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Original Research

TERT promoter mutations identify a high-risk group in metastasis-free advanced thyroid carcinoma

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Abstract ***Background:*** TERT promoter mutations are associated with adverse clinicopathological characteristics in thyroid carcinomas and considered as a major indicator of poor outcomes. Nevertheless, most studies have pooled heterogeneous types of thyroid carcinomas and have been conducted retrospectively. We investigated the association between TERT promoter mutations and recurrence in a prospective series of 173 intermediate- to high-risk patients with thyroid cancer.

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Patients: Patients referred for radioiodine treatment after thyroidectomy for intermediate- to high-risk differentiated thyroid carcinoma were included in a prospective observational study and tested for TERT promoter, BRAF, and RAS mutations of their primary tumours. We analysed the relationship between TERT promoter mutations and outcomes.
Results: The prevalence of TERT promoter mutations was 20.2% (35/173) in the total population. It was significantly higher in tumours harbouring aggressive histological features (poorly differentiated carcinoma, tall cell variant of papillary cancer or widely invasive follicular cancer) than in non-aggressive tumours: 32.7% (16/49) versus 15.3% (19/124; $p = 0.020$). TERT promoter mutations were also strongly associated with age $\geq$45 years ($p = 0.005$), pT4 stage ($p = 0.015$), metastatic disease ($p = 0.014$), and extrathyroidal extension ($p = 0.002$). TERT promoter mutations were associated with poor outcomes in the total population ($p < 0.001$) but not in the subgroup of non-metastatic patients ($p = 0.051$). However, they were associated with a worse outcome in patients both free of metastases and devoid of aggressive histological features. Neither BRAF nor RAS mutations were associated with event-free survival in non-metastatic patients.
Conclusion: Although their prognostic value does not seem to overcome that of histology, TERT promoter mutations may help to better define the prognosis of localized thyroid cancer patients without aggressive histology.

1. Introduction

Despite overall excellent prognosis, it is estimated that up to 20% of thyroid carcinomas relapse and 5–10% will evolve to metastases [1]. This proportion is increased among some subgroups of carcinomas including aggressive histological variants such as poorly differentiated thyroid carcinomas (PDTCs) or tall cell papillary carcinomas (TCPTCs) [2,3]. The development of targeted therapies has led to increased interest in the identification of molecular alterations present in thyroid cancer and their potential prognostic impact. BRAF and RAS mutations have been the most extensively studied. BRAF mutation is associated with advanced forms of thyroid cancer but is nowadays understood not to be an independent prognostic factor [4]. TERT promoter mutations have been described as predictive of poor prognosis in differentiated thyroid carcinoma (DTC) in most published studies [5–11], except in micropapillary cancer [12]. Nevertheless, these studies pooled heterogeneous types of thyroid carcinomas and most have been conducted retrospectively. The aim of the present study was to analyse the prognostic value of TERT mutations in a prospective series of intermediate- to high-risk patients with thyroid carcinoma.

2. Patients and methods

2.1. Patients

A total of 173 consecutive patients (105 females and 68 males; mean ± standard deviation [SD] age 50.8 ± 17.5 years, range: 18–93) with DTC (pT3$\geq$20 mm, pT4 or M1 according to the 2004 World Health Organization [WHO] classification) referred for radioiodine (RAI) therapy after thyroidectomy in one of the two nuclear medicine hospital departments of Lyon, France, were included between 2010 and 2013 in a prospective observational study and tested for molecular alterations of their primary tumour. The local ethical committee approved the study. Written informed consent for molecular analysis was obtained at the time of surgery.

In accordance with international guidelines available at the time of surgery [13], all patients had undergone a total or near total thyroidectomy, associated with lymph node dissection, depending on the results of preoperative ultrasonography (US). After RAI administration, follow-up and subsequent treatments, if necessary, were conducted as recommended by the American Thyroid Association (ATA) 2009 guidelines [13].

The patients were subjected to restaging procedures, as described by Tuttle *et al.* [14], and modified in the ATA 2016 guidelines [15] after a mean ± SD delay of 15.6 ± 6 months. Patients with excellent or indeterminate response to initial treatment were considered in remission, whereas those with biochemical or structural incomplete response were considered as having persistent disease.

