European Journal of Endocrinology, 2023, **188**, 1–8
https://doi.org/10.1093/ejendo/lvac011

Original Research

Target therapy for *BRAF* mutated anaplastic thyroid cancer: a clinical and molecular study

Tiago Nunes da Silva,[1,2,*] Ricardo Rodrigues,[2] Ana Saramago,[2] Carolina Pires,[2] Miguel Rito,[3] Mariana Horta,[4] Carmo Martins,[2] Valeriano Leite,[1,2,5] and Branca M. Cavaco[2]

[1]Serviço de Endocrinologia, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal
[2]Unidade de Investigação em Patobiologia Molecular (UIPM), Instituto Português de Oncologia de Lisboa Francisco Gentil, Rua Professor Lima Basto, Lisboa 1099-023, Portugal
[3]Serviço de Anatomia Patológica, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal
[4]Serviço de Radiologia, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal
[5]NOVA Medical School—Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisboa, Portugal

***Corresponding author:** Serviço de Endocrinologia, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal. Emails: tiago_fcml@yahoo.com; tlnsilva@ipolisboa.min-saude.pt

Abstract

Objectives: Anaplastic thyroid carcinoma (ATC) has a poor survival. The combination of Dabrafenib plus Trametinib (DT) had a significant impact in survival of *BRAF* p.V600E patients. However, durable responses may be compromised by resistance. We aim to present our experience with DT in *BRAF* positive ATC patients and compare the outcomes with usual therapy, and to study tumor molecular alterations in the DT group.

Methods: Patients treated between May 2018 and April 2022 in a tertiary referral center, assessed for *BRAF* status were included. Patients were divided in three groups: *BRAF* p.V600E treated with DT, *BRAF* wild type (WT) under multimodal therapy (MT), and *BRAF* WT under compassionate care (CC). Response was assessed monthly in the first 6 months and every 3 months afterwards, by RECIST 1.1. Overall survival (OS) and progression-free survival (PFS) were estimated with the Kaplan-Meier method and compared with the log-rank test.

Results: Twenty-seven ATC patients were included (DT = 9, MT = 8, and CC = 10). Median OS was 475 days for DT, 156 days for MT, and 39 days for CC ($P < .001$). At 12 months, only patients in the DT group were alive (71%). Median PFS was 270 days, in the DT group, compared with less than 32 days in *BRAF* WT ($P < .001$). No severe adverse events were reported. Molecular profiling showed that in one of the four clinical progressions, a pathogenic *NRAS* mutation was found.

Conclusions: Our results show a significant real-world efficacy of Dabrafenib plus Trametinib in both survival and recurrence compared with standard treatment, with a good safety profile.

Keywords: anaplastic thyroid cancer, targeted therapy, drug resistance, Dabrafenib, Trametinib, molecular profiling

Significance

Dabrafenib and Trametinib combination was recently approved by the FDA for anaplastic thyroid cancer. Our study, in a consecutive population of ATC patients, showed that in the *BRAF* p.V600E population (33%) it provides fast symptom control and significant survival benefit, in both localized and metastatic disease with minimal toxicity. This was achieved in a rapidly progressive disease, in an aged population (median 77 years old) with a fast *BRAF* screening and treatment initiation. The identification of resistance mechanisms, such as *RAS* mutations, may be useful to optimize treatment decisions both at start of therapy and at clinical resistance. This is the first European real-world study that shows the feasibility of this approach in an unselected population of ATC patients.

