Turkish Archives of Otorhinolaryngology
Türk Otorinolarengoloji Arşivi

Turk Arch Otorhinolaryngol 2016; 54: 74-8

# The Role and Importance of Molecular Tests in Approach to Thyroid Nodules

Review

Levent Gürbüzler

Department of Otorhinolaryngology, Gaziosmanpaşa University School of Medicine, Tokat, Turkey

Abstract

Although there is a significant increase in the detection of thyroid cancer because of the widespread utilization of ultrasound and fine needle aspiration biopsy, sometimes these techniques prove inefficient for diagnosis. Therefore, improved diagnostic tools are required. Increasing knowledge regarding thyroid cancer genetics has revived molecular testing. The possibility of thyroid malignancy can be considered or ruled out because of the high accuracy of results, such as 90% positive predictive value (PPV) and 96% negative predictive value (NPV), obtained from the molecular tests. Although the molecular biology of all thyroid cancers has not been completely understood, the remarkable progress done in this domain has widened the horizon of their diagnosis, prognosis and treatment.

**Keywords:** Thyroid cancer, thyroid nodule, molecular test, BRAF, RAS, RET/PTC

## Introduction

The incidence of thyroid cancers has increased approximately threefold in the past three decades (1). According to the findings of the United States of America's National Cancer Institute, thyroid cancer incidence was 4.85 per 100,000 in 1975 and 14.90 in 100,000 in 2012 (2). This increase is due to the increase of papillary thyroid carcinoma (PTC) incidence, rather than an increase in all types of thyroid cancers (1). Widespread usage of tools, such as thyroid ultrasonography (USG) and fine-needle aspiration biopsy (FNAB), has triggered more PTC diagnoses, and is considered to be the main factor in the increase in PTC incidence. According to the 2015 cancer statistics report compiled by the Cancer Department of the Public Health Agency of Turkey, the thyroid cancer incidence in Turkey in 2012 was determined to be 5.4 per 100,000 for the male population and 20.3 per 100,000 for the female population (3).

Approximately 5% of adults have palpable thyroid nodules, and this rate increases to 67% when considered in conjunction with thyroid USG in the elderly population (4, 5). Considering that 5-15% of these nodules are malignant, the importance of benign/malignant distinction becomes apparent (4, 6). Presently, the most valuable test in establishing this distinction is FNAB conducted on thyroid nodules. However, it is still unclear how one should proceed when faced with the biopsy results of category 3 (atypia of undetermined significance/follicular lesion of undetermined significance), category 4 (follicular neoplasm or suspicion of a follicular neoplasm), and category 5 (suspicious for malignancy) in the Bethesda System, which was introduced in order to standardize the terminology of thyroid cytopathologies. In a meta-analysis, 25,445 thyroid nodule aspirations were examined, and according to the Bethesda system, atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS) were observed in 9.6%, follicular neoplasm (FN) in 10.1%, and suspicion of malignancy in 2.7% (7). These three percentages add up to 22.4%, and they account for approximately a quarter of all biopsies. When evaluated according to their malignancy risks, AUS/FLUS, FN, and suspicion of malignancy have 5-15%, 15-30%, and 60-75% chance of being thyroid cancers, respectively (8). The alternatives for diagnosis/treatment after biopsies that result in these categories are repeat FNAB, diagnostic lobectomy and total thyroidectomy. The probability of the biopsy result returning as benign after the repeat FNAB option is approximately 50%, and the problem persists regarding how to proceed with the remaining patients (9). Diagnostic lobectomy and total thyroidectomy, on the other hand, include surgical complications, run the risk of under-or over-treatment, and are costly.

Understanding the molecular mechanisms that contribute to thyroid carcinogenesis and advances in diagnostic molecular technologies have begun

**Address for Correspondence:**
Levent Gürbüzler
**E-mail:** gurbuzler@yahoo.com

**Received Date:** 02.10.2015
**Accepted Date:** 19.12.2015
**Available Online Date:** 06.04.2016



DOI: 10.5152/tao.2015.1276

to be instructive in the diagnosis, treatment and prognosis of thyroid cancers. In general, our understanding of protein-structured molecular markers increases every day. In this study, we examine the genetic changes and molecular tests that affect thyroid carcinogenesis, and aim to present their contributions for diagnosis and treatment.

