




TERTp mutation is associated with a shorter progression free survival in patients with aggressive histology subtypes of follicular-cell derived thyroid carcinoma

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Abstract

Purpose Evaluate the impact of TERTp mutation on the outcomes after initial treatment of 45 patients with thyroid carcinomas derived from follicular cells (TCDFC) with aggressive histology, in which the role of this mutation is not yet well defined.

Methods Analysis of the presence of TERTp (−124C > T and −146C > T), BRAF (V600E), and NRAS (Q 61R) mutations by Sanger sequencing and analysis of their correlation with the patient's outcomes.

Results Forty-five patients with aggressive histopathologic variants were included in the study. Of these, 68.9% had aggressive variants of papillary thyroid cancer (PTC), 22.2% had poorly differentiated thyroid carcinoma (PDTC)/insular carcinoma, and 8.9% had invasive follicular thyroid cancer (FTC) with Hurthle cell features (Hurthle cell carcinoma). Lymph node metastases were present in 46.7% and distant metastases in 54.6%. The response to the initial therapy was excellent in 45.5% and structurally incomplete in 50%. During the follow-up period (median of 56 months; 5–360 months), 47.7% presented with disease progression and 17.8% experienced disease-related death. In 53.3% of the cases at least one molecular alteration (TERTp in 33.4%, BRAF in 24.5%, RAS in 8.9%) was detected. In the multivariate analysis, TERTp mutation was the factor associated with the highest risk (6 times) of having structural disease after initial therapy ($p = 0.01$), followed by vascular invasion ($p = 0.02$), gross extrathyroidal extension (ETE) ($p = 0.02$) and distant metastasis ($p = 0.04$). Regarding mutational status, only TERTp mutation was associated with disease progression, and diminished disease progression-free survival (PFS). The presence of distant metastasis, vascular invasion and gross ETE were significantly associated with the risk of disease progression.

Conclusions TERTp mutation appears to be an indicator of both persistence and progression of structural disease after initial therapy in aggressive variants of TCDFC, and associates with a shorter progression free survival regardless of the therapy employed.

Keywords TERT · Persistent · Progression · Aggressive variants · Thyroid cancer · Genetics

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Introduction

The TERT promoter (TERTp) gene mutation, recently reported in melanomas, gliomas, and thyroid cancer [1–4], occurs in two hot spot positions located on −124 and −146 upstream from the ATG (−124C > T also known as C228T, and −146C > T also called C250T). It confers an increased activity in the promoter of the TERT gene by creating a nucleotide ribbon with 11 bases 5'-CCCCCTCCGGGG-3' containing a consensus binding site, for the E-Twenty Six

(ETS) transcription factors family. Activation of the MAPK and/or PI3-Akt pathways by the BRAF/RAS mutations leads to an increased expression of the transcription factors of the ETS family and can potentiate the upregulation of the TERT expression [5–7] in this context [5–7].

Thyroid carcinomas derived from follicular cells (TCDFC) encompass well-differentiated thyroid carcinomas (WDTC) (papillary thyroid carcinoma—PTC and its variants—and follicular thyroid carcinoma—FTC), poorly differentiated thyroid carcinoma (PDTC) and anaplastic carcinoma. The aggressiveness in the aforementioned carcinomas and lethality are inversely correlated with cell differentiation. TERTp gene mutation was shown to be associated with aggressive features as well as with the presence of BRAF or BRAF/RAS mutations [1, 8–12] in WDTC. The role played by TERTp mutations on the clinical course of the non-aggressive forms of well-differentiated carcinoma (classic PTC and minimally invasive FTC) with regard to prognosis and survival has already been well established [6, 10, 12–15]. TERTp mutations is associated with worse prognosis, distant metastasis, and mortality. Cases with more aggressive histology types of TCDFC (aggressive variants of PTC, widely invasive FTC and PDTC/insular carcinoma), have more frequent TERTp mutation than non-aggressive well differentiated carcinomas [6, 8, 9, 12, 16, 17]. However, there is a lack of studies performed exclusively on patients with aggressive variants of TCDFC that evaluate the impact of TERTp mutation on the follow-up. To our knowledge, the studies that evaluated the frequency of TERTp mutation and its prognostic implications in such histological variants were addressed only a few subtypes such as PDTC and/or anaplastic tumors [17, 18], or with larger series enriched in classic variants of PTC and/or minimally invasive FTC [1, 6, 8, 9, 12, 16, 19]. Thus, the actual impact of the TERTp gene mutation in a cohort consisting exclusively of patients with aggressive histology of TCDFC (aggressive variants of PTC, widely invasive FTC and PDTC/insular carcinoma) remains to be addressed.

In this study, we intended to search for the presence of TERT promoter mutations in a specific group of thyroid tumors composed only by aggressive histological variants of TCDFC, and investigate if the mutation in the TERTp gene had any impact on clinical and prognostic features.

Material and methods

We performed a retrospective analysis of clinical and pathological data from a cohort of 45 patients, 21 years of age or older, diagnosed with thyroid cancer with aggressive histology and followed in two referral centers from 1998 to 2016. Aggressive histology variants include: aggressive

variants of PTC (tall cell ($n = 10$), PTC with oxyphilic cells ($n = 12$), aggressive forms of the follicular variant PTC displaying extensive invasive ($n = 4$), hobnail variant of PTC ($n = 1$), solid variant ($n = 3$) and columnar cells ($n = 1$)), widely invasive FTC ($n = 2$) (oxyphilic cells with—oncocyctic or Hurthle- and Warthin-like ($n = 2$)) and poorly differentiated thyroid cancer ($n = 10$). All patients underwent total thyroidectomy and therapeutic selective neck dissection when lymph node metastases were detected in pre operative and/or intraoperative evaluation.

