or insular (STI) architecture and lack of conventional PTC nuclear features. Consequently, the HGTC MSKCC criteria additionally capture a subgroup of tumors with necrosis and/or mitosis yet showing well-differentiated histology either because of conventional PTC nuclear features or because of a non-STI architecture (HGTC-nonPDTC). Indeed, several studies have shown that HGTC-PDTC virtually always meets the HGTC MSKCC, whereas 35% to 65% of HGTC classified using the MSKCC criteria do not fulfill the Turin consensus [7–10].

In the past decade, our understanding of the molecular pathogenesis and progression of thyroid carcinoma, including that of HGTC and PDTC, has increased significantly because of next generation sequencing [8, 9, 11–17]. It is now evident that there is a stepwise progression from well-differentiated to poorly differentiated or high grade, and finally to anaplastic thyroid carcinoma. While *BRAF* and *RAS* mutations remain the main

driver molecular alterations for all follicular cell-derived thyroid carcinoma, HGTC (and eventually anaplastic carcinoma) gain additional mutations leading to tumor progression and aggressiveness, e.g. *TERT* promoter mutation, *TP53* mutation, and alterations of PI3K-AKT-mTOR pathway [8, 9, 11–14, 17].

In this study, we gathered detailed characteristics of a large retrospective cohort of 364 patients with primarily resected HGTC. All tumors were further classified into HGTC-PDTC and HGTC-nonPDTC. The aims of the study were three-fold: 1) to describe the clinical behavior, pathologic features and molecular signatures of HGTC; 2) to identify the clinical, pathologic, and molecular prognostic factors in primary HGTC that may allow better risk stratification; 3) to define the genotype-phenotype correlation for HGTC-PDTC and HGTC-nonPDTC.

Materials and Methods

Characteristics of the study cohort and clinicopathologic review

The study was approved by the Institutional Review Board of MSKCC. Informed consent was not applicable for this specific study. A total of 364 patients with primary HGTC resected between 1981 and 2020 were included in this study. All cases were reviewed by at least one endocrine pathologist (BX or RG) blinded to the clinical outcome to confirm the HGTC diagnosis and gather detailed clinical and pathologic features.

HGTC was diagnosed using MSKCC criteria: the presence of elevated mitotic count ≥5/10 HPFs (total field size: 2.4 mm^2(i.e. >4 per 2 mm^2) and/or tumor necrosis (3) (Figure 1). Additionally, all tumors were further classified into HGTC-PDTC, i.e. high grade tumors that were poorly differentiated histologically according to Turin criteria [2], and HGTC-nonPDTC, i.e. high grade tumors that retained the distinctive architectural and/or cytologic features of well-differentiated histotypes of carcinoma of follicular cells throughout the tumor. Mitotic count was evaluated within the most mitotically active area (so called “hot spot”). Tumor necrosis was defined by the presence of clusters and sheets of ghost contours of dead tumor cells admixed with karyorrhectic debris. Infarct-type necrosis secondary to prior fine needle aspiration procedure was not considered tumor necrosis. Tumor necrosis was subdivided into focal (i.e. 1-2 spotty necrotic foci in not more than 2 tumor histology slides) or extensive (i.e. comedo or geographic-type necrosis, > 2 foci of necrosis per slide, and/or 1-2 spotty necrotic foci in more than 2 slides). Spotty necrosis was defined as a necrotic focus with less than 100 ghost tumor cells or less than 0.2 mm in greatest dimension.

Among the 220 patients with structural incomplete response who underwent radioactive iodine (RAI) scan post-operatively at our center, the results were reviewed by two endocrinologists (RMT and JD) and were classified as RAI-avid and not RAI-avid.

Molecular analysis

Molecular analysis was conducted in a subset of 166 cases using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT™) platform. MSK-IMPACT™ is a Food and Drug Administration (FDA)-approved deep-

coverage, targeted next-generation sequencing assay detecting single nucleotide variants (SNVs), small insertions/deletion (indels), copy number variants (CNVs) and select fusion/structural variants in 341 to 468 cancer related genes, using custom DNA probes designed for targeted sequencing of all exons and selected introns, including canonical and selected non-canonical transcripts [18, 19]. The molecular alterations of 76 of these 166 cases were previously reported by our group [9].

Clinical outcome and statistical analysis

Follow up data were available in 346 patients. The outcome collected included overall survival (OS), disease specific survival (DSS), distant metastasis free survival (DMFS), and locoregional recurrence free survival (LRRFS). The follow up was calculated from the date of primary resection. All statistical analyses were performed using the SPSS software 24.0 (IBM Corporation, Armonk, NY, U.S.). Univariate survival analysis was performed using log rank test for categorical variables and Cox proportional hazard model for continuous variables. Factors significant on univariate analysis were subsequently subjected to multivariate analysis using Cox proportional hazards model. Comparisons of clinicopathologic features between subgroups) were performed using Chi square test or Fisher's exact test for categorical variables and two-tailed Student's t test for continuous variables.

For the subgroup of 166 patients with molecular data, 165 had follow up data available. The prognostic values of the molecular alterations were determined using log rank test. Alterations significant on univariate analysis were subjected to multivariate analysis adjusted for age, gender, AJCC 8th edition pT stage and pN stage. P values less than 0.05 were considered to be statistically significant.

Results

Clinicopathologic characteristics

The clinical and pathologic features of the entire cohort are summarized in Table 1. The median age was 58 years (range: 5-90). There was a slight female predominance with a female to male ratio of 1.3:1. Most patients (303/364, 83.8%) underwent total thyroidectomy, whereas the remainder was subjected to lobectomy or hemithyroidectomy.

Given the study design, all tumors included in this study fulfilled the MSKCC criteria for HGTC. Consequently, all tumors showed at least one of the following two features: elevated mitotic count ≥5/10 HPFs (n=216, 59.3%) and/or tumor necrosis (n=277, 76.1%). Focal necrosis was found in 203 patients, and extensive necrosis in 74 patients.

Two hundred tumors (54.9%) were poorly differentiated histologically (HGTC-PDTC). The remaining 164 patients (45.1%) retained well-differentiated histology throughout, and could be classified as high grade PTC (n=155, 94.5%) or high grade follicular thyroid carcinoma (FTC, n=9, 5.5%). Among those tumors that may be regarded as high grade PTC, the most common variants in descending order were: tall cell variant (n=68), classic (n=29), infiltrative follicular variant (n=21), encapsulated follicular variant with capsular or vascular invasion (n=19), and encapsulated noninvasive follicular variant (n=3).The 9 cases of high-

grade FTC showed exclusive follicular architecture, lacked nuclear features of PTC, but demonstrated either tumor necrosis (n=7) and/or high mitotic index (n=6). Among them, 5 developed distant metastases, 4 suffered local recurrence, and 1 patient eventually died of the disease.

STI architecture was absent in 102 patients (38.6%). The median percentage of the STI component was 30% (range: 0 – 100%). Vascular invasion, microscopic extrathyroidal extension (ETE), gross ETE, high AJCC 8th pT stage (T3/T4), and nodal metastases were identified in 69.7%, 53.3%, 22.9%, 56.1% and 32.4%, respectively.

