Nodular Thyroid Disease and Thyroid Cancer in the Era of Precision Medicine

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Abstract

The management of thyroid nodules, one of the main clinical challenges in endocrine clinical practice, is usually straightforward. Although the most important concern is ruling out malignancy, there are grey areas where uncertainty is frequently present: the nodules labelled as indeterminate by cytology and the extent of therapy when thyroid cancer is diagnosed pathologically. There is evidence that the current available precision medicine tools (from all the “-omics” to molecular analysis, fine-tuning imaging or artificial intelligence) may help to fill present gaps in the future. We present here a commentary on some of the current challenges faced by endocrinologists in the field of thyroid nodules and cancer, and illustrate how precision medicine may improve their diagnostic and therapeutic capabilities in the future.

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Introduction

The concept of precision medicine implies that every patient is unique [1, 2]. Precision medicine emphasizes that physicians are not dealing with diseases but with particular individuals who are ill.

The easy access to health care resources and the wide use of periodic medical check-ups have led to the frequent diagnosis of thyroid nodules in many individuals who live in developed countries. There are no 2 identical thyroid nodules in the same way as there are no 2 identical thyroid cancers. A thyroid nodule (whatever it might be: benign or malignant) has a particular anatomical (size, echotexture, location, etc.) and molecular signature (harbour a number of specific gene mutations). Additionally that particular nodule exists in a distinctive individual, with a well-defined phenotypic and genotypic background. The molecular signature of 2 thyroid nodules might be very similar but the individuals in whom they are placed are precisely unique. Identifying these differences and tailoring their management are the main challenges that face precision medicine.

Fig. 1. Algorithm for the management of thyroid nodules. An accurate diagnosis will more precisely define the extent of treatment required for every thyroid nodule. This approach should compile information gathered from imaging techniques, cytological features, molecular tests, and artificial networks. FNA, fine-needle aspiration.

Thyroid Nodules

The development of highly sensitive thyroid imaging techniques in combination with the increased use of screening programmes has led to an overload in the number of patients with thyroid nodules visiting endocrine clinics. The challenge is to differentiate between malignant and benign lesions. The current assessment is based on cytology findings supported by ultrasound (US) features. Unfortunately, approximately one quarter of cytology results are either non-diagnostic or indeterminate [3]. Similarly, the information from US, or other techniques such as elastography, is far from an accurate identification of malignancy. Hence, we need better diagnostic methods with the aim to improve the precision in diagnosis and avoid unnecessary surgery (Fig. 1).

Refining Cytological Analysis

Categories III, IV, and V from the Bethesda classification comprise the spectrum of indeterminate cytological results [4]. Around 10–60% of these nodules are eventually confirmed to be malignant after surgery [5]. A number of ancillary approaches are under development with the aim to improve the diagnostic accuracy in these cases.

Immunocytochemistry

Several immunocytochemical markers have been proposed to differentiate benign from malignant nodules in fine-needle aspiration samples [6]. Some of them are listed in Table 1. Currently no immunomarker has demonstrated enough diagnostic accuracy to be used alone. However, the combined analysis of galectin-3 and Hector Battifora mesothelial-1 has shown acceptable sensitivity

Table 1. Immunocytochemistry markers studied to characterize thyroid nodules

TGF- α	Mig-6	XRCC4	Chromogranin A	PPAR- γ
LIg4	Ku80	NDRG2	CEA	HGF
WDR3	IGFBP7	ERs	Calcitonin	C-met
PDGF	FOXE1	β -Catenin	TROP-2	CD30
Galectin-3	TTF1	IL-6	p16	CaT12
u-PAR	MMPs	NAT2	p21	E-CAD
RAD2	IL-10	MT1G	p27	CD56
MUCs	CRABP1	Serpin-A	BCL-2	IGF-1
TIMP1	PTEN	NM23	BAX	IGF-1R
p63	KAI1	IGFBP5	BCL-XL	HMV-CK
NATH	CCND1	MYC	Cyclin-A	FRA-1
ERBB2	hNIS	c-FOS	Cyclin-D1	HMGGA2
Fibronectin-1	HBME1	CITED-1	Cyclin-B1	MRC2
CK-19	B-RAF	p53	EphB4	SFN
CD44	Ki-67	RET	EphB6	NF- κ B
PGK1	CD117	HER2	KAP-1	EGFR
MUC-1	COX-2	SSTR-2	CXCR4	PCNA
TPO	DPP4	HKIII	HMWCK	MIB-1
CD57	CD15	GLUT1	IL-6R	YY1
C1orf24	ITM-1	DDIT3	ARG2	HMGI(Y)

and specificity to identify malignant tumours [7]. In this regard, other promising markers are being studied, such as CD44 [8] or Ki-67 [9].

Molecular Tests

The advances in understanding the molecular pathways in tumorigenesis have identified many of the genetic abnormalities involved in thyroid cell transformation [10, 11]. The presence of a single mutation (such as *BRAF*) has shown high specificity but low sensitivity [12, 13]. The new approaches in diagnostics based on genetic abnormalities are testing a number of genetic alterations simultaneously. Several platforms are currently marketed to improve the malignancy risk assessment in indeterminate nodules [14]. These techniques are widely used in the USA but not in Europe.

- The *Afirma Gene expression classifier* analyses the mRNA expression of a panel of 167 genes. Fine-needle aspiration samples of the screened nodules are labelled “benign” or “suspicious” [15]. Gene expression classification has been proposed as a “rule-out” test [16], that is, a test with a high sensitivity and high negative predictive value.
- *ThyroSeq* is a dynamic panel that analyses a panel of specific thyroid cancer-related mutations. The ThyroSeq approach has been proposed as a “rule-in” test [16–18], that is, a test with a high specificity.