Data were obtained from the University Hospital Clementino Fraga Filho (UFRJ) and the Brazilian National Cancer Institute (INCA). A multidisciplinary team determined the initial treatment based on clinical, histopathological, and complementary tests such as post operative thyroglobulin and anti-thyroglobulin serum levels, post operative cross sectional images neck ultrasound, chest computed tomography or Magnetic resonance and/or PET/CT when performed. Prophylactic cervical dissection was not performed as routine in neither institution. In addition, samples from the primary tumor of all patients included in the study were obtained, reviewed, and studied as described below.

Samples

Fifty-six formalin-fixed paraffin-embedded (FFPE) samples from primary thyroid tumors of 45 patients corresponding to the patients followed were collected from the files of the National Institute of Cancer (INCA) and the Federal University of Rio de Janeiro, University Hospital Clementino Fraga Filho (HUCFF). The histology of all tumor samples was revised by thyroid-specialized pathologists (MSS) from the Institute for Research and Innovation in Health Sciences (I3S)/Institute of Molecular Pathology and Immunology at the University of Porto (IPATIMUP). Data on the clinicopathological characteristics of patients and tumors are summarized in Table 1.

Laboratory studies

Between 1998 and 2001, a thyroglobulin (Tg) assay with a functional sensitivity of 0.5 ng/ml was employed. From 2001 until 2010, serum Tg was quantified by an immunometric assay (Immulite) with a functional sensitivity of 0.2 ng/ml. From 2010 until the present, the functional sensitivity was reduced to 0.1 ng/ml.

Evaluation of outcomes

The clinicopathological characteristics of the patients, treatment details (surgery, RAI therapy), and postoperative follow-up (Tg, recurrence/persistence, deaths) were

Table 1 Characteristics of the patients

	<i>N</i> (<i>n</i> = 45)	%
Age (y)		–
Median (range)	54.0 (18–85)	
Mean ± DP	52.98 ± 16.57	
sex (F)	33	73.3
Histopathology		
Aggressive variants of PTC: tall cell, columnar, solid, trabecular, invasive FVPTC, clear cell, hobnail)	31	68.9
Invasive FTC and with Hurthle cell	4	8.9
PDTC/Insular carcinoma	10	22.2
Size (cm)		
Median (range)	3.0 (0.5–10)	
Mean ± SD	4.02 ± 2.64	
N1	21	46.7
M1	24	54.6
ATA risk stratification ²¹		
Intermediate	10	22.2
High	35	77.8
Response to therapy ^a		
Excellent	20	45.5
Indeterminate	2	4.5
Biochemical incomplete	0	0
Structural incomplete	22	50
Disease progression	21	47.7
Disease-related death	8	17.8
Follow-up (months)		
Median (range)	56.0 (5.0–360)	
Mean ± SD	75.2 ± 73.5	

SD standard deviation, *ATA* American Thyroid Association, *PTC* papillary thyroid cancer, *FTC* follicular thyroid cancer, *PDTC* poorly differentiated thyroid cancer

^aOne patient lost to follow-up within the first 2 years with not enough data to evaluate response to therapy

obtained. Patients were classified by AJCC/TNM 8th edition [20] and ATA risk classification [21]. Response to initial therapy was assessed by ATA and classified as follows: excellent response (undetectable thyroglobulin antibody –TgAb and negative imaging and suppressed Tg < 0.1 ng/ml or stimulated Tg < 1.0 ng/ml and negative imaging); indeterminate response (nonspecific findings on imaging studies, non-stimulated Tg detectable but < 1 ng/ml or stimulated Tg detectable but < 10 ng/ml or TgAb levels stable or); biochemical incomplete response (negative imaging and non-stimulated Tg > 1 ng/ml or stimulated Tg > 10 ng/ml or increasing TgAb levels); or structural incomplete response to therapy (structural or functional evidence of disease, with any Tg level) [21].

Patients were classified in the final follow-up as having no evidence of disease when the suppressed Tg was less than 0.1 ng/mL, no antibodies were present, and there was no structural evidence of the disease. Patients with suppressed Tg greater than 1 ng/mL and stimulated greater than 10 ng/mL or any evidence of structural disease (cross-sectional images or biopsy) were classified as biochemical or structural persistence, respectively. Cervical recurrence/persistence was defined as follows: positive cytology/histology, highly suspicious lymph nodes or thyroid bed nodules on ultrasound (hyper-vascularity, cystic areas, heterogeneous content, rounded shape and enlargement on follow-up), or cross-sectional imaging highly suspicious for metastatic disease. Distant metastases were assessed by cross sectional images and considered as present when there was iodine uptake and/or were highly suspicious on computed tomography and/or magnetic resonance imaging, even with no iodine uptake but with high thyroglobulin levels or if proven by biopsy [21].

Progression was determined by an increase in the sum of the longest diameters of the target lesions > 20% and/or the appearance of new metastatic foci on sequential imaging over 12–15 months of follow-up [21–24].

Time from initial therapy until the first disease progression was used to calculate progression free survival rate.

The ethical boards of both institutions involved in the present study approved this study.