Encapsulated HGTC accounted for 36.5% (n=132) of the study cohort. Among them, 17 patients had encapsulated noninvasive HGTC, whereas the remaining 115 tumors harbored capsular (n=95) and/or vascular invasion (n=99).

HGTC-PDTC patients were statistically associated with larger tumor size, higher mitotic index, extensive tumor necrosis, a higher percentage of STI architecture, encapsulation, vascular invasion, microscopic extrathyroidal extension, higher AJCC pT stage, lower rate of nodal metastasis, and a propensity to receive radiation therapy and kinase inhibitor therapy ($p<0.05$, Table 1) when compared with those diagnosed as HGTC-nonPDTC.

Outcome and prognostic factors

Follow up data was available on 346 patients with a median follow up of 5.3 years (range: 0.2 – 29.6 years). Overall, HGTC patients had poor long-term survival: 3-year, 5-year, 10-year and 20-year OS were 88%, 75%, 54%, and 28% respectively; 3-year, 5-year, 10-year and 20-year DSS were 89%, 76%, 60% and 35%, respectively.

Distant metastases were frequent, being observed in 216 (62.4%) patients, including 84 (23.1%) with distant metastases at the initial presentation (defined as metastases to distant sites identified prior to or within 3 months of the primary thyroid resection). Eleven patients developed distant metastases more than 10 years after the thyroid resection. The 3-year, 5-year, 10-year, and 20-year DMFS was 53%, 46%, 31% and 22%, respectively. The most common sites of metastasis were lung (n=159), bone (n=88) and brain (n=26). Kaplan Meier curves for DSS, DMFS, and LRRFS are shown in Supplementary Figure 1.

Results of univariate survival analysis on DSS, DMFS, and LRRFS are shown in Table 2, and selected Kaplan-Meier curves are illustrated in Figure 2. Prognostic factors identified on univariate analysis to impart worse DSS, DMFS, and LRRFS were older age, male gender, large tumor size, tumor necrosis, lack of encapsulation, microscopic ETE, gross ETE, high AJCC 8th edition pT category, and positive margin.

Extensive tumor necrosis was associated with decreased DSS and DMFS, while vascular invasion (regardless of its extent was associated with worse DSS and DMFS. The extent of VI failed to predict DMFS in the entire cohort as well as in the subgroups of HGTC-PDTC and encapsulated HGTC.

Additional prognostic factors identified on univariate analysis that correlated with worse outcome included: High AJCC 8th pN stage for DSS and LRRFS; diagnosis of poorly

differentiated carcinoma following the Turin criteria (HGTC-PDTC), and presence of STI component for DMFS; larger size of the largest nodal metastasis for LRRFS. The 3-year, 5-year, 10-year, and 20-year DMFS for HGTC-PDTC were 49%, 40%, 24%, and 14%; and for HGTC-nonPDTC was 59%, 52%, 40%, and 32%. DSS and LRRFS did not differ between HGTC-PDTC and HGTC-nonPDTC: the 3-year, 5-year, and 10-year DSS were 79%, 70% and 60% for HGTC-nonPDTC and 74%, 68%, and 56% for HGTC-PDTC; whereas the 3-year, 5-year, and 10-year LRRFS was 88%, 76%, and 65% for HGTC-nonPDTC, and 90%, 77%, and 57% for HGTC-PDTC.

Within the subgroup of HGTC-PDTC, the presence of oncocytic features did not significantly impact outcomes, e.g. OS (p=0.085), DSS (p=0.144), and DMFS (p=0.971).

Postoperative treatment (e.g. RAI, chemotherapy, radiation therapy, and kinase inhibitors) was associated with decreased survival due to selection bias of patients with aggressive/recurrent disease receiving additional therapy.

The results of multivariate survival analysis using Cox proportional hazards model are shown in Table 3. Independent prognostic factors for worse DSS included older age (p<0.001, hazard ratio HR=1.038, 95% confidence interval CI 1.022-1.055), extensive tumor necrosis (p=0.010, HR=1.370, 95% CI 1.077-1.744), and lack of encapsulation (p=0.027, HR=0.464, 95% CI 0.235-0.916). Factors associated with decreased DMFS on multivariate analysis were older age (p<0.001, HR=1.506, 95% CI 1.258-1.802), male sex (p=0.012, HR=1.505, 95% CI 1.094-2.071), and vascular invasion (p=0.029, HR=1.593, 95% CI 1.047-2.423). Lastly, independent predictors for worse LRRFS were older age (p=0.039, HR=1.015, 95% CI 1.001-1.029), extensive tumor necrosis (p=0.001, HR=1.484, 95% CI 1.170-1.883), high AJCC pN stage (p<0.001, HR=2.656, 95% CI 1.590-3.347), and positive margin (p=0.046, HR=1.671, 95% CI 1.009-2.769).

RAI avidity in HGTC

Among the subgroup of 220 patients with structural incomplete response who underwent post-operative RAI scan, 116 tumors (52.7%) were RAI-avid. Compared with HGTC that were not RAI-avid, RAI avidity was associated with HGTC-PDTC histology (RAI-avid: 65%, not RAI-avid: 51%, p=0.042), higher rate of vascular invasion (RAI-avid: 84%, not RAI-avid: 69%, p=0.014), lower frequency of nodal metastasis (RAI-avid: 28%, not RAI-avid 40%, p=0.047, Supplementary Table 1). Among RAI-avid HGTC-nonPDTC, the most frequent histotypes were PTC follicular variant (19/41, 46%). In contrast, the most common histotype of HGTC-nonPDTC that were not RAI-avid was PTC tall cell variant (23/51, 45%). No other clinicopathologic features were associated with RAI avidity (p>0.05).

MSK-IMPACT was performed in a subgroup of 113 tumors with known RAI avidity status. RAI avidity was associated with higher frequency of *RAS* mutations (RAI-avid: 71%, not RAI-avid 31%, p<0.001) and lower rate of *BRAF* V600E mutation (RAI-avid: 8%, not RAI-avid 26%, p=0.013).

OS, DSS, and DMFS did not differ according to RAI-avidity. In contrast, RAI-avid HGTC was associated with improved LRRFS on univariate survival analysis using the log rank test (p=0.013).

HGTC in pediatric patients

Fifteen patients with HGTC were 21 years or younger. Among them, 8 were histologically poorly differentiated (HGTC-PDTC), whereas the remaining 7 cases were HGTC-nonPDTC and could be classified as high grade PTC (classic PTC: 2; diffuse sclerosing variant: 2; follicular variant: 2; tall cell variant: 1). Five of 15 pediatric patients, 3 HGTC-PDTC and 2 HGTC-nonPDTC, developed distant metastasis. Five tumors were encapsulated, including 1 noninvasive case. A 17-year-old female with a HGTC-PDTC had distant metastasis at presentation and died of disease 1-year after the initial diagnosis. Only one pediatric patient underwent molecular profiling. The patient was a 19-year-old female who had a 5-cm pT4a pN1a infiltrative HGTC-PDTC that harbored *EML4-ALK* fusion and *TERT* promoter mutation.