**Main Pathways in Thyroid Carcinogenesis**

The mitogen-activated protein kinase pathway (MAPKP) is an important pathway in which vital mechanisms such as cell proliferation and cell differentiation are regulated, and therefore in which tumorigenesis is affected. It also has a pathway property in which growth factors, hormones, cytokines that interact with tyrosine kinase, which is one of the cell surface receptors, play a role. BRAF and RAS mutations, which have been demonstrated to play a role in PTC development, and RET/PTC rearrangements use MAPKP (10). As a result of thyroid cells being stimulated via this pathway with various growth factors and hormones, the G protein-coupled receptor in the plasma membrane is activated, and the activated G protein induces serine/threonine protein kinase in RAS. Stimulation of this cascade can lead to changes in the normal cell functions and the beginning of the tumorigenesis process.

The second pathway is the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. This pathway is associated with tumorigenesis, along with various cell processes such as growth, proliferation, and apoptosis. Transportation and usage of glucose, and protein biosynthesis are important physiological processes that are realized via this pathway. When the pathway is activated, cell proliferation accelerates and apoptosis is inhibited. Three Akt types are defined: Akt-1, Akt-2, and Akt-3. When compared with PTC, overexpression and overactivation of Akt (which uses the PI3K pathway) in follicular thyroid carcinoma (FTC) have been reported (11). On the other hand, genetic changes that use both the MAPK and the PI3K/Akt pathways are demonstrated in 81% of anaplastic thyroid carcinomas (ATC) (12).

**Genetic Changes That Affect Thyroid Carcinogenesis**

**BRAF mutation:**

BRAF, which is a member of the A/B/C RAF family in mammals, is a serine-threonine kinase. It is an effective gene in the MAPK pathway, and includes the important pathways of the cell cycle, RAS/RAF/MEK/ERK. It is the most commonly observed gene mutation in all PTCs (35-70%) (12). A BRAF mutation observed in biopsy specimens has a positive predictive value (PPV) of 99% for PTC. The BRAF V600E mutation, which occurs when the valine and glutamate amino acids substitute as a result of thymine-adenine mutation in the BRAF protein 1796 nucleotide, comprises more than 95% of all BRAF mutations that cause PTC. This mutation mostly leads to classic type and tall-cell PTC development. The second most common mutation, BRAF K601E, is reported to be mostly associated with follicular variant of papillary thyroid carcinoma (fvPTC) (13). According to a multicenter study that aimed to examine the clinical course and prognosis, BRAF mutation was found to be associated with extrathyroidal extension, lymph node metastasis, high-stage cancer risk, and recurrent/persistent disease. It was therefore argued that the thyroid cancers triggered by this mutation progress more aggressively and have poor prognoses (14). For this reason, whether or not central neck dissection ought to be included to total thyroidectomy in patients with this mutation is a controversial topic these days. On the other hand, BRAF mutation is investigated in recurrent PTCs as well, and it has been shown that it decreases radioactive iodine retention and that it is radioactive iodine-refractory (15). An aggressive progression associated with multifocality in papillary thyroid microcarcinomas is reported by Lin et al. (16).

**RAS Mutation:**

The RAS gene family includes 3 gene types: HRAS, NRAS, and KRAS. These genes encode for the 21k-Dalton cell membrane receptor G protein that sends signals from the cell membrane to intracellular targets using both the MAPK and PI3K pathways. RAS point mutations usually occur on codon numbers 12, 13, and 61. When the RAS proto-oncogene becomes an oncogene, a G protein which lacks the receptor subunit that increases the mitotic activity of the cell, and a mutant, uncontrollable GTPase are synthesized. RAS mutation is most commonly associated with FTC, which occurs in 40%–50% of all cases, and it is the most encountered mutation of FTC. Its frequencies in other cancer types are approximately 10% and 10-20% for PTC and ATC, respectively (12, 17). However, associating every RAS mutation with malignant lesions is not the right approach. Follicular adenomas (FA) are benign neoplastic formations in which RAS mutation can occur. If there is a RAS mutation that is associated with PTC, it is fvPTC. Only this mutation can lead to non-infiltrating, encapsulated fvPTC formation which can be treated more conservatively. Even though the RAS positivity in FNABs is not 100% indicative of malignancy, this ratio is high enough to make the decision to perform a total thyroidectomy in the first surgery (10, 18). Even if the histology results in a benign FA, potential malignant transformation risk due to RAS mutation will be an acceptable reason for the performed surgery.