DNA extraction

Genomic DNA from formalin-fixed paraffin embedded (FFPE) tissues was obtained after careful microdissection of 10 µm sections. The DNA extraction was performed using a digesting cell lysis solution (Citomed, Lisbon, Portugal) complemented with proteinase K (20 mg/dl). DNA precipitation was achieved through isopropanol. DNA was quantified using a Nanodrop N-100 Spectrophotometer (Thermo Scientific, Lithuania).

Genetic characterization

All the tumor samples were analyzed for the presence of mutations in the hotspot regions of the BRAF (exon 15), NRAS (codon 61), and TERT (promoter) genes. The primers and annealing temperatures used were previously described [1, 25, 26].

Briefly, genomic DNA (10–100 ng) was amplified by PCR using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany) according to the manufacturer instructions. The purified PCR product was sequenced by Sanger method using the ABI Prism Big Dye Terminator Kit (Perkin-Elmer, Foster City, California) and the fragments were run in an ABI prism 3100 Genetic Analyzer (Perkin-Elmer).

In all of the cases that were positive for the presence of mutation or inconclusive, a new independent PCR amplification/sequencing protocol was performed.

Statistical analysis

Statistical analysis was conducted with SPSS Inc. The results are expressed as a percentage or mean \pm SD. Statistical analysis was performed on the whole series of thyroid carcinomas and considering the different groups of tumors. Fisher's exact test, *t*-test (unpaired, two tailed), and ANOVA were used when appropriate.

The predictive value of TERTp mutations and other factors such as age, gender, histologic category, extra-thyroidal extension, vascular invasion, lymph node metastases, staging I–IV (Union for International Cancer Control/American Joint Committee on Cancer (AJCC)), BRAF mutations, RAS mutations, distant metastases, and disease-free status at the end of follow-up, were assessed using univariate and multivariate logistic regression models.

Survival curve was plotted by the Kaplan-Meier method with log-rank statistics. Multivariate survival analysis was performed using Cox regression. In the regression models, all of the variables significantly associated with the specified outcome in the univariate model were included in the multivariate analysis.

Results were considered statistically significant at $p < 0.05$.

Results

Clinicopathological characteristics (Table 1)

All patients in the study had tumors with aggressive histology being the most prevalent the aggressive variants of PTC (68.9%; $n = 31$), followed by PDTC/insular carcinoma (22.2%; $n = 10$), and widely invasive FTC composed by cells with Hurtle cell features (8.9%; $n = 4$). Based on the 2015 American Thyroid Association (ATA) risk classification (22), 77.8% were high risk and 22.2% were intermediate risk. The response to the initial therapy was excellent (45.5%) and structurally incomplete in half of the cases (50.0%). During the follow-up period (median 56 months; range 5–360 months), 47.7% ($n = 21$) of the patients showed disease progression and 17.8% ($n = 8$) experienced disease related death.

Genetic characterization

Among the 45 patients, 53.3% (24/45) showed at least one molecular alteration (Table 2). TERTp mutation was the most prevalent alteration, being present in 33.4% (15/45) of

the cases followed by the BRAFV600E mutation (24.4%, 11/45) and the RAS mutation (8.9%, 4/45). A total of 5 patients had concomitant mutational events (3 patients had TERTp and BRAF mutations and 2 patients had TERTp and RAS mutations), shown in Table 2.

The –124 C>T mutation was the most frequent TERTp mutation (14/15 TERTp mutated cases), followed by the –146 C>T (1/15 TERTp mutated). Among the three patients with the TERTp and BRAF mutation the mean age was 60 years, one male and 2 females, all had histopathology aggressive variants of PTC, high-risk ATA, and the mean total d of RAI ranged from 150–350 mCi. Two presented a pT4a tumor with gross ETE and M1 with vascular invasion, and evolved with incomplete structural response and disease progression. The 2 patients with TERT and RAS mutations, both presented vascular invasion and distant metastases and received an average RAI activity of 500 mCi. One of the cases had insular histology, T4a, N0, high risk ATA 2015, and evolved with incomplete structural response and disease progression. The other case had an aggressive histological variant of PTC, T3N1, intermediate risk ATA stratification, and incomplete structural response with progression during follow-up.

Response to therapy

In univariate analysis, we found that the factors associated with poor response to initial therapy in the first 6–24 months (Table 3) were ATA High risk ($p = 0.04$), aggressive histology PDTC/insular carcinoma and FTC, ($p = 0.005$), the presence of distant metastasis at diagnosis ($p = 0.004$), TERT mutation ($p = 0.001$), and NRAS mutation ($p = 0.02$).

Multivariate analysis for the risk of structural disease after initial therapy

In a multivariate analysis regarding the response to initial therapy (Table 4), TERTp mutation represents the highest risk (6 times) of having structural disease after initial therapy ($p = 0.01$). Also significant in multivariate analysis are the presence of vascular invasion ($p = 0.02$), gross ETE ($p = 0.02$) and distant metastasis ($p = 0.04$).