Outcomes of encapsulated noninvasive HGTC

In our cohort, 17 patients were diagnosed as encapsulated noninvasive HGTC based on the presence of nuclear features of PTC as well as high mitotic index and/or tumor necrosis. Eleven tumors had areas fulfilling Turin PDTC criteria except for the presence of invasion, while 6 were HGTC-nonPDTC. Among the 16 patients with follow up data, 3 developed distant metastases and 1 of them died of disease. The patient who died of disease was a 49-year-old male patient with an 8.0-cm HGTC-PDTC who developed distant metastases to bone, adrenal gland and liver 11.4 years after resection of the primary tumor, and eventually died 12.9 years after the initial diagnosis. The other two patients who developed distant metastasis harbored a 2.9 cm HGTC-nonPDTC NRASQ61R mutated and a 5.7 cm HGTC-PDTC. The tumor capsule of the 5.7 cm and 8.0 cm lesions was representatively sampled, whereas the 2.9 cm tumor was completely sampled for microscopic examination.

Molecular signatures of HGTC and their correlation with outcome

The molecular alterations detected in the subgroup of 166 patients whose tumors underwent molecular profiling using the MSK-IMPACT platform are shown in Figure 3 and Supplementary Figure 2.

Frequent molecular alterations in decreasing order of frequency were *TERT* 55%, *RAS* 40% (including *NRAS* 33%, *HRAS* 5% and *KRAS* 2%), *BRAF* 28%, *TP53* 11%, *PTEN* 10%, *E1F1AX* 8%, and *ATM* 6%.

Analysis for mutual-exclusivity showed that significantly mutually exclusive mutations in HGTC were *BRAF* and *NRAS* mutations ($p<0.001$, $q<0.001$); as well as *BRAF* and *PTEN* mutations ($p=0.003$, $q=0.039$). Significant co-concurrent alterations included *NRAS* and *E1F1AX* ($p<0.001$, $q=0.007$); *TP53* and *PTEN* ($p<0.001$, $q=0.007$); and *NRAS* and *TERT* mutations ($p<0.001$, $q=0.037$).

Mutations of *RMB10* occurred in seven HGTCs (2 HGTC-nonPDTC and 5 HGTC-PDTC). Among them, 5 had a co-existing *RAS* mutation, and none had *BRAF* alteration.

Fusion events were relatively uncommon, being detected in a total of 16 tumors (10%), including 5 with *RET* fusion (fusion partners: *CCDC6*, n=3; *NCOA4*, n=2), 4 with *PAX8-PPARG* fusion, 2 with *ALK* fusion (fusion partners: *EML4*, n=1; *CCDC149*, n=1), 1 with *NTRK3-EML4* fusion, 1 with *WDR89-NOTCH3* fusion, and intragenic fusions for *TSHR*, *TERT*, and *TP53* (one case each).

Compared with HGTC-nonPDTC, HGTC-PDTC were associated with significantly lower frequency of *BRAF* alterations (HGTC-nonPDTC: 53%, HGTC-PDTC 9%, Fisher's exact test, $p<0.001$), a higher rate of *RAS* (HGTC-nonPDTC: 30%, HGTC-PDTC: 48%, $p=0.021$), *TP53* (HGTC-nonPDTC: 4%, HGTC-PDTC 16%, $p=0.021$), *PTEN* (HGTC-nonPDTC: 1.4%, HGTC-PDTC: 16%, $p=0.001$), and *E1F1AX* mutations (HGTC-nonPDTC: 2.7%, HGTC-PDTC: 13%, $p=0.024$). *BRAF* alterations observed in the HGTC-PDTC group included V600E (n=5), K601E (n=1), V600_K601delinsE (n=1), and V600_S602del (n=1) variants.

When compared to *BRAF* V600E-mutated HGTC, *RAS*-mutants HGTC were associated with a HGTC-PDTC phenotype (67% vs. 11%, Fisher's exact test, $p<0.001$, Supplementary Table 2), higher frequency of vascular invasion (92% vs. 45%, Fisher's exact test, $p<0.001$), distant metastases (89% vs. 50%, log rank test, $p<0.001$), and a lower rate of nodal metastases at initial surgery (18% vs. 57%, Fisher's exact test, $p<0.001$). DSS (log rank test, $p=0.777$) and other clinicopathologic parameters ($p>0.05$, data not shown) did not differ between *RAS*- and *BRAF* V600E-mutants.

The results of univariate and multivariate survival analyses of various molecular alterations are shown in Tables 2 and 4. On univariate analysis, molecular markers associated with unfavorable outcome included: *RAS/BRAF* mutations for DMFS; *TERT* promoter mutation for DMFS and LRRFS; *TP53* and *PTEN* mutations for reduced DSS, DMFS and LRRFS. Multivariate analysis of those molecular markers associated with unfavorable outcome after univariate analysis showed independent prognostic value for the following mutations: *TP53* for DSS; *BRAF/RAS, TERT* promoter, *TP53* and *PTEN* for DMFS; *TERT*, *TP53*, and *PTEN* mutations for LRRFS ($p<0.05$).

Independent molecular markers after multivariate analysis adjusted for age, sex, AJCC pT and pN stage were: *TP53* mutation for DSS (HR=3.196, 95% CI 1.585-6.444, $p=0.001$, Figure 3) and LRRFS (HR=2.721,95% CI 1.353-5.472, $p=0.005$); *PTEN* mutation for DSS (HR=2.821, 95% CI 1.221-6.516, $p=0.015$), DMFS (HR=2.042, 95% CI 1.154-3.611, $p=0.014$) and LRRFS (HR=2.927, 95% CI 1.382-6.200, $p=0.005$); *TERT* promoter mutations for DMFS (HR=1.468, 95% CI 1.004-2.147, $p=0.048$). Additional molecular alterations failed to reach statistically significant values after multivariate analysis.

Lastly, we studied the combined impact of *RAS, BRAF* and *TERT* promoter mutation on DMFS. Tumors with *BRAF* V600E alone (not combined with *TERT* promoter mutation) carried a significant lower risk of DM when compared with all other subgroups after

univariate log rank pairwise test (compared with *RAS* group: p<0.001; *TERT*: p=0.001; *BRAF* V600E and *TERT*: p=0.002; *RAS* and *TERT*: p<0.001; none p=0.015) (Figure 3).

Discussion

In this large retrospective study, the largest published on the subject, we further confirm that HGTC MSKCC criteria identify a group of thyroid carcinomas with prognosis intermediate between that of well-differentiated and anaplastic carcinoma characterized by poor long-term survival and a high propensity for distant metastases. The disease specific mortality rate at 20 years is 65%, similar to the 57% to 86% mortality rate reported in HGTC-PDTC diagnosed following the Turin consensus [2, 20]. Additionally, we show that HGTC has a high propensity for distant metastases (including late metastases): the rate of distant spread is 47% at 3 years and 78% at 20 years, highlighting the need for long term follow up for the identification of distant metastases in these patients.

