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► **To cite this version:**

Segolene Hescot, Abir Al Ghuzlan, Theophraste Henry, Hala Sheikh Alard, Livia Lamartina, et al.. Prognostic of recurrence and survival in poorly differentiated thyroid cancer. *Endocrine-Related Cancer*, 2022, 10.1530/erc-22-0151 . hal-03860389

**HAL Id: hal-03860389**

**<https://cnrs.hal.science/hal-03860389>**

Submitted on 18 Nov 2022

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## RESEARCH

# Prognostic of recurrence and survival in poorly differentiated thyroid cancer

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M Schlumberger is a member of the editorial board. He was not involved in the review or editorial process for this paper, on which he is listed as an author.

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## Abstract

The prognosis of poorly differentiated thyroid carcinomas (PDTC) defined by the Turin criteria is variable. The aim of this study on 51 PDTC patients was to determine clinical, histological and molecular prognostic factors associated with recurrence in patients with localized disease at initial treatment and with overall survival in patients with distant metastases. Of 40 patients for whom next-generation sequencing (NGS) by ThyroSeq v3 was able to be performed on historical samples, we identified high-risk molecular signature (*TERT*, *TP53* mutations) in 24 (60%) cases, intermediate risk signature in 9 (22.5%) cases and low-risk signature in 7 (17.5%) cases. Potentially actionable mutations were identified in 10% of cases. After a median follow-up of 57.5 months, recurrence occurred in 11 (39%) of the 28 patients with localized disease. The American Thyroid Association (ATA) high risk of relapse, high mitotic count, high molecular risk signature and CD163 expression were associated with recurrence ( $P = 0.009, 0.01, 0.049, 0.03$  respectively). After a median follow-up of 49.5 months, thyroid cancer-related death occurred in 53% of the patients with distant metastases. There was no significant prognostic factor associated with death in univariate analysis. However, none of the patients with intermediate ATA risk of recurrence and none of the patients with low-risk molecular signature died from the disease. In addition, high molecular-risk signature was associated with the presence of synchronous or metachronous distant metastasis ( $P = 0.007$ ) and with poor overall survival ( $P = 0.01$ ). In conclusion, ATA risk of relapse and high mitotic count was associated with higher rate of recurrence in localized PDTC. High molecular-risk signature was associated with the presence of distant metastasis and poor overall survival. Further studies are needed to determine if molecular testing adds to ATA risk stratification or response to therapy in predicting outcomes.

## Key Words

- ▶ poorly differentiated thyroid carcinoma
- ▶ prognosis
- ▶ recurrence
- ▶ survival
- ▶ molecular signature
- ▶ ThyroSeq

*Endocrine-Related Cancer*  
(2022) **29**, 625–634

## Introduction

Poorly differentiated thyroid carcinoma (PDTC) is a relatively uncommon type of thyroid cancer with a rate of recurrence, distant spread and mortality that are significantly higher than in well-differentiated thyroid cancer (DTC) (Dettmer *et al.* 2012, Akaishi *et al.* 2019). Diagnostic histopathological criteria of PDTC were standardized by an international consensus in Turin in 2007 and include (i) vascular or capsular invasion, (ii) solid, insular or trabecular growth, (iii) presence of at least one of the following criteria:  $\geq 3$  mitoses/10 high-power field (HPF), necrosis or convoluted nuclei and (iv) absence of nuclear features of papillary thyroid carcinoma (Volante *et al.* 2007). Genetic profile of PDTC is different from DTC, with a higher mutation burden and more frequent occurrence of *TERT* and *TP53* mutations which are considered a late genetic hit. These mutations frequently coexist in PDTC with *NRAS* or *BRAF-V600E* mutations that are considered early hits, while chromosomal rearrangements are less frequently described (Landa *et al.* 2016). *TERT* promoter and *TP53* mutations may have a prognostic impact on patient overall survival (OS) (Volante *et al.* 2021). Other prognostic parameters for survival reported in cohorts that included both localized and metastatic PDTC are age, size and extra-thyroidal extension (Hiltzik *et al.* 2006, Asioli *et al.* 2010). However, little is known concerning prognostic parameters for recurrence in patients with localized PDTC. Recently, a molecular-based risk stratification using the ThyroSeq version 3 multi-gene assay was validated in DTC (Yip *et al.* 2021). However, its performance in PDTC prognostication has not been evaluated to date.

