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REVIEW

Poorly differentiated thyroid carcinoma: molecular, clinico-pathological hallmarks and therapeutic perspectives

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ABSTRACT

Poorly differentiated thyroid carcinoma (PDTC) is a rare and extremely aggressive tumor, accounting for about 2-15% of all thyroid cancer. PDTC has a distinct biological behavior compared to well-differentiated and anaplastic thyroid carcinoma and, in last years, it has been classified as a separate entity from both anatomopathological and clinical points of view. Nevertheless, there is still a lack of consensus among clinicians regarding inclusion criteria and definition of PDTC that affects its diagnosis and clinical management. Due to its rarity and difficulty in classification compared to other tumors, very few studies are available to date and series often include different histotypes in addition to PDTC. This review focuses on main studies concerning PDTC summarizing the evolution in the definition of its diagnosis criteria, clinicopathological features, management, and outcome. The data available confirm that the pathological evaluation and classification of PDTC are crucial and should therefore be standardized. Since the clinical presentation and prognosis of PDTC may vary widely depending on the different stage of the disease at diagnosis, the patient's management may differ in treatment and should be tailored to each patient. Finally, this review discusses advances in molecular insights of PDTC that, together with the implementation of both *in vitro* and *in vivo* models, will provide valuable insights into biological mechanisms of progression, metastasis, and invasion of this aggressive thyroid carcinoma. Further studies on larger, carefully selected series are needed to better assess the peculiar features of PDTC and to better define its management by focusing on the best diagnostic and therapeutic approaches.

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Poorly differentiated thyroid carcinoma (PDTC) is a rare and extremely aggressive tumor, accounting for about 2-15% of all thyroid cancer[1, 2] and with a mean survival after diagnosis of 3.2 years. PDTC originates from the tumor transformation of follicular thyroid cells that lose their typical features and acquire intermediate features between well-differentiated (WDTC) and anaplastic thyroid carcinoma (ATC).[3, 4] Since PDTC shows a distinct biological behavior with respect to WDTC and ATC, over the last two decades it has been classified as a separate entity. Nevertheless, there is still a lack of consensus among clinicians regarding inclusion criteria and defini-

tions to diagnose PDTC, thus limiting studies on this carcinoma. It is important for clinicians to recognize PDTC as a separate entity since it often shows a high incidence of recurrence despite appropriate treatments. Indeed, total thyroidectomy with lymph node dissection remains the major gold-standard treatment for PDTC, but with a 10-year survival rate lower than 50%.[5] Since more than 50% of patients affected with PDTC present with extensive cervical disease at diagnosis and up to 85% develop distant metastases during follow-up,[2] multimodality adjuvant treatments (radioactive-iodine-RAI, external beam radiotherapy and systemic chemotherapy) are usually required to treat these rare tumours, but with low effectiveness.[2] In the last decades, advances in the knowledge of genetic changes underlying the pathogenesis of thyroid cancer have been reached thanks to the improvement of sequencing techniques, such as the Next Generation Sequencing, able to investigate either the entire genome or exome, and transcriptome. The identification of new molecular markers led to the development of kinase-specific inhibitors for the treatment of patients with progressive RAI-refractory thyroid carcinoma, PDTC included. The understanding of the molecular biology of thyroid cancer, together with both *in vitro* and *in vivo* mouse models, will provide valuable insights into tumor biology, mechanisms of progression, metastasis, invasion, thus offering a more accurate diagnosis and prognosis and favoring the development of novel and better targeted therapeutic approaches in the future.

In this review, we have summarized the evolution in the diagnosis of PDTC that has allowed its recognition as separate entity, its clinicopathological features, management, and outcome. Furthermore, new molecular insights into genomic profile of PDTC and future perspectives for the improvement of the prognosis of PDTC patients have been also reviewed.

Clinical pathological features: what we know and what is still to be discovered

History of poorly differentiated thyroid carcinoma definition

Poorly differentiated thyroid carcinoma is a rare malignant follicular cell-derived tumor showing intermediate features between WDTC and ATC in terms of clinical and epidemiological aspects.[3, 4, 6, 7]

When comparing the prognosis of PDTC to other histological types, the definitions of PDTCs used by different authors should be noticed.[5, 8, 9] As shown in Figure 1, at the beginning of last century Teodor Langhans described a malignant thyroid carcinoma as "rampantly goiter," but only in the 60's the term "poorly differentiated thyroid

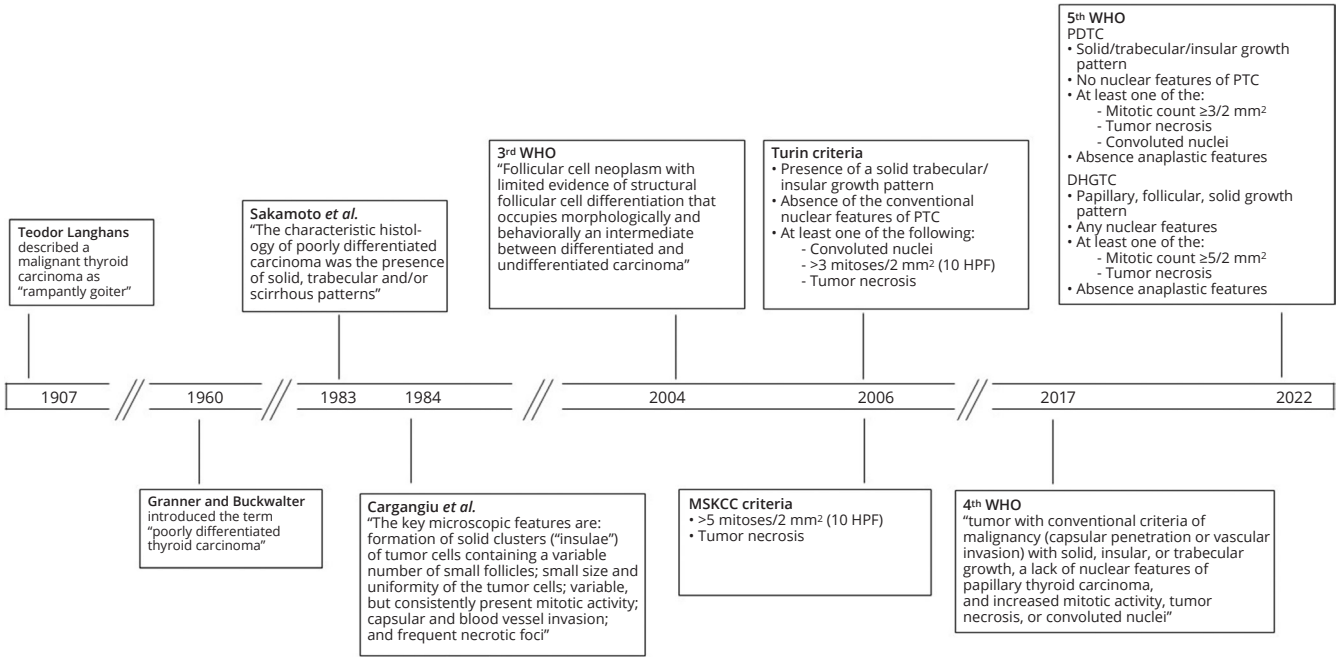

Figure 1.—Timeline depicting the changes in the histologic definition and classification of poorly differentiated thyroid carcinoma.

carcinoma" was introduced by Granner and Buckwalter. However, the first definition of the PDTC dates back to the papers of Sakamoto[10] and Cargangiu[11] in the early 1980s. Indeed, PDTC was initially proposed as a distinct entity[10] next defined as "insular" carcinoma in 1984.[11]

PDTC was considered a variant of WDTC until 2004, when the 3rd World Health Organization (WHO) Classification of Endocrine Tumors defined it a "follicular cell neoplasm with limited evidence of structural follicular cell differentiation that occupies morphologically and behaviorally an intermediate position between differentiated and undifferentiated carcinoma."[12] However, only in 2007 a study group in Turin, composed by pathologists from Italy, Japan and the United States, clearly defined the histologic features of PDTC as follows: 1) trabecular, insular, or solid growth pattern; 2) absence of the conventional nuclear patterns of papillary carcinoma; 3) presence of at least one of the following features: a) convoluted nuclei; b) mitotic activity ≥3×10 HPF; c) or necrosis.[13]

In the same year, Hiltzik *et al.* showed that mitotic rate and necrosis are the main determining factors of clinical behavior in 58 patients with PDTC, conversely the growth pattern and cell type did not influence the clinical outcome.[14] Indeed, the group of the Memorial Sloan Kettering Cancer Center (New York, NY, USA) provided a quite different definition of PDTC, known as MSKCC criteria: a thyroid carcinoma with follicular cell differentiation at the histologic and/or immunohistochemical levels exhibiting tumor necrosis or an elevated mitotic index of ≥3/10 HPFs (400×), regardless of the growth pattern and nuclear features.