Subsequently, once a year for 5 years, the status of the patients was re-evaluated on the basis of thyroglobulin (Tg) determination, cervical US, and, if appropriate, other imaging. No evidence of disease (NED) was defined by a suppressed Tg < 1 µg/l, no detectable Tg antibody, and no structural evidence of disease [15]. In case of recurrent disease, the type of relapse was specified: biological or morphological, local or metastatic. Cases and causes of death (from cancer or not) were recorded. The database also recorded every

thyroid-specific treatment administered, allowing the identification of iodine refractory disease, as defined by Schlumberger *et al.* [16].

2.2. Tumour sample analyses

All tumour samples were reviewed by a referent pathologist (M.D.-P.) and classified according to the 2004 WHO classification [17]. Patients with aggressive histological features including PDTC, TCPTC, and widely invasive follicular carcinomas were pooled and compared with those with non-aggressive tumours.

DNA was isolated from formalin-fixed paraffin-embedded tissue blocks of tumour thyroid tissue, using the QIAamp DNA FFPE Tissue Kit on a QIAcube instrument (Qiagen, Hilden, Germany). Mutations of TERT promoter were analysed using nested polymerase chain reaction (PCR) and sequencing as previously described [18]. Mutations of BRAF exon 15, NRAS exon 3 and HRAS exon 3 were screened using real-time PCR and fluorescence high-resolution melting curve analysis on a LightCycler 480 Instrument (Roche Diagnostics, Vienna, Austria). Positive samples were confirmed by Sanger sequencing [19].

2.3. Statistical analysis

Categorical variables are expressed as number (n) and percentage, and quantitative variables are expressed as mean ± SD. The hypothesis of normal distribution of quantitative variables was tested using the Kolmogorov–Smirnov test and graphically confirmed with a histogram.

Categorical variables were compared using the chi-square test or Fisher's exact test when the conditions of application of chi-square test were not met. Quantitative variables were compared between groups using Student's t test after verification of equality of variances when data were normally distributed and with the Wilcoxon nonparametric test when the hypothesis of normality of distribution was not verified.

Survival curves were obtained using a Kaplan–Meier model and compared using the log-rank test. Event was defined as diagnosis with metastasis or refractory disease or death during follow-up. Event-free survival was determined between the date of evaluation and date of event or date of the last known status.

Prognostic factors of event were determined using a Cox model after verification of the proportionality of risk hypothesis, first in a univariate analysis, and, if appropriate, in a multivariate analysis including factors found significant in the univariate analysis and adjusted on age.

The statistical tests were two tailed, and the level of significance was set to 5% ($p < 0.05$). Statistical analyses were conducted using SAS v9.4 (SAS Institute Inc, Cary, NC, USA).

3. Results

3.1. Clinical presentation and follow-up of the cohort

Clinical and histopathological data are presented in Table 1. Eighty-two percent of patients (142/173) had a well-differentiated thyroid cancer from papillary (PTC) or follicular (FTC) type and 18% (31/173), a PDTC. Among the 133 PTCs, most were classical or follicular variant, and 14 had tall cell features. Four of the nine FTCs had widely invasive features. As a whole, 49 tumours had aggressive histological features.

After thyroidectomy, 165 patients received a fixed activity of 3.7GBq of RAI; eight received a lower dose (range: 1.1–1.85GBq) either because of altered renal function or because of large remnant. Post-therapeutic whole-body scan (WBS) found extracervical uptake, indicative of metastatic disease in 30 patients, including the seven whose cancer had been diagnosed on a metastasis: metastatic sites were lung (n = 20), bones (n = 14), liver (n = 2) and brain (n = 1).

In total, 76.3% of the cohort had an excellent (n = 107) or indeterminate (n = 25) response to

Table 1
Clinical and histopathological data.