- *Rossetta genomics* is a platform recently developed that analyses the expression of a combination of micro-RNA (miRNA) species. The platform includes a set of 24 miRNAs and is reported to improve the malignancy risk assessment [19].

Developing Advances in Imaging Techniques

Advances in US technique have improved the ability to recognize suspicious nodules [20]. In addition, other radiological and molecular imaging methods have also been tested to differentiate benign from malignant thyroid nodules. Potentially these techniques could improve the accuracy in cancer diagnosis at earlier stages.

Liquid Biopsy

Innovations in genetic technologies have enabled the detection of circulating tumour cells, free DNA or even miRNA [21]. The detection of such abnormalities would help in the diagnosis of thyroid cancer in patients with thyroid nodules, or in the follow-up of patients with thyroid cancer. Pupilli et al. [22] investigated the presence of the DNA *BRAF* mutation in plasma from 103 patients with nodular goitre and reported 65% specificity and 80% sensitivity to discriminate papillary thyroid carcinoma (PTC) from benign nodules. miRNAs have also been quantified in serum, and differences have been found in blood samples from patients with PTC compared with

Table 2. Topics of debate on differentiated thyroid cancer treatment

Therapy	Topic
Surgery	Extent of thyroidectomy Surgery for microcarcinoma Prophylactic versus therapeutic lymph node dissection Major extension of lymph node dissection Number of nodes in lymph node dissection Use of selective sentinel lymph node biopsy
Radio-iodine	Indication Dose: high versus low Radio-iodine and survival Radio-iodine and the risk of second primary malignancy??
Other	TSH levels: suppressive versus replacement Long-term TSH-suppressive doses When to start TKI therapy Indications of external beam radiotherapy Imprecisions and interferences in thyroglobulin measurement

TSH, thyrotropin; TKI, tyrosine kinase inhibitors.

those from patients with benign thyroid nodules or controls [23, 24].

The Help of Artificial Intelligence

Artificial neural networks are statistical machine learning models that emulate the processing performance of biological neurons [25]. Artificial neural network models process input data, learn from experiences, and discover relationships between variables to generate a final decision output [26]. Artificial neural networks have been constructed with data from cytological [27, 28], clinical [29], or US [30] variables to differentiate benign from malignant thyroid nodules with accuracy up to 82%.

Differentiated Thyroid Cancer

The current management of differentiated thyroid cancer does not always tailor the treatment intensity to tumour aggressiveness. This imprecision in therapy challenges the current concept of precision medicine. The accurate characterization of the unit “patient-tumour” is the final goal to avoid unnecessary aggressive protocols in low-risk patients or to use the entire therapeutic arsenal in high-risk patients.

At present, more than 15 scoring systems have been proposed to characterize every individual case [31]. The most accepted prognostic factors are age, histological variant, initial extension of the disease, and size of the primary tumour. However, a significant percentage of patients are not correctly classified with these variables, indicating the necessity of better early markers of cancer risk assessment to obtain a fine-tuned prognostic characterization [32]. Furthermore, the currently used clinical scoring systems focus on disease-specific mortality, when, in fact, a system to predict recurrence is most relevant to the vast majority of thyroid cancer patients, since they will have low-risk disease.

Molecular Analysis

Molecular biology can offer the most effective way to achieve a personalized approach in differentiated thyroid carcinoma (DTC) patients.

Prognosis and Follow-Up

Currently, the significance of the *BRAF* mutation in PTC prognosis is a matter of debate [33–35]. New information illustrates that the combination of *BRAF* and *TERT* promoter mutations confers a particularly aggressive phenotype [36, 37]. Other genetic alterations such as *RET* (rearranged during transfection)/PTC rearrangements [38], *TP53* [17, 39] or *PI3K-AKT* signalling pathway changes have been reported to influence the outcome of PTC as well [40]. In a meta-analysis Pak et al. [41] concluded that the determination of a single genetic mutation is a poor prognostic marker. The whole-genome characterization of PTC offers a more sophisticated tumour classification and a list of new potential prognostic markers [10].

Recent studies show that deregulation of miRNAs may be implicated in a number of thyroid cancer characteristics. Some miRNAs have been associated with aggressive features for recurrence in PTC [42–44].

Serum thyroglobulin is the widely used tumour marker in patients with DTC but its utility is frequently hampered by the presence of antithyroglobulin antibodies [45]. The analysis of thyroglobulin free DNA can bypass the antibodies and provide a non-invasive approach to assess tumour evolution [46]. This technique could more reliably detect postoperative residual disease and identify patients who should receive further therapy [47].

Treatment

Several issues related to thyroid treatment are the subject of intense debate, such as the extent of thyroidecto-

Fig. 2. Topics that remain to be elucidated in the treatment of thyroid cancer with TKIs. TKI, tyrosine kinase inhibitor; Tg, thyroglobulin; TSH, thyrotropin.

my, the indication for prophylactic neck lymph node dissection, or the usefulness of radio-iodine ablation (Table 2). At present we are not able to identify the “iodine-refractory thyroid cancer” (IRTC) tumours from the outset [48]. Initial tumour molecular characterization could tip the balance between conservative and aggressive management.

The targeted therapy era for thyroid cancer started nearly a decade ago. To date, 2 tyrosine kinase inhibitors (TKIs), sorafenib and lenvatinib, have been approved for treatment of IRTCs. Both compounds have demonstrated a significant improvement in progression-free survival compared to placebo [49]. It is not clear whether the mutational tumour status modifies the effectiveness of these drugs. In theory, a more specific target-based therapy might improve its efficacy. A number of clinical trials with different TKIs is currently in progress in patients with IRTCs [50].