These findings led to the question whether thyroid tumors with high mitotic rate or necrosis and papillary or follicular pattern, and solid tumors with papillary nuclear features can be considered PDTC.

Gnemmi *et al.* found that among 82 selected thyroid carcinomas, 46 and 50 were diagnosed as PDTC using the Turin and the MSKCC criteria, respectively.[15] Despite the two criteria showed similar prognostic performance, the concordance between them was of 75.6%. The authors suggested that PDTC defined according to MSKCC criteria, based only on the proliferative grading, should be better acknowledged as carcinomas 'with high-grade features', rather than PDTC.[15]

Though the diagnostic criteria were not used univocally, and many pathologists were following criteria of Turin and other criteria for long-time, the major revision of the 2017 WHO Classification of Tumors of Endocrine Organs (4th edition) adopted the Turin criteria for PDTC and indicated that any poorly differentiated component should be mentioned in the pathology report.[14, 16]

The last edition of WHO Classification of Endocrine Tumors (fifth edition),[6] released in 2022, classified high-grade non-anaplastic follicular cell-derived carcinomas in two categories:

- an intermediate entity of 'differentiated high-grade thyroid carcinoma' (DHGTC) for PTCs and FTCs (follicular thyroid carcinomas) with ≥5 mitoses per 2 mm^2 and/or tumor necrosis; these tumors maintain PTC-related nuclear atypia or a follicular growth pattern, features not satisfactory for a PDTC diagnosis;
- PDTC, reflecting the Turin criteria.

Anatomopathological features of PDTC at diagnosis

Poorly differentiated thyroid carcinomas are tumors with more aggressive clinical features and behavior compared to differentiated thyroid carcinomas.[5, 8, 9] At the time of diagnosis, PDTCs are typically already at an advanced stage of disease, showing large dimension (≥4 cm), extrathyroidal extension (ETE) and extensive local invasion characterized by infiltration of perithyroidal soft tissues, vessels, nerves, and skeletal muscles of the neck.[5, 8, 9]

In a recent paper,[17] the tumor size of 200 PDTCs, diagnosed according to Turin criteria, was >4 cm in 120 patients (60.3%), and the gross extra-thyroidal extension (gETE) was detected in 38 cases (20.2%). Higashino *et al.* compared 67 PDTCs with 209 WDTCs, and found a significant difference in T stage, being higher in PTDCs, both for T3 (83.6% *vs.* 46.4%) and T4 (26.9% *vs.* 13.4%).[18]

In the work of Ibrahimpasic *et al*. 30% of 91 PDTCs, diagnosed according to MSKCC criteria, had gETE with invasion of adjacent structures (T4a according to the AJCC Cancer Staging Manual 7th edition) at diagnosis.[19]

On the other hand, in the work of Wong *et al.* although 16 out of 47 PDTCs (34%) had gETE, there were also cases with encapsuled PDTCs (38% with extensive vascular invasion and 28% with focal capsular or vascular invasion).[20] Interestingly, the authors demonstrated that the extent of capsular and vascular invasion has an important impact on the clinical outcome. Indeed, in patients without distant metastases at diagnosis, the 5-year disease-free survival (DFS) was 100% if there was present focal capsular or vascular invasion, 73% in those with encapsulated tumors with extensive vascular invasion and 11% in those with widely invasive tumors. However, no differences in the clinical behavior of tumors with gETE were observed regardless of histology (PDTC or WDTC).

Moreover, Higashino *et al.* demonstrated that the recurrent laryngeal nerve infiltration was not different between PDTCs and WDTCs (11.7% *vs.* 6.7%, P=0.45).[18]

It is noteworthy that, except for few cases,[21] most PDTCs arises from WDTCs. This origin could explain the existence of tumors with different amount of poorly differentiated cells.[22] Since it has been demonstrated that even the presence of small amounts of poorly differentiated component can have a prognostic impact, the threshold >10% is enough to justify the diagnosis of PDTC.[22]

Sugitani *et al.* divided 376 cases of PTC or FTC into two groups according to the amount of solid, trabecular, and insular components, and found that tumors with ≥10% of solid, trabecular, and insular components were related to older age, larger size, and distant metastases at diagnosis than tumors with <10% of these components.[23]

The presence of poorly differentiated component is an independent negative predictive factor for the outcome, regardless of histology. Indeed, in an Indian paper, the authors compared 27 PDTCs, 27 PTCs and 88 FTCs both with poorly differentiated component.[24] The three groups differed for a higher rate of ETE, lymph nodes metastatic involvement and distant metastases in PDTCs, although the final outcome was similar.[24]

In addition, oncocytic variant of PDTCs, more frequently involved older patients, showed a larger median tumor size, a more frequently extensive vascular invasion, a higher rate of 5- to 10-year incidence of locoregional and distant metastases, resulting in a worse prognosis.[25, 26]

However, data about the comparison of oncocytic and non-oncocytic variants of PDTC are controversial. Dettmer and colleagues, comparing 16 non-oncocytic and 18 oncocytic PDTC, found that the oncocytic PDTC had a significant low overall survival and tumor specific survival than non-oncocytic PDTC.[27]

Wong *et al.* described a cohort of 47 PDTCs, including 15 cases of oncocytic variant: among patients with at least 5 years of follow-up, those who died for disease were more likely having oncocytic morphology (71% *vs.* 29%; P=0.011).[20] Conversely, Asioli *et al.* did not show significant differences in overall survival, disease-free survival, and metastasis-free survival between oncocytic and non-oncocytic PDTCs in a cohort of 152 cases from Mayo Clinic (Rochester, MN, USA) and University of Turin (Italy), despite having analyzed a similar rate of oncocytic PDTC (32%).[28] These tumors have a greater predisposition than WDTCs to metastasize at locoregional level and to distant sites. Indeed, PDTCs are also frequently associated with high rate of regional lymph node metastases, that usually exceed 70%,[29, 30] and to distant metastases compared to WDTCs.[31] Ibrahimpasic *et al.* showed that 24 out of 91 patients with PDTC (26%) had distant metastases at diagnosis and other 14 (15%) developed it during the follow-up.[31] Lung seems to be the most frequent site affected by distant metastases in PDTC (14-54%), followed by bone (18-33%).[1, 32]

At immunohistochemistry analysis, PDTC shows positivity for TTF-1 (thyroid transcription factor-1), Pax8 (paired box gene 8), CK7 (cytokeratin 7), and thyroglobulin, although this latter has a tendency to be weak and focal.[28, 33, 34] Asioli *et al.* found positivity for thyroglobulin in 95 of 103 (92%) cases of PDTC, and they observed a peculiar pattern of dot-like paranuclear staining in most cases (77%).[28] The same staining pattern was previously reported by Pietribiasi *et al.*[35]

Conversely, Bejarano *et al.* reported a lower rate of positivity for thyroglobulin in PDTCs (57%), but an immunoreactive staining for both TTF-1 and CK7 in 86% of cases.[34] Nonaka *et al.* showed diffuse and strong Pax8, TTF-1 and TTF-2 (thyroid transcription factor-2) stainings in all seven cases of PDTC analyzed.[33]

The expression of Ki67 proliferative index, as expected, is highest in PDTCs and lowest in WDTCs.[36, 37] In the cohort of 103 PDTCs analyzed by of Asioli *et al.* Ki67 ranged from 3% to 40%, with a mean of 13%.[28]

Clinical picture and diagnosis of PDTCs: various potential outcomes and prognosis

Epidemiological criteria

To date, data regarding epidemiological, clinical, prognostic features and patients' management are not yet conclusive, given the rarity of PDTC and the different pathological classifications over the years. Thus, it is difficult to give accurate indications, guidelines, and unique consensus regarding this peculiar category of thyroid carcinoma.[5]