**RET/PTC Rearrangement:**

The RET proto-oncogene encodes for the tyrosine kinase receptor protein, which is only present in the parafollicular C cells in the thyroid gland and is effective depending on the cell membrane. Rearrangement occurs when a specific region of a gene switches places with a region of another donor gene. RET/PTC rearrangement typically occurs with the fusion of the 3'-portion of RET proto-oncogene tyrosine kinase and 5'-portion of a different gene. 15 RET/PTC rearrangement types, which consist of RET and 10 different genes, have been defined, and among these, RET/PTC1 and RET/PTC3 are particularly associated with PTC (19). RET/PTC rearrangement is observed in 10-20% of all PTCs. In particular, findings of RET/PTC rearrangement with the history of radiation exposure in young PTC patients after Chernobyl, leads to the belief that radiation contributes to the development of this rearrangement. It is not a definitive malignancy indicator for PTC, given that it is observed in benign nodules as well. However, it is obvious that these patients must be closely monitored. On the other hand, all

subtypes of RET/PTC rearrangements are reported to be high risks for neck lymph node metastasis, and it is emphasized that the possibility of aggressive progression must be considered (20).

**PAX8/PPARγ Rearrangement:**
The PAX8 gene encodes for a transcription factor that contributes to the formation of thyroid follicle cells. PPARγ (peroxisome proliferator-activated receptor gamma) is also a transcription factor that belongs to the hormone nuclear receptor family such as thyroid hormone, retinoic acid, androgen and estrogen receptors that are underexpressed in normal thyroid cells. This rearrangement occurs due to the fusion of the PPARγ gene, which is associated with lipid metabolism as well, with the PAX8 gene as a result of translocation. Even though its mechanism in oncogenesis has not been properly revealed, it is suspected that the tumorigenesis process begins with the inactivation of PPARγ, which is actually a tumor suppressor gene. It is the second most common molecular agent in FTCs, after RAS mutation, and it is responsible for approximately 35% of all cases (21). It is responsible for less than 5% of fvPTCs, and its incidence in FA is between 4-13% (21). PAX8/PPARγ positivity is characterized by younger FTC patients. These tumors are solid featured, and lead to vascular invasion. A low positivity ratio observed in FA should warn (for capsule and vascular invasion) histopathologists to conduct examinations in order not to overlook a potential FTC diagnosis, and if needed, should lead them to reevaluation.

**New Molecular Markers:**
PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene that is localized in chromosome 10q. It is the negative regulator of the PI3K/Akt pathway. Various mutations in this gene contribute to the development of several cancers. It is especially noteworthy in ATC, with a frequency of 20%, and it is observed with the frequencies of 15% and 1-2% in FTC and PTC, respectively (22, 23).

The 2013 research article on Telomerase Reverse Transcriptase (TERT) gene mutation emphasizes the occurrence of this mutation in aggressively progressing thyroid cancer types such as ATC, tall-cell PTC, BRAF mutation (+)PTC, and poorly-differentiated thyroid carcinomas (PDTC) (24). For this reason, TERT mutation is recommended to be a molecular marker that can be used for prognosis and clinical course (24).

