

Emerging Therapeutics for Radioiodide-Refractory Thyroid Cancer

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Abstract: Although uncommon, thyroid cancer constitutes the main endocrine neoplasia with an incidence rate that has been increasing steadily over the past decades. Recently, remarkable advances have occurred in understanding the biology of thyroid cancer. Novel germline and somatic point mutations as well as somatic chromosomal rearrangements associated with thyroid carcinogenesis have been discovered. Strikingly, acquired knowledge in the genetics of thyroid cancer has been translated into clinical practice, offering better diagnostic and prognostic accuracy and enabling the development of novel compounds for the treatment of advanced thyroid carcinomas.

Even after 70 years, radioiodide therapy remains as the central treatment for advanced or metastatic differentiated thyroid cancer. However, the mechanisms leading to reduced radioiodide accumulation in the tumor cell remain partially understood. Radioiodide-refractory thyroid cancer metastasis constitutes a central problem in the management of thyroid cancer patients. In recent years, the antiangiogenic tyrosine kinase inhibitors sorafenib and lenvatinib have been approved for the treatment of advanced radioiodide-refractory thyroid carcinoma. Moreover, still on clinical phase of study, oncogene-specific and oncogene-activated signaling inhibitors have shown promising effects in recovering radioiodide accumulation in radioiodide-refractory thyroid cancer metastasis. Further clinical trials of these therapeutic agents may soon change the management of thyroid cancer.

This review summarizes the latest advances in the understanding of the molecular basis of thyroid cancer, the mechanisms leading to reduced radioiodide accumulation in thyroid tumors and the results of clinical trials assessing emerging therapeutics for radioiodide-refractory thyroid carcinomas in the era of targeted therapies.

Keywords: Radioiodide therapy, sodium iodide symporter, radioiodide-refractory thyroid cancer, MAPK pathway inhibitors, multi-targeted tyrosine kinase inhibitors.

INTRODUCTION

Thyroid cancer is the most common endocrine neoplasia, constituting approximately 1–5% of all cancers in females and less than 2% in males [1]. Every year, more than 300,000 new cases of thyroid cancer are diagnosed and ~40,000 people die from this disease [1]. The incidence of thyroid cancer has increased significantly over the past three decades and, if this trend continues, thyroid cancer will replace colorectal cancer as the fourth leading cancer diagnosis by 2030 in the United States [2]. A considerable extent, although not all, of the augmented incidence is generally believed to result from increased access to high-resolution imaging and the use of fine-needle aspiration biopsy of small nodules. In the last decade, major advances in early diagnosis, treatment standardization, and novel therapeutic options have maintained the annual number of thyroid cancer-derived death worldwide [1].

Thyroid cancers of follicular-cell origin account for more than 95% of all cases of thyroid cancer, with the

remaining cancers originate from calcitonin-producing parafollicular cells (medullary thyroid carcinomas). Differentiated thyroid cancer, the most common type of follicular cell-derived thyroid cancer including papillary (80-85%) and follicular carcinoma (15-20%), is generally associated with a favorable prognosis. Nevertheless, 10-30% of patients thought to be disease free after initial treatment will develop recurrence and/or metastases. Patients with differentiated thyroid cancer have a survival rate at 10 years of 85-95%. However, at the time of diagnosis, over 50% of cases show regional lymph node involvement and ~10% of patients have distant metastases thus reducing the survival rate and constituting the main cause of mortality [3]. In sharp contrast to differentiated carcinomas, undifferentiated thyroid cancers—poorly differentiated and anaplastic carcinomas—are responsible for 2–5% of all thyroid tumors, but accounts for over 50% of thyroid cancer-attributable deaths. Anaplastic carcinoma, one of the most aggressive cancer in humans, has a typically rapid and lethal progression; the 1-year survival rate is below 20% with a median survival of 5 months after diagnosis [4].

MOLECULAR PATHWAYS INVOLVED IN FOLLICULAR CELL-DERIVED THYROID CANCER

The last decade has been characterized by significant advances in the understanding of the

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molecular basis of thyroid cancer. The identification of molecular alterations leading to thyroid carcinogenesis, including constitutive activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway and the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, has started a new era in thyroid cancer medicine. The knowledge of molecular mechanisms that promote thyroid carcinogenesis has provided unprecedented opportunities for the identification of molecular markers to establish diagnostic and prognostic criteria, and rationally design of therapeutic strategies.

The cancer genome atlas research network has provided the most comprehensive characterization of the genomic landscape of papillary carcinomas [5]. The study demonstrated that papillary carcinomas have a low frequency of somatic mutations, carrying at least one driver alteration in over 96% of the cases. Not surprisingly, over 70% of the carcinomas are conducted by mutually-exclusive oncogenes leading to constitutive activation of the MAPK/ERK pathway involving point mutations of BRAF and RAS genes, as well as chromosomal rearrangements involving RET and TRK tyrosine kinases. Notably, the cancer genome project showed that papillary carcinomas have the highest frequency of chromosomal rearrangements [6]. Moreover, the study identified novel low frequency mutated genes related to thyroid carcinogenesis including EIF1AX, PPM1D, and CHEK2, and somatic copy number alterations often involving the chromosome 22q in the absence of known genetic lesions. Consistently, recently Garcia-Rendueles *et al.* [7] demonstrated that loss of function of the 22q neurofibromatosis-2 gene, encoding the tumor suppressor merlin, is implicated in thyroid carcinogenesis. Furthermore, the genomic analysis proposed a reclassification of papillary carcinomas into two major genetic subtypes (BRAF^{V600E}-like or RAS-like carcinomas) to better reflect their underlying signaling and differentiation properties [5]. Importantly, such genetic classification may tackle existing dilemmas in clinical risk stratification and decision making for the management of papillary carcinomas since different tumor signaling properties can mean the cancer responds differently to particular therapies.