As well as ATC, PDTC is a rare tumor developing as rapidly growing masses and representing from 2% to 15% of all thyroid cancers.[1, 2] The variation in its incidence seems to be influenced to environmental factors or differences in histopathological interpretation.[28]

PDTC usually occurs in adults significantly older at diagnosis (usually over age 50) compared to WDTC.[15, 28] Ibrahimpasic *et al.* described a cohort of 91 patients with PDTC, defined according to the MSKCC criteria, and found that the median age at diagnosis was 59 years with most of them ≥45 years (75%).[31] In childhood and adolescence PDTC is a very rare tumor.[38] Regarding sex distri-

bution, PDTC is slightly more frequent in females with a female to male ratio 1.1:1.[31, 39]

The prevalence of PDTC may vary depending on geographical variations.[6, 28] Indeed, its incidence is higher in Europe and South America than in the United States and Japan, suggesting the involvement of either ethnic or dietary (iodide) factors in the development of PDTC.[6]

Clinical features of PDTC

At diagnosis, PDTC frequently presents as a rapidly growing neck nodule and, in some cases, the patient may already show compressive and infiltrative symptoms of the neck structures, such as dyspnea, dysphagia, and dysphonia.[40] Sometimes PDTC is detected during a screening ultrasound examination and, in these cases, the diagnosis may be done luckily early.

Nevertheless, the diagnosis of PDTC must be confirmed by cytological and histopathological examinations. It is worth to mention that the occurrence and development of PDTC is correlated to the presence of DTC or ATC foci,[41] which has a significant impact on tumor growth and on the probability of developing metastases. It was hypothesized that PDTC can represent a continuum of disease that progresses from WDTC to the fatal ATC.[41]

Molecular data show an increase in DNA copy number abnormalities from WDTC to PDTC that could be involved in chromosomal instability favoring the progression to more aggressive thyroid cancers.[41] A recent study suggested that PTC with high-grade features should be considered a distinct group from carcinomas with PDTC components, which show a more aggressive behavior and for which different treatment strategies must be adopted.[42]

The ultrasound (US) pattern is usually represented by nodules of significant size (>2 or 3 cm), often with significant size increase. US suspicion criteria, according to international ultrasound classifications, are represented by the presence of marked hypoechogenicity, irregular margins, and microcalcifications.[43]

Although US is an important diagnostic tool, no peculiar ultrasound features for PDTCs, except for the presence of an irregular vascularization (seen on Doppler US as a "sword sign") or jugular vein invasion, are known to date and prospective studies will be needed to deepen this point.

It must be considered that often thyroid nodules, later confirmed as PDTCs on histologic examination, are not recognized as suspicious by cytologic evaluation. Indeed, only in about 30% of cases the cytology is suspicious or positive for malignancy.[40]

Some immunocytochemical markers related to thyroid differentiation have been found to be useful in the pre-surgical diagnosis of PDTCs, *e.g.*, Thyroglobulin, TTF1, PAX8, cytokeratins (usually cytokeratin 7), Ki67, Galectin 3, PanCK, but further studies are needed to validate their efficacy in pre-surgical diagnosis.[6, 40]

Clinical management and disease outcome

The first step for the management of patients with PDTC is an adequate disease staging by the endocrinologist and the surgeon in a short time, to establish a correct treatment plan and to obtain the best recurrence-free survival outcome.[40] Although surgery remains the best therapeutic approach, disease control is still poor, and disease specific deaths is mainly related to local recurrence and presence of distant metastases.[5]

Usually, PDTC does not satisfyingly respond to ^{131}I therapy despite iodine avidity in some foci, and remission rate by lesion resection followed by ^{131}I therapy is limited to one-third of patients.[44] However, there are not enough studies to confirm the impact of ^{131}I therapy on prolonging disease-specific survival or overall survival rates.

Chemotherapy and external radiotherapy are not effective treatments for this category of carcinomas. However, in recent years, multityrosine kinase drugs (TKIs) have been found to be very effective in advanced radioiodine refractory DTC, and in PDTC too.[45]

In terms of follow-up, postoperative thyroglobulin, and anti-thyroglobulin antibodies levels in subset of patients with PDTC following total thyroidectomy and RAI were reported to predict potential rate for recurrence.[46] These data suggest the use of a suppressive hormone therapy until the disease remission is observed during the follow-up. When a structural and biochemical remission of disease is obtained, a switch to replacement therapy can be performed.[40]

Being a rare thyroid cancer, there are not many studies on PDTC without considering other histotypes, such as anaplastic carcinoma, and in the case require the collection of multicenter cases. In this review, we summarized the results about the disease outcome of PDTC obtained from the most important studies (Supplementary Digital Material 1: Supplementary Table I).[10, 31, 47-49]

One of the first studies, performed by a Japanese group, carried out a detailed evaluation of the histological features and survival rates of 258 cases treated at the Cancer Institute Hospital in Tokyo between 1965 and 1980.[10] The 5-year survival rate for PDTC was 65.0%, while that of WDTC was 95.1% with survival curves significantly dif-

ferent both in the whole series and in cases without extrathyroidal extension (P<0.01).[10]

In 2004, the Italian group of Volante carried out a study on 183 patients affected with PDTC (trabecular-insular-solid) and showed that 79/183 (43%) tumors had an aggressive evolution. These patients showed average 5-year and 10-year survival rates of 85% and 67%, respectively.[47] The overall survival of patients with PDTC resulted to be intermediate between those of patients with WDTC and ATC (P<0.0001). The authors of this study proposed a scoring system based on the combination of a number of clinicopathological parameters able of predicting a more aggressive behaviour.[47]

In the same year, Luna-Ortiz *et al.* evaluated the outcome in 13 patients with PDTC with respect to 71 cases affected with papillary thyroid carcinoma (PTC) in a Mexican population. Metastases were significantly more frequent in the PDTC group than in the PTC group (53.8% *vs.* 5.3%; P=0.001). The Kaplan-Meier Curve revealed that survival was lower in patients with PDTC than in those with PTC.[48]

Approximately 10 years after these studies, one of the most relevant studies was performed at Memorial Sloan-Kettering Cancer on 91 patients with PDTC treated from 1986 to 2009 with initial surgery and in some cases with additional treatments.[31] The 5-year overall survival and disease-specific survival (DSS) of patients who died of disease and those who did not die during a median follow-up of 50 months, were 62 and 66%, respectively. Of 27 disease-specific deaths, 23 (85%) were due to distant metastases. At univariate analysis, factors correlated with worse DSS were age ≥45 years, tumor size >4 cm, extrathyroidal extension, higher pathological T-stage, surgical margins involved by neoplastic extension, and distant metastases. On multivariate analysis, however, the prognostic predictors of worse DSS were pT4a stage and the presence of distant metastases.[31]

Another study was a retrospective analysis conducted on a large series of patients with ATC and PDTC followed at Seoul National University Hospital from January 1985 to December 2013.[49] The authors compared 38 PDTCs with 98 ATCs and 48 DTCs. They found that DTC patients with foci of anaplastic carcinoma or PDTC had a significantly higher five-year disease-specific survival rate than ATC patients (81.3% and 65.8%, respectively *vs.* 14.3%; P<0.001). Moreover, in the series analyzed, subsequent treatments with external beam radiation or radioactive iodine increased the survival time in PDTC and DTC with anaplastic foci.[49]

Prognostic factors

PDTCs usually have a better prognosis than ATCs, even if we sometimes face patients with PDTC that arises or becomes radio-iodine refractory for whom the prognosis worsens severely.[2, 40] Survival rates at 5, 10 and 15 years and 15 years are significantly lower in patients with PDTC (50%, 34% and 0%) than in patients with WDTCs (95%, 86%, and 81%).[50]

A strong relationship between tumor architecture and prognosis has not been found. In fact, some architectural patterns, such as solid growth, do not lead to an unfavorable outcome *per se* and are not sufficient criteria for the diagnosis of PDTC. The survival data in patients with PDTC showed the outcomes of PDTC and DTC patients did not differ regardless of the extent of poorly differentiated (PD) area, which makes us wonder the presence of any PD area should be enough to label a tumor as PDTC rather than the proportion.[22] Furthermore, it has been reported that high-grade features, such as mitosis, necrosis, nuclear pleomorphism and invasiveness, provide better clinical and prognostic significance.[47, 51] PDTC cases diagnosed based on these high-grade features have a worse prognosis than those defined on an architectural basis.