TP53 point mutations are important due to encoding for the p53 protein, which plays an important role in cell metabolism. As well as repairing damaged cell DNA, this protein participates in apoptosis. These TP53 point mutations are associated with direct dedifferentiation, and are observed in high percentages in ATC and PDTC, 25-30% and 70-80%, respectively, rather than in well-differentiated thyroid carcinomas (10-23). However, in recent years, studies have focused on PDTCs and ATCs, in addition to the commonly observed well-differentiated thyroid carcinomas, and several molecular markers that are suspected to lead to dedifferentiation have been discovered. Among these, the frequencies of PIK3CA point mutations, mutations of CTTNB1 that encode for beta-catenin, and AKT1 mutations are 10-20%, 25%, and 15% in PDTC, respectively (10, 23).

**Molecular Test Panels**
Detecting, through research, genetic changes such as mutations and rearrangements that cause thyroid tumorigenesis, brings up several test panels regarding risk assessment. Nikiforov et al. (18) reported initially that the 44% malignancy risk of sole PNAB increases to 80% with the mutation panel positivity and that a considerable gain is achieved. Examining the presently commonly used test panels, it can be seen that an ideal panel does not exist. Therefore, these tests must, first of all, be divided into "ruling in" (those that demonstrate malignancy) and "ruling out" (those that rule out malignancy) categories.

The mutational panel can be cited as an example for the "ruling in" test. In this panel, prepared by the Pittsburgh University Health Center, a total of 7 gene changes in DNA, consisting of BRAF, H/K/N RAS, RET/PTC1, RET/PTC3, and PAX8/PPARγ, are being investigated. This panel appears to show malignancy due to high specificity and PPV (Table 1). On the other hand, it appears to not rule out malignancy due to low sensitivity and negative predictive value (NPV) (18).

**Table 1.** Diagnostic performance percentages of published test panel data

| | Sensitivity | Specificity | NPV | PPV |
|---|---|---|---|---|
| GC | 87% | 53% | 90% | 47% |
| *Mutation panel | 57-68% | 96-99% | 72-94% | 87-95% |
| Thyrosec2 | 90% | 93% | 96% | 83% |

NPV: negative predictive value; PPV: positive predictive value; GC: gene classifier.
*In mutation panel, values for Bethesda 3/4/5 are stated separately.

The Gene Classifier (Afirma; Veracyte, South San Francisco, USA) can be given as an example for the "ruling out" test. mRNA analysis of 142 different genes is performed via the Gene Classifier (GC). It has a reasonably high NPV (90%) and sensitivity (87%) (Table 1) (25). On the other hand, the same cannot be said for its PPV and specificity (47%, 53%). Another disadvantage is its cost of 3,200 USD, which is the most expensive test panel.

Additionally, Thyrosec2, which is another next generation panel produced by the Pittsburgh University Health Center, examines 12 gene changes. It conducts BRAF, H/K/N RAS, RET, AKT1, TP53, PTEN, GNAS, CTTNB1, TSHR, and PIK3CA analyses as part of the examination. It is a candidate for an ideal test in the future, given its high PPV (83%) and NPV (96%) (Table 1).

**Targeted Therapies**
Understanding the molecular mechanisms of thyroid cancer development enables the investigation of new treatment agents and conducting new studies. In particular, these agents are beacons of hope for ATC, PDTC, metastatic, and radioactive iodine-refractory thyroid cancers, which are presently difficult to

treat. Distant metastases in thyroid cancers mostly occur in the lungs or bones. Unfortunately, metastatic thyroid cancers progress with resistant radioactive retention, and lead to shorter lives.

Tyrosine kinases play important roles in tumor angiogenesis, proliferation, and metastases. New drugs that establish the inhibition of VEGFR1-2, EGFR, PDGFR, FGFR, RAF, and RET are generally named as tyrosine kinase receptor inhibitors (TKRI). Motesanib is an orally-administered TKRI that is effective on receptors such as VEGFR1-2-3, PDGFR. In a phase II study where locally-advanced stage, metastatic, and progressing thyroid cancer patients were treated with Motesanib, 14% of patients had a partial response, and the disease stabilized for more than 24 weeks in 35% of patients (26).

SU11248 (Sunitinib) is a TKRI that targets VEGFR, PDGFR, RET, and fms-related tyrosine kinase 3. In the study conducted by Carr et al. (27), in which Sunitinib treatment was administered on patients with iodine-refractory, differentiated thyroid cancer, metastatic medullary thyroid carcinoma, and FDG-PET positivity, 3% showed complete response, 28% showed partial response, and 46% showed stabilization.