Characteristic	Total n = 173	TERT wild-type, n = 138	TERT mutated, n = 35	p
Sex, female, n (%)	105 (60.7)	86 (62.3)	19 (54.3)	0.385[a]
Age at surgery, n (%)				0.005[a]
<45 years	71 (41.0)	64 (46.4)	7 (20.0)	
≥45 years	102 (59.0)	74 (53.6)	28 (80.0)	
Histological features, n (%)				0.011[a]
Non-aggressive	124 (71.7)	105 (76.1)	19 (54.3)	
Aggressive	49 (28.3)	33 (23.9)	16 (45.7)	
Initial pT stage (M0 patients), n (%)				0.015[b]
pT3	136	116 (97.5)	20 (14.7)	
pT4	7	3 (42.9)	4 (57.1)	
Initial M stage, n (%)				0.014[a]
M0	143 (82.7)	119 (86.2)	24 (68.6)	
M1	30 (17.3)	19 (13.8)	11 (31.4)	
Tumour size (cm), mean ± SD	4.15 ± 1.9	4.06 ± 1.8	4.51 ± 2.1	0.205[c]
Extrathyroidal extension, n (%)				0.002[a]
Yes	92 (53.8)	65 (47.8)	27 (77.1)	
No	79 (46.2)	71 (52.2)	8 (22.9)	
Quality of resection, n (%)				0.100[a]
R0	136 (84.0)	113 (86.3)	23 (74.2)	
R1–R2	26 (16.0)	18 (13.7)	8 (25.8)	
Vascular embolism invasion, n (%)				0.438[a]
No	105 (65.2)	86 (66.7)	19 (59.4)	
Yes	56 (34.8)	43 (33.3)	13 (40.6)	

SD, standard deviation.
[a] Chi-square test.
[b] Fisher's exact test.
[c] Wilcoxon test.

therapy, whereas 23.7% had a biochemical (n = 7) or structural (n = 34) incomplete response (Table 2). Among the 30 initially metastatic patients, eight (26.7%) had an excellent response to therapy, five (16.7%) an indeterminate response and 17 (56.7%) remained with structural disease. Among the 143 non-metastatic patients, 99 (69.2%) had an excellent response to therapy, 20 (13.9%) an indeterminate response, seven (4.8%) a biochemically incomplete response, and 17 (11.8%) a structurally incomplete response.

Regarding treatments, 114 (65.9%) patients received a single course of RAI after surgery, whereas 59 (34.1%) received at least one subsequent course of RAI either for initially metastatic disease or for subsequent relapse. Twenty patients were reoperated, for cervical disease (n = 14) or for bone metastases (n = 6). Local treatments were administered to 12 patients (external beam n = 8, cryotherapy n = 2, cementoplasty n = 1 and radiofrequency n = 1), and ten patients received systemic treatments (chemotherapy, n = 2 and tyrosine kinase inhibitors, n = 8).

At the end of follow-up, after a mean ± SD delay of 46 ± 19 months, 28 patients (16.2%) were metastatic (n = 22, including 14 with refractory disease) or had died from thyroid cancer (n = 6; Table 2). Among the 132 patients with excellent or indeterminate response at the time of restaging, 119 (90.1%) remained in remission. Twelve (9.1%) experienced relapse from thyroid carcinoma, five with isolated elevated Tg levels, three with cervical recurrence and four with metastatic disease. One patient died from pulmonary embolism.

3.2. Prevalence of mutations

A TERT mutation was found in 35 (C228T, n = 31 and C250T, n = 4) of the 173 tumours (20.2%). There was a trend towards greater prevalence of TERT mutations in PDTC (10/31, 32.3%) than in DTC (25/142, 17.6%; p = 0.084). The prevalence of TERT mutations was 32.7% (16/49) in tumours with aggressive histological features, and this was significantly higher than in non-aggressive tumours (15.3%, 19/124; p = 0.020).

A BRAF mutation was found in 50 tumours (28.9%). Prevalence was 34.5% (49/142) in DTC, and this was significantly higher than that in PDTC (1/31, 3.2%; p < 0.001); there was no significant difference between aggressive (12/49, 24.5%) and non-aggressive tumours (38/124, 30.6%; p = 0.462).

A RAS mutation was found in 27 (NRAS, n = 17 and HRAS, n = 10) tumours (15.6%). RAS mutations were identified in 12.0% (17/142) of DTC tumours, and this was significantly lower than in PDTC tumours (32.3%, 10/31; p = 0.011).