Several retrospective studies found that the following factors correlated negatively with survival rates: patient age greater than 45 years, tumor size greater than 4 cm, absence of radioactive iodine therapy, and cervical lymph node involvement.[14, 31, 51]

Single- and multicenter studies have shown that with appropriate initial surgical treatment and, in patients with radioiodine uptake, tailored radiometabolic therapy, it is possible to achieve excellent loco regional control in PDTC and, thus, a good prognosis.

In cases with advanced AJCC stage and distant metastases present at diagnosis, the prognosis is significantly worse and therefore more extensive effective treatments should be planned to achieve a prolonged DSS as much as possible.[31] Recently, the use of TKI drugs has significantly improved the survival of these patients by extending life expectancy though with a less efficacy than observed in DTCs.

From genomic to epigenomic profiling: known and new potential alterations

As mentioned, PDTCs and ATCs represent a major clinical challenge and the understanding of their molecular biology may help obtaining a more accurate diagnosis and de-

veloping better targeted therapies. Most PDTCs and ATCs are thought to arise from preexisting WDTCs based on their frequent co-occurrence in the same tumor specimen and the sharing of common driver mutations. Untreated PDTCs could eventually progress to ATCs by dedifferentiation process.[8]

In the last decades, advances in the knowledge of genetic changes underlying the pathogenesis of these aggressive and fatal cancers have been reached thanks to the improvement of sequencing techniques, such as the Next Generation Sequencing (NGS), able to investigate either the entire genome or exome, and transcriptome. After that The Cancer Genome Atlas (TCGA) network reported a comprehensive genomic landscape of papillary thyroid cancer in 2014, other seven groups have published genomic findings in PDTCs and ATCs using NGS or whole exome sequencing.[52-60]

Regarding PDTCs, the study of Landa and colleagues remains the one with the largest series analyzed (84 PDTCs) to date. They adopted an ultra-deep sequencing strategy (MSK-IMPACT cancer exome panel), which targets all exons and selected introns of 341 cancer-related genes allowing an average depth of coverage of 584.[55] A targeted sequencing approach of selected exons and hotspot of cancer-related genes was used by other research groups too, but on smaller PDTC series (Supplementary Digital Material 2: Supplementary Table II).[52, 53, 55, 57-60]

Interestingly, the median number of mutations observed in PDTCs is two, but the mutation burden increases with increasing age and associate with a larger tumor size, higher frequency of distant metastasis and shorter overall survival.[55]

Overall, these studies demonstrate that the activation of RAS-RAF-MEK signaling pathway is important for thyroid cancer initiation and progression, and that *BRAF*V600E and *RAS* mutations are the commonest main drivers also in PDTCs. Additional late events are gained such as *TERT* promoter and *TP53* mutations, as far as alterations in PIK3CA-PTEN-AKT-mTOR pathway (*i.e.*, *PTEN* and *CTNNB1* mutations), SWI-SNF complex, histone-methyltransferases (HMTs), mismatch repair genes (MMR) and chromosome 1q gain.[61]

Alterations in RAS-RAF-MEK signaling pathway

Mutations in *BRAF* and *RAS* genes are the main early genetic changes of WDTC contributing to its dedifferentiation into PDTC and ATC. The proto-oncogene *BRAF* encodes a protein belonging to the RAF family of serine/threonine protein kinases and plays a role in regulating the MAP kinase/ERK signaling pathway, which affects cell division and differentiation. The most common change reported in several cancers including TC is a point mutation in exon 15, that leads to a substitution of valine 600 with a glutamate (V600E). This mutation causes the stabilization of the active form of BRAF protein leading to the continuous stimulation of MAP kinase/ERK signaling pathway.[62]

RAS genes (*H, N, K-RAS*) encode for proteins that are members of the small GTPase superfamily involved in both MAPK and the PI3K/AKT pathways.[8] Point mutations exhibit either increased affinity for GTP (codons 12 and 13) or inhibition of autocatalytic GTP-ase function (codon 61). Both mechanisms result in constitutive, aberrant activation of the downstream MAPK and PI3/AKT signaling pathways, a critical event in thyroid tumorigenesis.

Recent molecular studies on aggressive thyroid carcinomas using NGS confirmed that *BRAF*V600E and *RAS* mutations are mutually exclusive main driver mutations not only in DTCs, but also in PDTCs occurring in 10-86% and 10-39% of tumor samples, respectively.[52, 53, 57-60]

Interestingly, Landa *et al.* observed a relationship between PDTC mutational status and histological features: 92% of PDTCs diagnosed according to the Turin criteria harbored *RAS* alterations, whereas 81% of those diagnosed using the MSKKCC criteria had *BRAF*V600E mutation. *BRAF*-mutated PDTCs preferentially metastasize to regional lymph nodes, while those harboring *RAS* mutations have a higher rate of systemic metastases.[55, 63] Moreover, PDTCs harboring *BRAF* alterations also showed a decreased ability to trap radioiodine and are more likely to be RAI resistant.[8] Some discrepancies in the frequency of both *BRAF* and *RAS* mutations found in these studies may be due to small sample size or to the presence of many patients with PTC components, as discussed by Duan *et al*.[60]

Chromosomal rearrangements

Only two studies reported the analysis of chromosomal rearrangements in PDTCs.[55, 60] *RET* fusions are the most common rearrangements observed in PDTCs (6-15%) involving mainly *CCD6* and *NCOA* as partners and were observed especially in young patients.[55]

The other rearrangements found are *PAX8-PPARG* (2-4%), *ALK/STRN*, *ALK/ELM4* and *ALK/CCDC149* (2-4%), but also *BRAF*, *NTRK1* and *NTRK3* fusions have been observed in some cases.[55, 60] It is likely that chromosomal rearrangements are detected at a low frequency because they give a less aggressive phenotype to tumor clones, which are thus supplanted by those with more advantageous mutations.

Eukaryotic translation initiation factor 1A X-linked (EIF1AX) mutations

EIF1AX gene encodes an essential translation initiation factor required for binding of the 43S complex and it has been recently proposed as a cancer driver gene able to trigger protein translation and cell proliferation. Mutations in this gene have been firstly described in 1% of PTC among the TCGA cohort, and thereafter in 5-11% of PDTCs. In the latter case, *EIF1AX* mutations co-occur with those of *RAS* genes and predict a shorter survival.[55, 60]

Alterations in PI3KCA-PTEN-AKT-mTOR pathway

Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), phosphatase and tensin homolog (PTEN) and AKT serine/threonine kinase 1 and 3 (AKT1 and 3) are members of the oncogenic PI3K/PTEN/AKT/mTOR signaling pathway altered in several malignancies, aggressive TC included. Mutations in the corresponding genes are associated with thyroid tumor progression and dedifferentiation and are reported to be mutually exclusive.

Duan *et al.* found *PIK3CA* mutations in 20% of PDTCs,[60] a frequency quite high than those reported by the other groups (range 2-14%).[52, 53, 55, 57-59] As reported above, this finding may be due to the large number of patients with PTC components analyzed.[60] Interestingly, a high mortality risk has been reported for PDTC patients with coexisting *TERT* and *PIK3CA* mutations.[60]

PIK3CA mutations were also found in metastatic/recurrent tumor tissues indicating the need to test both primary and metastatic sites to avoid the underestimation of these alterations.

A great variability has been observed in the frequency of *PTEN* mutations (from 4 to 33%)[53, 55, 57] which often co-occur with neurofibromin1 (*NF1*) or RB transcriptional repressor 1 (*RB1*) alterations.[55]

Finally, *AKT1* and *AKT3* mutations have been found only in a small portion of PDTCs[55, 57, 60] and with those of *AKT1* gene co-occurring with *BRAFV600E*.

Alterations in the WNT/β-catenin pathway

The WNT/β-catenin signaling pathway is an evolutionarily conserved pathway that regulates crucial aspects of cell fate determination, migration, polarity, neural patterning, and organogenesis during embryonic development.