Introduction

Anaplastic thyroid carcinoma (ATC) is a highly aggressive and undifferentiated tumor.[1–3] ATC molecular alterations include MAPK pathway-related genes [*RAS* (24%–43%), *BRAF* p.V600E (40%–70%)], the PI3K/AKT pathway [*PIK3CA* (10%–18%), *PTEN* (10%–15%)], *TERT* promoter (*TERTp*) (65%–75%), and *TP53* (60%–80%).[4–6]

Traditionally, multimodality therapy (MT) with surgery, external beam radiation, and systemic chemotherapy, has been recommended in patients with good performance status and with localized or oligometastatic disease. Nevertheless, clinical response is poor with an overall survival (OS) between 3 and 5 months and progression-free survival (PFS) up to 4 months.[7–9]

Strategies targeting angiogenesis with multikinase inhibitors, such as sorafenib and pazopanib, achieved low overall response rate (ORR) (up to 10%) and median PFS and OS of 1.9 and 5 months, respectively.[10] Lenvatinib showed an ORR of 24% and median PFS of 7.4 months in the Japanese population.[11] Nevertheless, a subsequent study done with a

Received: September 7, 2022. **Revised:** October 25, 2022. **Editorial Decision:** November 28, 2022. **Accepted:** December 6, 2022

majority of caucasian population (80%) was terminated early for low efficacy (ORR of 5%).[12]

Due to a high prevalence of *BRAF* p.V600E mutation in ATC (40%–70%, depending on the series),[4–6,9] targeted therapy was studied with vemurafenib, a BRAF inhibitor, which had modest efficacy, with an ORR of 29% (2/7 patients).[13] These results suggest the activation of alternative pathways, decreasing the efficacy of single BRAF inhibitors.[14,15]

Other targeted therapies, such as NTRK and RET inhibitors, have also been explored in *BRAF* wild-type (WT) ATC, when *NTRK* and *RET* fusions are present (1%–3% of the ATC cases).[4] Despite good responses, such gene fusions are rare, which might limit the number of patients who can benefit from them [one ATC patient with *RET* fusion had a partial response (PR) in LIBRETTO-001 and, in another study, 3/7 patients with *NTRK* fusions reached PR and stable disease (SD)].[16–18]

In 2018, a breakthrough phase II, open-label basket trial, with the BRAF inhibitor dabrafenib (150 mg twice daily) plus the MEK1/2 inhibitor trametinib (2 mg once daily) (DT) for patients with *BRAF* p.V600E rare malignancies, including ATC patients, reported for the first time an ORR of 69%, an estimated 12-month OS of 80%, and a PFS of 79%.[19] This led to FDA approval of DT in *BRAF* p.V600-mutant ATC patients, as well as to *BRAF* p.V600E mutation testing and treatment recommendations in updated ESMO, NCCN, and ATA guidelines.[9,10] More recently, in the updated cohort, which included 36 DT-treated patients, the ORR was 56%, with a median OS of 14.5 months and a median PFS of 6.7 months.[20]

Nevertheless, patients eventually develop resistance to DT therapy,[21] as previously described in *BRAF* mutated melanoma,[22,23] colorectal cancer,[24,25] and non-small cell lung cancer (NSCLC).[26] The resistance mechanisms can be primary/intrinsic, due to the presence of resistant clones, or secondary/acquired, which derive from the selective pressure of the targeted therapy.[21,27] Resistance mechanisms can also be on-target and off-target. On-target resistance has been predominantly described in the *BRAF* gene, that affects the binding of the inhibitors to their specific targets, such as *BRAF* splice variants, amplifications, dimerization, epigenetic, or overexpression.[21,27,28] In a minority of cases, in melanoma, resistance has been attributed to *MAP2K1/2* gene mutations,[29,30] which encode for MEK1 and MEK2, respectively. Off-target resistance mechanisms include *RAS* mutations or in downstream mediators of MAPK, alterations in tyrosine kinase receptors (RTKs), PI3K/AKT/mTOR signaling pathway, and epigenetics, decreasing BRAF signaling dependency.[23,31] Nevertheless, only approximately half of melanoma and NSCLC patients, studied by whole-genome sequencing, RNA sequencing and comparative genomic hybridization (CGH) arrays, had alterations detected at progression.[26,32] Recently, BRAF off-target resistance mechanisms in ATCs, such as *RAS* mutations,[21,33] *RAC1* mutations, and copy number variations,[34] have been described. However, due to the recent introduction of DT in ATC, these resistance mechanisms are less well characterized. To the best of our knowledge, only one patient series of four ATC treated with DT described *RAS* mutations at progression (two with *KRAS* p.G12V and two with *NRAS* p.Q61K).[33]

We present our experience with DT treatment in nine *BRAF* p.V600E mutated ATC patients and compare the outcomes with contemporary ATC patients under multimodal therapy (MT) or compassive care (CC). In addition, we evaluated the molecular alterations at baseline and during progression under DT.