Co-occurrence of BRAF and TERT mutations were found in 13 tumours (7.5%) and of TERT and RAS in nine (5.2%); 22 of the 35 (62.8%) tumours with a TERT mutation also had another mutation.

3.3. Relationship among TERT mutations, clinicopathological features and outcomes

The presence of a TERT mutation was significantly more frequent among patients aged ≥45 years at time of surgery (p = 0.005), pT4 stage (p = 0.015), metastatic disease (p = 0.014), extrathyroidal extension (p = 0.002; Table 1), occurrence of extracervical RAI uptake on post-therapeutic WBS (p = 0.011) or of structural incomplete response at restaging (Table 2).

Among M0 patients, the proportion of excellent/indeterminate response to initial treatment was higher in TERT wild-type patients (86.6%) than in those with mutated TERT (66.7%; p = 0.042, Table 2). At the last follow-up, the proportion of NED patients was higher in TERT wild-type M0 patients (79.1%) than in TERT-

Table 2
Results of post-therapeutic whole-body scan (post-Tx WBS) and follow-up.

Status	Total population (n = 173)	Total population			M0		
		TERT wild-type (n = 138)	TERT mutated (n = 35)	p	TERT wild-type (n = 119)	TERT mutated (n = 24)	p
Results of post-Tx WBS, n (%)				0.011[a]			0.351[a]
Thyroid bed only uptake or no uptake	128 (74.0)	108 (78.3)	20 (57.1)		107 (89.9)	20 (83.3)	
Cervical lymph node or extracervical uptake	45 (26.0)	30 (21.7)	15 (42.9)		12 (10.1)	4 (16.7)	
Restaging, n (%)				0.002[b]			0.042[b]
Excellent or indeterminate response	132 (76.3)	113 (81.9)	19 (54.3)		103 (86.6)	16 (66.7)	
Biochemical incomplete response	7 (4.0)	5 (3.6)	2 (5.7)		5 (4.2)	2 (8.3)	
Structural incomplete response	34 (19.7)	20 (14.5)	14 (40.0)		11 (9.2)	6 (25.0)	
Final status, n (%)				<0.001[b]			0.003[b]
No evidence of disease	127 (73.4)	111 (80.4)	16 (45.7)		102 (79.1)	13 (54.2)	
Biological persistent disease	10 (5.8)	7 (5.1)	3 (8.6)		4 (3.4)	2 (8.3)	
Cervical persistent disease	8 (4.6)	6 (4.3)	2 (5.7)		5 (4.2)	2 (8.3)	
Metastases or death from cancer	28 (16.2)	14 (10.1)	14 (40.0)		8 (6.7)	7 (29.2)	

[a] Chi-square test.
[b] Fisher's exact test.

mutated M0 patients (54.2%; p = 0.003). The proportion of metastatic or dead from thyroid carcinoma patients was lower in TERT wild-type M0 patients (6.7%) than in TERT-mutated patients (29.2%).

The final status of initially metastatic patients did not differ according to the TERT status: 9/19 (47.4%) of TERT wild-type achieved NED compared with 3/11 (27.3%) TERT mutated; and 6/19 (31.6%) of TERT wild-type patients remained metastatic or had died from thyroid carcinoma, versus 7/11 (63.7%) of TERT-mutated patients.

Among patients with aggressive histology, the proportion of remission and metastases were similar, irrespective of the TERT mutation status as presented in Table 3. In the group of patients with non-aggressive histology (n = 124), the proportion of excellent or indeterminate response to initial treatment (p = 0.006) and the proportion of NED at the end of follow-up were higher in TERT wild-type than in TERT-mutated patients (p = 0.001; Table 3).

3.4. Predictive factors

In the total population (M0+M1), TERT mutations were significantly associated with poor prognosis (log-rank test, p < 0.001; Fig. 1A); the difference did not remain statistically significant when only M0 patients were considered (log-rank test, p = 0.051; Fig. 1B). Aggressive histological features were significantly associated with poor event-free survival even among non-metastatic patients (log-rank test, p < 0.001; Fig. 2).