Molecular-based risk stratification of papillary carcinomas according to the presence of the oncogene BRAF^{V600E} has been proposed based on its association with poor clinicopathologic outcomes [8,9] and radioiodide-refractory metastases [10,11]. However, the genetic background underlying the aggressive

group of papillary carcinomas remains obscure. Recently, telomerase reverse transcriptase (TERT) promoter mutations were identified in aggressive papillary carcinomas. Xing *et al.* [12] performed a retrospective study to evaluate the correlation between the oncogene BRAF^{V600E} and TERT promoter mutations with the clinicopathological outcomes of papillary carcinomas in a cohort of 507 patients. The study showed that coexisting BRAF^{V600E} and TERT^{C228T} mutations are commonly associated with high-risk papillary carcinomas. Tumor recurrence rates were 69% in patients with coexistence of both mutations versus 16% and 19% in patients harboring independent mutations in BRAF or TERT, respectively. These findings indicate that coexisting mutations in BRAF and TERT genes may develop a unique genetic background that defines aggressive subtypes of papillary carcinomas, providing a novel clinical stratification criterion.

Familial nonmedullary thyroid cancer, which accounts for 3-9% of all cases of thyroid cancer, has an autosomal dominant pattern of inheritance. Recently, Gara *et al.* [13] identified the germline mutation G534E HAP2 in seven affected members of a kindred with familial papillary thyroid cancer. Analysis of the cancer genome atlas database showed that only 4.7% of 423 sporadic papillary thyroid tumors harbored the mutation G534E HAP2. Thyroid tumors from family members harboring G534E HAP2 were associated with increased protein levels of HAP2, as compared to normal adjacent thyroid tissue. Functional studies showed that HAP2 bears tumor suppressor activity, whereas the mutant G534E HAP2 results in loss of function with dominant-negative properties. All together, these observations suggest that HAP2 is a novel susceptibility gene for familial thyroid cancer of follicular-cell origin. Further studies are still required to understand the role of G534E HAP2 in thyroid carcinogenesis.

As mentioned above MAPK/ERK pathway plays a fundamental role in the tumorigenesis of papillary carcinomas, whereas activation of the PI3K/AKT pathway plays a central role in follicular carcinomas. Particularly, the most prominent molecular features of follicular carcinomas are the high prevalence of point mutations in the proto-oncogene RAS (30-45%) and the chromosomal rearrangement PAX8-PPAR γ (30-35%). Noteworthy, both oncogenes are also frequently observed in follicular adenomas and the follicular variant of papillary carcinomas [14]. In addition, epigenetic gene silencing or somatic mutation of

important molecules in the regulation of RAS activation, such as RASSF1A and RASAL1, are frequent events in follicular carcinomas [15]. Opposite to BRAF^{V600E}-mutated carcinomas that show robust activation of ERK feedback-insensitive MAPK signaling leading to higher output of the ERK transcriptional program, RAS-triggered gene expression is sensitive to ERK inhibitory feedback to dampen MAPK/ERK signaling resulting in robust activation of PI3K/AKT signaling to promote the development of follicular carcinomas. Consistently, the importance of PI3K/AKT pathway in follicular thyroid carcinomas is highlighted by the recurrent presence of somatic mutations and copy number gain of the PI3K/AKT signaling effector PIK3CA and epigenetic gene silencing of the negative regulator of the pathway PTEN [16].

Undifferentiated thyroid cancers can develop *de novo*, although many of them originate from dedifferentiation of preexisting differentiated thyroid cancers. Accordingly, genetic events involved in the carcinogenesis of differentiated thyroid neoplasia are commonly found in undifferentiated carcinomas. Indeed, mutations in the proto-oncogenes BRAF and RAS are found in 30-40% and 20-40% of anaplastic carcinomas, respectively [17]. Alterations of PI3K signaling effectors and regulators, such as PIK3CA, AKT1, and PTEN are recurrent events in anaplastic carcinomas (10-20%, 5-10%, and 5-15%, respectively) [10,17]. In addition, anaplastic carcinomas frequently present additional genetic alterations, which are uncommon in differentiated carcinomas, and represent late events in tumor dedifferentiation. Point mutations in the TP53 gene, which encodes the tumor suppressor p53, and the CTNNB1 gene, which encodes β -catenin that is a pivotal player in the Wnt signaling pathway, are found in 50–80% and 5-60% of cases of anaplastic carcinoma, respectively [18,19]. Importantly, acquisition of loss of function p53 mutations is an important step in the progression to anaplastic carcinomas. Using transgenic mouse models, McFadden *et al.* [20] demonstrated that p53 tumor suppressor activity constrains the progression from BRAF^{V600E}-positive papillary to anaplastic carcinoma.