Materials and methods

Patients

This is a retrospective, real-world, single-center (Instituto Português de Oncologia de Lisboa Francisco Gentil—IPOLFG) study that included all ATC patients treated in our department between May 2018 and April 2022. This department is the referral center for aggressive thyroid tumors for the southern region of the country (approximately 4 million people). A special approval was granted for compassionate use of DT in *BRAF* p.V600E cases. IPOLFG Institutional Review Board (IRB) approval was obtained before data collection. ATC diagnoses were confirmed by pathologists dedicated to endocrine tumor pathology.

For DT-treated patients, inclusion criteria were: (i) availability of adequate tumor tissue for confirmatory *BRAF* p.V600E mutation analysis, (ii) ability to take orally medication with at least a nasogastric tube (NGT), and (iii) adequate organ function.

Treatment strategy

DT group IVB and IVC patients were started on continuous dabrafenib (150 mg twice daily) and trametinib (2 mg once daily) preferably in neoadjuvancy (six cases) or after surgery and/or radiotherapy (three cases). Treatment was pursued until unacceptable toxicity or death. In the context of lack of treatment alternatives, DT was continued during disease progression if considered to slow progression and/or death (patient 4). DT was withheld 7 days before and after surgery and radiotherapy.[19]

Patients on MT were treated with surgery, external radiation, chemotherapy (either carboplatin/paclitaxel or doxorubicin/docetaxel), or lenvatinib after multidisciplinary board decision.

BRAF WT patients, who were considered to get no benefit from active treatments after multidisciplinary decision, due to low performance status, high probability of R2 surgery, total laryngectomy with significant M1 disease, or who preferred supportive care, were included in the CC group and treated symptomatically, as described in guidelines.[9]

Response assessment

Neck and chest computed tomography (CT) was performed before treatment, every month during the first 6 months and every 3 months afterwards. Radiological evaluation was reviewed according to RECIST 1.1 by the same expert head and neck radiologist. Positron emission tomography (PET)–CT using [18F] fluorodeoxyglucose (FDG) (PET/CT) was performed to confirm radiological complete remission.

Treatment efficacy was evaluated by OS, PFS, duration of response (DOR), and clinical benefit (CB).

Molecular analyses

Cases and biological samples

The collection of biological samples was performed after the written informed consent was given by the patient (27 patients with ATC). Nine consecutive *BRAF* p.V600E ATC patients treated with the DT were included. ATC samples, pre-DT

(nine patients) and post-DT (two patients) therapy, were collected by surgery and/or biopsy, and were preserved in formalin-fixed paraffin-embedded (FFPE) tissue blocks. For molecular analysis, an expert pathologist selected regions containing >90% tumor cells.

BRAF p.V600E mutational testing

All 27 ATC cases were initially analyzed for *BRAF* p.V600E mutation by automated real-time PCR, using the Cobas® 4800 System (analytical sensitivity ≥95%). Later, in the ATC *BRAF* positive subgroup, *BRAF* p.V600E was confirmed by next-generation sequencing (NGS), as described below.

DNA and RNA extraction from FFPE samples

Nucleic acid extraction from FFPE thyroid tumor samples was performed using Maxwell® RSC Instrument, as detailed in Material and Methods section of Supplementary Data.

Next-generation sequencing

NGS analysis was performed in the *BRAF* positive (p.V600E) ATC samples, from the 9 patients who underwent DT therapy, using the AmpliSeq™ for Illumina Focus Panel, a 52 genes panel, which allows the analysis of SNVs, indels, and gene fusions (Table S1), in an Illumina MiSeq™. With a minimal coverage of 500×, the used methodology allows, with a sensibility >95%, a detection limit of 5% in the analyzed DNA. Bioinformatic analysis is detailed in Material and Methods section of Supplementary Data.