In non-metastatic patients, event-free survival was associated in univariate analysis with age equal to 45 years or above at surgery (p = 0.034), histological aggressive features (p < 0.001) and vascular invasion (p < 0.001). Neither TERT nor BRAF or RAS was associated with event-free survival (Table 4). In multivariate analysis, histological aggressive features remained significantly associated with worse event-free survival (Table 5).

TERT mutations were not significantly associated with event-free survival in M0 patients with aggressive histology (Fig. 3A) but were strongly associated with this among M0 patients with non-aggressive histological features (no event in the Wild Type group; Fig. 3B).

4. Discussion

In the present study, TERT mutations were found to be strongly associated with adverse pathological features such as older age at surgery, aggressive histological features, pT4 stage, metastatic disease and extra-thyroidal extension, which is in accordance with the literature [5–8,10,20–23]. TERT mutations were associated with poor outcomes in the total population but were not predictive of outcomes in initially non-metastatic patients or among those harbouring aggressive histologic features. Nevertheless, TERT mutations were strongly associated with a poor outcome in patients free of metastases and devoid of aggressive histological features.

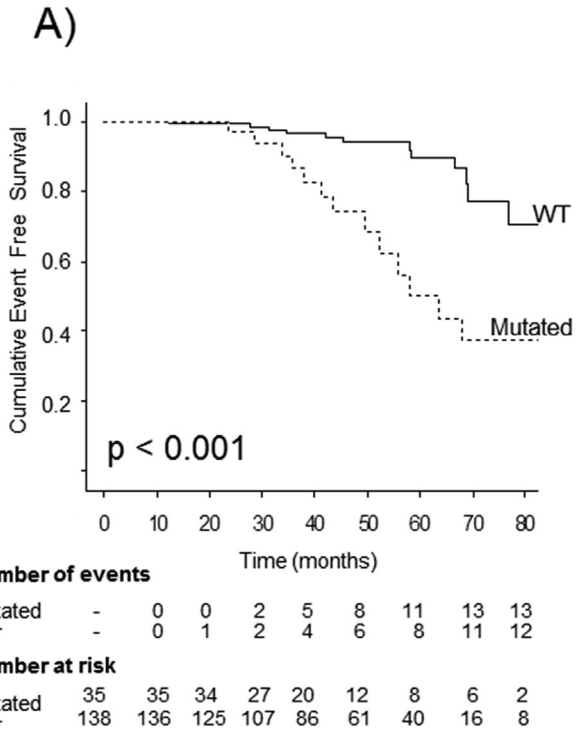
Patients included herein were selected on the histological criteria (the pT stage, size or initial metastatic spread) that, in our experience, are the most frequently observed among RAI refractory patients. All were classified at either intermediate or high risk of relapse according to the ATA 2009 classification [13]. Importantly, response to initial treatment and status at the end of follow-up were those expected in patients with such clinical and histological features [14]. We, therefore, believe that this cohort is representative of intermediate- to high-risk patients with thyroid cancer. The mean duration of follow-up of 46 months seems acceptable as it has been reported that more than 75% of papillary thyroid carcinoma recurrence occurs within the first 5 years after the initial treatment [24]. Furthermore, by contrast with most published studies, this was a prospective cohort which may avoid some classical biases.

TERT mutations were identified among a fifth of the tumours herein, a relatively high proportion compared with the pooled frequency of 14.3% in a recent meta-analysis [25], that is explained by the selection of intermediate- to high-risk DTC. While it is well established that PDTCs more frequently harbour TERT mutations

Table 3
Response to treatment according to the histology and TERT promoter mutation status.

Status	Aggressive histology			Non-aggressive histology		
	Non-mutated (n = 33)	Mutated (n = 16)	p[a]	Non-mutated (n = 105)	Mutated (n = 19)	p[a]
Restaging, n (%)			0.410			0.006
Excellent or indeterminate response	22 (66.7)	8 (50.0)		91 (86.7)	11 (57.9)	
Biochemical incomplete response	1 (3.0)	1 (6.3)		4 (3.8)	1 (5.3)	
Structural incomplete response	10 (30.3)	7 (43.8)		10 (9.5)	7 (36.8)	
Final status, n (%)			0.057			0.001
No evidence of disease	20 (60.6)	5 (31.3)		91 (86.7)	11 (57.9)	
Biological persistent disease	0 (0.0)	2 (12.5)		7 (6.7)	1 (5.3)	
Cervical persistent disease	1 (3.0)	1 (6.3)		5 (4.8)	1 (5.3)	
Metastases or death from cancer	12 (36.4)	8 (50.0)		2 (1.9)	6 (31.6)	

[a] Fisher's exact test.