Constitutive activation of WNT pathway is a crucial step in the development and progression of aggressive cancers, included thyroid cancer, and is caused by mutations in various components of the pathway, leading either to inactivation of APC or to oncogenic activation of beta-catenin codifies by *CTNNB1* gene.

In addition, recent evidence suggests that the activity of the WNT pathway may also be influenced by the status of the E-cadherin cell adhesion protein, encodes by *CDH1* gene.

CTNNB1 mutations have been reported in a small portion of PDTCs (2%),[60] whereas those of *APC* gene in a higher percentage of patients (17%).[57]

Moreover, *CDH1* mutations have been detected in these patients with a great variability in their frequency (4-33%),[53, 57] supporting the hypothesis that the loss of E-cadherin expression rather than of beta-catenin induces the process of thyroid tumor dedifferentiation.[64]

Telomerase reverse transcriptase (TERT) promoter mutations

TERT gene encodes for the reverse transcriptase subunit of telomerase, a ribonucleoprotein polymerase that maintains telomere ends length. Deregulation of telomerase expression is one of hallmarks of cancer and a role in the dedifferentiation of WDTCs has been suggested.[65] *TERT* mutations occur in two exclusive hotspots, C228T and C250T, within the promoter region and have been firstly described in aggressive thyroid cancer by Liu *et al.* in 2013.[65] In following studies, a higher rate of clonal *TERT* mutations (22-40%) were reported in PDTCs compared to PTCs.[55, 60] Thirty-three percent of cases harbored the C228T mutation, while only 7% had the alteration at the position 250.[55] Interestingly, *TERT* promoter mutations co-occur with those of *BRAF* and *RAS* genes, consistent with the proposed mechanism whereby *TERT* mutations create new binding sites for ETS transcription factors activated by MAPK signaling thus enhancing the expression of *TERT*.[65]

Finally, *TERT* mutations have been reported to correlate with aggressive clinical behavior, regional and distant metastases, and greater mortality. Moreover, the co-occurrence of *TERT* mutations with those of either *RAS* or *BRAF* exerts a negative synergic effect on cancer prognosis.[55, 61, 65]

Mutations in DNA repair and Mismatch Repair pathway

DNA repair pathway normally protect cells from damage that can lead to DNA breaks. The tumor suppressor gene *TP53* encodes a protein which plays a vital role in preserving DNA integrity and controlling cell cycle. It is activated by several stress signals inducing cell cycle arrest if it is

possible to repair DNA or leading to apoptosis and senescence in case of unrepairable DNA damages.

TP53 is the mostly mutated gene in human cancers and its inactivating mutations are reported to be involved in thyroid tumor dedifferentiation process. Indeed, *TP53* mutations are frequently found in ATC cases (70%) and at lower and variable frequency also in PDTCs.[8]

Four NGS studies reported either a low combined *TP53* mutations frequency of 10%[55, 58, 59] or no mutations[52] in patients affected with PDTC. On the other hand, three other groups reported a high combined frequency of *TP53* mutations in 46% PDTCs.[53, 57, 60]

Landa *et al.* also reported mutations in genes codifying checkpoint kinases belonging to the DNA Damage Response pathway. *CHEK2* and *ATM* result to be mutated in 33% and 7-13% of PDTCs, respectively.[55, 57]

Finally, alterations in the Mismatch Repair gene *MSH2* were detected in 2% of PDTCs. Tumors harboring mutations in DNA repair and Mismatch Repair genes show a hypermutator phenotype.[55]

Other mutated genes

Recently, mutually exclusive alterations in genes encoding components of the SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodeling complexes have been detected.[55] SWI/SNF is a subfamily of ATP-dependent proteins which form complexes involved in the remodeling and packaging of DNA. Loss of function mutations in *ARID1A*, *ARID1B*, *ARID2*, *ARID5B*, *SMARCB1*, *PBRM1* and *ATRX* have been detected in 6% of PDTCs.[55] Mutations in *SMARCB1* gene were also confirmed by Gerber *et al.* in their PDTCs cohort with a similar frequency (4%).[57]

Other genes found to be mutated in 75 of PDTCs are histone methyltransferases (HMTs) genes *KMT2A*, *KMT2C*, *KMT2D*, and *SETD2*. HMTs are histone-modifying enzymes that catalyze the transfer of one, two, or three methyl groups to lysine and arginine residues of histone proteins. Additional mutations affecting the histone acetyltransferase *CREBBP* and sporadic inactivating mutations in other epigenetic regulators, such as *EP300*, *BCOR*, and *BCL6*, were also reported.[55]

A small proportion of PDTCs shows mutations in genes codifying epidermal growth factor receptors (*ERBB1*and *4*), vascular endothelial growth factor receptors (*FLT1*, *FLT4* and *KDR)*, and NOTCH receptors (*NOTCH1-4*).[55, 57]

Infrequent truncating mutations have been also found in *RB1* gene (1-4%), encoding a negative regulator of the cell cycle, and MEN1 (1%), which codifies a scaffold protein named Menin involved in histone modification and epigenetic gene regulation.[55, 57] Low-frequency mutations were found also in other genes (*i.e.*, *TSHR*, *STK11*, *MED12*, *RBM10*, *SMAD4*, *KIT*) listed in Supplementary Table II.[55, 57]

Somatic copy number alterations (CNAs)

The comparative genomic hybridization (CGH) array showed the presence of several chromosomal number abnormalities (CNAs) in thyroid cancers. CNAs are common and widespread in advanced thyroid carcinomas with respect to PTC which is generally diploid. CNAs represent important markers of thyroid cancer aggressiveness and have been well characterized as prognostic factors of recurrence and death.

Chromosome 1p, 13q and 17p losses are frequently observed in PDTCs that lacked driver mutations, whereas loss of 22q is strongly associated with *RAS*-mutated PDTCs.[55]

The loss of 22q tumor suppressor gene *NF2* is likely responsible for the transcriptional activation of both oncogenic and wild-type *RAS*. Moreover, patients with PDTCs with gains in chromosome 1q showed worse survival.[55] Opposite to these findings, no high level of gene/chromosomal amplifications were identified in the study of Chen *et al*.[58]

Gene expression profiling

Loss of expression of thyroid differentiation markers, such as NIS and other genes required for iodine incorporation, is one of the hallmarks of advanced thyroid cancers leading to refractoriness to radioiodine therapy. Landa and colleagues determined the transcriptomic profiling of PDTCs using the TCGA's *BRAF*V600E-*RAS* score (BRS) for which DTCs can be classified into *BRAF*V600E-like (with negative BRS) and *RAS*-like (with positive BRS) tumors. They found that the expression profile of thyroid differentiation markers did not differ greatly in PDTCs compared to PTCs. *BRAF*-mutated PDTCs, defined based on the presence of high mitotic rate, retain a *BRAF*-like signature and are less differentiated. On the other hand, those *RAS*-mutated are more differentiated and show a high thyroid differentiation score following the standard histological definition of the Turin criteria.[55]

Epigenetic modifications: DNA methylation, microRNA, and long noncoding RNA deregulation

Epigenetic modifications include DNA methylation and histone deacetylation regulating genes expression. In thy-

roid cancer, aberrant methylation of thyroid-specific tumor suppressors drives dedifferentiation and occurs in the initial phase of tumorigenesis. For example, the *RASSF1* tumor suppressor silencing by hypermethylation was detected in one case with PDTC.[66] On the contrary, the promoter hypomethylation of *SERPINB5*, codifying the mammary serine protease inhibitor (Maspin), has been detected in 41% of PDTCs. This led to the overexpression of Maspin with a metastatic promoting function.[67]

Non-coding RNAs (ncRNAs) are a class of RNAs transcribed, but not translated into proteins. They include both small RNAs, such as microRNAs, and long ncRNAs. The improper epigenetic regulation by these RNA molecules contributes to tumor progression in multiple human cancer, including PDTCs and ATCs.