Decreased expression of the sodium iodide symporter is the main cause of the IRTC phenotype. Although the aetiology of sodium iodide symporter loss of function is not completely understood, hyperactivation of some molecular pathways, such as the MAPK and the PI3K pathways, plays a primordial role [51]. Several approaches

have been evaluated to restore the sodium iodide symporter action with the aim to reverse the refractoriness [52, 53]. In this scenario, TKIs are used only for a few days, with the objective of increasing iodine uptake and permitting ^{131}I therapy.

Despite the important role of TKIs in the management of advanced thyroid cancer, several concerns remain to be addressed (Fig. 2).

Some new exciting approaches are on the therapeutic horizon, including immunotherapy [54] or locally directed treatments such as radiofrequency and cryo-ablation for solid tumours or cementoplasty for bone metastasis [55].

Molecular Imaging

Several molecular imaging technologies will improve the accuracy of the radiological follow-up of DTC patients. Specific targeted probes will help to characterize the outcome of specific targeted drugs in a precise tandem model. The dual role (diagnostic and therapeutic or theranostics) of such molecular imaging techniques will expand the contribution of this to thyroid oncology.

Current positron emission tomography (PET) modalities are designed to visualize specific molecular and cel-

Table 3. Risk stratification and recommendations for prophylactic thyroidectomy in children with *RET* mutations

MTC risk level	<i>RET</i> mutation	Exon	Age for prophylactic surgery in children
Highest	M918T ¹	16	First year or the first months of life
High	C634F/G/R/S/W/Y A883F ¹	11 15	At or before 5 years of age based on serum CT levels
Moderate	G553C C609F/G/R/S/Y C611F/G/S/Y/W C618F/R/S, C620F/R/S C630R/Y, D631Y, K666E E768D, L790F V804L/M S891A, R912P	8 10 11 13 14 16	To be performed when the serum CT level becomes elevated, or in childhood if parents do not wish to embark on a lengthy period of periodic evaluation

Adapted from Wells et al. [68]. MTC, medullary thyroid carcinoma; RET, rearranged during transfection; CT, calcitonin. ¹ Mutations associated with multiple endocrine neoplasia type 2B.

lular processes such as glucose uptake (¹⁸FDG-PET), cell proliferation (¹⁸F-fluorothymidine) and, more recently, tumour hypoxia (¹⁸F-fluoromisonidazole) [56]. A step forward in PET technology is the development of probes with specific molecular targets such as monoclonal antibodies, pro-angiogenic molecules, or somatostatin receptors [57]. The role of these new techniques has not been established in thyroid cancer, beyond anecdotal cases [58–60]. These findings have prompted the development of peptide receptor radionuclide therapy with yttrium- or lutetium-labelled somatostatin analogues to treat advanced DTC tumours [60–62]. Additionally TKI-PET tracers have shown encouraging results [63]. Finally, ¹²⁴I-PET has demonstrated clinical and functional value in the analysis of DTC metastases [64, 65].

Magnetic resonance imaging has also been used to assess tumour angiogenesis by targeting integrin $\alpha\beta 3$ expression, and as a measure of the efficacy of anti-angiogenesis drugs [66]. With the same purpose, contrast-enhanced US with microbubbles targeting angiogenesis markers has been studied in a preliminary mouse model of thyroid cancer [67].

Medullary Thyroid Carcinoma

Currently, it is difficult to predict the course of medullary thyroid carcinoma (MTC); thus, it is challenging to tailor the appropriate treatment to every individual case of MTC. Usually MTC clinical behaviour is more aggres-

sive than that of DTC, although some MTC patients with distant metastases have an indolent course. Total thyroidectomy, central neck dissection, and therapeutic dissection of involved lateral neck compartments are the current recommended surgical treatment for patients with MTC [68]. Recent data have shown that bilateral disease is identified in 5.6% of patients with sporadic disease, while multifocal disease was noted in 16.0% of patients, suggesting that total thyroidectomy should remain the standard of care for initial surgery, as less complete thyroid surgery may fail to address fully the primary site of disease [69].

The Question of Prophylactic Thyroidectomy

The specific *RET* mutation enables us to intervene in multiple endocrine neoplasia type 2 patients with a prophylactic thyroidectomy in susceptible individuals (Table 3) [68]. Although decisions based on the known genotype-phenotype are established on a solid rationale, there is great heterogeneity in the age of onset and aggressiveness of MTC among individuals with the same *RET* mutation. Undoubtedly, other epigenetic, metabolomic, and environmental factors will contribute to making more precise decisions on the timing of thyroidectomy.

Contribution of Genetics

A precision approach to patients with MTC implies accurate identification of the molecular signature. To date prediction of phenotype through the genotype is far from perfect.

Somatic *RET* mutations in sporadic tumours may not necessarily drive tumorigenesis but rather appear to be important for the progression of disease. Often, tumours show mutational heterogeneity, that is, *RET* mutations may be found in subpopulations of tumour cells rather than the entire tumour [70, 71]. Different amino acid substitutions at a particular *RET* codon may be associated with phenotypes with different tumour aggressiveness [72]. An important limitation of genomic analysis is epigenetic influences. Therefore our current practice is far from the accuracy that could be achieved by in-depth biological, psychosocial, environmental, and lifestyle information about an individual patient.

Imprecisions and Troubles in the Diagnosis and Follow-Up

Although the serum calcitonin (CT) level is considered a sensitive but non-specific tool for the diagnosis of MTC in patients with thyroid nodules, no clear CT threshold has been identified. Thus, there is a need for accurate markers to rule out MTC in these patients. Some studies have reported better performance of procalcitonin in comparison with CT [73].