Very recently, Landa *et al.* [21] reported a comprehensive genomic characterization of 341 cancer-associated genes from a large series of undifferentiated thyroid tumors—84 poorly differentiated and 33 anaplastic thyroid carcinomas—. The study revealed that advanced thyroid tumors correlate with a higher number of mutations in cancer-associated genes. The analysis confirmed the common

occurrence of mutually exclusive BRAF and RAS mutations, present in 33% and 28% of poorly differentiated carcinomas and 45% and 24% of anaplastic carcinomas, respectively. Surprisingly, chromosomal rearrangements were only observed in poorly differentiated carcinomas (14%). Mutations of genes encoding different members of the PI3K/AKT pathway were particularly prevalent in anaplastic carcinomas (39% of anaplastic versus 11% of poorly differentiated carcinomas). Although most mutations were identified in PIK3CA and PTEN, mutations in other members of the cascade—PIK3C2G, PIK3CG, PIK3C3, PIK3R1, PIK3R2, AKT3, TSC1, TSC2, MTOR—were also present. Mutations in the translation initiation factor EIF1AX, initially reported in 2.3% of papillary carcinomas but absent in the follicular variant [22], showed a higher frequency in undifferentiated thyroid tumors (11% of poorly differentiated and 9% of anaplastic carcinomas). Furthermore, the study confirmed the common occurrence of TERT promoter mutations in undifferentiated carcinomas (40% of poorly differentiated and 73% of anaplastic carcinomas versus 9% of papillary carcinomas), and the high prevalence of TP53 mutations in anaplastic carcinomas (73%) but its relatively rare presence in poorly differentiated carcinomas (8%). A conclusive finding has been the identification of low frequency mutations of genes encoding members of the Wnt signaling pathway—CTNNB1, AXIN1, and APC—in undifferentiated carcinomas. Finally, the study uncovers genetic defects that implicate functional programs not previously associated with thyroid cancer, such as mutations in genes encoding components of the SWI/SNF chromatin remodeling complex, mutations in the cell-cycle checkpoint and DNA damage response gene ATM, mutations of the DNA mismatch excision repair genes MSH2, MSH6, MLH1 and mutations of the histone methyltransferases KMT2A, KMT2C, KMT2D, SETD2. Altogether, the genomic characterization of advanced carcinomas provides unprecedented knowledge to improve the molecular diagnosis of these aggressive tumors and also opens new avenues to further characterize novel genetic associations that can be exploited therapeutically.

Regardless of the progress in understanding the etiology of thyroid cancer, the current knowledge of genetic factors that trigger certain variants of differentiated carcinomas such as Hürthle cell carcinomas and follicular variant of papillary carcinomas remains incomplete. A comprehensive genomic characterization of infrequent thyroid

carcinomas will possibly lead to the identification of the molecular events driving these neoplastic processes in order to provide novel possibilities for diagnostic and identification of additional therapeutic targets.

DIAGNOSIS AND TREATMENT OF DIFFERENTIATED THYROID CANCER

Thyroid carcinomas usually present as a solitary thyroid nodule. Cytological examination of a fine-needle aspirate of the nodule constitutes the cornerstone in the diagnosis of thyroid cancer [23]. However, 5-30% of thyroid nodules cannot be conclusively diagnosed as benign or malignant after cytological evaluation using the morphologic criteria of The Bethesda System for reporting thyroid cytopathology [23]. The unclear diagnosis prevents optimal management of these patients and frequently results in avoidable diagnostic surgeries. The characterization of the genomic landscape of thyroid cancer has provided the basis for the rational design of multigene mutational panels for the detection of cancer in thyroid nodules. Last year, Nikiforov *et al.* [24] reported the validation of a multigene mutational panel on cancer diagnosis in thyroid nodules with atypia of undetermined significance or follicular lesion of undetermined significance. The analysis showed 91% sensitivity and 92% specificity for cancer detection, which resulted in a 97% negative predictive value and a 77% positive predictive value in the studied cohort. Although further studies are required, multigene mutational panels may provide accurate diagnosis of thyroid cancer without unnecessary diagnostic surgery. Recently, the importance of these molecular markers was reflected in the guidelines for the management of patients with thyroid nodules and differentiated thyroid cancer, published by the American Thyroid Association [25].

Therapeutic management of differentiated thyroid tumors comprises lobectomy or total thyroidectomy mostly depending on tumor size. Imaging studies prior to surgery to inspect the lymph node compartments in the lateral and central neck for metastases are required to evaluate sentinel lymph node dissection. Most current guidelines recommend the subsequent removal of normal remnant tissue and/or tumor metastatic cells using radioiodide therapy in (near) totally thyroidectomized patients. Significantly, radioiodide remnant ablation improves monitoring for any possible recurrence by assessing thyroglobulin levels. The debate regarding the use of radioiodide therapy for patients without lymph-node or distant metastases and low stages of the primary tumor is still ongoing.