Multivariate analysis for the risk of having structural disease progression

Among the mutations studied, only the TERTp mutation was associated with the risk of disease progression (Table 5). The disease progression-free survival (PFS) was significantly lower in the TERTp mutated group (Fig. 1). In four out of eight patients that died from the disease we could obtain enough material for genetic studies and all of

Table 2 Molecular analyses in tumors displaying aggressive histology

	Aggressive variants of PTC (tall cell, columnar, solid, trabecular, invasive FVPTC, clear cell, hobnail) (<i>n</i> = 31)	Invasive FTC and Hurtle cell variant (<i>n</i> = 4)	Insular/PDTC (<i>n</i> = 10)
TERT mutation alone (<i>n</i> = 10)	5 (50%)	3 (30%)	2 (20%)
BRAF mutation alone (<i>n</i> = 8)	8 (100%)	0 (0%)	0 (0%)
TERT + BRAF mutations (<i>n</i> = 3)	3 (100%)	0 (0%)	0 (0%)
TERT + NRAS mutations (<i>n</i> = 2)	1 (50%)	0 (0%)	1 (50%)
NRAS mutation alone (<i>n</i> = 2)	0 (0%)	0 (0%)	2 (100%)

Table 2 shows the distribution of the mutations among the different groups, for example, all the isolated BRAF mutations were found in the aggressive variants of papillary thyroid cancer

them had TERT mutation. Distant metastasis, vascular invasion and gross ETE were also significantly associated with the risk of having structural disease progression (Table 5).

Discussion

In the present study, we demonstrate that the TERTp gene mutation is associated with persistent structural disease after initial treatment and with structural disease progression in aggressive variants of TCDCF (aggressive variants of PTC, widely invasive FTC, and PDTC/insular carcinoma). The frequency of the TERTp mutation in our cohort (33.4%) is consistent with that reported in tumors with aggressive behavior in previous studies [6, 14, 17], the $-124 C > T$ mutation being the most frequent TERTp mutation.

In our cohort, 21/45 (47.5%) of the patients presented disease progression throughout the follow-up, and among these patients the presence of the TERTp gene mutation increased by 5.9 times the risk of such progression. We also showed a significant decrease in PFS among TERT mutated cases vs TERT wild type (WT) (72.7 months vs 97.7 months, respectively; $p = 0.001$). Among the existing studies that evaluated TERTp gene in aggressive histological types [1, 6, 8, 12, 15–19], none evaluated this progression exclusively in a series composed solely by tumors exhibiting such of aggressive histologies.

Five patients showed concomitance of TERTp mutation along with other studied mutations: three cases with BRAFV600E and two cases with NRAS. Although four out of the five (80%) patients who had two mutations had disease progression, the small number of patients with two mutations does not drawing a solid conclusion from this finding. The association between TERTp and BRAFV600E among the aggressive variants was also not significantly prevalent in previous variants [1, 6, 8, 17]. The same holds true for the association between TERTp and RAS in similar variants [1, 6, 8]. The BRAFV600E mutation was not associated with a worse response to the initial treatment or to disease progression in this group of patients. This is in agreement with several studies carried out in well-differentiated, non-aggressive variants, and suggests that this mutation only has a role in the first step of tumorigenesis and therefore it should not be considered per se as a predictor of worse outcome [27–31].

In the present study we were not able to analyze the association of TERTp mutation with mortality but it was associated with mortality in other studies [6, 16, 19, 30]. The small size of our sample and/or the relatively short time of follow-up together with the small number of deaths during the follow-up period have probably impaired our analysis compared to others [32].

Table 3 Response to therapy in the first 6–24 months (univariate analysis)

	Excellent (<i>n</i> = 20)	Indeterminate (<i>n</i> = 1)	Biochemical incomplete (<i>n</i> = 1)	Structural incomplete (<i>n</i> = 22)	<i>p</i> -value [#]
Age	48 (18–73)	46	45	58(22–85)	0.58
Gender (F)	65%	100%	100%	77.3%	0.35
Size (cm)	2.93 (2–7)	2.0	2.1	5.23 (1–10)	0.38
Gross ETE	10%	0	0	40.9%	0.01
Vascular invasion	30%	0	0	81.8%	0.01
<i>ATA risk</i>					
Intermediate	35%	100%	100%	13.6%	0.04
High	65%	0	0	86.4%	
<i>Histology</i>					
<i>Aggressive</i>					
Variants PTC	90%	100%	100%	45.5%	0.005
Invasive FTC and with Hurthle cell	5%	0	0	13.6%	
PDTC/insular carcinoma	5%	0	0	40.9%	
Lymph node mets	40%	100%	100%	45.5%	0.18
Distant mets	25%	0	100%	81.8%	0.004
TERT mutation	10%	0	0	59.2%	0.01
BRAF V600E	35%	100%	100%	9.1%	0.007
NRAS mutation	0	0	0	22.7%	0.02
Follow-up (months)	50.95(6–137)	61	74	98 (5–360)	0.35

^aOne patient lost to follow-up within the first 2 years with not enough data to evaluate response to therapy
[#]*p*-values express the comparison between excellent response and structural incomplete response to initial therapy

The bold value indicates statistically significant

Table 4 Multivariate analysis for the risk of having structural incomplete response after initial therapy

	HR	IC 95%	<i>p</i> -value
Distant metastases	2.0	1.56–21.2	0.04
Vascular Invasion	4.8	2.6–13.7	0.02
Gross ETE	5.0	1.9–6.9	0.02
TERT mutation	6.6	3.3–29.3	0.01
BRAF V600E mutation	7.0	0.49–99.0	0.15
RAS mutation	0.9	0.7–12.3	0.9

ETE extra-thyroidal extension, *HR* hazard ratio

^aOne patient lost to follow-up within the first 2 years with not enough data to evaluate response to therapy. All factors that were significant in the univariate analysis were chosen to be part of the multivariate analysis, performed step wise