Surveillance for hyperparathyroidism and pheochromocytoma is necessary in multiple endocrine neoplasia type 2A or familial MTC patients. Today it is not possible to predict who might suffer from these diseases [74]. Precision medicine should be able to discriminate patients requiring lifelong surveillance.

The follow-up of patients with persistent or recurrent MTC poses a major problem. Frequently, patients have elevated postsurgical tumour markers with negative imaging. A precise assessment of postsurgical serum CT elevations requires consideration of the particular conditions of the patient (presurgical CT values, renal function, thyroid auto-immune status, hypergastrinaemia, heterophilic antibodies, interfering medications, etc.), an accurate pre-analytical assessment (time from surgery and time of the day), as well as the performance of the particular assay.

In patients with elevated serum CT values, whole-body imaging evaluation is recommended [68]. Unfortunately, the sensitivity of the conventional imaging techniques is poor [75]. A precision medicine approach implies the use of radiotracers specifically captured by MTC cells. Nevertheless, results of the few comparative studies have been variable, and we still do not know whether PET imaging using new radiotracers, such as ^{18}F -dihydrophenylalanine and ^{68}Ga -labelled somatostatin analogues, will offer us better performance in clinical practice [76].

Need to Improve Our Current Treatment Options

Today 2 TKIs, vandetanib [77] and cabozantinib [78], are offered to patients with advanced metastatic MTC. Both drugs are not selective and target multiple kinases. Other TKIs are being tested in clinical trials in patients with advanced MTC. These drugs target several tyrosine kinases and their effects are not precise and specific. Additionally a characteristic of cancer cells is instability. Targeting a single pathway results in the development of drug resistance, and patients may then need a number of synergistic or sequential therapies.

Several unanswered questions still remain [68, 79]. It is not known whether targeted agents improve overall survival. Specific criteria for selection of the appropriate drug in a particular patient are lacking, and the dose and treatment timing are not clearly established. In the future, progress in pharmacogenomics will allow us to anticipate what drug will be suitable for a given patient. Nowadays, a wide range of pharmacogenomic tests are beginning to be recognized as having significant potential to alter standard medical practice [80].

Innovative Options for Treatment Need More Precise Knowledge

Information about tumour mutations can allow a more precise selection of the target therapy. *RAS* and *RET* mutations appear to be mutually exclusive [81]. The Raf-1/MEK/ERK pathway has been implicated in the metastatic phenotype [82], whereas inactivation of glycogen synthase kinase-3 has been reported to be associated with growth suppression in MTC cells [83]. The deregulation of the PI3K/Akt/mTOR pathway seems to contribute to the tumorigenic activity of *RET* proto-oncogene mutations. Targeting this pathway may represent an attractive potential therapeutic approach.

Targets for therapeutic agents could be independent of the tumour mutational status. The abnormal expression or deregulation of angiogenesis and cell proliferation factors, including VEGFR, EGFR, cMET, and FGFR4, may contribute to the progression and divergent responses to different drugs. An interesting approach might be to change the methylation status of the tumour, since it is known that MTC is characterized by general hypomethylation [84]. Histone deacetylase inhibitors have also been shown to suppress proliferation of MTC cell lines [85].

In patients with advanced tumours that do not respond to currently accepted therapies, a personalized approach will be an option in the future. Novel strategies include the use of tumour vaccines [86], anti-CEA-pretargeted radioimmunotherapy [87], and radiolabelled octreotide [88].

Conclusions

The correct diagnostic identification of malignant nodules is a major challenge in thyroidology. Although some interesting advances have been made in the last 2 decades, novel and promising imaging techniques, combined with more accurate molecular examination of tissue samples, will reduce the diagnostic uncertainty of this prevalent entity. This approach will also allow tailoring the treatment to the patients' needs.

Personalized medicine also depends on using specialized approaches and multidisciplinary health care teams to promote health, patient education and satisfaction, and customized disease prevention, detection, and treatment strategies.

Disclosure Statement

The authors declare no conflict of interests.