Polymerase chain reaction

Polymerase chain reaction (PCR) was performed using the Platinum Taq DNA polymerase High Fidelity protocol, as described in Table S2.

Automated Sanger sequencing

Sanger sequencing was used to confirm some variants identified by NGS and to sequence specific genes (*TERT*p, *RAC1*, and *PTEN*). The sequencing products were analyzed in a Genetic analyzer 3500 sequencer, and examined with Variant Reporter v3.0 software, as detailed in Material and Methods section of Supplementary Data.

High-resolution comparative genomic hybridization

High-resolution comparative genomic hybridization (HR-CGH) analysis was performed in patient 4's recurrence sample, as previously described.[35]

For image acquisition and analysis, a Zeiss Epifluorescence microscope, linked to a Cytovision HR-GCH software (Cytovision version 7.4; Leica Biosystems, Richmond, VA, USA), was used.

Statistical analysis

Results were presented as frequencies and, in the DT group, differences in target lesion from baseline at 1 month and best tumor response were evaluated by Wilcoxon test. Total survival, OS, PFS, and DOR were estimated with the Kaplan-Meier method and compared with the log-rank test. Statistical analysis was performed with SPSS v22.

Results

Following real-time PCR analysis for *BRAF* p.V600E, the ATC cohort ($n = 27$) was divided in three groups: *BRAF* p.V600E patients, who underwent DT therapy ($n = 9$), *BRAF* WT treated with MT ($n = 8$), and *BRAF* WT, who underwent CC ($n = 10$). Groups' clinical characteristics are detailed in Table 1.

In the DT group, the median age was 77 years (56–83), 5 patients were stage IVC (lung = 5, pleura = 1, bone = 1) and presented with a median primary tumor size of 7.4cm (6.3–8.4 cm). Six patients were treated with neoadjuvant intention after which three underwent total thyroidectomy and definitive radiotherapy, one underwent definitive radiotherapy and two progressed under DT. Three patients, who started DT after R1/R2 surgery, were being treated for persistent disease: two for metastatic lung disease (patient 1 after R1 surgery and definitive radiotherapy and chemotherapy with doxorubicin and docetaxel, and patient 9 after R2 surgery), and the third (patient 2) for rapid locoregional progression after R2 surgery and definitive radiotherapy. In these three patients, the ATC diagnosis was made in the surgical specimen.

Patients in the MT group had a median age of 62 years (49–80), 5 were stage IVC (lung = 5 and bone = 1) and had a median primary tumor size of 7.7 cm (5.8–9.1 cm). Multimodal treatment included surgery (5/8), definitive radiotherapy (5/8), and systemic chemotherapy (combination doxorubicin/docetaxel in five patients, carboplatin/paclitaxel in two patients, paclitaxel in one patient and lenvatinib in two patients).

Treatment efficacy

Treatment outcomes are detailed in Table 2. At the data cutoff (April 30, 2022), the median follow-up (days) of the DT group was 530 (range 88–1060) compared with 96 (MT) and 24 (CC).

In the DT group, a rapid improvement of the compressive symptoms (median 3.5 days, range 3–5 days) and of the median size of the target lesion during the first month (7.2–4.8 cm) (33% reduction, $P = .012$) was observed. At best CT evaluation, median target lesion size further decreased to 3.9 cm (50% reduction, $P = .028$). Therapy was well tolerated with no discontinuation due to adverse events. Patient 1 had a dabrafenib dose reduction to 225 mg day^{-1} due to recurrent fever, seven patients (78%) reported a grade 1 adverse event (fever and hypertension in three, fever in two, and hypertension in two) and patient 7 had no symptoms.