Independent adverse prognostic clinicopathologic factors identified include older age (for DSS, DMFS, and LRRFS), extensive tumor necrosis (for DSS and LRRFS), lack of encapsulation (for DSS), the presence of vascular invasion (for DMFS), positive margin (for LRRFS), and nodal metastasis (for LRRFS). Two previous studies from our group showed that encapsulated HGTC were associated with a more favorable outcome compared with unencapsulated HGTC [3, 4], which is further confirmed by the results of the current study. However, it is worthwhile to mention that even encapsulated HGTC devoid of invasion still carry a risk of distant metastasis and mortality. In our cohort, among the 16 patients with encapsulated non-invasive HGTC and follow up data, 3 developed distant metastases whereas 1 eventually died of the disease.

Contrary to previous studies from our group which demonstrated that extensive vascular invasion was associated with decreased disease free survival in encapsulated low and high grade thyroid carcinoma [4, 21], we herein report that the presence but not the extent of vascular invasion is an independent prognostic factor for distant metastasis free survival. Such prognostic difference for vascular invasion may be related to different tumor biology (low grade vs. high grade thyroid carcinoma) and the presence vs. absence of encapsulation. Recently, Wong et al. showed that poorly differentiated carcinoma with extensive vascular invasion or those which were widely invasive were associated with decreased disease free survival compared with similar tumors with capsular or focal vascular invasion in a cohort of 47 cases classified as poorly differentiated using the Turin criteria [22]. These discrepant results may be explained by several differences between the two studies: 1) the cohort size differed (364 vs. 47); 2) the current study included HGTC according to the MSKCC criteria whereas Wong et al. only included HGTC-PDTC; 3) the outcome measures studied were OS, DSS, DMFS, and LRRFS in the current study; and disease free survival in Wong et al.; and 4) we performed univariate pairwise comparisons between any of the two groups as well as multivariate survival analysis, whereas Wong et al. performed univariate survival analysis across all groups studied. Further studies are needed to determine the prognostic significance of the extent of vascular invasion in PDTC and HGTC.

Pediatric HGTC is rare [23, 24]. In our cohort, 15 HGTCs occurred in patients with an age of 21 years or younger accounting for 5.7% of the entire cohort. Balanchandar et al. showed that pediatric differentiated thyroid carcinoma, including HGTC following MSKCC criteria, carries an overall excellent prognosis [23]. Similarly, we demonstrate the important prognostic significance of age in HGTC: the pediatric age group and young adults (22 to 34-year-old) have an improved DSS, DMFS, and LRRFS compared to other age groups. Patients ≥55-year-old have worst prognosis among all age groups studied. Nevertheless, pediatric HGTC does not always follow an indolent clinical course. Our data highlight the potential of pediatric HGTC to develop distant metastases (5 of 15 cases) and even cause tumor-related death (1 of 15 cases).

In the current cohort of 364 patients, we demonstrate that extensive tumor necrosis is an independent adverse prognostic factor, whereas there was no prognostic difference between tumors with no necrosis and those with focal necrosis. Our group previously reported that the extent of tumor necrosis did not impact OS and progression free survival in a cohort of 58 HGTC [3]. This difference may be explained by the large difference in cohort size between our previous and the current study. Based on these findings, we recommend documenting the presence of extensive tumor necrosis in the pathology report of HGTC.

Over the past decade, the molecular profile of over 200 HGTC has been reported using targeted next generation sequencing [8, 9, 11–14]. Cumulative data indicate that both HGTC-PDTC and HGTC-nonPDTC represent a progression from well-differentiated thyroid carcinomas. *BRAF* V600E and *RAS* mutations remain mutually exclusive main drivers in HGTC, each being detected in approximately 25% of HGTC. Furthermore, HGTC accumulates additional mutations and aggressive molecular signatures, e.g. *TERT* promoter mutation in 42%, *TP53* mutation in 17%, *E1F1AX* mutation in 14%, *PIK3CA* mutation in 6%, and *PTEN* mutation in 3% [8, 9, 11–14]. We herein presented the largest cohort of HGTC sequenced to date, and our data validate many that have been previously reported. The frequency of *BRAF*, *RAS*, *TERT*, *TP53*, *PTEN*, *E1F1AX*, and *PIK3CA* alteration in this HGTC cohort is 28%, 40%, 55%, 11%, 10%, 8%, and 3% respectively. *RBM10* is an RNA binding protein which participates in alternative pre-mRNA splicing. Mutation of *RBM10, a molecular event* which occurs at high frequency (11%)in fatal non-anaplastic thyroid carcinoma, indicates tumor virulence [25], was detected in 4% of HGTC and was mutually exclusive with *BRAF* V600E.

Several similarities and differences emerge when comparing the genotype and phenotype of HGTC-nonPDTC and HGTC-PDTC. Evidently, both subgroups are clinically aggressive, with similar disease specific survival and locoregional recurrence rates. Similarly, Wong et al. reported that high grade PTC was associated with aggressive clinical behaviors and worse DSS compared to HGTC-PDTC [26]. Certain clinical features (e.g. age and sex) do not differ significantly between the two groups. *TERT* promoter mutation, a molecular marker of poor prognosis, is identified at similar frequency in both groups. On the other hand, HGTC-PDTC is characterized by a significantly higher frequency of vascular invasion, tumor encapsulation, *RAS*, *TP53*, *PTEN*, and *E1F1AX* mutations, a lower rate of *BRAF* V600E, large tumor size, higher pT stage, lower rate of nodal metastases, and an increased propensity for distant metastasis (86% vs. 68% at 20 years) compared with

HGTC-nonPDTC. Landa et al. also previously reported that HGTC-PDTC was associated with frequent *RAS* mutation, rare *BRAF* mutations and a RAS-like *BRAF-RAS score* (BRS); while the HGTC-nonPDTC were enriched with *BRAF* V600E mutation, had a low frequency of *RAS* mutations and a BRAF-like *BRS* [9]. Together, these genomic data strongly suggest that HGTC-PDTC is the aggressive poorly differentiated counterpart of follicular carcinoma and follicular variant of PTC, whereas HGTC-nonPDTC is the aggressive form of papillary thyroid carcinoma, in particular its tall cell variant. Taken together, our data support a new classification under the umbrella of high-grade follicular cell-derived thyroid carcinomas to include two subgroups: 1) Poorly differentiated thyroid carcinomas based on the Turin consensus (HGTC-PDTC) and 2) HGTC based on MSKCC criteria that do not meet the Turin criteria because the tumor retains well differentiated histology (HGTC-nonPDTC). A better term for the latter would be high grade differentiated thyroid carcinoma. This is the first study to report a definite correlation between RAI avidity and various pathologic features in a large cohort of 220 HGTC. We show that RAI-avidity in HGTC is associated with HGTC-PDTC, high grade PTC follicular variant, *RAS* mutation, and vascular invasion, as well as a decreased frequency of *BRAF* V600E mutation, high grade PTC tall cell variant, nodal metastasis, and locoregional recurrence. These results further support our previous observation that *RAS* mutation is prevalent in RAI-avid metastatic differentiated thyroid carcinoma [27] and that *RAS*-mutated HGTC are associated with high thyroid differentiation score (9), which preserves in principle their ability to concentrate iodine.