References

- Galofré JC, Díez JJ, Cooper DS: Thyroid dysfunction in the era of precision medicine. *Endocrinol Nutr* 2016;63:354–363.
- Ladenson PW: Precision medicine comes to thyroidology. *J Clin Endocrinol Metab* 2016;101:799–803.
- Misiakos EP, Margari N, Meristoudis C, Machairas N, Schizas D, Petropoulos K, Spathis A, Karakitsos P, Machairas A: Cytopathologic diagnosis of fine needle aspiration biopsies of thyroid nodules. *World J Clin Cases* 2016;4:38–48.
- Cibas ES, Ali SZ: NCI Thyroid FNA State of the Science Conference. The Bethesda System for Reporting Thyroid Cytopathology. *Am J Clin Pathol* 2009;132:658–665.
- Hsiao SJ, Nikiforov YE: Molecular approaches to thyroid cancer diagnosis. *Endocr Relat Cancer* 2014;21:T301–T313.
- Gómez Sáez JM: Diagnostic usefulness of tumor markers in the thyroid cytological samples extracted by fine-needle aspiration biopsy. *Endocr Metab Immune Disord Drug Targets* 2010;10:47–56.
- Wu G, Wang J, Zhou Z, Li T, Tang F: Combined staining for immunohistochemical markers in the diagnosis of papillary thyroid carcinoma: improvement in the sensitivity or specificity? *J Int Med Res* 2013;41:975–983.
- Das DK, Al-Waheeb SKM, George SS, Haji BI, Mallik MK: Contribution of immunocytochemical stainings for galectin-3, CD44, and HBME1 to fine-needle aspiration cytology diagnosis of papillary thyroid carcinoma. *Diagn Cytopathol* 2014;42:498–505.
- Su JJ, Hui LZ, Xi CJ, Su GQ: Correlation analysis of ultrasonic characteristics, pathological type, and molecular markers of thyroid nodules. *Genet Mol Res* 2015;14:9–20.
- Cancer Genome Atlas Research Network: Integrated genomic characterization of papillary thyroid carcinoma. *Cell* 2014;159:676–690.
- Fagin JA, Wells SA Jr: Biologic and clinical perspectives on thyroid cancer. *N Engl J Med* 2016;375:1054–1067.
- Fnaiss N, Soobiah C, Al-Qahtani K, Hamid JS, Perrier L, Straus SE, Tricco AC: Diagnostic value of fine needle aspiration BRAF (V600E) mutation analysis in papillary thyroid cancer: a systematic review and meta-analysis. *Hum Pathol* 2015;46:1443–1454.
- Jia Y, Yu Y, Li X, Wei S, Zheng X, Yang X, Zhao J, Xia T, Gao M: Diagnostic value of BRAF (V600E) in difficult-to-diagnose thyroid nodules using fine-needle aspiration: systematic review and meta-analysis. *Diagn Cytopathol* 2014;42:94–101.
- Nishino M: Molecular cytopathology for thyroid nodules: a review of methodology and test performance. *Cancer Cytopathol* 2016;124:14–27.
- Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, Friedman L, Kloos RT, LiVolsi VA, Mandel SJ, Raab SS, Rosai J, Steward DL, Walsh PS, Wilde JI, Zeiger MA, Lanman RB, Haugen BR: Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. *N Engl J Med* 2012;367:705–715.
- Eszlinger M, Hegedüs L, Paschke R: Ruling in or ruling out thyroid malignancy by molecular diagnostics of thyroid nodules. *Best Pract Res Clin Endocrinol Metab* 2014;28:545–557.
- Nikiforova MN, Wald AI, Roy S, Durso MB, Nikiforov YE: Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. *J Clin Endocrinol Metab* 2013;98:E1852–E1860.
- Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, Hodak SP, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN: Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. *Cancer* 2014;120:3627–3634.
- Benjamin H, Schnitzer-Perlman T, Shtabsky A, Vanden Bussche CJ, Ali SZ, Kolar Z, Pagni F; Rosetta Genomics Group; Bar D, Meiri E: Analytical validity of a microRNA-based assay for diagnosing indeterminate thyroid FNA smears from routinely prepared cytology slides. *Cancer Cytopathol* 2016;124:711–721.
- McQueen AS, Bhatia KSS: Thyroid nodule ultrasound: technical advances and future horizons. *Insights Imaging* 2015;6:173–188.
- Ignatiadis M, Lee M, Jeffrey SS: Circulating tumor cells and circulating tumor DNA: challenges and opportunities on the path to clinical utility. *Clin Cancer Res* 2015;21:4786–4800.
- Pupilli C, Pinzani P, Salvianti F, Fibbi B, Rossi M, Petrone L, Perigli G, De Feo ML, Vezzosi V, Pazzagli M, Orlando C, Forti G: Circulating BRAFV600E in the diagnosis and follow-up of differentiated papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2013;98:3359–3365.
- Yu S, Liu Y, Wang J, Guo Z, Zhang Q, Yu F, Zhang Y, Huang K, Li Y, Song E, Zheng XL, Xiao H: Circulating microRNA profiles as potential biomarkers for diagnosis of papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2012;97:2084–2092.
- Graham ME, Hart RD, Douglas S, Makki FM, Pinto D, Butler AL, Bullock M, Rigby MH, Trites JR, Taylor SM, Singh R: Serum microRNA profiling to distinguish papillary thyroid cancer from benign thyroid masses. *J Otolaryngol Head Neck Surg* 2015;44:33.
- Manning T, Sleator RD, Walsh P: Biologically inspired intelligent decision making: a commentary on the use of artificial neural networks in bioinformatics. *Bioengineered* 2014;5:80–95.
- Sheikhtaheri A, Sadoughi F, Hashemi Dehaghi Z: Developing and using expert systems and neural networks in medicine: a review on benefits and challenges. *J Med Syst* 2014;38:110.
- Shapiro NA, Poloz TL, Shkurupij VA, Tarkov MS, Poloz VV, Demin AV: Application of artificial neural network for classification of thyroid follicular tumors. *Anal Quant Cytol Histol* 2007;29:87–94.