The aim of this study was to examine clinical, pathological, immunohistochemical and molecular prognostic markers of PDTC recurrence in patients with localized disease at initial presentation and of OS in patients with distant metastases.

## Materials and methods

### Clinical data

Consecutive patients with PDTC with available primary thyroid tumor samples who were followed at Gustave Roussy between January 1, 1995, and December 31, 2015, were included in the study. Informed consent was obtained from all patients. The medical files of each patient were reviewed by one investigator (SH) to record clinical parameters at the time of initial diagnosis and outcome.

The study was approved by Gustave Roussy's local ethical committee.

### Pathology review

All slides of primary tumor were reviewed by two experienced thyroid pathologists (AAG and HSA), and a total of 51 cases of poorly differentiated carcinoma were identified according to the 2017 WHO classification of thyroid tumors which is based on the Turin diagnostic criteria for PDTC (Lloyd *et al.* 2017). The following features were analyzed: tumor size, necrosis, mitoses count on 2 mm<sup>2</sup> ( $\times 400$ ) and vascular invasion. The presence of a tumor capsule and extrathyroid extension was recorded. Vascular and capsular invasion was subdivided into three categories: absent, focal ( $< 4$  invasive foci) and extensive ( $\geq 4$  invasive foci). Tumor necrosis was classified as absent, focal or extensive. The percentage of a well-differentiated component within the other PDTC tumor was recorded. Finally, the predominant cell type was categorized as oncocytic vs non-oncocytic.

### Interpretation of immunohistochemical stains

Immunohistochemical techniques were carried out by the Laboratory of Experimental and Translational Pathology (PETRA), Gustave Roussy Cancer Campus. Staining platforms, antibody clones, dilutions and relevant patterns for interpretation are detailed in Supplementary Table 1 (see section on [supplementary materials](#) given at the end of this article). The immunohistochemical expression of IMP3 (insulin-like growth factor II mRNA-binding protein-3) was scored by considering both the intensity and the extent of the staining using a semi-quantitative scoring system based on a previous study (Asioli *et al.* 2010). The mitotic index (per 2 mm<sup>2</sup>) and Ki67 proliferation index were evaluated in the area showing the highest proliferative activity (so-called hot spots).

### Molecular analysis and risk groups

ThyroSeq was only performed in 40 patients that either had tissue or nucleic acids available for molecular analysis or had acceptable quality of nucleic acids isolated from FFPE tissue. Nucleic acids were isolated either from formalin-fixed, paraffin-embedded tissues ( $n=20$ ) or snap-frozen tissues obtained from surgically excised tumor samples ( $n=26$ ) and were tested using the ThyroSeq v3 Genomic Classifier (GC)-targeted next-generation sequencing assay (University of Pittsburgh Medical Center), as previously described (Nikiforova *et al.* 2018, Steward *et al.* 2019).

The test analyzes 112 genes for mutations, gene fusions, chromosomal copy number alterations (CNAs) and gene expression alterations (GNA). Detected molecular alterations were classified into low, intermediate and high molecular risk groups (MRGs). The risk stratification was based on the associations between molecular alterations and the probability of disease recurrence or distant metastasis and OS (Xing *et al.* 2013, Yip *et al.* 2015, 2021, Haugen *et al.* 2016, Song *et al.* 2016, Patel *et al.* 2017). Specifically, the low-risk group includes isolated *RAS* or *RAS*-like alterations and low-risk CNA. The intermediate risk group includes *BRAF* V600E, *BRAF* V600E-like alterations and Hurthle-type CNAs. The high-risk profile included the presence of an early mutation (*BRAF* or *RAS*), and a late-hit mutation, including *TERT*, *TP53*, *AKT1* and *PIK3CA*.

### Statistical analysis

Quantitative variables were presented as mean (s.d.) and qualitative variables as count (percentage). Recurrence-free survival (RFS) and OS were calculated according to the Kaplan–Meier method from the time of initial diagnosis to the date of recurrence for RFS and from the time of stage IV diagnosis to the date of death from any cause for OS. Prognostic parameters for recurrence in the non-metastatic subpopulation and for OS in the metastatic subpopulation were first explored using univariate Cox regression analysis. We reported hazard ratios and their 95% CI. *P*-value for each variable was extracted from the likelihood ratio test of each fitted model. Multivariate analysis was not performed due to small sample sizes. Association between metastatic