It is worthwhile to mention that there were histologic heterogenicity within each group of HGTC. HGTC-PDTC can be further subdivided into those with and those without Oncocytic (Hurthle cell) features [28], whereas HGTC-nonPDTC may be additionally classified using cytologic features and architecture into those with follicular architecture (e.g. follicular variant of PTC and FTC) and those of PTC classic and tall cell phenotypes.

This study is also the first to report several independent adverse prognostic molecular events in HGTC after multivariate analysis. *TP53* and *PTEN* mutations predict worse DSS and LRRFS, and *TERT* promoter mutation decreased DMFS after adjusting for common clinicopathologic prognostic factors, e.g. age, gender, and stage. Previously, Landa et al. showed that high mutation count, *EIF1AX* mutation, *TERT* promoter mutations, and chromosome 1q gain are associated with decreased OS [9]. However, the authors did not perform multivariate analysis, possibly due to the small cohort size. We herein confirm the prognostic significance of *TERT* promoter mutation in HGTC.

In our cohort, the rate of distant metastases differs according to the underlying molecular signature, being highest in the *RAS*-mutants, lowest in the *BRAF* V600E-mutants, and intermediate in those without either mutations, confirming the reported association between *RAS* mutation and hematogenous spread in HGTC reported by Landa et al. [13]. Interestingly, the addition of *TERT* promoter mutation seems to promote distant spread in *BRAF* V600E-mutated HGTC. While *BRAF* V600E-mutated HGTC is associated with better DMFS, those HGTC with concomitant *BRAF* V600E and *TERT* promoter mutations have similar risk of distant metastasis compared with *RAS*-mutated HGTC (with or without *TERT* promoter mutation).

In conclusion, in this comprehensive retrospective study of 364 patients with resected primary HGTC, we show that HGTC is associated with poor long-term survival and high risk of distant metastasis. Independent prognostic factors of poor outcome in HGTC include older age, male sex, lack of encapsulation, extensive tumor necrosis, vascular invasion, positive margin, and nodal metastasis. Additionally, we identified *TP53*, *PTEN* and *TERT* promoter mutations as independent adverse prognostic molecular signatures in HGTC. Although the overall prognosis is similar between HGTC-PDTC and HGTC-nonPDTC, HGTC-PDTC is associated with higher propensity for distant metastasis, as well as a higher frequency of *RAS*, *TP53*, *PTEN*, and *EIF1AX* mutations. Although pediatric and encapsulated non-invasive HGTC have significantly better outcomes compared to other HGTC, these rare subgroups of HGTC can give rise to distant metastases and lead to the patients' demise. A new classification is needed under the category of high grade non-anaplastic follicular cell-derived thyroid carcinoma that will emphasize the aggressive behavior of these tumors. Further subdividing them into HGTC-PDTC for those tumors that have poorly differentiated histology (Turin criteria) and high grade differentiated thyroid carcinoma for those that are high grade but retain well-differentiated cytoarchitectural features will reflect the clinical, histological and molecular differences between the 2 subtypes of these high grade thyroid carcinomas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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BX: study design, case review, database management, statistics, and manuscript drafting, JD: chart review for RAI data, SD and IL: molecular analysis and MSK-IMPACT, NK and MS: manuscript editing, AK: database management, EJS and IG: clinical inputs and manuscript editing, RMT: clinical input, RAI data collection, and manuscript editing, GT: conception and manuscript editing, JAF: molecular analysis, clinical input and manuscript editing, RAG: study design, case review, and manuscript editing.

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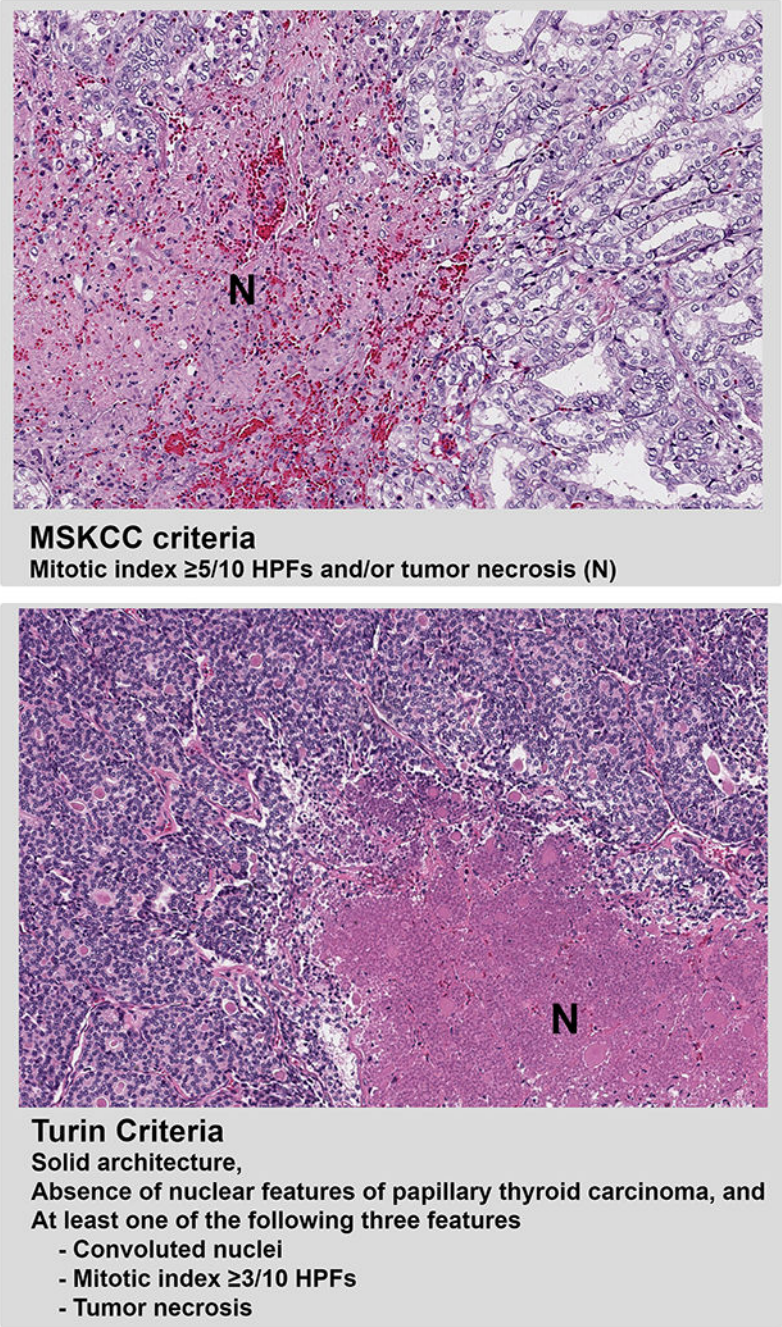

Figure 1. Diagnostic criteria for high grade thyroid carcinoma (HGTC) using MSKCC criteria and poorly differentiated thyroid carcinoma using Turin proposal.