The bold value indicates statistically significant

Table 5 Multivariate analysis for the risk of having structural progression

	HR	IC 95%	<i>p</i> -value
Distant metastases	2.0	1.1–3.7	0.05
Vascular invasion	9.5	2.2–9.4	0.01
Gross ETE	5.7	2.0–7.8	0.01
TERT mutation	5.9	2.34–10.4	0.01
BRAF V600E mutation	7.0	0.49–26.0	0.40
RAS mutation alone	1.0	0.7–3.2	0.8

ETE extra-thyroidal extension, *HR* hazard ratio

^aOne patient lost to follow-up within the first 2 years with not enough data to evaluate response to therapy. All factors that were significant in the univariate analysis were chosen to be part of the multivariate analysis, performed step wise

The bold value indicates statistically significant

Our study shows the association of the mutation of TERTp with the persistence and progression of structural disease. As noted above, studies conducted to evaluate the TERTp mutation in aggressive histology cell types histology [1, 6, 8, 9, 12, 15–19] were not designed to evaluate the

progression of disease in this specific subgroup of disease. Two studies have demonstrated association between TERTp mutated cases and persistence of disease [12, 17]. Shi [17] showed that among anaplastic thyroid cancer, the presence of structural disease was significantly associated with

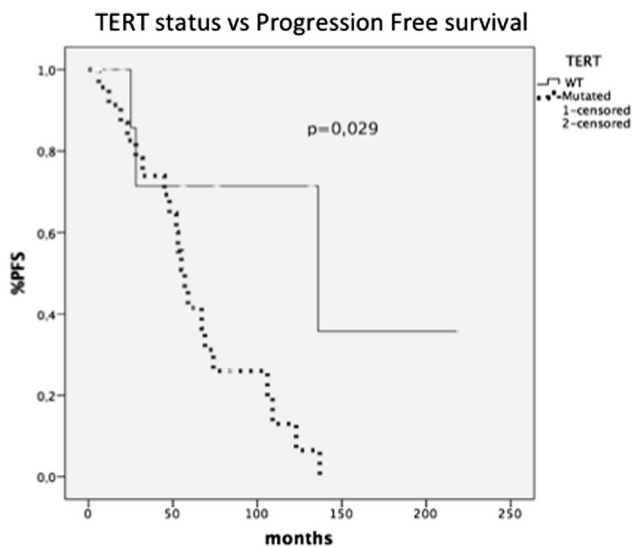


Fig. 1 PFS progression-free survival. Median PFS for TERT mutated cases: 57 (47.9–66) months. Median PFS for TERT WT cases: 136 (69–not reached)

TERTp mutated cases (15/18; 83.3%) vs WT TERT cases. (8/26;30.8%). Qasem [12] also found a significant association between persistence or recurrence of disease and TERTp mutated cases in a group described as “all types of thyroid cancer except conventional PTC” (tall cell PTC, diffuse sclerosing variant of PTC, PDTC, Hurthle cell, follicular thyroid carcinoma); this study showed persistence/recurrence of the disease in 85% of TERTp mutated cases vs 46.7% in WT TERT cases. The other studies [1, 6, 8, 9, 12, 15–19], however, only assessed the frequency of the TERTp gene mutation in such TCDFC subgroups [1, 8, 9] or showed no difference between the presence of structural disease in the TERTp mutated cases and WT TERT cases among one or more of these variants of TCDFC [6, 16, 19].

Therefore, the association of the TERTp gene mutation with the persistence and progression of structural disease in patients with thyroid tumors displaying aggressive histology may suggest that the mutation of the TERTp gene plays a major role in the pathogenesis and aggressiveness of TCDFC. These results indicate that this mutation may serve as a predictor of poorer prognosis in thyroid cancer, regardless of the histologic type and level of differentiation, as also reported by Shi X et al. [17].

If our data are confirmed in upcoming studies, then may prove useful for tailoring the management of patients with aggressive histology and TERTp mutation, both for decision making regarding therapeutic choices (extension of the surgery, RAI therapy) and for choice of models (frequency and tools) used for follow-up (frequency of follow-up, laboratory and imaging surveillance, TSH suppression level). Also, these findings would be clinically important for patients with aggressive histologic TERTp-mutated types of TCDFC since they could be selected early for new treatment options.

The present study suggests that regardless of the type of thyroid cancer, whether non-aggressive forms of well-differentiated carcinoma (classic PTC and minimally invasive FTC) or aggressive forms of well-differentiated carcinoma as well as PTDC/insular carcinoma, the TERTp mutation may be associated with poorer response to initial treatment and structural disease progression. These results are in accordance with previous evidence obtained by from our [6] and others [33] groups that showed that patients harboring tumors with TERTp mutation are subjected to a significant higher number of treatments (including higher radioiodine doses) [6, 33], and that TERTp mutated PTC express very low levels of NIS mRNA [34].

Further studies with a higher number of patients, will be very important to clarify the findings of our study which is limited by the relatively small number of patients included.