Median survival (days) since diagnosis was 537 in the DT group, 184 in the MT, and 39 in the CC group ($P < .001$; Figure 1A). OS (days) was 475 (DT) vs 156 (MT) and 39 (CC) ($P < .001$; Figure 1B). At 12 months, only patients in the DT group were alive (71%). Median PFS (days) was 270 in the DT group vs 32 in the MT (in the CC group all patients progressed) (Figure 1C). DOR was 215 days in the DT group and 0 days in the MT due to quick progression (Figure 1D). At 3- and 6-month time points, the ORR and CB were 78% and 50% in the DT group, and 12.5% and 0%, respectively, in the multimodal group.

Tumor mutational profiling through NGS and Sanger sequencing

Results of NGS mutational profiling of ATC samples from the nine patients treated with DT are described in Table 3 (and

Table 1. Clinical characteristics of ATC patients.

Variable	Group 1 Compassionated care ($n = 10$)	Group 2 Multimodal treatment ($n = 8$)	Group 3 DT ($n = 9$)
Female	5	3	5
Male	5	5	4
Median age at diagnosis in years (range)	85.5 (60–92)	62 (49–80)	77 (56–83)
Stage IVB	3	3	4
Stage IVC	7	5	5
Range of the size of the target lesion (cm)	6.6–9.6	5.8–9.1	6.3–8.4
Median size of the target lesion (cm)	7.7	7.7	7.4
Surgery	0	5	6
Radiotherapy	0	5	6
Systemic chemotherapy	0	5	2
Multikinase inhibitor	0	2 (lenvatinib)	0

ATC, anaplastic thyroid cancer; DT, combined dabrafenib trametinib therapy.

Table 2. Treatment outcomes of ATC cases.

Clinical outcome	Group 1 Compassionated care ($n = 10$)	Group 2 Multimodal treatment ($n = 8$)	Group 3 DT ($n = 9$)
Median follow-up (days)	24	96	530
Survival since diagnosis (days)	39	184	537
OS (days)	39	156	475
12-month OS (%)	0	0	71[a]
PFS (days)	NA—all progressed	32	270
12-month PFS (%)	0	0	43[a]
DOR (days)	0	0	215
12-month DOR (%)	0	0	43[a]
Clinical benefit (CB)			
30 days (n)	0	2	8
90 days (n)	0	1	7
ORR (CR + PR)			
30 days (n)	0	1	7
90 days (n)	0	1	7
Alive at the end of follow-up (n)	0	0	5

ATC, anaplastic thyroid cancer; CB, Clinical benefit; CR, Complete response; DOR, Duration of response; DT, combined dabrafenib trametinib therapy; NA, not available; ORR, Overall response rare; OS, Overall survival; PFS, Progression-free survival; PR, Partial response.
[a]Two patients with ongoing responses and under treatment for less than 12 months.

detailed in Table S3). All ATC samples from the nine patients had *BRAF* p.V600E and *TERT*p mutations [C228T (c.−124C > T) or C250T (c.−146C > T)] (100%). In 5/9 cases (55.5%), *TP53* mutations were detected, and had a predicted heterogeneous impact in the protein function: loss of function in 4/5 mutations (80%) and gain of function in 1/5 mutations (20%). In patient 2, an additional mutation in the MAPK pathway, a novel mutation in *MAP2K1* (c.358G > C, p.E120Q), was detected, which was classified as likely pathogenic by *in silico* analysis. Mutations in genes related to the PI3K/AKT/mTOR pathway were detected in four cases: *PIK3CA* in 3/4 (patients 2 and 4: *PIK3CA* c.1633G > A, p.E545K; patient 6: *PIK3CA* c.3140A > G, p.H1047R) and *MTOR* in 1/4 (patient 9: *MTOR* c.722C > G, p.A1459D).