**Table 1** Patient characteristics.

Variable	Category	N evaluable	Total, n (%)
Age at initial diagnosis (years)	Median (IQR)	51	59.00 (51.00, 72.00)
Sex	F	51	24 (47.1)
	M		27 (52.9)
Modality of diagnosis	Fortuitous	51	6 (11.8)
	Goiter/nodule		32 (62.7)
	Clinical		32 (62.7)
	Metastasis		9 (17.6)
Cytology (Bethesda)	B1	33 (64.7)	1 (3.0)
	B2		2 (6.1)
	B3		6 (18.2)
	B4		3 (9.1)
	B5		12 (36.4)
	B6		9 (27.3)
Lymph node dissection	Yes	51	13 (27.7)
	No		34 (72.3)
pT	2	50 (98)	6 (12)
	3a		24 (48)
	3b		9 (18)
	4		7 (14)
pN	0	34 (72.3)	22 (64.7)
	1a		5 (14.7)
	1b		7 (20.6)
M1 at initial diagnosis	No	51	28 (54.9)
	Yes		23 (45.1)
ATA risk	Intermediate	51	21 (41.2)
	High		30 (58.8)
Mitosis count (/2 mm <sup>2</sup> )	≤5	49 (96)	20 (40.8)
	>5		29 (59.2)
Extra thyroid extension	No	42 (82.3)	17 (40.5)
	Yes		25 (59.5)
Vascular invasion	No	50 (98)	21 (42.0)
	Yes		29 (58.0)
Necrosis	No	48 (94.1)	28 (58.3)
	Yes		20 (41.7)
Ki67 (%)	<20	46 (90.2)	21 (45.7)
	≥20		25 (54.3)
Molecular signature risk group	Low	40 (78)	7 (18)
	Intermediate		9 (23)
	High		24 (60)



disease and molecular risk signature was evaluated according to Fisher's exact test. Statistical analysis was realized with R version 4.0.3 on linux platform.