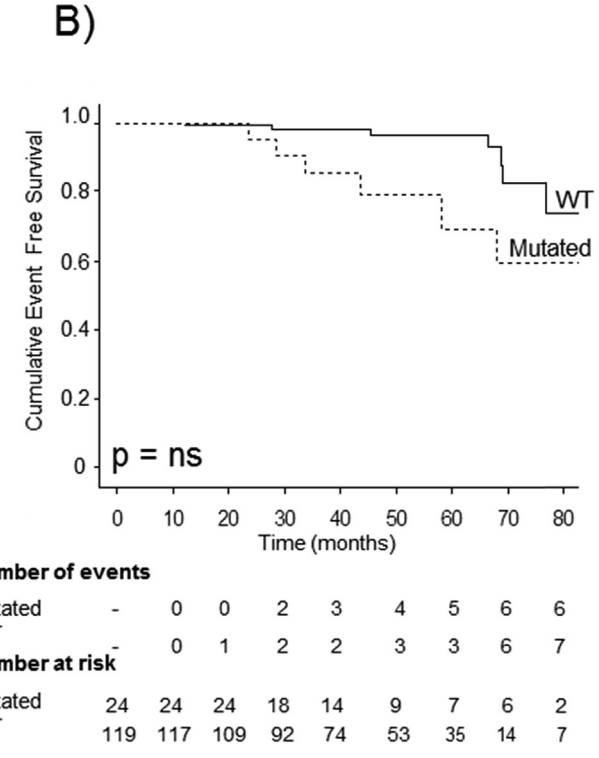


Fig. 1. Kaplan–Meier analysis of the impact of TERT promoter mutation on event-free survival of the total population (A) and of the initially metastasis-free patients (B).

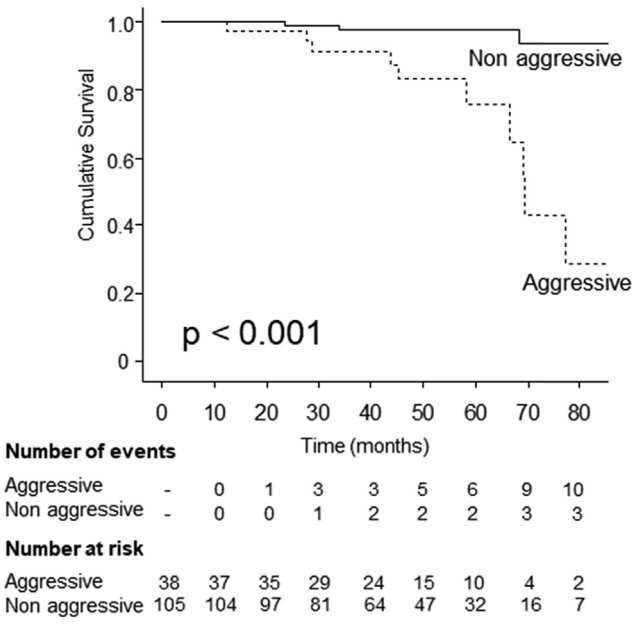


Fig. 2. Kaplan–Meier analysis of the impact of histological features on event-free survival of initially metastasis-free patients.

Table 4
Univariate analysis of prognostic factor for event-free survival among patients without metastasis.