Generally, the recommended therapeutic for patients with unresectable persistent, recurrent, and metastatic differentiated carcinoma consists of radioiodide therapy—only for patients with demonstrable metastatic radioiodide uptake—and levothyroxine replacement therapy aimed at suppressing circulating thyroid-stimulating hormone (TSH) levels. Significantly, these methods provide complete remission in one-third of patients with metastatic disease [3]. However, safe and effective therapies for patients with differentiated thyroid carcinoma who do not respond to radioiodide treatment are lacking, with conventional chemotherapy proving relatively ineffective.

RADIOIODIDE THERAPY FOR THE TREATMENT OF DIFFERENTIATED THYROID CANCER

The ability of thyroid cells to concentrate iodide has long constituted the molecular basis for the treatment of thyroid cancer based on radioiodide therapy. For over 70 years, radioiodide therapy using ^{131}I -iodide has been the mainstay therapy for radioiodide-avid locally advanced and metastatic thyroid carcinomas. Retrospective studies have demonstrated that the ability of tumor cells to accumulate radioiodide is the best indicator of disease-free survival [26-28]. However, 10-20% of differentiated thyroid tumors and 30% of metastases derived from radioiodide-avid tumors lose the ability to accumulate radioiodide, causing them to become resistant to radioiodide therapy. Loss of iodide accumulation is associated with poor prognosis; patients with thyroid cancer metastases that accumulate iodide showed a survival rate at 10 years of ~56%, while the survival is drastically reduced to ~10% in patients with radioiodide refractory metastases [26]. Therefore, understanding the cause of reduced iodide accumulation in thyroid tumors will certainly have major implications for radioiodide therapy.

Active iodide accumulation in thyrocytes constitutes a key step in the biosynthesis of iodine-containing thyroid hormones. The transporter responsible for iodide uptake into the thyroid cell is the sodium iodide symporter (NIS), an integral plasma membrane glycoprotein located at the basolateral membrane [29]. Radioiodide therapy efficiency is ultimately dependent on functional NIS expression at the plasma membrane of thyroid tumor cells, as deficient radioiodide accumulation is the major cause of treatment failure. Although thyroid tumors often exhibit reduced iodide transport compared to normal thyroid tissue, most thyroid tumors overexpress NIS compared to the

surrounding normal tissue. Surprisingly, NIS expression is usually increased although mainly intracellularly retained, suggesting the presence of defects in the traffic of the protein to the cell surface [30-32]. The paradoxical co-occurrence of decreased iodide transport and NIS overexpression (although intracellularly retained) highlights the importance of uncovering the mechanisms involved in NIS trafficking to and retention at the cell surface under physiological conditions.

In normal thyroid cells, TSH enhances iodide uptake through a cAMP-mediated increase in NIS gene expression. The human NIS gene contains a full TSH-responsive enhancer region located between -9,847 and -8,968 bp. Different b-Zip molecules interact with a CRE-like element within the NIS enhancer [33]. Although the CRE-like element plays a key role in mediating cAMP-induced NIS expression, full TSH/cAMP-dependent NIS gene transcription requires the cooperation of the thyroid transcription factor Pax8 [33]. In addition, TSH regulates iodide uptake by modulating the stability and subcellular distribution of NIS without apparently influencing the intrinsic functional status of the protein [34]. Therefore, adequate TSH stimulation is required to enhance radioiodide accumulation in thyroid tumor cells, and this is achieved either through thyroid hormone withdrawal or recombinant TSH administration. Despite the unfavorable cost-effectiveness analysis, recombinant TSH injection avoids the transient thyroid hormone withdrawal-induced deterioration of health-related quality-of-life due to the induction of hypothyroidism [35]. Although the majority of differentiated thyroid cancers often respond to TSH stimulation with an increase in radioiodide accumulation, 30–40% of advanced metastatic thyroid carcinomas does not respond to radioiodide therapy, even after TSH stimulation [36].

A majority of differentiated thyroid carcinomas (68–86%) partially retains functional NIS expression, thus allowing radioiodide therapy [30,37]. However, differentiated thyroid cancer cells often lose their ability to accumulate iodide, thus becoming resistant to radioiodide therapy. Several mechanisms have been postulated to explain a transcriptional repression of NIS gene expression in thyroid tumors. Ectopic oncogene expression into well-differentiated thyroid cell lines has long been used to assess the transcriptional mechanisms repressing NIS expression in thyroid cancer. Venkateswaran *et al.* [38] reported that RET/PTC1 overexpression down-regulates NIS

expression by interrupting TSHR/cAMP signaling, thus suppressing the nuclear localization of catalytic protein kinase A (PKA). Reduced PKA activity is associated with down-regulation of bZIP proteins that regulate NIS gene expression in response to increased cAMP levels [33]. Similarly, Baratta *et al.* [39] demonstrated that HRAS^{G12V} oncogenic activity blocked PKA activity causing a reduction of Pax8 transcriptional activity that, in turn, results in a down-regulation of NIS gene expression.