Four patients (patients 3, 4, 6, and 8) had progressive disease, and in patients 3 and 4, who had surgery after neoadjuvant DT, tissue samples were obtained at the time of progression. In patient 3, a pathogenic *NRAS* p.Q61K mutation, which probably represents an acquired off-target resistance mechanism to DT, was detected.[25,34] In patient 4, no new alterations were observed in the surgical specimen compared with baseline, by NGS, or by Sanger sequencing analysis of *RAC1* and *PTEN* genes. No gene fusions in *BRAF*, *ALK*, *RET*, and *NTRK* were detected. However, HR-CGH analysis of patient's 4 post-DT sample revealed a highly aneuploid tumor, showing total and partial gains and losses in chromosomal regions where several thyroid cancer-related oncogenes and tumor suppressor genes are located (detailed description in Results section of Supplementary Data and Table S4).

Discussion

Treatment strategies for a significant number of ATC patients have been largely unsuccessful until the use of BRAF/MEK target therapy in the ROR trial.[19,20] In 2020, the MD Anderson group further published a retrospective series of 477 ATC patients, from 2000 to 2019 (56 patients treated with DT), which included 20 ATC treated in neoadjuvancy. This study showed an important benefit in the OS of these patients, who underwent surgery following *BRAF* target therapy, compared with no surgery (Hazard Ratio 0.29 with 12-month survival of 94% vs 52%). These results lead to a treatment algorithm where *BRAF* p.V600E positive ATC patients in stage IVB and IVC receive BRAF target therapy followed by surgery, if feasible.[36,37] In 2021, a real-world study of treatment outcomes in ATC from Korea reported that in the 19 patients in the TKI group, 5 were treated with DT and experienced a PR.[38]

Nevertheless, DT has not yet been approved by EMA and, as to the best of our knowledge, no European case series has been published.

Here, the results of our real-world experience with DT in patients with ATC are presented together with contemporary multimodal therapy and compassionate treatment groups. In the DT group, patients showed a very fast and significant clinical and radiological response, in a rapidly progressive disease, associated with an important increase in meaningful clinical endpoints, such as median OS, PFS, and median PFS. These results were achieved with little toxicity. The chosen treatment strategy was based on MD Anderson results, discussed above, to start DT in the neoadjuvant setting in all IVB and IVC stages as soon as possible,[39] which was achieved in most cases (6/9).