Sorafenib is another TKRI that inhibits VEGFR1-2-3, PDGFR beta, RET (including RET/PTC), and RAF (including BRAF V600E). Differently from the other agents, there is a phase III study involving Sorafenib, regarding locally-advanced, metastatic, and radioactive iodine-refractory thyroid cancer patients (28). According to the results of this study, the median progression-free survival period in the patient group that received Sorafenib treatment was found to be five months longer than the placebo group (28). On the other hand, this exciting result was accompanied by drug-related side effects such as skin reactions in the hands and face, fatigue, diarrhea, dyspnea, hypertension, weight loss, and desquamation.

Vemurafenib, which is also known as PLX4032, is a BRAF V600E kinase inhibitor molecule. According to a phase III study conducted in malignant melanoma patients with BRAF V600E mutations, prolonged survival is achieved, along with successful results in treatment response rates, and the USA Food and Drug Administration has approved this drug for use in melanoma patients (29). This has led to studying the effects of Vemurafenib on other cancers related to BRAF V600E. To that end, Vemurafenib was administered to three patients with BRAF V600E positive metastatic thyroid cancer, and one of them showed partial response and the other two showed stabilization (30). These results paved the way for a phase II study as well.

## Conclusion

Better understanding of the communication pathways of thyroid cancer genetics in time and detecting the genetic changes that affect these pathways are instructive both in the diagnosis stage and in prognosis. On the other hand, new molecular agents emerge as beacons of hope in the treatments of ATC, PDTC, metastatic, and radioactive iodine-refractory thyroid cancers, which our current methods are having difficulty treating. As studies on thyroid molecular biology continue, and as our incomplete understanding improves, it is clear that more useful target molecules will be found.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** No conflict of interest was declared by the author.

**Financial Disclosure:** The author declared that this study has received no financial support.

## References

1. Davies L, Welch HG. Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surg 2014; 140: 317-22. [CrossRef]
2. Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, et al. SEER Cancer Statistics Review, 1975-2012, National Cancer Institute. http://seer.cancer.gov/ csr/1975_2012/ Erişim Tarihi: 29.06.2015.
3. 2012 Yılı Türkiye Kanser İstatistikleri. http://kanser.gov.tr/ daire-faaliyetleri/kanser-istatistikleri/1710-2012-türkiye-kans- er-istatistikleri.html. Erişim Tarihi: 17.11.2015.
4. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, et al. Revised American Thyroid A ssociation m anagement g uidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009; 19: 1167-214. Erratum in: Thyroid. 2010; 20: 942. Hauger, Bryan R [corrected to Haugen, Bryan R]. Thyroid. 2010; 20: 674-54. [CrossRef]
5. Ezzat S, Sarti DA, Cain DR, Braunstein GD. Thyroid incidetalo- mas. Prevalance by palpation and ultrasonography. Arch Intern Med 1994; 154: 1838-40. [CrossRef]
6. Frates MC, Benson CB, Doubilet PM, Kunreuther E, Contreras M, Cibas ES, et al. Prevalence and distribution of carcinoma in patients with solitary and multiple thyroid nodules on sonography. J Clin Endocrinol Metab 2006; 91: 3411-7. [CrossRef]
7. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. Acta Cytol 2012; 56: 333-9. [CrossRef]
8. Cibas ES, Ali SZ. The Bethesda System for Reporting Thyroid Cytopathology. Am J Clin Pathol. 2009; 132: 658-65. [CrossRef]
9. Heller M, Zanocco K, Zydowicz S, Elaraj D, Nayar R, Sturgeon C. Cost-effectiveness analysis of repeat fine-needle aspiration for thyroid biopsies read as atypia of undetermined significance. Surgery 2012; 152: 423-30. [CrossRef]
10. Yip L, Ferris RL. Clinical application of molecular testing of fine needle aspiration specimens in thyroid nodules. Otolaryngol Clin North Am 2014; 47: 557-71. [CrossRef]
11. Ringel MD, Hayre N, Saito J, Saunier B, Schuppert F, Burch H, et al. Overexpression and overactivation of akt in thyroid carcinoma. Cancer Res 2001; 61: 6105-11.
12. Omur O, Baran Y. An update on molecular biology of thyroid cancers. Crit Rev Oncol Hematol 2014; 90: 233-52. [CrossRef]
13. Ohori NP, Singhal R, Nikiforova MN, Yip L, Schoedel KE, Coyne C, et al. BRAF mutation detection in indeterminate thyroid cytology specimens: underlying cytologic, molecular, and pathologic characteristics of papillary thyroid carcinoma. Cancer Cytopathol 2013; 121: 197-205. [CrossRef]
14. Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, et al. BRAF Mutation Predicts a Poorer Clinical Prognosis for Papillary Thyroid Cancer. J Clin Endocrinol Metab 2005; 90: 6373-9. [CrossRef]