Considering the high prevalence of BRAF^{V600E}-positive metastatic papillary carcinomas refractory to radioiodide therapy, Riesco-Eizaguirre *et al.* [11] investigated NIS expression by immunohistochemistry in a cohort of 60 papillary carcinomas, and reported a significant reduction of NIS expression and impaired targeting to the plasma membrane in tumors harboring the oncogene BRAF^{V600E}. Furthermore, *in vitro* experiments demonstrated that BRAF^{V600E} expression sharply impairs NIS targeting to the cell surface and progressively decreases NIS protein expression in a similar fashion as does TSH withdrawal [34], suggesting that BRAF^{V600E}-induced NIS transcriptional and posttranslational modifications may be dependent upon impairment of cAMP-mediated response. Concomitantly, BRAF^{V600E} overexpression induces transforming growth factor (TGF)- β secretion from thyroid cancer cells, resulting in its paracrine action on the tumor tissue [40]. Increased TGF- β signaling in thyroid cells is associated with suppression of NIS gene expression through SMAD3 signaling which negatively regulates Pax8 transcriptional activity [41]. Very recently, Riesco-Eizaguirre *et al.* [42] described a novel posttranscriptional regulation of NIS gene expression involving miR-146b-3p which is among the most abundantly expressed miRNAs in papillary thyroid tumors. The authors described that miR-146b-3p binds to the 3'-untranslated region of Pax8 and NIS, leading to impaired protein translation and a subsequent reduction in iodide uptake [42].

Previously, we evidenced the role of the nuclear factor (NF)- κ B subunit p65 cooperating with Pax8 in the transcriptional regulation of NIS gene expression [43]. Interestingly, recently, we provided novel evidence suggesting that S-nitrosylation of p65 at cysteine 38 which prevents its binding to DNA thus suppressing p65-dependent gene expression, represses TSH-induced NIS expression in thyroid cells [44]. Future studies assessing S-nitrosylation in radioiodide-refractory thyroid tumors may provide novel therapeutic

strategies for increasing NIS expression, thereby making possible more effective radiotherapy regimens.

The pituitary tumor-transforming gene (PTTG)-binding factor (PBF) has been characterized as the only NIS-interacting protein in the thyroid tissue. Smith *et al.* [45] reported that exogenous PBF overexpression represses iodide uptake in thyroid cells by binding to NIS and leading to its internalization into clathrin-coated CD63-positive late endosomes. Proto-oncogene tyrosine kinase Src-mediated PBF phosphorylation at tyrosine 174 is required for its physical interaction with NIS; abrogation of Src kinase activity restores NIS expression at the plasma membrane and radioiodide uptake in thyroid cancer cells [46]. Further identification of NIS-interacting protein(s), such as PBF, may provide novel targets to stimulate cell surface NIS expression in thyroid tumors where NIS expression remains intracellular.

Very recently, Darrouzet *et al.* [47] reported the first systematic evaluation of potential NIS intracellular sorting motifs involved in plasma membrane targeting. The authors identified an internal PDZ-binding motif comprising residues 118-121, within the intracellular loop 2, which plays a crucial role in NIS trafficking to the plasma membrane; disruption of this PDZ-binding motif by the substitution L121A completely abolished NIS expression at the plasma membrane. Along the same line, Paroder *et al.* [48] evaluated the importance of the position 124—located in the intracellular loop 2 but outside the mentioned PDZ-binding domain—after the R124H NIS mutant was identified in a patient with congenital hypothyroidism due to an iodide transport defect [49]. Amino acid substitutions at position 124 revealed a key structural role for the δ -amino group of R124 in NIS targeting to the plasma membrane. Indeed, an intramolecular interaction between the δ -amino group of R124 and the thiol group of C440, located in the intracellular loop 6, is critical for the local folding required for NIS sorting out through endoplasmic reticulum quality-control system.

Overall, functional NIS expression can be enhanced by up-regulation of both the transcriptional and post-translational pathways. Dissection of such signaling pathways should lead to novel strategies to further increase radioiodide accumulation in thyroid cancer, thus expanding the application of radioiodide therapy to radioiodide-refractory thyroid cancer. Importantly, any therapeutic approach aiming to recover NIS gene transcription should consider the fact that a deficient traffic of the protein to the plasma membrane could

result in therapeutic failure to increase radioiodide accumulation.

TYROSINE KINASE INHIBITORS FOR RADIOIODIDE-REFRACTORY DIFFERENTIATED THYROID CARCINOMAS

Traditionally, patients with locally advanced or metastatic radioiodide-refractory differentiated thyroid carcinomas who experience rapid disease progression have had limited therapeutic options. During recent years, several multi-targeted tyrosine kinase inhibitors have shown considerable effectiveness against metastatic radioiodide-refractory thyroid cancers. On the basis of remarkable results in phase III clinical trials, the antiangiogenic inhibitors sorafenib and lenvatinib have been approved for the clinical intervention of patients with metastatic radioiodide-refractory thyroid cancers [50,51].