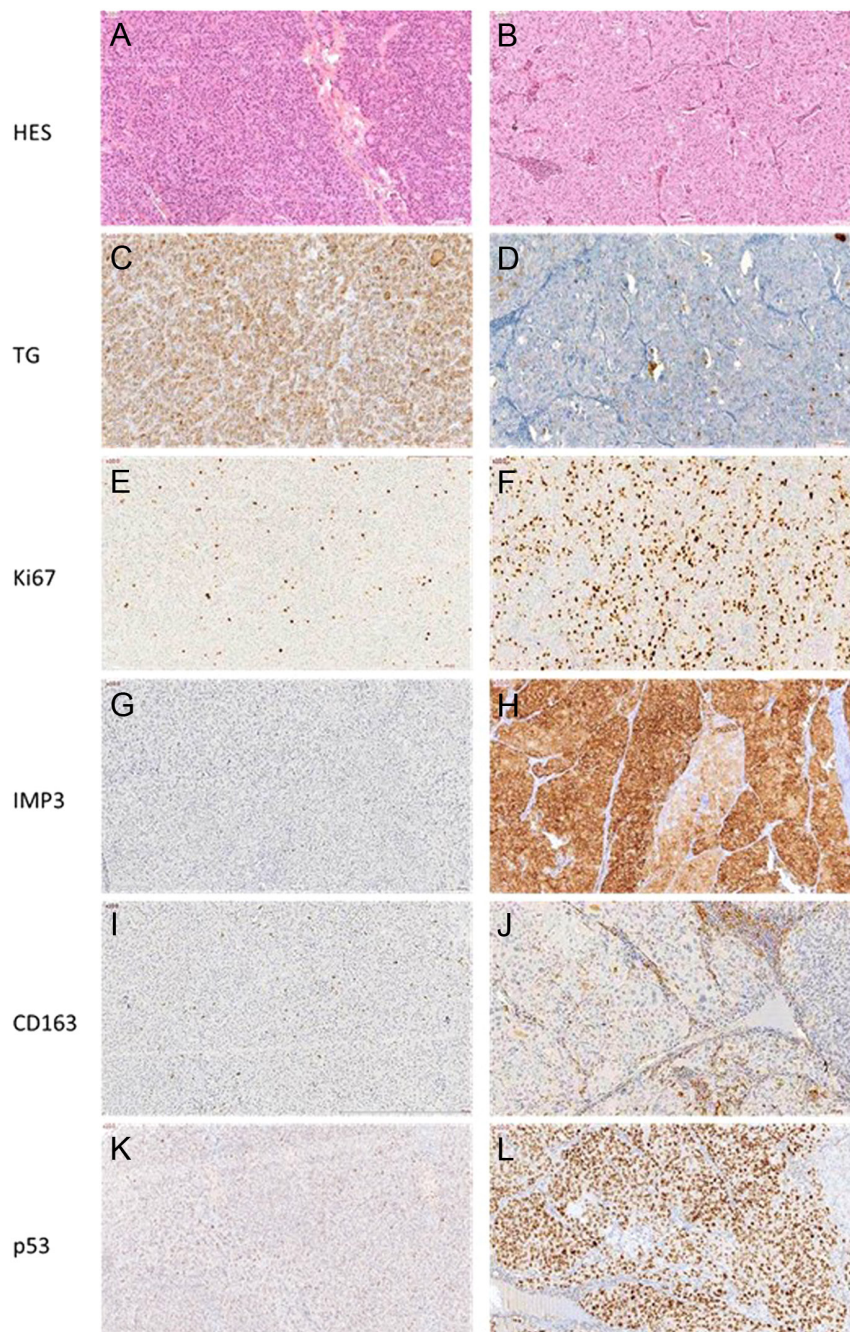
## Results

### Clinicopathological characteristics

The clinical and pathological characteristics of the 51 patients (24 females, median age: 59 years) are

summarized in [Table 1](#). At initial diagnosis, 28 patients (55%) had disease localized to the neck (M0) and the other 23 (45%) patients had synchronous distant metastases (M1). According to the American Thyroid Association (ATA) classification, among the 28 M0 patients, 21 were intermediate risk and 7 were high risk (defined in PDTC by the presence of gross extrathyroidal extension, incomplete resection or lymph node metastases > 3 cm).

On histologic examination, the mean tumor size was 42.8 mm (range: 21–85 mm). Fifty-eight percent



**Figure 1**

Histologic features and immunohistochemical staining in two cases of poorly differentiated thyroid carcinoma ( $\times 10$ ): (1) A case with an NRAS mutation, patient alive and free of disease (low-grade lesion): (A) (hematoxylin and eosin), trabecular and microfollicular growth pattern, mild atypia and low mitotic index, (C) diffusely expressed Tg, a peculiar pattern of dot-like paranuclear staining is observed, (E) low proliferation index (Ki67: 5–10%), (G) negative IMP3, (I) few inflammatory cells positive with CD163, (K) wild type pattern of P53. (2) A metastatic lesion with PTEN and P53 mutations, the patient died from the disease (high-grade lesion): (B) (hematoxylin and eosin), solid growth with marked atypia and high mitotic index, (D) focal and weak TG staining, (F) high proliferation index (Ki67: 40%), (H) diffuse and strong cytoplasmic staining for IMP3, (J) more important inflammatory cells infiltration, positive with CD163, (L) mutated pattern of P53.

of tumors (29/50) had vascular invasion, mostly extensive (four or more foci). Extrathyroidal extension was present in 59.5% (25/42), macroscopic in 14% (6/42) and necrosis in 41.7% (20/48). The necrosis was extensive in 15 cases. Oncocytic features were present in 37% of cases (19/51). A well-differentiated component was present in 27.5% of cases (14/51). Mitosis count was >5/mm<sup>2</sup> in 29 (56.7%) patients, ≤ 5/mm<sup>2</sup> in 20 (39.2%) patients and unknown in 2. Expression of each protein studied by immunohistochemistry (Fig. 1) is reported in Supplementary Table 1. All cases had ≥5 % of Ki67-positive cells (5–90%). Ki67 index was ≥ 20% in 17/47 cases (36 %) with available index (Fig. 1). Eighteen of 49 (37%) cases showed prevalent cytoplasmic staining for IMP3 (Fig. 1). Six of them (12%) with an immunohistochemical cumulative score for IMP3 more than 2 showed only focal thyroglobulin (Tg) expression and a high proliferation index (Ki67). Tg immunostaining was positive in most cases and 16% (8/50) showed only focal expression (Fig. 1). All cases but one was positive for TPO (5–100% of cells). Sixty percent of cases (30/49) were positive for GLUT1 (5–100% of cells), with membranous staining in all that was associated with cytoplasmic staining in 9 (18%). Forty-two cases showed inflammatory cells positive for