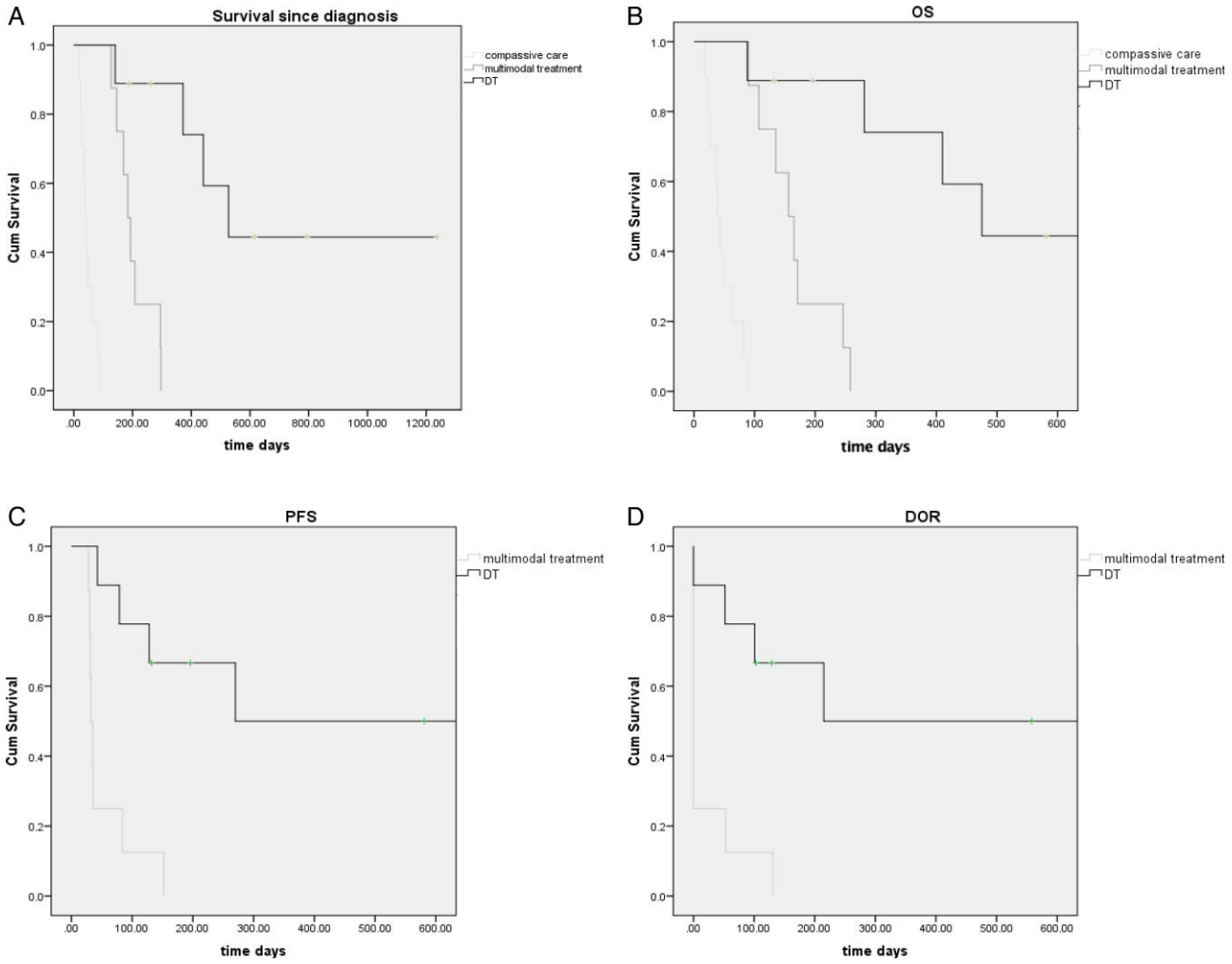

Figure 1. Kaplan-Meier curves for ATC groups. A, Disease-specific survival since diagnosis; B, overall survival (OS); C, progression-free survival (PFS); D, duration of response (DOR). Kaplan-Meier curves of the anaplastic thyroid cancer groups showed that DT treatment promoted an increase of all presented parameters.

This strategy was made possible due to the fast *BRAF* screening and DT approval from the date of ATC diagnosis at our institution (less than one week). Nevertheless, three patients started DT after surgery, since the pre-surgical diagnosis was an aggressive papillary thyroid cancer, and ATC was diagnosed subsequently on the surgical specimen.

Molecular analysis demonstrated that all *BRAF* p.V600E mutated ATC patients (33%) had a *TERT*p mutation (c.−124C > T or c.−146C > T). *TERT*p mutations represent a late genetic event and are found at higher frequency in aggressive thyroid cancer cases (65%–75% in ATC).[4–6,40–42] The high frequency of *TERT*p mutations in *BRAF* p.V600E ATC in the present series is in accordance with that reported by Landa *et al.*,[4] who found that 14/15 *BRAF* p.V600E mutated ATC had *TERT*p mutations (93.3%). However, a recent series showed in 123 tested ATC (total cohort 202) a co-existence of both mutations in just 38% of *BRAF* p.V600E cases.[36] Our results might be due to the small sample size of *BRAF* positive patients. Nevertheless, and despite a reported worst prognosis of these two mutations, it has recently been demonstrated in an *in vitro* and *in vivo* study that this "genetic duet" could improve therapeutic sensitivity to BRAF/MEK inhibitors.[43] This study further suggested that tumor cells harboring the two mutations relied on *BRAF* + *TERT*p to promote *TERT*-mediated cell survival, and proliferation, through the BRAF/MAPK-c-FOS-*TERT*p axis.[43] Consequently, these cells are more susceptible to BRAF inhibition, causing tumor cell death.[43]

Molecular analysis also identified mutations in other genes related to MAPK and PI3K pathways, which may also have a role in DT efficacy.