Brose *et al.* [50] conducted a placebo-controlled, phase 3 trial to evaluate efficacy and safety of the multi-targeted tyrosine kinase inhibitor sorafenib in patients with radioiodide-refractory locally advanced or metastatic differentiated thyroid cancer. Enrolled patients received either sorafenib 400 mg twice daily (n=207) or matched placebo (n=210). Sorafenib treatment significantly improved progression-free survival over that of placebo (10.8 versus 5.8 months, respectively). Sorafenib-induced adverse events occurred in 98.6% patients. Most adverse events were grade 1 or 2, and usually occur early in treatment. The most common adverse events were hand-foot skin reaction (76%), diarrhea (69%), alopecia (67%), and rash or desquamation (50%). The rate of drug discontinuation due to adverse events was 19%, indicating that the drug was mostly well tolerated. However, dose reduction was required in 64% patients. Consistently with the importance of effective adverse event management in maintaining patients on sorafenib therapy, Worden *et al.* [52] described a detailed analyses of the incidence, prevalence, and severity of sorafenib-evoked adverse effects, as well as the interventions (e.g. dose interruption/reduction, medication) used to manage them over time.

Last year, Schlumberger *et al.* [51] conducted a placebo-controlled, phase 3 trial to evaluate efficacy and safety of the multi-targeted tyrosine kinase inhibitor lenvatinib in patients with progressive radioiodide-refractory thyroid cancer. Eligible patients were randomly assigned to receive lenvatinib 24 mg daily (n=261) or placebo (n=131). Lenvatinib treatment

significantly improved progression-free survival compared with placebo (18.3 versus 3.6 months, respectively), and was associated with significant improvement in the response rate (64.8% versus 1.5%, respectively). Treatment-related adverse events were reported in 97.3% patients receiving lenvatinib. Most often, these were hypertension (68%), diarrhea (59%), fatigue or asthenia (59%), and reduced appetite (50%). A total of 68% of the patients on lenvatinib required dose reduction, 82% required dose interruption, and 14% of patients discontinued the drug. Significantly, in 7.7% patients in the lenvatinib group, adverse effects that developed during treatment were fatal. However, no specific pattern of fatal adverse events was evidenced.

Other multi-targeted tyrosine kinase inhibitors (i.e., motesanib, axitinib, pazopanib) have been evaluated in patients with advanced differentiated thyroid cancer showing 15–60% response rates [53–55]. Further placebo-controlled phase III trials evaluating the efficacy and safety of these inhibitors are eagerly awaited.

ONGOING STUDIES ON ONCOGENE-SPECIFIC INHIBITORS FOR RADIOIODIDE-REFRACTORY DIFFERENTIATED THYROID CARCINOMAS

Mutually exclusive somatic genetic alterations leading to constitutive activation of the MAPK/ERK signaling pathway have been reported in up to 70% of patients with papillary carcinomas. Consequently, the inhibition of MAPK/ERK pathway represents an attractive therapeutically target to treat papillary carcinoma. Hayes *et al.* [56] conducted a small phase II trial to evaluate the efficacy, safety, and tolerability of selumetinib in radioiodide-refractory papillary thyroid cancer. Selumetinib is a potent, selective MEK1/2 kinase inhibitor. Enrolled patients (n=39) received selumetinib 100 mg twice daily. The most frequent adverse effects associated to selumetinib treatment were rash, fatigue, diarrhea, and peripheral edema. A small portion of patients experienced side effects of grade 3/4 toxicity and 16% of patients discontinued the therapy due to adverse events. Best responses in 32 evaluable patients were 3% partial response, 54% stable disease, and 28% progressive disease. Although the drug showed limited single-agent activity in unselected patients with papillary carcinoma, the cohort of patients with BRAF^{V600E}-positive tumors showed a trend to have better median progression free survival compared with patients harboring BRAF wild-type tumors (33 versus 11 weeks, respectively). However,

this data should be viewed with caution until further studies with more patients could confirm these findings.

Based on preclinical observations demonstrating that chemical inhibition of the MAPK/ERK signaling restores radioiodide accumulation in BRAF^{V600E}-driven mouse models of differentiated thyroid cancer [57], Ho *et al.* [58] conducted a phase II trial to assess the efficacy of selumetinib to restore radioiodide accumulation in patients with metastatic radioiodide-refractory differentiated thyroid cancer. The study quantified TSH-stimulated radioiodide accumulation using positron emission tomography-computed tomography in patients (n=20) before and 4 weeks after selumetinib 75 mg twice daily administration. Interestingly, selumetinib treatment increased radioiodide accumulation in 60% of the patients enrolled in the study. The dosimetry threshold for radioiodide therapy was reached in 8 out of 12 patients, and these patients were treated with radioiodide. Of the 8 patients treated with radioiodide, 5 had confirmed partial response and 3 had stable disease. Surprisingly, the study showed a marked dissimilar response to selumetinib treatment between tumors harboring the oncogenes NRAS^{Q61R/K} or BRAF^{V600E}, as all 5 patients with NRAS-mutant tumors exceeded the dosimetry threshold to receive radioiodide therapy, but only 1 out of 9 patients with BRAF-mutant tumors exceeded the threshold.