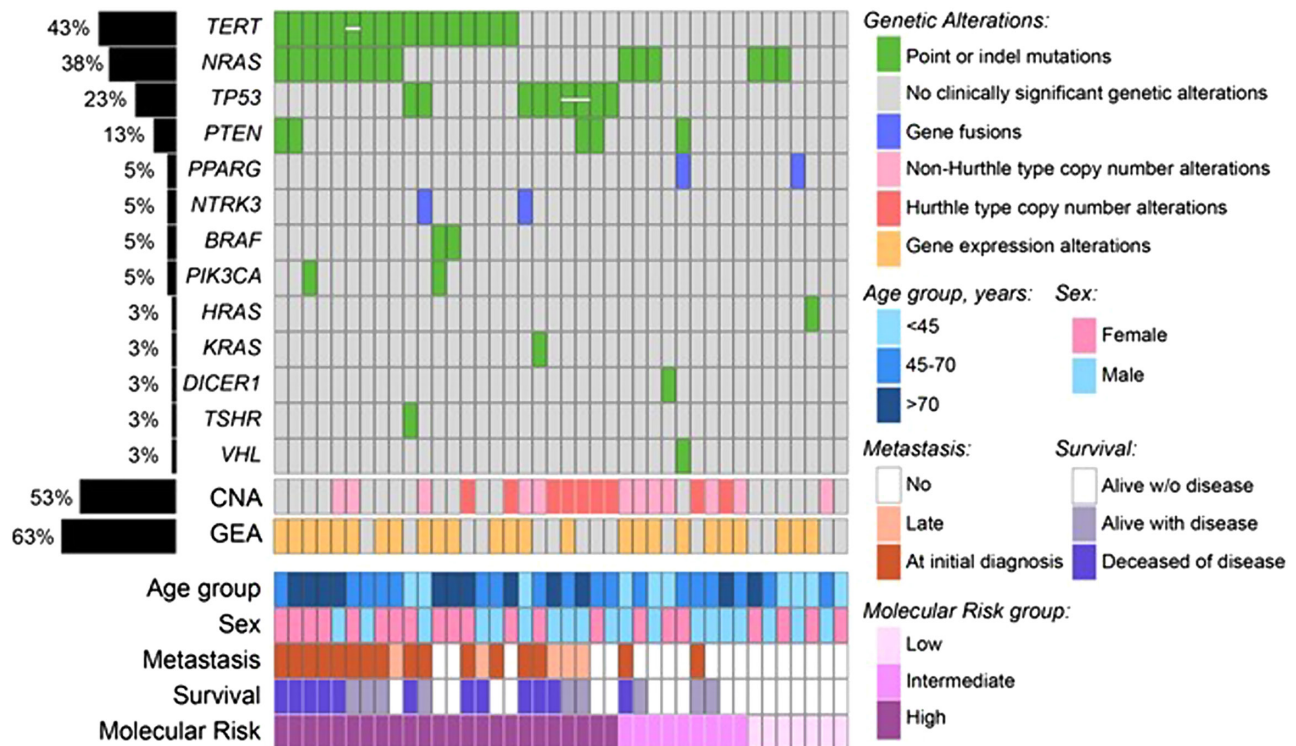
CD163 in intra-tumoral localization (Fig. 1) that were associated in 31% of them (13/42) with positive cells in the fibrous tissue surrounding the tumor. P53 was overexpressed in 100% of cells in four cases (Fig. 1).

Surgery was performed on 47 patients and 44 patients received radioactive iodine therapy. In the 28 patients with localized PDTC, following initial treatment (total thyroidectomy and RAI), ATA response to treatment at 6–12 months was excellent in 20, indeterminate in 4, biochemically incomplete in 1, structurally incomplete in 2 and not available in 1.

**Patient follow-up**

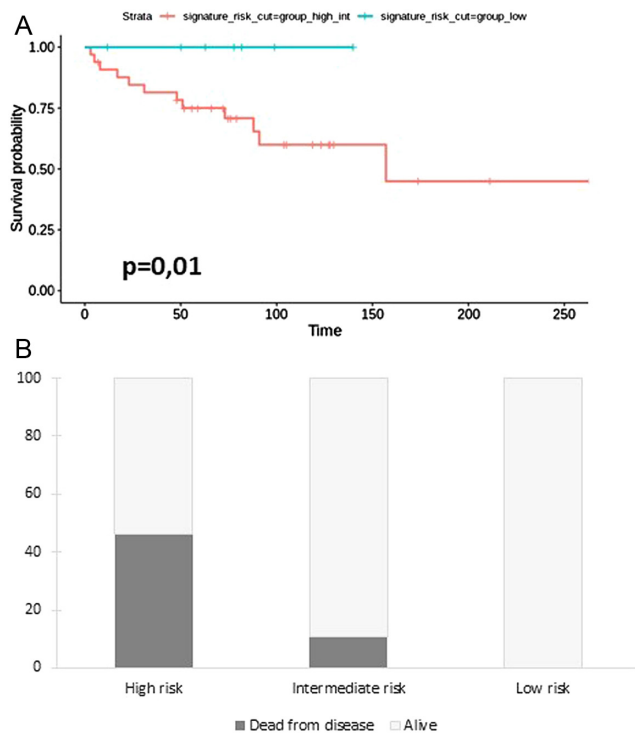
Median follow-up of the whole cohort was 73 months (range: 3–263) with a median survival not reached. One- and 5-year survival rates were 94.1 (95% CI: 88–100%) and 77.4% (95% CI: 67–90%), respectively.