MicroRNAs can function as oncogenes or tumor suppressor genes by regulating the expression of targets genes. To date, about 10-15 miRNA have been found to be downregulated in PDTCs, including miR-23 and miR-150 which target *FGFR3* and *TP53*, respectively. The overexpression of miR-146b, which target *SMAD4*, and miR-221 and -222, which target *PTEN*, *CDK1A*, *CDK1B*, *TIMP3*, *KIT* and *IQGAP1*, has been also observed in PDTCs. Interestingly, miR-150, miR-183-3p, miR221 and miR-222 result to be the most dysregulated microRNAs, able to distinguish WDTCs from PDTCs.[68]

Long non-coding RNAs are non-protein coding transcripts longer than 200 nucleotides that regulate proliferation and tumor recurrence. They may be involved in thyroid progression as proposed for *MALAT1* which is downregulated in PDTCs.[69]

DICER1 mutations in childhood- and adolescent-onset PDTCs

Hotspot mutations in *DICER1* gene have been identified in 83% childhood- and adolescent-onset PDTCs by NGS. These mutations fall within the metal-ion binding sites of the RNase IIb domain of the protein. No other mutations or gene fusions typical of adult WDTC and PDTC were detected, indicating that these tumors are genetically distinct from adult-onset PDTCs.[38]

In-vitro/in-vivo studies: current knowledge and future developments

Studying PDTC in *in vitro* settings and using mouse models can provide valuable insights into tumor biology, mechanisms of progression, metastasis, invasion, and potential therapeutic approaches. While both *in vitro* and *in vivo* studies have limitations, modelling poorly differentiated thyroid cancer is particularly challenging due to the high heterogeneity of these tumors and the fact that PDTC represents an intermediate stage in the natural evolution of thyroid cancer.

Out of more than fifty established and characterized thyroid cancer cell lines,[70] only five lines have been utilized in studies focused on PDTC (Supplementary Digital Material 3: Supplementary Table III).[1, 71-91] While T243, T351, and THJ529 cells were established from histologically confirmed poorly differentiated thyroid carcinomas,[71, 72] they are not widely used in *in vitro* experiments.

On the other hand, KTC-1 and BCPAP cells have been extensively studied, but they were derived from PTC or PTC with poorly differentiated component, respectively.[73, 74] Although both cell lines exhibit a poorly differentiated phenotype, caution must be exercised when extrapolating findings from these lines.

Compared to *in vitro* settings where tumor cells lack specific microenvironment and may exhibit paradoxical silencing of thyroid differentiation program,[92] modelling thyroid cancer using transgenic animals is more relevant, biologically, and clinically.

Since the 1990s, transgenic mouse models have significantly contributed to our understanding of the mechanisms of development and progression of thyroid tumors, as well as preclinical testing of new therapeutic strategies. While most established murine models are primarily focused on characterization of WDTC and/or ATC, a limited number of studies have identified and investigated PDTC as a distinct entity (Supplementary Table II).

Mouse models of PDTC employ transgenic thyroglobulin (*Tg)* or thyroid peroxidase genes promoters for thyroid-specificity and utilize conditional/inducible approaches to express oncogenes and to alter tumor-suppressor genes. Similar to PDTC in humans, the development of murine poorly differentiated tumors commonly requires genetic alterations in more than one cancer-related genes, often involving a combination of early event, such as oncogenic mutation of *BRAF* or *RAS*, and late event, typically alteration of *Trp53*.[55]

Several models have reported the development of PDTC driven by a single oncogenic event, including *BRAF*V600E,[75, 93] *NRAS*Q61K,[76] *STRN-ALK*,[77] *Pten* knockout (KO),[78] and aberrant expression of *ALK*F1174L.[79] Interestingly, in three of these models, the exogenous bovine *Tg* promoter was used to drive the expression of oncogene starting from the prenatal period. The use of the *Tg* promoter-dependent oncogenes may enable capturing

PDTC before they fully dedifferentiate and transform to ATC. With exception of short-latency PDTC model driven by *BRAF*V600E,[93] another common observation from single oncogene models is that the penetrance of PDTC correlates with age, which may be linked to accumulation of additional mutations required for transformation of normal follicular cells or pre-existing WDTC to PDTC. Indeed, an elegant study using mice with thyroid specific homozygous *HRAS*G12V demonstrated that the induction of additional mutations *via* transposon mutagenesis led to formation of poorly differentiated carcinomas.[80]

The requirement of several genetic hits for the development of murine PDTC with a shorter latency was also evident in models combining early and late driver events: *BRAF*V600E + *Tp53*KO/*Trp53*R270H,[81] *BRAF*V600E + *Pten* KO,[82] *BRAF*V600E + *AridA1/2* KO,[83] *HRAS*G12V + splice*EiF1AX*,[84] *HRAS*G12V + *Pten*KO,[85, 86] *HRAS*G12V + *Nf2* KO,[87] *HRAS*G12V + *Trp53* KO,[87] *STRN-ALK* + *Trp53* KO,[88] *RET/PTC3* + *Trp53* KO.[89] Besides the type of the oncogenic event, the number of affected alleles can also influence PDTC development, as was demonstrated in mouse models utilizing *HRAS*G12V and *Tp53*KO/*Trp53*R270H.[81, 86]

Non-genetic factors contributing to PDTC development in animal models include elevated blood levels of thyroid-stimulating hormone (TSH), and specific fibroinflammatory stroma. While many mouse models of PDTC exhibit elevated TSH levels due to oncogene-induced impairment of thyroid function[79, 81, 90, 93] or due to goitrogen treatment,[77, 88] it is worth noting that *Pten* KO results in suppressed TSH.[78]

Studies on murine WDTC have demonstrated the dependence of tumor penetrance and growth on TSH signalling.[94, 95] Elevated levels of TSH have been also found to accelerate the initiation and progression of PDTC.[88] Conversely, suppression of TSH can improve survival of PDTC-bearing animals, although no effects on phenotypes of established PDTC were observed.[79] Interestingly, some murine PDTCs retain partial expression of TSH receptors (TSHR), what may explain higher penetrance and aggressive behavior of these tumors in the presence of stimulating TSH.[88] However, once PDTC is established, it may become less reliant on TSH signaling, possibly due to acquiring additional mutations (such as genetic alterations in *Trp53*) and profound suppression of thyroid differentiation program leading to complete loss of TSHR. The involvement of the tumor microenvironment in PDTC has also been investigated, revealing that the development of PDTC is associated with infiltration of tumors by macrophages (M2), myeloid-derived tumor suppressor cells, T-regs, and cancer-associated fibroblasts, particularly in *BRAF*-driven PDTCs.[82, 86, 90, 91]

Similar to poorly differentiated thyroid tumors in humans, approaches to histological diagnosis of PDTC in mice vary among institutions. Only a limited number of studies reported application of Turin criteria and performed detailed histological descriptions of tumor phenotypes. It is important to consider that in some animal studies (not covered in this review) poorly differentiated tumors may have been classified as ATC. Murine PDTCs typically demonstrate local extrathyroidal extension, capsular and vascular invasion, and metastasis to the lungs, with less frequent involvement of the bones and liver. Interestingly, lymph node metastases were reported only in *RET-PTC3*/Trp53KO transgenic mouse model.[89] The growth of PDTC in mice, especially those with *Trp53* alterations, is rapid and leads to early removal of mice from experiments due to the protrusion of tumor beneath the thyroid cartilage and compression of the airways.

Poorly differentiated thyroid carcinomas in mice not only recapitulate histopathological and genetic features of human PDTC, but also demonstrate a comparable degree of heterogeneity in the context of dedifferentiation. Murine PDTCs display varying levels of decreased or lost expression of key thyroid-specific transcription factors (TTF1, FOXE1, and PAX8), as well as genes involved in iodine metabolism and transport (TG, NIS, TPO, Duox1/2). There is a possibility that PDTC may undergo consecutive stages of tumor dedifferentiation before transforming into anaplastic carcinoma, as was suggested in the recent study involving *STRN-ALK;Trp53*KO mouse model of multistep progression of thyroid cancer.[77] This study has identified two distinct types of PDTC, designated as type 1 (PDTC1) and type 2 (PDTC2). Although both PDTC1 and PDTC2 met the Turin diagnostic criteria and shared the same primary genetic drivers, they exhibited distinct cellular and molecular characteristics. PDTC1 retained some level of expression of thyroid transcriptional factors and iodine metabolism/transport genes, while PDTC2 showed more profound inhibition of these genes. Preserving the expression of genes involved in iodine metabolism and transport, such as NIS and TPO, is critical for tumor cell's ability to accumulate radioactive iodine (RAI), making the study of the mechanisms underlying the natural evolution of PDTC clinically relevant.