The limitations of the study were (i) the small number of patients, although it should be emphasized that it is a group formed by patients with more rare histological variants; (ii) all the studied material comes from blocks of paraffin, some of which are already very old (more than 10 years), which is known to impair the DNA quality studied. However, it is worth noting that the selection of all the blocks was very careful, only the blocks were chosen exhaustively and rigorously evaluated by the pathologists of the two services of origin and also reviewed all the material by an expert pathologist, analysis of this material (Professor Manuel Sobrinho-Simões); and the extraction of formalin-embedded paraffin-embedded DNA was performed after careful microdissection (10 μ m) and quantified using the Nanodrop N-100 spectrophotometer; (iii) still within the quality of the material, it is also known that in a material of impaired quality “false” C/T exchanges can be detected, leading to a false positive result of the TERTp mutations found, which occur precisely because of these exchanges in the position –124 C>T from the site of the start of transcription in the promoter region of the gene. However, the prevalence that we found of this mutation is in agreement with that reported in the literature, in these more aggressive histological variants (between 30–40%); therefore, we did not think that this possible interference was relevant in our study.

We conclude that TERTp mutation may be an indicator of both, persistence and progression of structural disease after initial therapy in aggressive variants of thyroid cancer. Therefore, detection of this mutation appears to be a promising tool as a prognostic indicator and may be useful to guide the management of patients with aggressive variants of thyroid cancer derived from follicular cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. J. Vinagre, A. Almeida, H. Populo, R. Batista, J. Lyra, V. Pinto, R. Coelho, R. Celestino, H. Prazeres, L. Lima, M. Melo, A.G. da Rocha, A. Preto, P. Castro, L. Castro, F. Pardal, J.M. Lopes, L.L. Santos, R.M. Reis, J. Cameselle-Teijeiro, M. Sobrinho-Simoes, J. Lima, V. Maximo, P. Soares, Frequency of TERT promoter mutations in human cancers. *Nat. Commun.* **4**, 2185 (2013). <https://doi.org/10.1038/ncomms3185>
2. S. Horn, A. Figl, P.S. Rachakonda, C. Fischer, A. Sucker, A. Gast, S. Kadel, I. Moll, E. Nagore, K. Hemminki, D. Schadendorf, R. Kumar, TERT promoter mutations in familial and sporadic melanoma. *Science* **339**(6122), 959–961 (2013). <https://doi.org/10.1126/science.1230062>
3. F.W. Huang, E. Hodis, M.J. Xu, G.V. Kryukov, L. Chin, L.A. Garraway, Highly recurrent TERT promoter mutations in human melanoma. *Science* **339**(6122), 957–959 (2013). <https://doi.org/10.1126/science.1229259>
4. P.J. Killela, Z.J. Reitman, Y. Jiao, C. Bettegowda, N. Agrawal, L. A. Diaz Jr., A.H. Friedman, H. Friedman, G.L. Gallia, B.C. Giovanella, A.P. Grollman, T.C. He, Y. He, R.H. Hruban, G.I. Jallo, N. Mandahl, A.K. Meeker, F. Mertens, G.J. Netto, B.A. Rasheed, G.J. Riggins, T.A. Rosenquist, M. Schiffman, M. Shih Ie, D. Theodorescu, M.S. Torbenson, V.E. Velculescu, T.L. Wang, N. Wentzensen, L.D. Wood, M. Zhang, R.E. McLendon, D.D. Bigner, K.W. Kinzler, B. Vogelstein, N. Papadopoulos, H. Yan, TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc. Natl. Acad. Sci. USA* **110**(15), 6021–6026 (2013). <https://doi.org/10.1073/pnas.1303607110>
5. A. Pestana, J. Vinagre, M. Sobrinho-Simoes, P. Soares, TERT biology and function in cancer: beyond immortalisation. *J. Mol. Endocrinol.* **58**(2), R129–R146 (2017). <https://doi.org/10.1530/JME-16-0195>
6. M. Melo, A.G. da Rocha, J. Vinagre, R. Batista, J. Peixoto, C. Tavares, R. Celestino, A. Almeida, C. Salgado, C. Eloy, P. Castro, H. Prazeres, J. Lima, T. Amaro, C. Lobo, M.J. Martins, M. Moura, B. Cavaco, V. Leite, J.M. Cameselle-Teijeiro, F. Carrilho, M. Carvalheiro, V. Maximo, M. Sobrinho-Simoes, P. Soares, TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. *J. Clin. Endocrinol. Metab.* **99**(5), E754–E765 (2014). <https://doi.org/10.1210/jc.2013-3734>