The median follow-up of the 28 patients with initially localized PDTC (cohort M0) was 57.5 months (range: 8–263). Eleven patients had recurrence with a median time to recurrence of 24 months. Median recurrence-free survival was not reached, while 1-year and 5-year recurrence-free survivals were 92.9 (95% CI: 83.8–100) and 62.5% (95% CI: 46.5–84.0), respectively.



**Figure 2**  
Genomic alterations of the 40 cases informative for ThyroSeq sequencing analysis.



**Figure 3**

(A) Overall survival of patients according to molecular risk signature.  
(B) Percentage of deaths according to molecular risk signature.

Among the 32 metastatic patients (cohort M1, including 9 localized PDTC who developed M1 during follow-up and 23 PDTC patients with synchronous metastasis), distant metastases were located in mediastinal lymph nodes in 17, in lungs in 20, in bones in 14 and other sites in 6. The median follow-up of the M1 patients was 49.5 months (range: 3–222). Eleven patients died, all from thyroid cancer with a median time from diagnosis of M1 of 28 months (range: 3–157). Median OS was 68 months (lower bound of the 95% CI: 47) with a 1- and 5-year OS of 90.5 (95% CI: 80.9–100%) and 52.4% (95% CI: 37.0–74.4%), respectively.

### Genomic alterations and molecular risk stratification

ThyroSeq molecular analysis was informative in 40 cases and detected discrete genomic alterations in 39 (97.5%) (Fig. 2). Most common mutations identified were *TERT* promoter and *TP53*, both high-risk mutations that were found in 24 (60%) cases, all in combination with other molecular alterations. RAS and RAS-like molecular profile was detected in 19 (48%) cases. Potentially targetable alterations were detected in four (10%) cases

(two BRAF V600E and two NTRK3 fusions) (Fig. 2). Chromosomal copy number alterations were detected in 21 (53%) cases, including 9 cases with genome haploidization type (GH-type) CNA characteristic of Hürthle cell tumors. One tumor from the 11-year-old patient had *DICER1* p.E1813D mutation together with multiple copy number alterations, which is a molecular profile recently described in childhood and adolescence PDTC (Chernock *et al.* 2020). Based on the detected genomic alterations, 24 (60%) cases were classified as high molecular risk, 9 (22.5%) as intermediate and 7 (17.5%) as low molecular risk.

In this cohort of 40 informative cases, high molecular risk was associated with the presence of distant metastasis at presentation or later during follow-up ( $P = 0.007$ , Fisher's exact test) with worse OS ( $P = 0.01$ , Fig. 3A). On the other side, all the seven patients with low-risk molecular signature had initial localized disease and intermediate ATA risk of recurrence. Death occurred in 46% (11/24) of the patients in the high-risk group, in 11% (1/9) intermediate-risk group, and in none (0/7) of the patients with low-risk molecular signature (Fig. 3B).

### Predictive markers of recurrence and overall survival

We looked for predictive markers of recurrence in the M0 population. The univariate analysis showed that age  $\geq 55$  years, high risk in the ATA risk of recurrence stratification, mitosis count  $>5/2$  mm<sup>2</sup>, high molecular risk signature and CD163 expression (both high intensity and peripheral infiltration) were associated with recurrence (Table 2). The two most significant covariates were high ATA risk of recurrence and Mitosis count  $> 5/2$  mm<sup>2</sup> (Fig. 4).

No predictive factor of OS was found in the univariate analysis in the 32 M1 patients.