Parameter	n	Event	HR	95% CI	p
Age					
<45	64	1	1	/	
≥45	79	14	9.14	1.19–70.40	0.034
Sex					
Female	83	8	1	/	
Male	60	7	0.73	0.24–2.25	0.584
Vascular embolism invasion					
No	95	4	1	/	
Yes	38	11	19.00	4.17–86.48	<0.001
Extrathyroid extension					
No	65	4	1	/	
Yes	76	10	2.48	0.76–8.13	0.133
Histologic features					
Non-aggressive	105	4	1	/	
Aggressive	38	11	10.32	2.86–37.25	<0.001
TERT					
Wild-type	119	8	1	/	
Mutation	24	7	2.81	0.95–8.30	0.061
BRAF					
Wild-type	95	11	1	/	
Mutation	48	4	0.65	0.20–2.07	0.461
TERT + BRAF					
Both wild type	82	7	1	/	
At least one mutation	50	5	0.66	0.17–2.56	0.549
Both mutation	11	3	2.17	0.55–8.57	0.270
RAS					
Wild-type	126	13	1	/	
Mutation	17	2	1.08	0.24–4.87	0.923

HR, hazard ratio; CI, confidence interval.
Event includes metastasis, refractory disease and death.

Table 5
Multivariate analysis of prognostic factor for event-free survival among patients without metastasis.

Parameter	n	Event	HR[a]	95% CI	p
TERT					
Wild-type	119	8	1	/	0.111
Mutation	24	7	2.50	0.81–7.69	
Histological features					
Non-aggressive	105	4	1	/	<0.001
Aggressive	38	11	9.43	2.51–35.42	

HR, hazard ratio; CI, confidence interval.
[a] Adjusted on age in continuous variable.

than differentiated carcinomas [10,21,26,27], the difference did not reach statistical significance in the present study. This discrepancy may be attributed to the low number of PDTCs, or to the relatively high proportion of TCPTC, among which up to 30% harbour TERT mutations [25]. Indeed, herein, TERT mutations were significantly more frequent in tumours with aggressive histological features than in non-aggressive tumours, a result that is concordant with the literature [21–23].

BRAF mutation was initially identified as a major prognostic factor of papillary thyroid carcinomas [28]. Herein, even in the univariate analysis, BRAF was not associated with event-free survival. One potential explanation could be the relatively low prevalence of BRAF mutation in the present study that pooled PTC, FTC and PDTC. But it must also be underlined that the prognostic value of BRAF mutation has been questioned, some studies failing to demonstrate that BRAF mutation was an independent prognostic factor [29].

Most studies have evaluated the impact of TERT only on long-term outcomes, finding an increased risk of death and of RAI courses [10,30], whereas the prospective design of the present study allowed the analysis of the patient status at various times of follow-up. At the time of restaging, only structural incomplete responses were considered as it has been reported by others that most patients with biological residual disease will finally evolve without any relapse [31]. We found that TERT mutations were associated with incomplete response to initial treatment and a lower probability of NED at the end of follow-up, even in the subgroup of initially non-metastatic patients, a finding which suggests that these patients need reinforced monitoring and treatment. Penna *et al.* [23] recently found an association between TERT mutations and both initial response to treatment and progression of structural disease among patients with aggressive variants of thyroid carcinomas, data not confirmed in our study. Nevertheless, their cohort differs from our subgroup of patients with aggressive histologic features in terms of definition (for example, we did not include follicular variant of PTC displaying extensive invasion in that subgroup) and initial stage (54% of their patients were M1), and this may have influenced the results. In both studies, the number of