The study cohort includes 364 patients with resected primary HGTC defined using the MSKCC criteria. Among them, 200 (54.9%) additionally meet the Turin criteria. HPFs: high power fields, N necrosis.

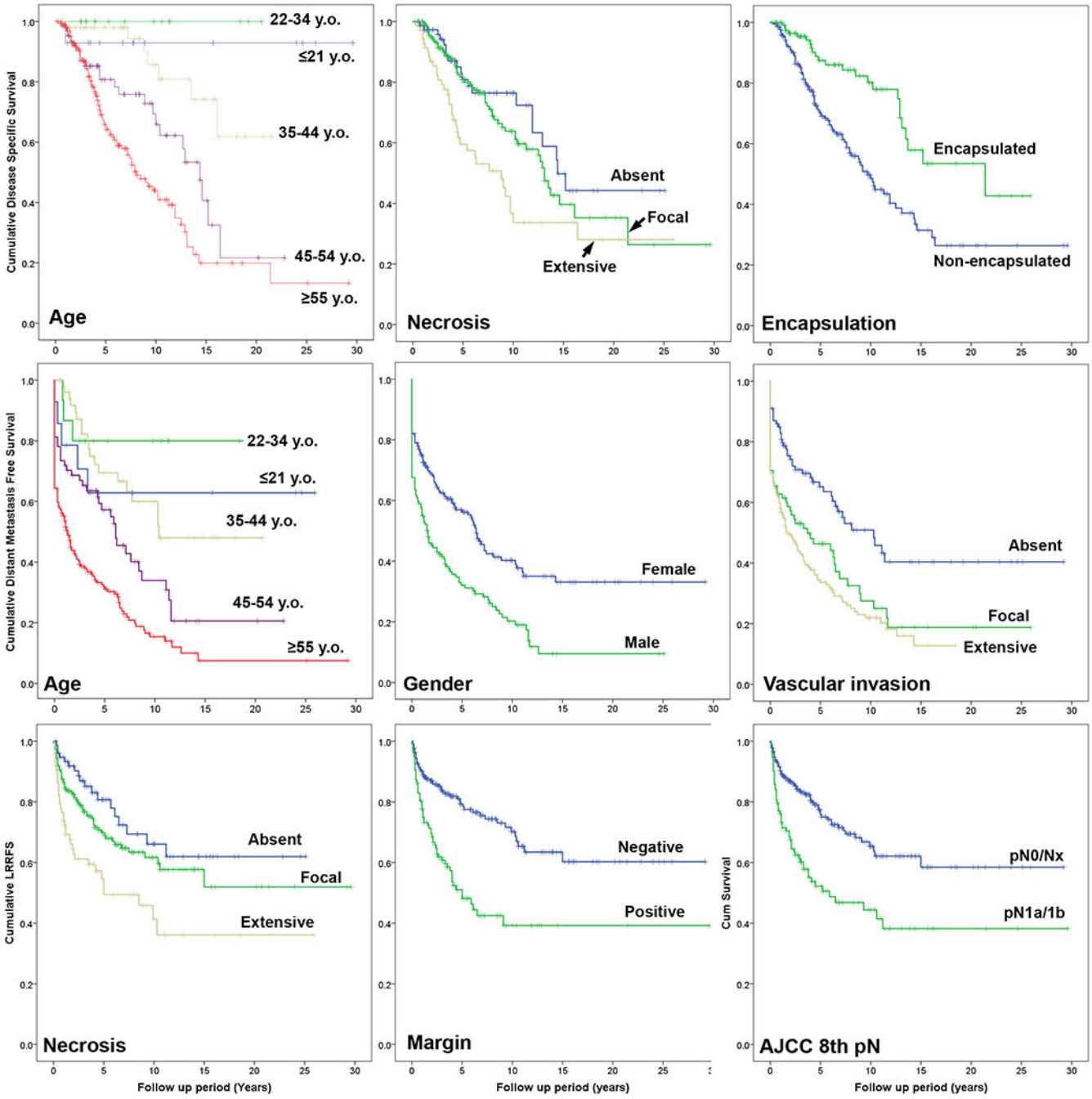

Figure 2. Kaplan-Meier curves for disease specific survival (top row), distant metastasis free survival (middle row), and locoregional recurrence free survival (LRRFS, bottom row).

$P < 0.05$ on univariate survival analysis using log rank test and multivariate analysis using Cox proportional hazards model (see Tables 2 and 3 for details). Y.o: year-old.