### Discussion

In this study, we report a cohort of 51 patients with PDTC followed at Gustave Roussy with detailed clinical and pathological characterization, including an immunohistological assessment of relevant biomarkers, and a comprehensive molecular characterization of 40 tumors. Prognostic factors of survival have been described in cohorts of PDTC that included both localized and metastatic PDTC and tumor size and

**Table 2** Univariate and multivariate analysis of prognostic factors for recurrence-free survival in the M0 population.

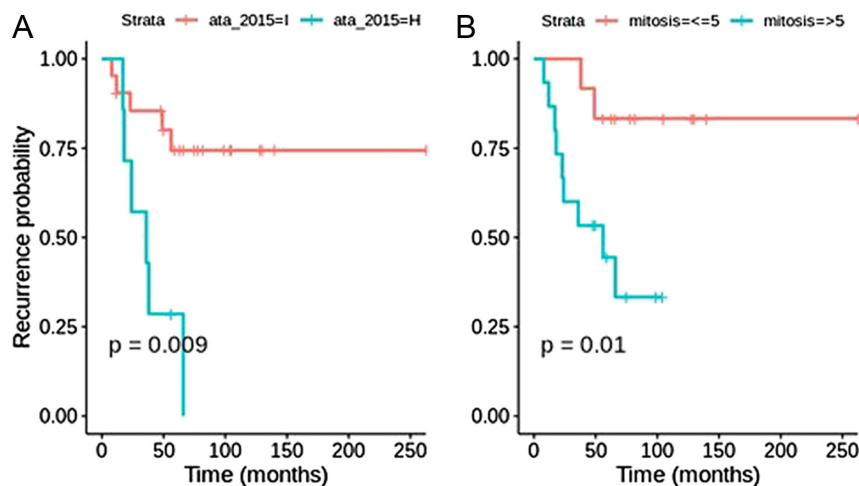
Parameter (number of subjects/number of events)	Univariate analysis	
	HR (95% CI)	P
Sex (28/11)		
Male (15/ 8)	2.7 (0.7–10.1)	0.12
Age (28/11)		
≥ 55 years (17/9)	4.3 (0.9–20.1)	<b>0.03</b>
Size (28/11)	1 (0.9–1.03)	0.61
pN (28/11)		
N1 (6/3)	2.1 (0.5–7.9)	0.64
ATA risk (28/11)		
High (vs intermediate) (7/6)	5.3 (1.6–17.9)	<b>0.009</b>
Mitosis (/2 mm <sup>2</sup> ) (27/11)		
>5 (15/9)	1.5 (0.3–7.8)	<b>0.01</b>
Presence of vascular invasion, <i>n</i> = 28 (yes : 14/6)	1.3 (0.4–4.2)	0.68
Presence of necrosis, <i>n</i> = 27 (yes : 8/3)	7.5 (0.2–2.8)	0.66
Ki67 (%) (26/10)		
≥20 (17/7)	1.2 (0.3–4.8)	0.76
Immunohistochemistry		
Tg, <i>n</i> = 28	9.9 (0.9–1)	0.43
TPO, <i>n</i> = 28	1 (0.9–1)	0.86
GLUT1, <i>n</i> = 27	9.9 (0.9–1)	0.76
ERK, <i>n</i> = 28	9.9 (0.9–1)	0.58
P53, <i>n</i> = 27 (mutated : 2/2)	4 (0.8–19)	0.13
CD163, <i>n</i> = 28 (2–3 : 14/8)	4 (1.1–15.2)	<b>0.03</b>
IMP3, <i>n</i> = 9 (>2 : 3/1)	2.8 (0.2–47.1)	0.48
Molecular risk signature (24/7)		
High (vs intermediate) 0.46 (0.09–2.42)		
High (vs low) 2e-9 (0–∞)		<b>0.049</b>

Bold indicates statistical significance.

extent of invasion (Hiltzik *et al.* 2006, Asioli *et al.* 2010, Gnemmi *et al.* 2014, Wong *et al.* 2019). We decided to specifically examine separately the predictive factors of recurrence in patients with localized disease and predictive factors of survival in patients with distant metastases because these are the main questions that patients and clinicians face.

Our findings reveal several new prognostic markers for this aggressive tumor type that may further help by managing these patients.

Clinical presentation of patients with PDTC reported in this study was overall similar to that of previously published series (Wong *et al.* 2019, 2021). Regarding molecular profiling, *BRAF* and *TP53*

**Figure 4**

Recurrence-free survival of patients according to (A) ATA risk and (B) mitosis count.



mutations were less frequent in our series as compared to PDTC reported in the study by [Landa \*et al.\* \(2016\)](#), while *TERT* promoter mutations, present in 48% of the cohort, were present at similar rates ([Landa \*et al.\* 2016](#), [Wong \*et al.\* 2021](#)).