Consistently with the high frequency of BRAF^{V600E} in thyroid carcinogenesis, Dadu *et al.* [59] reported the efficacy and safety of the potent BRAF inhibitor vemurafenib in patients (n=17) with BRAF^{V600E}-positive metastatic papillary thyroid cancer outside of a clinical trial. Vemurafenib was given orally at different starting doses (240–960 mg twice daily). Best response rates with vemurafenib were partial response in 47% patients and stable disease in 53% patients. However, 13 of the 15 patients showed disease progression within 12 months of starting vemurafenib treatment. Drug discontinuation owing to significant adverse effects was required in 29% patients. The reasons for discontinuation were skin lesions (17.6%), fatigue (5.9%), and atrial fibrillation (5.9%).

As previously mentioned, vemurafenib showed limited efficacy as monotherapy in phase II trials of patients with BRAF^{V600E} positive thyroid cancer [59]. Regarding this point, Montero-Conde *et al.* [60] found that most thyroid cancer cell lines harboring the mutation BRAF^{V600E} are refractory to growth inhibition by vemurafenib. The inhibition of RAF effectors in

BRAF^{V600E}-positive thyroid cells is transient, with a rebound in MAPK signaling beginning few hours after drug treatment. Vemurafenib treatment increases the expression of the human epidermal growth factor receptor 3 (HER3) by relief of feedback mechanisms, leading to reactivation of MAPK/MEK signaling upon autocrine-secreted neuregulin-1 activation of HER3 signaling, thus promoting resistance to growth inhibition. Furthermore, the addition of the HER kinase inhibitor lapatinib prevented the rebound effect in cells exposed to vemurafenib. Overall, targeted therapies may relieve tumor lineage-specific negative feedback events and reengage pathways that limit the antitumor response. Further knowledge regarding functional consequences of reactivated pathways upon treatment with signaling inhibitors will allow the appropriate selection of combination therapies.

Last year, Rothenberg *et al.* [61] evaluated the selective BRAF inhibitor, dabrafenib, to induce radioiodide accumulation in BRAF^{V600E}-positive radioiodide-refractory papillary carcinomas. A reduced number of patients (n=10) harboring BRAF^{V600E}-positive thyroid tumors were enrolled to receive dabrafenib 150 mg orally twice daily for 25 days before TSH-stimulated radioiodide whole body scan. Interestingly, 6 out of 10 patients (60%) showed new radioiodide-avid foci of disease, and remained on dabrafenib treatment for another 17 days before receiving ablative radioiodide therapy. Six months after radioiodide therapy, 5 out of 6 patients showed a reduction in the size of radioiodide-avid target lesions on computed tomography scan. Moreover, two patients met criteria for partial responses, including one with a nearly 60% reduction in the size of the target lesion. The remaining 4 patients showed stable disease, 3 of them demonstrated a reduction in the size of the target lesions (12-20%) and one a slight increase (3%). Significantly, all patients completed dabrafenib therapy without dose modification and unexpected adverse effects.

As previously discussed, RET/PTC chromosomal rearrangements play a causative role in the pathogenesis of papillary carcinomas. Leboulleux *et al.* [62] undertook a randomized phase 2 trial to evaluate efficacy and safety of the multi-targeted tyrosine kinase inhibitor vandetanib in patients with metastatic radioiodide-refractory differentiated thyroid carcinomas. Enrolled patients received either vandetanib 300 mg daily (n=72) or matched placebo (n=73). At data cutoff, 72% patients in the vandetanib group and 84% in the placebo group discontinued the therapy due to disease

progression. Patients who received vandetanib showed longer median progression free survival than did those who received placebo (11.1 month vs. 5.9 months, respectively). The most common grade 3 or worse adverse events related to vandetanib exposure were QTc prolongation (14%), diarrhoea (10%), asthenia (7%), and fatigue (5%). Patients who received vandetanib required dose reduction to 300 mg every other day (n=16) or dose interruption (n=28), mainly because of adverse events. Significantly, further studies assessing the efficiency of vandetanib would require the evaluation of progression-free survival in different genetic biomarker cohorts, as papillary carcinomas harboring RET rearrangements might be significantly more sensitive to vandetanib treatment.

The central role of PI3K/AKT signaling in tumor progression has prompted an uncommon effort to develop specific inhibitors targeting PI3K and its downstream kinases such as mammalian target of rapamycin (mTOR). Several clinical trials involving inhibitors of this pathway have been or are being conducted in different tumor types. During an ongoing phase 2 trial with the allosteric mTOR inhibitor everolimus in thyroid cancer, Wagle *et al.* [63] identified a nonsense mutation in TSC2, a negative regulator of mTOR, in a patient with metastatic anaplastic thyroid cancer with a high sensitivity to the drug. However, after an extraordinary 18-month response, the tumor developed resistance to everolimus as result of *de novo* mutation in mTOR that confers resistance to allosteric mTOR inhibitors [63]. Genetic alterations in components of the PI3K/AKT pathway or in other genes that, in turn, activate the PI3K/AKT signaling such as RAS and RET/PTC are frequent events in follicular and anaplastic carcinomas, rendering the PI3K/AKT pathway as an attractive therapeutic target for thyroid cancer. Indeed, preclinical data support the therapeutic potential of the AKT inhibitor MK2206 to block the proliferation of thyroid cancer cells that harbored PI3K/AKT pathway activating mutations [64]. Genetic-guided clinical trials assessing the efficacy of inhibitors targeting PI3K/AKT pathway (as single-agent or in combination with MEK inhibitors) in the treatment of thyroid cancer are eagerly awaited.