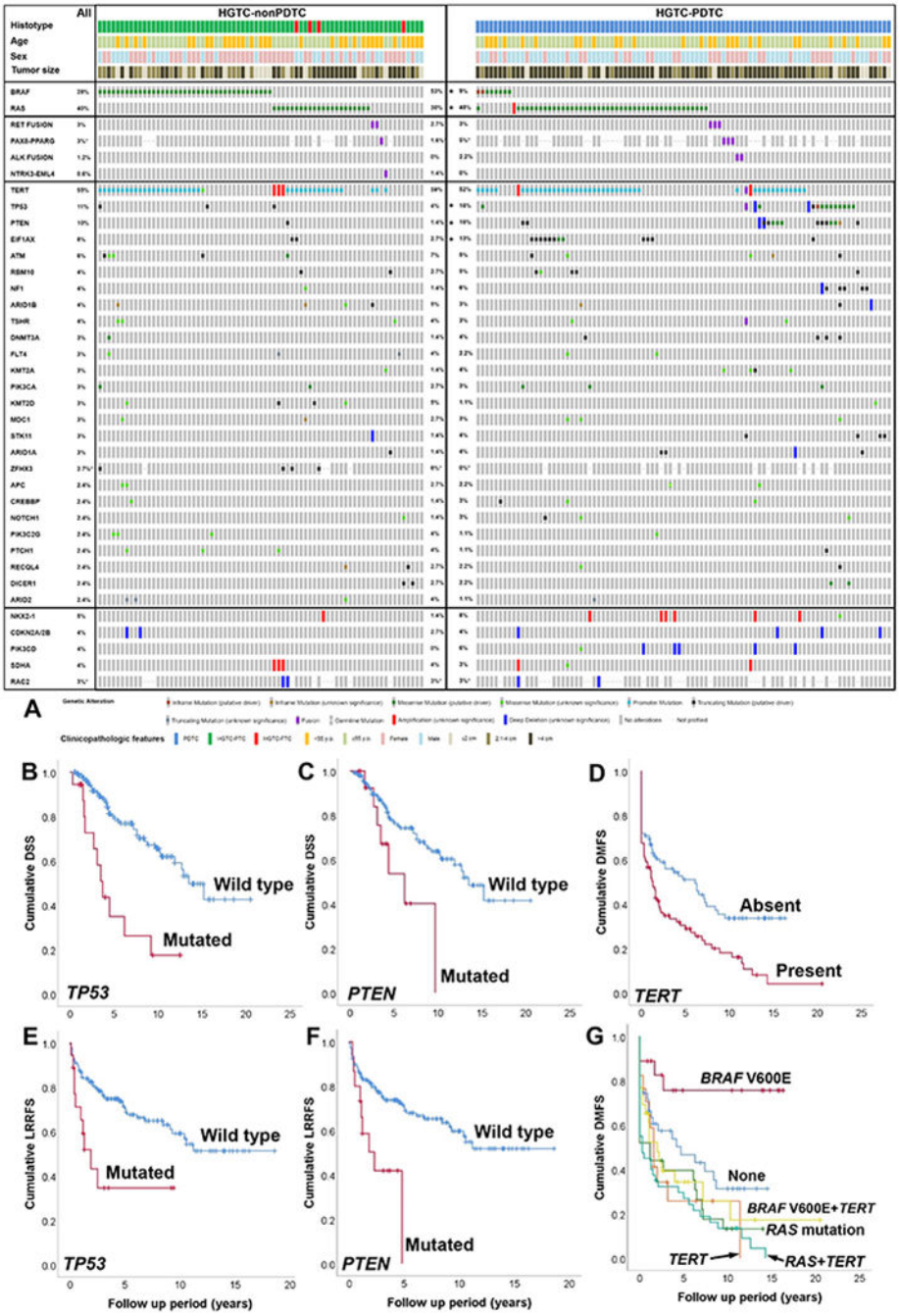

Figure 3. Molecular signatures of high grade thyroid carcinoma (HGTC).

(A) Oncoprint of 166 patients with HGTC diagnosed using MSKCC criteria. Genes are grouped from top to bottom as *BRAF/RAS* signature, fusions, mutations, and copy number alteration. All tumors are further classified as HGTC-PDTC and HGTC-nonPDTC using Turin criteria. *: Significant difference between HGTC-PDTC and HGTC-nonPDTC ($p<0.05$). PTC: papillary thyroid carcinoma, FTC: follicular thyroid carcinoma. (B-G) Kaplan Meier curves for disease specific survival (DSS), distant metastasis free survival (DMFS), and locoregional recurrence free survival (LRRFS). *TP53* (B and E) and *PTEN* (C

and F) mutations are independent prognostic factors for DSS and LRRFS, whereas *TERT* promoter mutation independently predicts DMFS (D, $p<0.05$, see Table 4). HGTC with $BRAF^{V600E}$ mutation alone is associated with an improved DMFS when compared with the other HGTCs (log rank pairwise test, $p<0.05$) (G).

Table 1.

Clinicopathologic characteristics of 364 patients with resected primary high grade non anaplastic thyroid carcinoma (HGTC).

		All (n=364)	HGTC-nonPDTC (n=164)	HGTC-PDTC (n=200)	P value
Clinicopathologic parameters					
Age (years)	Median (range)	58 (5-90)	55 (9-90)	59 (5-87)	NS
	≤21	15 (4.1%)	7 (4.3%)	8 (4.0%)	
	22-34	18 (4.9%)	9 (5.5%)	9 (4.5%)	
	35-44	57 (15.7%)	32 (19.5%)	25 (12.5%)	
	45-54	68 (18.7%)	33 (20.1%)	35 (17.5%)	
	≥55	206 (56.6%)	83 (50.6%)	123 (61.5%)	
Sex	Female	208 (57.1%)	103 (62.8%)	105 (52.5%)	NS
	Male	156 (42.9%)	61 (37.2%)	95 (47.5%)	
Distant metastasis at presentation		84 (23.1%)	31 (19.9%)	53 (27.9%)	
RAI avidity (n=220)		116/220 (52.7%)	41/92 (42%)	75/128 (64.7%)	0.042
Surgical type	Lobectomy/ hemithyroidectomy	59 (16.2%)	26 (15.9%)	33 (16.5%)	
	Total/subtotal thyroidectomy	303 (83.8%)	138 (84.1%)	167 (83.5%)	
Tumor size (cm)	Median (range)	4.0 (0.3-14.5)	3.0 (0.3-10.5)	4.7 (1.0-14.5)	<0.001
	≤2cm	57 (16.0%)	37 (23.4%)	20 (10.1%)	
	2.1-4cm	125 (35.0%)	66 (41.8%)	59 (29.6%)	
	>4cm	175 (49.0%)	33 (34.8%)	120 (60.3%)	
Classification of HGTC-nonPDTC	PTC tall cell variant	68	68	NA	NA
	PTC follicular variant	43	43		
	PTC classic type	29	29		
	Follicular carcinoma (FTC)	9	9		
	PTC columnar variant	5	5		
	PTC diffuse sclerosing variant	5	5		
	PTC hobnail variant	3	3		
	PTC solid variant	2	2		
STI pattern, %, median (range)		30% (0-100%)	0% (0-100%)	70% (0-100%)	<0.001
Mitotic index	<5/10 high power fields	148 (40.7%)	80 (48.8%)	68 (34.0%)	0.005
	≥5/10 high power fields	216 (59.3%)	84 (51.2%)	132 (66.0%)	
Tumor necrosis	Absent	87 (23.9%)	40 (24.4%)	47 (23.5%)	0.010
	Focal	203 (55.8%)	102 (62.2%)	101 (50.5%)	
	Extensive	74 (20.3%)	22 (13.4%)	52 (26.0%)	
Encapsulation	Non-encapsulated	230 (63.5%)	126 (77.3%)	104 (52.3%)	<0.001
	Encapsulated	132 (36.5%)	37 (22.7%)	95 (47.7%)	
Capsular invasion in encapsulated lesions (CI, n=130)	Absent	35 (26.9%)	11 (29.7%)	24 (25.8%)	NS
	Focal CI (1-3 foci)	53 (40.8%)	15 (40.5%)	38 (40.9%)	
	Extensive CI (≥4 foci)	42 (32.3%)	11 (29.7%)	31 (33.3%)	
Vascular invasion (VI, n=363)	Absent	110 (30.3%)	81 (49.7%)	29 (14.5%)	<0.001