7. N.W. Kim, M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L. Ho, G.M. Coviello, W.E. Wright, S.L. Weinrich, J.W. Shay, Specific association of human telomerase activity with immortal cells and cancer. *Science* **266**(5193), 2011–2015 (1994)
8. I. Landa, I. Ganly, T.A. Chan, N. Mitsutake, M. Matsuse, T. Ibrahimasic, R.A. Ghossein, J.A. Fagin, Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. *J. Clin. Endocrinol. Metab.* **98**(9), E1562–E1566 (2013). <https://doi.org/10.1210/jc.2013-2383>
9. X. Liu, J. Bishop, Y. Shan, S. Pai, D. Liu, A.K. Murugan, H. Sun, A.K. El-Naggar, M. Xing, Highly prevalent TERT promoter mutations in aggressive thyroid cancers. *Endocr. Relat. Cancer* **20**(4), 603–610 (2013). <https://doi.org/10.1530/ERC-13-0210>
10. X. Liu, S. Qu, R. Liu, C. Sheng, X. Shi, G. Zhu, A.K. Murugan, H. Guan, H. Yu, Y. Wang, H. Sun, Z. Shan, W. Teng, M. Xing, TERT promoter mutations and their association with BRAF V600E mutation and aggressive clinicopathological characteristics of thyroid cancer. *J. Clin. Endocrinol. Metab.* **99**(6), E1130–E1136 (2014). <https://doi.org/10.1210/jc.2013-4048>
11. M. Xing, R. Liu, X. Liu, A.K. Murugan, G. Zhu, M.A. Zeiger, S. Pai, J. Bishop, BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. *J. Clin. Oncol.* **32**(25), 2718–2726 (2014). <https://doi.org/10.1200/JCO.2014.55.5094>
12. E. Qasem, A.K. Murugan, H. Al-Hindi, M. Xing, M. Almohanna, M. Alswailem, A.S. Alzahrani, TERT promoter mutations in thyroid cancer: a report from a Middle Eastern population. *Endocr. Relat. Cancer* **22**(6), 901–908 (2015). <https://doi.org/10.1530/ERC-15-0396>
13. M. Bullock, Y. Ren, C. O’Neill, A. Gill, A. Aniss, M. Sywak, S. Sidhu, L. Delbridge, D. Learoyd, F. de Vathaire, B.G. Robinson, R.J. Clifton-Bligh, TERT promoter mutations are a major indicator of recurrence and death due to papillary thyroid carcinomas. *Clin. Endocrinol.* **85**(2), 283–290 (2016). <https://doi.org/10.1111/cen.12999>
14. J.R. George, Y.C. Henderson, M.D. Williams, D.B. Roberts, H. Hei, S.Y. Lai, G.L. Clayman, Association of TERT promoter mutation, but Not BRAF mutation, with increased mortality in PTC. *J. Clin. Endocrinol. Metab.* **100**(12), E1550–E1559 (2015). <https://doi.org/10.1210/jc.2015-2690>
15. M. Melo, A. Gaspar da Rocha, R. Batista, J. Vinagre, M.J. Martins, G. Costa, C. Ribeiro, F. Carrilho, V. Leite, C. Lobo, J.M. Cameselle-Teijeiro, B. Cavadas, L. Pereira, M. Sobrinho-Simoes, P. Soares, TERT, BRAF, and NRAS in primary thyroid cancer and metastatic disease. *J. Clin. Endocrinol. Metab.* **102**(6), 1898–1907 (2017). <https://doi.org/10.1210/jc.2016-2785>
16. T. Liu, N. Wang, J. Cao, A. Sofiadis, A. Dinets, J. Zedenius, C. Larsson, D. Xu, The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. *Oncogene* **33**(42), 4978–4984 (2014). <https://doi.org/10.1038/nc.2013.446>
17. X. Shi, R. Liu, S. Qu, G. Zhu, J. Bishop, X. Liu, H. Sun, Z. Shan, E. Wang, Y. Luo, X. Yang, J. Zhao, J. Du, A.K. El-Naggar, W. Teng, M. Xing, Association of TERT promoter mutation 1,295,228 C T with BRAF V600E mutation, older patient age, and distant metastasis in anaplastic thyroid cancer. *J. Clin. Endocrinol. Metab.* **100**(4), E632–E637 (2015). <https://doi.org/10.1210/jc.2014-3606>
18. I. Landa, T. Ibrahimasic, L. Boucai, R. Sinha, J.A. Knauf, R.H. Shah, S. Dogan, J.C. Ricarte-Filho, G.P. Krishnamoorthy, B. Xu, N. Schultz, M.F. Berger, C. Sander, B.S. Taylor, R. Ghossein, I. Ganly, J.A. Fagin, Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. *J. Clin. Invest.* **126**(3), 1052–1066 (2016). <https://doi.org/10.1172/JCI85271>
19. G. Gandolfi, M. Ragazzi, A. Frasoldati, S. Piana, A. Ciarrocchi, V. Sancisi, TERT promoter mutations are associated with distant metastases in papillary thyroid carcinoma. *Eur. J. Endocrinol.* **172**(4), 403–413 (2015). <https://doi.org/10.1530/EJE-14-0837>