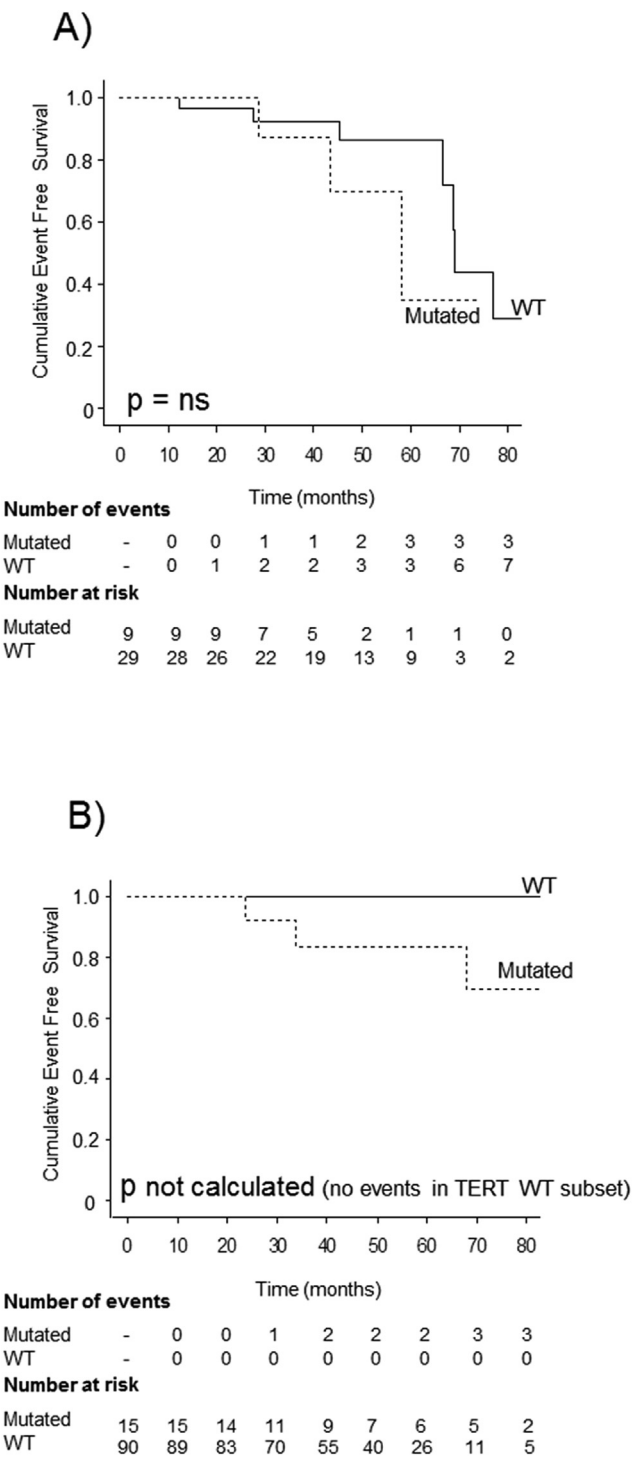


Fig. 3. Kaplan–Meier analysis of the impact of TERT promoter mutation on event-free survival of initially metastasis-free patients with aggressive histological features (A) and without aggressive histological features (B).

patients with aggressive variants of thyroid cancer remains limited, and prospective studies on larger groups remain necessary.

In this study, the limited length of follow-up and the rarity of deaths precluded the analysis of the influence of

TERT mutations on overall survival. For the event-free survival analysis, patients with metastatic disease at diagnosis were excluded. In univariate analysis, older age at diagnosis, vascular invasion and aggressive histology were significant indicators of higher risk of event, but TERT mutations were not. The literature is currently divided as to the association between TERT mutations and recurrence. For instance, there are studies that have found TERT mutations to be an independent predictor of poor outcomes [8,10,32,33]. Conversely, in accordance with the results presented herein other authors failed to demonstrate that TERT mutations were an independent prognostic factor [5,27,34]. The present study suggests that the prognosis of patients with mutated tumours is not independent from histological features. This was also suggested in one study in which TERT mutations were associated with distant metastases in multivariate analysis, but only after exclusion of vascular invasion which was not considered in the multivariate model [10]. Furthermore, in patients with aggressive clinicopathological characteristics, the utility of molecular markers can be questioned.

Interestingly, when focussing on M0 patients without aggressive histological features, the subgroup of the cohort less prone to experience recurrence, TERT mutations were strongly associated with a worse event-free survival. These results, in concordance with those recently published by Xu *et al.* who report a small retrospective study of PTC with initially low-risk histological features who developed metastases, suggest that TERT mutations should help to identify patients requiring more intensive treatment and close follow-up [35].

5. Conclusion

The prognostic value of TERT mutations did not seem to overcome that of histology, but it was associated with worse outcomes in metastasis-free patients without aggressive histological features and could, thus, help to identify a high-risk subset of patients who could benefit from intensive treatment and follow-up.

Conflict of interest statement

None declared.

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