- 28 Ippolito AM, De Laurentiis M, La Rosa GL, Eleuteri A, Tagliaferri R, De Placido S, Vigneri R, Belfiore A: Neural network analysis for evaluating cancer risk in thyroid nodules with an indeterminate diagnosis at aspiration cytology: identification of a low-risk subgroup. *Thyroid* 2004;14:1065–1071.
- 29 Saylam B, Keskek M, Ocak S, Akten AO, Tez M: Artificial neural network analysis for evaluating cancer risk in multinodular goiter. *J Res Med Sci* 2013;18:554–557.
- 30 Zhu LC, Ye YL, Luo WH, Su M, Wei HP, Zhang XB, Wei J, Zou CL: A model to discriminate malignant from benign thyroid nodules using artificial neural network. *PLoS One* 2013;8:e82211.
- 31 Lang B, Lo C, Chan W, Lam K: Staging systems for papillary thyroid carcinoma. A review and comparison. *Ann Surg* 2007;245:366–378.
- 32 Zafon C: Papillary thyroid microcarcinoma – do classical staging systems need to be changed? In Ward LS (ed): *Thyroid and Parathyroid Diseases – New Insights into Some Old and Some New Issues*. Rijeka, Intech, 2012, DOI: 10.5772/25354.
- 33 Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, Kim KW, Hahn SK, Youn YK, Kim KH, Cho BY, Park DJ: The association of the BRAF (V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis. *Cancer* 2012;118:1764–1773.
- 34 Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, Elisei R, Bendlová B, Yip L, Mian C, Vianello F, Tuttle RM, Robenshtok E, Fagin JA, Puxeddu E, Fugazzola L, Czarniecka A, Jarzab B, O'Neill CJ, Sywak MS, Lam AK, Riesco-Eizaguirre G, Santisteban P, Nakayama H, Clifton-Bligh R, Tallini G, Holt EH, Šýkorová V: Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. *J Clin Oncol* 2015;33:42–50.
- 35 Henke LE, Pfeifer JD, Ma C, Perkins SM, DeWees T, El-Mofty S, Moley JF, Nussenbaum B, Haughey BH, Baranski TJ, Schwarz JK, Grigsby PW: BRAF mutation is not predictive of long-term outcome in papillary thyroid carcinoma. *Cancer Med* 2015;4:791–799.
- 36 Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, Pai S, Bishop J: BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. *J Clin Oncol* 2014;32:2718–2726.
- 37 Song YS, Lim JA, Choi H, Won JK, Moon JH, Cho SW, Lee KE, Park YJ, Yi KH, Park do J, Seo JS: Prognostic effects of TERT promoter mutations are enhanced by coexistence with BRAF or RAS mutations and strengthen the risk prediction by the ATA or TNM staging system in differentiated thyroid cancer patients. *Cancer* 2016;122:1370–1379.
- 38 Romei C, Elisei R: RET/PTC Translocations and clinico-pathological features in human papillary thyroid carcinoma. *Front Endocrinol* 2012;3:54.
- 39 Zafon C, Obiols G, Castellví J, Tallada N, Baeña JA, Simó R, Mesa J: Clinical significance of RET/PTC and p53 protein expression in sporadic papillary thyroid carcinoma. *Histopathology* 2007;50:225–231.
- 40 Petrulea MS, Plantinga TS, Smit JW, Georgescu CE, Netea-Maier RT: PI3K/Akt/mTOR: a promising therapeutic target for non-medullary thyroid carcinoma. *Cancer Treat Rev* 2015;41:707–713.
- 41 Pak K, Suh S, Kim SJ, Kim IJ: Prognostic value of genetic mutations in thyroid cancer: a meta-analysis. *Thyroid* 2015;25:63–70.
- 42 Aragon Han P, Weng CH, Khawaja HT, Nagarajan N, Schneider EB, Umbricht CB, Witter KW, Zeiger MA: MicroRNA expression and association with clinicopathologic features in papillary thyroid cancer: a systematic review. *Thyroid* 2015;25:1322–1329.
- 43 Sondermann A, Andreghetto FM, Moullet AC, da Silva Victor E, de Castro MG, Nunes FD, Brandão LG, Severino P: MiR-9 and miR-21 as prognostic biomarkers for recurrence in papillary thyroid cancer. *Clin Exp Metastasis* 2015;32:521–530.
- 44 Chruściak A, Lam AK: Clinical pathological impacts of microRNAs in papillary thyroid carcinoma: a crucial review. *Exp Mol Pathol* 2015;99:393–398.
- 45 Netzel BC, Grebe SK, Carranza Leon BG, Castro MR, Clark PM, Hoofnagle AN, Spencer CA, Turcu AF, Algeciras-Schimmich A: Thyroglobulin (Tg) testing revisited: Tg assays, TgAb assays, and correlation of results with clinical outcomes. *J Clin Endocrinol Metab* 2015;100:E1074–E1083.