Furthermore, an important question arises regarding the reversal of dedifferentiation in PDTC, and enhancement or restoration of tumor avidity to RAI. Existing

mouse models have shown that the degree of tumor dedifferentiation, particularly the loss of NIS, correlates with the level of activation of the MAPK signaling pathway.[77, 96, 97] The pharmacological inhibition of MAPK results not only in structural response but also in restoration of the differentiation program and increased RAI uptake in short-latency *BRAF*V600E-driven PDTC.[93] However, once PDTC is established, it may further evolve and, depending on gained mutations, become resistant to treatment, and acquire irreversible silencing of thyroid differentiation genes. For instance, while poorly differentiated tumors with *BRAF*V600E or HRAS/Nf2 mutations respond to the MEK inhibitor AZD6244, PDTC driven by *BRAF*V600E+*AridA1/2*KO or *HRASV12*+*Trp53*KO show diminished response. Furthermore, although MEK inhibition can induce redifferentiation in *BRAF*V600E-driven PDTC, restoration of NIS is abolished in these tumors after additional inactivation SWI/SNF.[83, 87]

Thus, existing mouse models of PDTC successfully replicate the histopathological and molecular characteristics of their human counterparts and together with established cell lines represent an excellent tool for revealing the mechanisms of thyroid cancer dedifferentiation and for development of new therapeutic approaches.

Standardized diagnostic criteria for PDTC in murine models should be established to ensure consistency and accuracy in identifying and categorizing these tumors. Further studies should focus on characterizing the genetic alterations and molecular pathways associated with PDTC development and progression, with particular attention to the role of additional mutations in tumor stepwise dedifferentiation. Moreover, studying the microenvironment of PDTC in animal models can provide valuable insights into the role of fibroinflammatory stroma in thyroid cancer progression and identification of new therapeutic targets. Ultimately, these future directions will contribute to improving the clinical management and outcomes for patients with PDTC.

Treatments of PDTCs: conventional therapies, new drugs, and prospective treatments

Poorly differentiated thyroid carcinoma carries a dismal prognosis with 10-year survival rates lower than 50% with conventional therapies.[5] However, treatment protocols have not been established due to the rarity of this entity and the inconsistency of diagnostic criteria between centers.[2] Therefore, most centers treat PDTC patients according to radioactive iodine-refractory differentiated thyroid carcinoma guidelines. In Figure 2 it has been represented the flow chart to be followed to choose the most appropriate therapeutic strategy for PDTC.

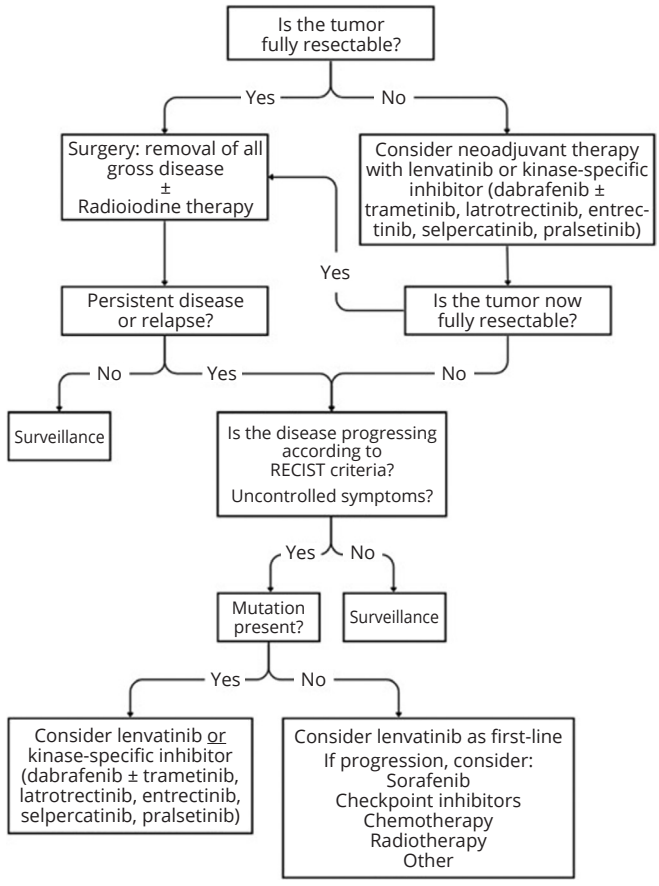

Figure 2.—Schematic representation of the flow chart to be followed to choose the most appropriate therapeutic strategy for PDTC.

Conventional therapies

Total thyroidectomy with lymph node dissection remains the major gold-standard treatment of PDTC, with five-year locoregional control rates of 81%.[2, 5, 31] However, more than 50% of patients with PDTC present with extensive cervical disease at diagnosis and up to 85% develop distant metastasis during follow-up.[2] Therefore, adjuvant treatments are frequently necessary. Adjuvant therapies include radioactive-iodine (RAI), external beam radiotherapy (EBRT) and systemic chemotherapy. However, effectiveness in patients with PDTC tend to be significantly lower and overall survival benefit has not been proven.[2, 45]

RAI has been administered to the vast majority of PDTC patients, considering the positive RAI-avidity in most met-

astatic lesions, the favorable tolerability of the treatment and also the lack of more effective alternatives.[31] However, the presence of less differentiated areas significantly reduces RAI retention and treatment effectiveness.[31]

To date, most studies have failed to prove overall survival benefit of RAI in PDTC patients.[5]

EBRT has been recommended for patients with tumors with extensive extrathyroidal extension, cervical lymph node metastasis, incomplete surgery, or unresectable disease,[2, 5] but its benefit in PDTC patients is controversial. Indeed, it does not seem to improve survival and local disease control is not clear.[2]

The experience with systemic chemotherapy in PDTC patients is scarce. So far, no clinical benefit has been reported and this intervention is generally not advised due to the significant adverse effects associated.[5]

Systemic therapies

Tyrosine multikinase inhibitors

Most recently, tyrosine kinase inhibitors (TKIs) emerged as a promising treatment modality for patients with progressive RAI-refractory thyroid carcinoma.[98] TKIs can block one or multiple tyrosine receptor kinases involved in tumorigenesis, particularly in angiogenesis and cellular proliferation. However, they are also responsible for inducing tumor resistance/development of new mutations. As so, careful selection of patients with RAI-refractory rapidly progressive disease is of utmost importance. The role of TKIs in PDTC patients is not definitively established but can be extrapolated from the results of randomized trials and real-world studies with RAI-refractory thyroid carcinoma which included some patients with PDTC.

European Medicines Agency (EMA) has approved Sorafenib and Lenvatinib as first-line therapies for patients with RAI-refractory DTC, based on the encouraging results of DECISION and SELECT studies, respectively. In SELECT, Lenvatinib has also shown favorable results as a second-line TKI.[99] Both studies included a significant subset of patients with PDTC (11.6% and 10.7% in DECISION and SELECT, respectively).[99, 100] Furthermore, PDTC subgroup analysis was performed in SELECT, with remarkable greater progression-free survival (PFS) in the treatment group (14.8 *vs.* 2.1 months in the placebo group).[99] Initial daily doses were 24 mg and 800 mg for Lenvatinib and Sorafenib, respectively.[99, 100] Noteworthy, one posterior study did not show noninferiority of Lenvatinib 18 mg comparing to 24 mg, reinforcing the relevance of maximal tolerated starting dose.[101] Regarding tolerability, any-grade adverse effects (AEs) were reported in 97.3% and 98.6% with Lenvatinib and Sorafenib, respectively.[99, 100] The majority were grade 1 and 2 AEs, and tended to emerge shortly after treatment start.[99, 100] The most frequent AEs reported with Lenvatinib were hypertension (67.8%), diarrhea (59.4%), fatigue, weight loss and nausea, while Sorafenib was mainly associated with hand-foot syndrome, diarrhea, alopecia, rash, fatigue, weight loss and hypertension.[99, 100] In SELECT, AEs led to treatment definite suspension in 14.2% of patients, while treatment interruption and dose reductions occurred in 67.8% and 82.4%, respectively.[99] In DECISION, treatment definite suspension, treatment interruption and dose reductions occurred in 66.2%, 64.3% and 18.8% of patients, respectively.[100] As a consequence of treatment-related AEs, mean daily doses were 17.2 mg (28% reduction) for Lenvatinib and 651 mg (19% reduction) for Sorafenib.[99, 100] After the release of these results, several real-life studies have been published. One recent retrospective study has specifically addressed the results of Lenvatinib in PDTC patients. The authors reported a median PFS of 12 months with an overall survival rate of 57% at 18 months of follow-up.[102] The remaining studies have included mostly DTC patients with variable proportions of PDTC, with PFS ranging from 13.3 to 17.3 months with Sorafenib[103-105] and 10.0 to 35.3 months with Lenvatinib.[104, 106-112] Patients of real-world studies are significantly more heterogenous comparing to randomized controlled trials, which should be taken into consideration while interpreting the results. AEs of TKIs remain the main challenge in clinical practice, which can compromise compliance, mean daily doses and treatment effectiveness if not timely address.