20. L. Lamartina, G. Grani, E. Arvat, A. Nervo, M.C. Zatelli, R. Rossi, E. Puxeddu, S. Morelli, M. Torlontano, M. Massa, R. Bellantone, A. Pontecorvi, T. Montesano, L. Pagano, L. Daniele, L. Fugazzola, G. Ceresini, R. Bruno, R. Rossetto, S. Tumino, M. Centanni, D. Meringolo, M.G. Castagna, D. Salvatore, A. Nicolucci, G. Lucisano, S. Filetti, C. Durante, 8th edition of the AJCC/TNM staging system of thyroid cancer: what to expect (ITCO#2). *Endocr. Relat. Cancer* **25**(3), L7–L11 (2018). <https://doi.org/10.1530/ERC-17-0453>
21. B.R. Haugen, E.K. Alexander, K.C. Bible, G.M. Doherty, S.J. Mandel, Y.E. Nikiforov, F. Pacini, G.W. Randolph, A.M. Sawka, M. Schlumberger, K.G. Schuff, S.I. Sherman, J.A. Sosa, D.L. Steward, R.M. Tuttle, L. Wartofsky, 2015 American Thyroid Association Management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid* **26**(1), 1–133 (2016). <https://doi.org/10.1089/thy.2015.0020>
22. M. Schlumberger, M. Brose, R. Elisei, S. Leboulleux, M. Luster, F. Pitoia, F. Pacini, Definition and management of radioactive iodine-refractory differentiated thyroid cancer. *Lancet Diabetes Endocrinol.* **2**(5), 356–358 (2014). [https://doi.org/10.1016/S2213-8587\(13\)70215-8](https://doi.org/10.1016/S2213-8587(13)70215-8)
23. M. Melo, A.G. da Rocha, J. Vinagre, R. Batista, J. Peixoto, C. Tavares, R. Celestino, A. Almeida, C. Salgado, C. Eloy, P. Castro, H. Prazeres, J. Lima, T. Amaro, C. Lobo, M.J. Martins, M. Moura, B. Cavaco, V. Leite, J.M. Cameselle-Teijeiro, F. Carrilho, M. Carvalheiro, V. Máximo, M. Sobrinho-Simões, P. Soares, TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. *J. Clin. Endocrinol. Metab.* **99**(5), E754–E765 (2014)
24. P. Soares, V. Trovisco, A.S. Rocha, J. Lima, P. Castro, A. Preto, V. Máximo, T. Botelho, R. Seruca, M. Sobrinho-Simões, BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. *Oncogene* **22**(29), 4578–4580 (2003)
25. J.H. Kim, Comparison of the RECIST 1.0 and RECIST 1.1 in patients treated with targeted agents: a pooled analysis and review. *Oncotarget* **7**(12), 13680–13687 (2016). <https://doi.org/10.18632/oncotarget.7322>
26. M. Ruan, Y. Shen, L. Chen, M. Li, RECIST 1.1 and serum thyroglobulin measurements in the evaluation of responses to sorafenib in patients with radioactive iodine-refractory differentiated thyroid carcinoma. *Oncol. Lett.* **6**(2), 480–486 (2013). <https://doi.org/10.3892/ol.2013.1424>
27. V. Trovisco, J.P. Couto, J. Cameselle-Teijeiro, I.V. de Castro, E. Fonseca, P. Soares, M. Sobrinho-Simoes, Acquisition of BRAF gene mutations is not a requirement for nodal metastasis of papillary thyroid carcinoma. *Clin. Endocrinol.* **69**(4), 683–685 (2008). <https://doi.org/10.1111/j.1365-2265.2008.03243.x>
28. V. Trovisco, P. Soares, A. Preto, I.V. de Castro, J. Lima, P. Castro, V. Maximo, T. Botelho, S. Moreira, A.M. Meireles, J. Magalhaes, A. Abrosimov, J. Cameselle-Teijeiro, M. Sobrinho-Simoes, Type and prevalence of BRAF mutations are closely associated with papillary thyroid carcinoma histotype and patients' age but not with tumour aggressiveness. *Virchows. Arch.* **446**(6), 589–595 (2005). <https://doi.org/10.1007/s00428-005-1236-0>
29. C. Eloy, J. Santos, P. Soares, M. Sobrinho-Simoes, The pre-eminence of growth pattern and invasiveness and the limited influence of BRAF and RAS mutations in the occurrence of papillary thyroid carcinoma lymph node metastases. *Virchows. Arch.* **459**(3), 265–276 (2011). <https://doi.org/10.1007/s00428-011-1133-7>
30. V. Sancisi, D. Nicoli, M. Ragazzi, S. Piana, A. Ciarrocchi, BRAFV600E mutation does not mean distant metastasis in thyroid papillary carcinomas. *J. Clin. Endocrinol. Metab.* **97**(9), E1745–E1749 (2012). <https://doi.org/10.1210/jc.2012-1526>
31. C. Gouveia, N.T. Can, A. Bostrom, J.P. Grenert, A. van Zante, L. A. Orloff, Lack of association of BRAF mutation with negative prognostic indicators in papillary thyroid carcinoma: the University of California, San Francisco, experience. *JAMA Otolaryngol. Head. Neck Surg.* **139**(11), 1164–1170 (2013). <https://doi.org/10.1001/jamaoto.2013.4501>
32. R. Bu, A.K. Siraj, S.P. Divya, Y. Kong, S.K. Parvathareddy, M. Al-Rasheed, K.A.S. Al-Obaisi, I.G. Victoria, S.S. Al-Sobhi, M. Al-Dawish, F. Al-Dayel, K.S. Al-Kuraya, Telomerase reverse transcriptase mutations are independent predictor of disease-free survival in Middle Eastern papillary thyroid cancer. *Int. J. Cancer* (2017). <https://doi.org/10.1002/ijc.31225>
33. C. de la Fouchardiere, M. Decaussin-Petrucci, J. Berthiller, F. Descotes, J. Lopez, J.C. Lifante, J.L. Peix, A.L. Giraudet, A. Delahaye, S. Masson, C. Bournaud-Salinas, F. Borson Chazot, Predictive factors of outcome in poorly differentiated thyroid carcinomas. *Eur. J. Cancer* **92**, 40–47 (2018). <https://doi.org/10.1016/j.ejca.2017.12.027>
34. C. Tavares, M.J. Coelho, C. Eloy, M. Melo, A.G. da Rocha, A. Pestana, R. Batista, L.B. Ferreira, E. Rios, S. Selmi-Ruby, B. Cavadas, L. Pereira, M. Sobrinho Simoes, P. Soares, NIS expression in thyroid tumors, relation with prognosis clinicopathological and molecular features. *Endocr. Connect.* **7**(1), 78–90 (2018). <https://doi.org/10.1530/EC-17-0302>

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