- 46 Ma M, Zhu H, Zhang C, Sun X, Gao X, Chen G: “Liquid biopsy”-ctDNA detection with great potential and challenges. *Ann Transl Med* 2015;3:235.
- 47 Cradic KW, Milosevic D, Rosenberg AM, Erickson LA, McIver B, Grebe SKG: Mutant BRAF (T1799A) can be detected in the blood of papillary thyroid carcinoma patients and correlates with disease status. *J Clin Endocrinol Metab* 2009;94:5001–5009.
- 48 Schlumberger M, Brose M, Elisei R, Leboulleux S, Luster M, Pitoia F, Pacini F: Definition and management of radioactive iodine-refractory differentiated thyroid cancer. *Lancet Diabetes Endocrinol* 2014;2:356–358.
- 49 Llaveró-Valero, Guillén-Grima F, Zafon C, Galofré JC: The placebo effect in thyroid cancer: a meta-analysis. *Eur J Endocrinol* 2016;174:465–472.
- 50 <https://clinicaltrials.gov/ct2/results?term=differentiated+thyroid+cancer+&Search=Search> (accessed August 24, 2016).
- 51 Spitzweg C, Bible KC, Hofbauer LC, Morris JC: Advanced radioiodine-refractory differentiated thyroid cancer: the sodium iodide symporter and other emerging therapeutic targets. *Lancet Diabetes Endocrinol* 2014;2:830–842.
- 52 Wong K-P, Lang BH-H: New molecular targeted therapy and redifferentiation therapy for radioiodine-refractory advanced papillary thyroid carcinoma: literature review. *J Thyroid Res* 2012;2012:818204.
- 53 Vaisman F, Carvalho DP, Vaisman M: A new appraisal of iodine refractory thyroid cancer. *Endocr Relat Cancer* 2015;22:R301–R310.
- 54 Ilyas S, Yang JC: Landscape of tumor antigens in T cell immunotherapy. *J Immunol* 2015;195:5117–5122.
- 55 Kim JH, Yoo WS, Park YJ, Park DJ, Yun TJ, Choi SH, Sohn CH, Lee KE, Sung MW, Youn YK, Kim KH, Cho BY: Efficacy and safety of radiofrequency ablation for treatment of locally recurrent thyroid cancers smaller than 2 cm. *Radiology* 2015;276:909–918.
- 56 Teng FF, Meng X, Sun XD, Yu JM: New strategy for monitoring targeted therapy: molecular imaging. *Int J Nanomed* 2013;8:3703–3713.
- 57 Woelfl S, Bogner S, Huber H, Salaheddin-Nassr S, Hatzl M, Decristoforo C, Virgolini I, Gabriel M: Expression of somatostatin receptor subtype 2 and subtype 5 in thyroid malignancies. *Nuklearmedizin* 2014;53:179–185.
- 58 Nakajo M, Nakajo M, Kajiya Y, Jinguji M, Mori S, Aridome K, Suenaga T, Tanaka S: High FDG and low FLT uptake in a thyroid papillary carcinoma incidentally discovered by FDG PET/CT. *Clin Nucl Med* 2012;37:607–608.
- 59 Traub-Weidinger T, Putzer D, von Guggenberg E, Dobrozemsky G, Nilica B, Kendler D, Bale R, Virgolini IJ: Multiparametric PET imaging in thyroid malignancy characterizing tumour heterogeneity: somatostatin receptors and glucose metabolism. *Eur J Nucl Med Mol Imaging* 2015;42:1995–2001.
- 60 Versari A, Sollini M, Frasoldati A, Fraternali A, Filice A, Froio A, Asti M, Fioroni F, Cremonini N, Putzer D, Erba PA: Differentiated thyroid cancer: a new perspective with radiolabeled somatostatin analogues for imaging and treatment of patients. *Thyroid* 2014;24:715–726.
- 61 Budiawan H, Salavati A, Kulkarni HR, Baum RP: Peptide receptor radionuclide therapy of treatment-refractory metastatic thyroid cancer using (90)yttrium and (177)lutetium labeled somatostatin analogs: toxicity, response and survival analysis. *Am J Nucl Med Mol Imaging* 2013;4:39–52.
- 62 Salavati A, Puranik A, Kulkarni HR, Budiawan H, Baum RP: Peptide receptor radionuclide therapy (PRRT) of medullary and nonmedullary thyroid cancer using radiolabeled somatostatin analogues. *Semin Nucl Med* 2016;46:215–224.
- 63 Slobbe P, Poot AJ, Windhorst AD, van Dongen GA: PET imaging with small-molecule tyrosine kinase inhibitors: TKI-PET. *Drug Discov Today* 2012;17:1175–1187.
- 64 Gulec SA, Kuker RA, Goryawala M, Fernandez C, Perez R, Khan-Ghany A, Apaza A, Harja E, Harrell M: (124)I PET/CT in patients with differentiated thyroid cancer: clinical and quantitative image analysis. *Thyroid* 2016;26:441–448.