Cabozantinib has been approved by the EMA and FDA as second-line therapy for the treatment of RAI-refractory DTC with evidence of progression with a first-line TKI (Lenvatinib or Sorafenib). This recommendation was based on the results of COSMIC-311 trial,[113] which reported a significantly increase PFS in the treatment group (11.0 *vs.* 1.9 months in the placebo group) in patients with RAI-refractory DTC (including PDTC) and evidence of progression under Lenvatinib and/or Sorafenib treatment. PDTC population sub-analysis was not performed.

Kinase-specific inhibitors and redifferentiation therapy

The advent of molecular studies allowed the identification of mutations involved in tumorigenesis, which was the launch point for the development of kinase-specific inhibitors. The rationale for the development of specific target TKIs was to improve effectiveness and reduce AEs com-

paring to multikinase inhibitors. Some kinase-specific inhibitors have also shown the capability of inducing tumor redifferentiation, increasing the membrane expression of sodium-iodine symporter (NIS) and subsequently improving radioiodine avidity and treatment response.

BRAF is the most commonly mutated gene in thyroid carcinoma. Therefore, BRAF-inhibitors are under intense investigation for the treatment of BRAFV600E-mutated RAI-refractory thyroid carcinoma. The combination of BRAF-inhibitor Dabrafenib and MEK-inhibitor Trametinib has been approved by FDA for the treatment of BRAFV600E-mutated anaplastic thyroid cancer (ATC). Also, this association induced remarkable redifferentiation in one patient with PDTC.[114] On the other hand, Dabrafenib monotherapy seems similarly effective in DTC patients, in which it is generally reserved as second-line therapy after multikinase inhibitors lenvatinib and sorafenib.[115, 116]

Dabrafenib and MEK-inhibitor selumetinib are under investigation for their capacity of RAI uptake restoration.[116-119]

Another BRAFV600E-inhibitor, vemurafenib, has shown anti-tumor activity in a phase II study[120] and redifferentiation capacity in a small group of patients with BRAFV600E-mutated RAI-refractory DTC.[121]

RET alterations account for a small proportion of follicular cell-derived thyroid carcinomas.[116] Selpercatinib is a selective *RET* inhibitor approved by the EMA for the treatment of *RET* mutated follicular-cell thyroid cancer, based on the encouraging results of the phase 1/2 LIBRETTO-001 trial.[122] Pralsetinib is also a selective RET inhibitor which is approved by the FDA for the treatment of RAI-refractory thyroid cancer. This is based on the positive results of the phase 1/2 ARROW trial.[123]

NTRK gene fusions are also responsible for a small percentage of follicular-cell thyroid carcinomas.[116] Larotrectinib is an inhibitor of tropomyosin kinase receptors TRKA, B and C which has shown encouraging results in phase 1/2 clinical trials with *NTRK* fusion-positive thyroid carcinoma patients.[124] A small case series has also demonstrated its redifferentiation potential.[125] Entrectinib is a multikinase inhibitor which targets TRKA, B and C, but also ALK, ROS1, JAK2 and TNK2.[116] One patient with *NTRK* gene fusion ATC has been reported to have an impressive response to entrectinib in a neoadjuvant setting.[126] However, randomized clinical trials results are still not available.

Noteworthy, RET and NTRK inhibitors have shown a very low rate of grade 3 or higher AEs, which reinforces this benefit of targeted therapy but also establishes an important difference to BRAF-inhibitors. The development of other kinase-specific inhibitors directed to other molecular targets like TERT or ALK are now under investigation. It is also hypothesized that the combination of inhibitors of different pathways (*e.g.*, MAPK inhibitor in association with PI3K/AKT inhibitor) can have a synergistic effect and reduce treatment resistance.[116] With the widespread of molecular studies, new molecular targets will also probably be identified.

Future prospects

Checkpoint inhibitors

The inhibition of several proteins in the checkpoint cascade is under intense investigation for cancer treatment. Recently, these have also been proposed as potential agents for the treatment of thyroid carcinoma, particularly in synergic combination with TKIs.[127] Furthermore, the overexpression of PD-L1 in PDTC cells has been proven, reinforcing the rationale for the use of these drugs.[128]

Pembrolizumab, an anti-PD-1 monoclonal antibody, has shown interesting results in combination with Lenvatinib for the treatment of PDTC and ATC patients.[129]

Also, nivolumab and spartalizumab showed interesting results in ATC patients, and cemiplimab is currently under investigation.[116, 130, 131]

Virotherapy

Virotherapy represents a potential new treatment approach for multiple cancer types, including thyroid cancer. This is based on the development of viruses which selectively infect and destroy tumor cells. Some pre-clinical experiments have been performed in PDTC and ATC models with interesting results.[132, 133] However, safety concerns are still a significant limitation of this potential treatment modality.[132]

Other therapies

Panobinostat, a histone deacetylase inhibitor, has been tested in PDTC and ATC cell lines and was associated with a significant cytotoxic effect and an increased expression of NIS.[134] Bortezomib, a proteasome inhibitor, has shown synergistic cytotoxic effect with vemurafenib in BRAFV600E-mutated papillary-thyroid carcinoma cell lines.[135]

Conclusions

Poorly differentiated thyroid carcinoma (PDTC) is a rare and extremely aggressive tumor that accounts for approxi-

mately 2-15% of all thyroid cancers and shows a distinct biological behavior compared to WDTC and ATC. Therefore, it represents a distinct entity from anatomic-pathological and clinical points of view. Due to its rarity and difficulty in classification compared to other tumors, very few studies are available to date and series often include different histotypes in addition to PDTC.

This review has, thus, shown the main studies concerning this category of thyroid carcinomas from different perspectives. The data available to date confirm that the pathological evaluation and classification is crucial and should therefore be standardized in the multiple centers of reference.

Moreover, the clinical presentation and prognosis of PDTC may vary widely depending on the different stage of the disease at diagnosis. Therefore, patient management may differ in treatment and should be tailored to each patient.

It emerged that further studies on larger, carefully selected series are needed to better assess the special features of this carcinoma. These studies would help to better define the management of these carcinomas by focusing on the best diagnostic procedure and the best therapeutic approach.

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Conflicts of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions

All authors have participated to drafting the manuscript. Valentina Cirello wrote the paragraph "From genomic to epigenomic profiling: known and new

potential alterations", the abstract, the introduction. She prepared Supplementary Table II and revised critically all the manuscript. Carla Gambale wrote the paragraph "Clinical pathological features: what we know and what is still to be discovered" and prepared Figure 1. Chie Masaki wrote the paragraph "Clinical picture and diagnosis of PDTCs: various potential outcomes and prognosis" and prepared Supplementary Table I. Alyaksandr V. Nikitski wrote the paragraph "*In-vitro/in-vivo* studies: current knowledge and future developments" and prepared Supplementary Table III. João Roque wrote the paragraph "Treatments of PDTCs: conventional therapies, new drugs, and prospective treatments" and prepared Figure 2. Carla Colombo contributed to paragraphs "Clinical pathological features: what we know and what is still to be discovered" and "Clinical picture and diagnosis of PDTCs: various potential outcomes and prognosis" and wrote the conclusions. She has given substantial contributions to the conception or the design of the manuscript. All authors read and approved the final version of the manuscript.

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Supplementary data
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