In the near future, the use of tumor biomarkers to design personalized therapies will improve the survival of a disease that not long ago was hardly treatable.

CONCLUSIONS AND PERSPECTIVES

Over the last decade, a remarkable progress in understanding the molecular basis of thyroid cancer

has been achieved. Particularly, the papillary thyroid cancer genome atlas and recently, the genomic characterization of advanced thyroid cancer—poorly differentiated and anaplastic thyroid tumors—have provided the most comprehensive description of the genomic landscape of thyroid carcinomas to date [5,21]. Activation of MAPK/ERK and PI3K/AKT pathways constitute the major oncogenic driver that promotes the development and progression of thyroid carcinomas. Acquired knowledge in the molecular pathogenesis of thyroid cancer has now opened unprecedented opportunities for the rational design of multigene mutational panels for the detection of malignant thyroid nodules and novel therapeutic strategies for thyroid cancer clinical management.

Over 70 years of clinical experience validate the effectivity and safety of radioiodide therapy in most differentiated thyroid carcinomas. Stimulation of thyroid cancer cells with TSH maximizes radioiodide accumulation, likely by enhancing NIS expression at transcriptional level and its targeting to the plasma membrane. Recent progress in understanding the molecular mechanisms controlling NIS gene expression under physiological and pathological conditions has brought about possibilities of new therapeutic approaches, which may decrease the dose of radioiodide as well as expanding the application of radioiodide therapy to radioiodide-refractory thyroid cancers. Indeed, emerging therapies, still on clinical phase of study, using small-molecule inhibitors—dabrafenib and selumetinib—have shown promising effects enhancing radioiodide accumulation in radioiodide-refractory differentiated thyroid cancer metastasis. Furthermore, the paradoxical observation of decreased iodide uptake and increased intracellularly retained NIS protein expression in differentiated thyroid tumors remain unsolved. The mechanisms regulating NIS trafficking to and removal from the plasma membrane under physiological and pathological conditions constitutes still a major open question in the thyroid field.

In recent years, new targeted therapies based on the antiangiogenic tyrosine kinase inhibitors sorafenib and lenvatinib have been approved for the treatment of patients with advanced radioiodide-refractory thyroid carcinomas. Although these therapies are encouraging, these drugs may not be appropriate for all patients after the evaluation of the possible risks against the potential for benefit. Particularly, therapies based on tyrosine kinase inhibitors are not recommended for patients with asymptomatic and slow-growing radioiodide-refractory

thyroid carcinomas. Though currently off-label, the anti-oncogene directed signal transduction inhibitors dabrafenib or selumetinib have emerged as an interesting first-line option for patients with indolent radioiodide-refractory carcinomas. Although single-agent therapy have shown poor long-term responses, dabrafenib and selumetinib treatment overcome radioiodide resistance, thus allowing subsequent radioiodide ablation protocols. A significant advantage of this combination therapeutic strategy over long-term treatment with antiangiogenic tyrosine kinase inhibitors is that only a short course of drug therapy is required to elicit a durable clinical effect, thus reducing drug-associated adverse effects. Future phase 3 studies evaluating the clinical benefit from the combination of dabrafenib or selumetinib and radioiodide therapy in larger cohort of patients and, perhaps, in particular subgroups of patients according to the oncogenic driver event are eagerly awaited.

Clinical trials assessing the efficacy of tyrosine kinase inhibitors and anti-oncogene directed signal transduction inhibitors have demonstrated notable difference in outcome according to the driver oncogene. Particularly, the subgroup of patients harboring the oncogene NRAS^{Q61R/K}—not BRAF^{V600E}—was much more likely to benefit from the combination of selumetinib and radioiodide ablation. Altogether, these observations strongly suggest that mutation screening to identify driving oncogenes should be performed on a routine basis in patients with radioiodide-refractory thyroid tumors because it may lead to the selection of a specific inhibitor in the presence of a driver mutation.

To date, no adequate treatment is available for anaplastic thyroid cancer, the most aggressive and rapidly fatal thyroid disease. Very recently, the largest mutational landscape of advanced thyroid tumors has identified a significant number of druggable genetic alterations [21]. Moreover, the biological consequences of a previously unsuspected group of genetic alterations in the context of thyroid tumorigenesis remain to be further explored. Significantly, off-label treatment with vemurafenib and everolimus has shown an exquisite response in patients with anaplastic thyroid cancer harboring the oncogenes BRAF^{V600E} and TSC2^{Q1178X}, respectively [63,65]. Importantly, genomic screening of anaplastic thyroid cancer may assist the identification of subgroups of patients for enrollment in clinical trials assessing the efficacy of established oncogene-directed therapies. The identification of effective therapies for anaplastic thyroid tumors

remains as a major challenge in the thyroid cancer field.

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AUTHORS' CONTRIBUTIONS

Dr. Nicola made significant contributions to the conception, literature review, writing, reviewing and editing the manuscript. Dr. Masini-Repiso made significant contributions to reviewing and editing the manuscript. Both authors read and approved the final version of the manuscript.

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DISCLOSURE STATEMENT

The authors have nothing to disclose.

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