- 65 Van Nostrand D, Moreau S, Bandaru VV, Atkins F, Chennupati S, Mete M, Burman K, Wartofsky L: (124)I positron emission tomography versus (131)I planar imaging in the identification of residual thyroid tissue and/or metastasis in patients who have well-differentiated thyroid cancer. *Thyroid* 2010;20:879–883.
- 66 Debergh I, Van Damme N, De Naeyer D, Smeets P, Demetter P, Robert P, Carme S, Pattyn P, Ceelen W: Molecular imaging of tumor-associated angiogenesis using a novel magnetic resonance imaging contrast agent targeting $\alpha v\beta 3$ integrin. *Ann Surg Oncol* 2014;21:2097–2104.
- 67 Streeter JE, Gessner RC, Tsuruta J, Feingold S, Dayton PA: Assessment of molecular imaging of angiogenesis with three-dimensional ultrasonography. *Mol Imaging* 2011;10:460–468.
- 68 Wells SA Jr, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, Machens A, Moley JF, Pacini F, Raue F, Frank-Raue K, Robinson B, Rosenthal MS, Santoro M, Schlumberger M, Shah M, Waguespack SG: American Thyroid Association Guidelines Task Force on Medullary Thyroid Carcinoma: Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid* 2015;25:567–610.
- 69 Essig GF Jr, Porter K, Schneider D, Arpaia D, Lindsey SC, Busonero G, Fineberg D, Fruci B, Boelaert K, Smit JW, Meijer JA, Duntas LH, Sharma N, Costante G, Filetti S, Sippel RS, Biondi B, Topliss DJ, Pacini F, Maciel RM, Walz PC, Kloos RT: Multifocality in sporadic medullary thyroid carcinoma: an international multicenter study. *Thyroid* 2016;26:1563–1572.
- 70 Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, van Amstel HK, Lips CJ, Nishisho I, Takai SI, Marsh DJ, Robinson BG, Frank-Raue K, Raue F, Xue F, Noll WW, Romei C, Pacini F, Fink M, Niederle B, Zedenius J, Nordenskjöld M, Komminoth P, Hendy GN, Mulligan LM, et al: The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. *JAMA* 1996;276:1575–1579.
- 71 Dvorakova S, Vaclavikova E, Sykorova V, Vcelak J, Novak Z, Duskova J, Ryska A, Laco J, Cap J, Kodetova D, Kodet R, Krskova L, Vlcek P, Astl J, Vesely D, Bendlova B: Somatic mutations in the RET proto-oncogene in sporadic medullary thyroid carcinomas. *Mol Cell Endocrinol* 2008;284:21–27.
- 72 Valdés N, Navarro E, Mesa J, Casterás A, Alcázar V, Lamas C, Tébar J, Castaño L, Gaztambide S, Forga L: RET Cys634Arg mutation confers a more aggressive multiple endocrine neoplasia type 2A phenotype than Cys634Tyr mutation. *Eur J Endocrinol* 2015;172:301–307.
- 73 Trimboli P, Seregini E, Treglia G, Alevizaki M, Giovannella L: Procalcitonin for detecting medullary thyroid carcinoma: a systematic review. *Eur J Endocrinol* 2015;22:R157–R164.
- 74 Milos IN, Frank-Raue K, Wohllk N, Maia AL, Pusiol E, Patocs A, Robledo M, Biarnes J, Barontini M, Links TP, de Groot JW, Dvorakova S, Peczkowska M, Rybicki LA, Sullivan M, Raue F, Zosin I, Eng C, Neumann HP: Age-related neoplastic risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germ line RET Cys634Trp (TGC>TGG) mutation. *Endocr Relat Cancer* 2008;15:1035–1041.
- 75 Hu MI, Ying AK, Jimenez C: Update on medullary thyroid cancer. *Endocrinol Metab Clin North Am* 2014;43:423–442.
- 76 Verbeek HH, Plukker JT, Koopmans KP, de Groot JW, Hofstra RM, Muller Kobold AC, van der Horst-Schrivers AN, Brouwers AH, Links TP: Clinical relevance of ^{18}F -FDG PET and ^{18}F -DOPA PET in recurrent medullary thyroid carcinoma. *J Nucl Med* 2012;53:1863–1871.
- 77 Wells SA Jr, Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, Baudin E, Elisei R, Jarzab B, Vasselli JR, Read J, Langmuir P, Ryan AJ, Schlumberger MJ: Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. *J Clin Oncol* 2012;30:134–141.
- 78 Elisei R, Schlumberger MJ, Müller SP, Schöffski P, Brose MS, Shah MH, Licitra L, Jarzab B, Medvedev V, Kreissl MC, Niederle B, Cohen EE, Wirth LJ, Ali H, Hessel C, Yaron Y, Ball D, Nelkin B, Sherman SI: Cabozantinib in progressive medullary thyroid cancer. *J Clin Oncol* 2013;31:3639–3646.
- 79 Sherman SI: Lessons learned and questions unanswered from use of multitargeted kinase inhibitors in medullary thyroid cancer. *Oral Oncol* 2013;49:707–710.
- 80 Voora D, McLeod H, Eby C, Gage B: The pharmacogenetics of coumarin therapy. *Pharmacogenomics* 2005;6:503–513.
- 81 Alonso-Gordoa T, Díez JJ, Durán M, Grande E: Advances in thyroid cancer treatment: latest evidence and clinical potential. *Ther Adv Med Oncol* 2015;7:22–38.
- 82 Ning L, Kunnimalaiyaan M, Chen H: Regulation of cell-cell contact molecules and the metastatic phenotype of medullary thyroid carcinoma by the Raf-1/MEK/ERK pathway. *Surgery* 2008;144:920–924.
- 83 Kunnimalaiyaan M, Vaccaro AM, Ndiaye MA, Chen H: Inactivation of glycogen synthase kinase-3beta, a downstream target of the raf-1 pathway, is associated with growth suppression in medullary thyroid cancer cells. *Mol Cancer Ther* 2007;6:1151–1158.
- 84 Rodríguez-Rodero S, Fernández AF, Fernández-Morera JL, Castro-Santos P, Bayon GF, Ferrero C, Urdinguio RG, Gonzalez-Marquez R, Suarez C, Fernández-Vega I, Fresno Forcelledo MF, Martínez-Cambor P, Mancikova V, Castelblanco E, Perez M, Marrón PI, Men-diola M, Hardisson D, Santisteban P, Riesco-Eizaguirre G, Matias-Guiu X, Carnero A, Robledo M, Delgado-Álvarez E, Menéndez-Torre E, Fraga MF: DNA methylation signatures identify biologically distinct thyroid cancer subtypes. *J Clin Endocrinol Metab* 2013;98:2811–2821.
- 85 Lin SF, Lin JD, Chou TC, Huang YY, Wong RJ: Utility of a histone deacetylase inhibitor (PXD101) for thyroid cancer treatment. *PLoS One* 2013;8:e77684.
- 86 Papewalis C, Wuttke M, Jacobs B, Domberg J, Willenberg H, Baehring T, Cupisti K, Raffel A, Chao L, Fenk R, Seissler J, Scherbaum WA, Schott M: Dendritic cell vaccination induces tumor epitope-specific Th1 immune response in medullary thyroid carcinoma. *Horm Metab Res* 2008;40:108–116.
- 87 Chatal JF, Campion L, Kraeber-Bodéré F, Bardet S, Vuillez JP, Charbonnel B, Rohmer V, Chang CH, Sharkey RM, Goldenberg DM, Barbet J; French Endocrine Tumor Group: Survival improvement in patients with medullary thyroid carcinoma who undergo pre-targeted anti-carcinoembryonic-antigen radioimmunotherapy: a collaborative study with the French Endocrine Tumor Group. *J Clin Oncol* 2006;24:1705–1711.
- 88 Salaun PY, Campion L, Bournaud C, Faivre-Chauvet A, Vuillez JP, Taieb D, Ansquer C, Rousseau C, Borson-Chazot F, Bardet S, Oudoux A, Cariou B, Mirallié E, Chang CH, Sharkey RM, Goldenberg DM, Chatal JF, Barbet J, Kraeber-Bodéré F: Phase II trial of anti-carcinoembryonic antigen pretargeted radioimmunotherapy in progressive metastatic medullary thyroid carcinoma: biomarker response and survival improvement. *J Nucl Med* 2012;53:1185–1192.