		All (n=364)	HGTC-nonPDTC (n=164)	HGTC-PDTC (n=200)	P value
	Focal VI (1-3 foci)	83 (22.9%)	38 (23.3%)	45 (22.5%)	
	Extensive VI (≥4 foci)	170 (46.8%)	33 (27.0%)	126 (63.0%)	
Microscopic extrathyroidal extension (ETE)	Absent	168 (46.7%)	55 (33.7%)	113 (57.4%)	<0.001
	Fibroadipose tissue	113 (31.4%)	60 (36.8%)	53 (26.9%)	
	Skeletal muscle	62 (17.2%)	38 (23.3%)	24 (12.2%)	
	Other organs	17 (4.7%)	10 (6.1%)	7 (3.6%)	
Gross ETE (n=340)	Absent	262 (77.1%)	114 (75.0%)	150 (79.8%)	NS
	Present	78 (22.9%)	38 (25.0%)	38 (20.2%)	
Margin status (n=350)	Negative	227 (64.9%)	101 (63.9%)	126 (65.6%)	
	Positive	123 (35.1%)	57 (36.1%)	66 (34.4%)	
AJCC 8th pT stage	pT1	59 (16.3%)	37 (22.7%)	22 (11.1%)	<0.001
	pT2	100 (27.6%)	49 (30.1%)	51 (25.6%)	
	pT3	155 (42.8%)	53 (32.5%)	102 (51.3%)	
	pT4	48 (13.3%)	24 (14.7%)	24 (12.1%)	
AJCC 8th pN stage	pN0/pNx	246 (67.6%)	82 (50.0%)	164 (82.0%)	<0.001
	pN1a/pN1b	118 (32.4%)	82 (50.0%)	36 (18.0%)	
Number of positive lymph nodes (n=118)	1-4	69 (58.5%)	45 (54.9%)	24 (66.7%)	NS
	≥5	49 (41.5%)	37 (45.1%)	12 (33.3%)	
Size of largest metastatic focus (n=115)	<1 cm	37 (32.2%)	27 (34.2%)	10 (27.8%)	NS
	≥1cm	78 (67.8$)	52 (65.8%)	26 (72.2%)	
Extranodal extension (n=118)	Absent	52 (48.1%)	37 (49.3%)	15 (45.5%)	NS
	Present	56 (51.9%)	38 (50.7%)	18 (54.5%)	
Follow up and treatments (n=346)					
Follow up period, years, median (range)		5.3 (0.2-29.6)	5.4 (0.2-29.2)	5.1 (0.3-29.6)	NS
Radioactive iodine therapy (RAI)		271 (78.3%)	121 (77.6%)	150 (78.5%)	NS
Radiation therapy		144 (41.6%)	55 (35.3%)	89 (46.8%)	0.036
Kinase inhibitors		94 (27.2%)	29 (17.7%)	65 (32.5%)	0.002
Chemotherapy		60 (17.3%)	25 (16.0%)	35 (18.4%)	NS
Anaplastic transformation in recurrence		10 (2.9%)	5 (3.0%)	5 (2.5%)	NS

HGTC-PDTC: poorly differentiated thyroid carcinomas based on Turin criteria; HGTC-nonPDTC: high grade thyroid carcinoma with well-differentiated histology; PTC: papillary thyroid carcinoma; RAI: radioactive iodine; NS: not significant; NA: not applicable; STI: solid, trabecular or insular growth.

Table 2.

Univariate survival analysis for disease specific survival (DSS), distant metastasis free survival (DMFS), and locoregional recurrence free survival (LRRFS) in high grade non-anaplastic thyroid carcinoma.

	DSS	DMFS	LRRFS
Clinical parameters			
Age (as continuous variable)	<0.001	<0.001	0.002
Sex	<0.001	<0.001	0.018
RAI avidity	NS	NS	0.013
RAI	NS	0.023	0.035
Chemotherapy	<0.001	<0.001	<0.001
Radiation	<0.001	<0.001	<0.001
Kinase inhibitors	<0.001	<0.001	0.002
Surgery type	NS	<0.001	NS
Pathologic parameters			
Size (as continuous variable)	<0.001	<0.001	0.004
Mitosis (<5/10 HPFs. Vs. ≥5/10 HPFs)	NS	NS	NS
Necrosis			
Absent vs. Focal	NS	NS	NS
Absent vs. extensive	0.003	<0.001	0.001
Focal vs. extensive	0.005	0.005	0.005
Encapsulation	<0.001	0.001	<0.001
Capsular invasion	NS	NS	NS
Vascular invasion			
Absent vs. focal	NS	0.002	NS
Absent vs. extensive	0.018	<0.001	NS
Focal vs. extensive	NS	NS	NS
Turin criteria	NS	0.006	NS
Percentage of STI component (as continuous variable)	NS	0.009	NS
Microscopic ETE	<0.001	0.001	<0.001
Gross ETE	<0.001	<0.001	0.015
AJCC 8th pT (T1/T2 vs. T3/T4)	<0.001	<0.001	0.009
AJCC 8Th pN (N0/Nx vs. N1a/N1b)	0.022	NS	<0.001
Margin status	<0.001	<0.001	<0.001
Number of positive lymph nodes (1-4 vs. ≥5)	NS	NS	NS
Size of largest metastasis (<1 cm vs. ≥1 cm)	NS	NS	0.017
ENE	NS	NS	NS
Molecular signatures (n=165)			
RAS or $BRAF^{V600E}$ mutation	NS	0.001	NS
TERT promoter mutation	NS	0.001	0.003
TP53 alteration	<0.001	0.010	0.002
PTEN mutation	0.012	0.003	0.002
Other alterations	NS	NS	NS

Values are p values obtained using log rank test for categorical variables and Cox proportional hazards model for continuous variable. RAI: radioactive iodine therapy. NS: not significant, HPFs: high power fields, ETE: extrathyroidal extension, PTC: papillary thyroid carcinoma; STI: solid, trabecular or insular growth.

Table 3.

Multivariate survival analysis using Cox proportional hazards model.

	P value	Hazard ratio (95% CI)
Disease specific survival		
Age (as continuous variable)	**<0.001**	1.038 (1.022-1.055)
Tumor size (as continuous variable)	0.736	1.016 (0.926-1.115)
Sex	0.095	1.464 (0.936-2.292)
Tumor necrosis (absent/focal vs. extensive)	**0.010**	1.370 (1.077-1.744)
Encapsulation	**0.027**	0.464 (0.235-0.916)
Vascular invasion (present vs. absent)	0.948	1.018 (0.585-1.772)
Microscopic ETE	0.633	1.184 (0.591-2.373)
Gross ETE	0.979	1.008 (0.571-1.779)
AJCC 8th pT	0.089	1.344 (0.956-1.890)
N stage	0.251	1.339 (0.813-2.206)
Margin status	0.617	1.134 (0.693-1.858)
Distant metastasis free survival		
Age (as continuous variable)	**<0.001**	1.506 (1.258-1.802)
Tumor size (as continuous variable)	0.695	0.985 (0.911-1.064)
Sex	**0.012**	1.505 (1.094-2.071)
Tumor necrosis (absent/focal vs. extensive)	0.212	1.122 (0.937-1.343)
Encapsulation	0.113	0.698 (0.447-1.089)
Vascular invasion (present vs. absent)	**0.029**	1.593 (1.047-2.423)
Turin criteria	0.902	1.025 (0.690-1.522)
Microscopic ETE	0.409	0.840 (0.555-1.271)
Gross ETE	0.197	1.354 (0.854-2.147)
AJCC 8th pT	0.110	1.205 (0.959-1.514)
Margin status	0.466	1.154 (0.785-1.694)
Locoregional recurrence free survival		
Age (as continuous variable)	**0.039**	1.015 (1.001-1.029)
Tumor size (as continuous variable)	0.073	1.094 (0.992-1.207)
Sex	0.851	1.042 (0.678-1.601)
Tumor necrosis (absent/focal vs. extensive)	**0.001**	1.484 (1.170-1.883)
Encapsulation	0.230	0.668 (0.345-1.291)
Microscopic ETE	0.845	0.934 (0.472-1.847)
Gross ETE	0.128	0.646 (0.368-1.134)
AJCC 8th pT	0.244	1.214 (0.876-1.683)
N stage	**<0.001**	2.656 (1.590-3.347)
Margin status	**0.046**	1.671 (1.009-2.769)

Bold p values: significant p values. CI: confidence interval.