Four (10%) tumors with informative molecular profiles in this study revealed the presence of molecular markers that can be potentially used for targeted therapies. Among them, two tumors carried *NTRK3* fusions, which are targetable by specific and *NTRK* inhibitors (entrectinib, larotrectinib) with high efficacy ([Capdevila \*et al.\* 2022](#)). The other two tumors were positive for *BRAF* V600E mutations that can be targeted by anti *BRAF* (dabrafenib +/-, trametinib and vemurafenib) ([Brose \*et al.\* 2016](#), [Capdevila \*et al.\* 2022](#)).

Transcriptomic studies performed in our study (data not shown) did not bring new findings on molecular PDTC characterization, but molecular risk signature was established for the first time in a cohort of PDTC: more than half of these tumors were found to be at high risk, while the risk was intermediate in 22.5% and low in 17.5%. Finding intermediate- and low-risk molecular signature in 40% of these tumors, which overall are known to be aggressive, was unexpected. We found that the molecular signature was associated with aggressiveness of PDTC, with a molecular high-risk signature being associated with the presence of metastasis at diagnosis and death from disease. On the opposite, a low-risk molecular signature was associated with a better prognosis. Indeed, all seven patients with low-risk molecular signature had initial localized disease and intermediate ATA risk of recurrence. Moreover, we observed that after a significant follow-up of roughly 5 years, none of the patients with low-risk molecular signature and only 11% of patients with intermediate-risk molecular signature died of the disease, as compared to 46% of patients in the high-risk molecular signature.

Regarding the risk of recurrence in localized disease, age and proliferation (Ki67) have been previously reported as risk factors for recurrence ([Gnemmi \*et al.\* 2014](#), [Akaishi \*et al.\* 2019](#)). We also identified on the univariate analysis that age  $\geq 55$  years, high ATA relapse risk, mitotic count  $>5/2$  mm<sup>2</sup>, high molecular-risk signature and CD163 expression were all associated with disease recurrence. The potential role of CD163 staining found in the study confirms the association of tumor-associated macrophages with the prognosis of PDTC ([Ryder \*et al.\* 2008](#)). At the immunohistochemistry level, we could not confirm the association between

tumor recurrence and IMP3 and GLUT1 expressions as reported by others ([Asioli \*et al.\* 2010](#), [Grabellus \*et al.\* 2012](#)). At the molecular level, *TERT* promoter mutation and *RAS* mutation on their own were not prognostic, in contrast to the previous reports ([Volante \*et al.\* 2009](#), [Landa \*et al.\* 2013](#)). This may be due to the limited sample size of patients with recurrence. Given the number of patients studied and the number of events, we did not perform a multivariate analysis.

Regarding the risk of death in patients with distant metastases, we were not able to identify reliable prognostic factors for OS, although death only occurred in patients with high- or intermediate-risk molecular signature, whereas all seven patients with low-risk molecular signature were alive at the end of follow-up period. The limitation of the study is the small number of samples studied. Indeed, it was not possible to do multivariate analysis and to analyze the added value of molecular analysis to ATA response to therapy.

In conclusion, the results of this study demonstrate that ATA risk that includes relevant pathological features and mitosis count can be used to predict the risk of recurrence in PDTC. Moreover, molecular signature was found to be relevant to establish prognosis for PDTC patients and also to identify molecular alterations that could be used for targeted therapy ([Chen \*et al.\* 2018](#)). Further studies are needed to determine if molecular testing adds to ATA risk stratification or response to therapy in predicting outcomes.

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#### Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/ERC-22-0151>.

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#### Declaration of interest

Y E N and M N N own intellectual property and receive royalties related to ThyroSeq test through the University of Pittsburgh. All other authors declare no conflict of interests.

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#### Funding

This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

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#### Author contribution statement

S Hescot and A Al Ghuzlan contributed equally to this work as first authors. M N Nikiforova and S Lebouleux contributed equally as last authors.

### Acknowledgement

The authors acknowledge the Platform of Experimental and Translational Pathology – CNRS UMS3655 – INSERM US23 AMMICA – Gustave Roussy, for their help.

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Received in final form 24 August 2022

Accepted 30 August 2022

Accepted Manuscript published online 30 August 2022