15. Capelli L, Marfisi C, Puccetti M, Saragoni L, De Paola F, Zaccaroni A, et al. Role of BRAF molecular analysis in the management of papillary thyroid carcinoma: analysis of cytological and histological samples. Cytopathology 2015; 26: 297-302. [CrossRef]
16. Lin KL, Wang OC, Zhang XH, Dai XX, Hu XQ, Qu JM. The BRAF mutation is predictive of aggressive clinicopathological characteristics in papillary thyroid microcarcinoma. Ann Surg Oncol 2010; 17: 3294-300. [CrossRef]
17. Esapa CT, Johnson SJ, Kendall-Taylor P, Lennard TW, Harris PE. Prevalence of Ras mutations in thyroid neoplasia. Clin Endocrinol (Oxf ) 1999; 50: 529-35. [CrossRef]
18. Nikiforov YE, Ohori NP, Hodak SP, Carty SE, LeBeau SO, Ferris RL, et al. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. J Clin En-docrinol Metab 2011; 96: 3390-7. [CrossRef]
19. Tallini G, Asa SL. RET oncogene activation in papillary thyroid carcinoma. Adv Anat Pathol 2001; 8: 345-54. [CrossRef]
20. Adeniran AJ, Zhu Z, Gandhi M, Steward DL, Fidler JP, Giordano TJ, et al. Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. Am J Surg Pathol 2006; 30: 216-22. [CrossRef]
21. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW 2nd, Tallini G, et al. RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab 2003; 88: 2318-26. [CrossRef]
22. Özdemir S, Özdemir Ö. Tiroid kanserinde moleküler etyolojik faktörler. Cumhuriyet Tıp Dergisi 2014; 36: 128-46. [CrossRef]
23. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 2013; 13: 184-99. [CrossRef]
24. Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, et al. Highlyprevalent TERT promoter mutations in aggressive thyroid cancers. Endocr Relat Cancer 2013; 20: 603-10. [CrossRef]
25. Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, et al. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med 2012; 367: 705-15. [CrossRef]
26. Sherman SI, Wirth LJ, Droz JP, Hofmann M, Bastholt L, Martins RG, et al. Motesanib diphosphate in progressive differentiated thyroid cancer. N Engl J Med 2008; 359: 31-42. [CrossRef]
27. Carr LL, Mankoff DA, Goulart BH, Eaton KD, Capell PT, Kell EM, et al. Phase II study of daily sunitinib in FDG-PET-positive, iodine-refractory differentiated thyroid cancer and metastatic medullary carcinoma of the thyroid with functional imaging correlation. Clin Cancer Res 2010; 16: 5260-8. [CrossRef]
28. Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, dou- bleblind, phase 3 trial. Lancet 2014; 384: 319-28. [CrossRef]
29. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011; 364: 2507-16. [CrossRef]
30. Kim KB, Cabanillas ME, Lazar AJ, Williams MD, Sanders DL, Ilagan JL, et al. Clinical responses to vemurafenib in patients with metastatic papillary thyroid cancer harboring BRAFV600E mutation. Thyroid 2013; 23: 1277-83. [CrossRef]