Regarding other proteins from the MAPK signaling pathway, we found, in the tumor of patient 2, a novel likely pathogenic mutation in *MAP2K1* gene (p.E120Q), located in the kinase domain of MEK1. Despite being present in the kinase domain, and possibly contributing to ATC pathogenesis, it does not appear to affect trametinib response, as the patient has a long-term complete response (CR) (>2 years).

Mutations in the PI3K pathway (*PIK3CA* or *MTOR*) were present in patients 2, 4, 6, and 9. These mutations could potentially lead to MAPK independence of the tumor, leading to decreased apoptosis, increased cell proliferation,[44] possibly decreasing DT efficacy, as previously described in melanoma and colorectal cancer studies.[45,46] However, in these four patients, these PI3K pathway mutations do not seem to have an identical role in DT efficacy, particularly *PIK3CA* mutations, detected in refractory cases (patient 4 with early resistance in a soft tissue metastasis and patient 6 with PR and progression),

Table 3. Molecular profiling of DT-treated group ($n = 9$).

Patient	Treatment response	*TERT*p[a]	NGS mutational analysis
Baseline study (pre-DT)			
P1	CR	C228T (c.−124C > T)	*BRAF* p.V600E
P2	CR	C228T (c.−124C > T)	*BRAF* p.V600E *PIK3CA* p.E545K *TP53* p.E285ter *MAP2K1* p.E120Q
P3	PR and progression	C228T (c.−124C > T)	*BRAF* p.V600E
P4	PR primary tumor New soft tissue metastasis	C228T (c.−124C > T)	*BRAF* p.V600E *PIK3CA* p.E545K *TP53* p.K132N
P5	CR	C228T (c.−124C > T)	*BRAF* p.V600E *TP53* p.R175H
P6	PR and progression	C228T (c.−124C > T)	*BRAF* p.V600E *PIK3CA* p.H1047R
P7	PR	C250T (c.−146C > T)	*BRAF* p.V600E
P8	PR and progression	C228T (c.−124C > T)	*BRAF* p.V600E *TP53* p.G293RfsTer13
P9	PR	C228T (c.−124C > T)	*BRAF* p.V600E *TP53* p.S241C *MTOR* p.A1459D
Post-DT samples (during progression)			
P3	PR and progression	C228T (c.−124C > T)	*BRAF* p.V600E *NRAS* p.Q61K
P4	PR primary tumor New soft tissue metastasis	C228T (c.−124C > T)	*BRAF* p.V600E *PIK3CA* p.E545K *TP53* p.K132N

All patients except P6 and P8 were sequenced for *RAC1* mutations with the results being wild type; *PTEN* exon 5 sequencing analysis was performed in patients P2 and P4 (pre-DT and post-DT samples) with the results being wild type; High-resolution comparative genomic hybridization was performed in P4 post-DT. CR, Complete response; DT, combined dabrafenib trametinib therapy; PR, Partial response.
[a]Sanger sequencing.

but also in patient 2, who had a long term (>2 years) CR. Furthermore, patient 9 with *MTOR* p.A1459D mutation is also showing an ongoing PR.

As previously stated, targeted therapy is prone to resistance mechanisms. In the four patients who progressed under DT (3, 4, 6, and 8), tissue samples at progression were only available in the two patients who underwent surgery (patients 3 and 4). While a known pathogenic *NRAS* p.Q61K mutation[33] was found in the post-DT surgical specimen of patient 3, patient's 4 sample showed no acquired mutations in the NGS analysis. However, CGH analysis in patient's 4 sample revealed chromosomal gains and losses in regions where thyroid cancer-related genes are located. Indeed, the gain of 7p22-q22 encompasses *RAC1* and *EGFR* genes. *EGFR* and *RAC1* upregulation may represent off-target resistance mechanisms in melanoma and thyroid cancer under BRAF inhibitors.[23,34] Epigenetic mechanisms, that modulate DNA methylation, histone, and chromatin structure, may also have a role in DT resistance, because they can promote differential expression of genes, which could be associated with a decreased efficacy of BRAF inhibitors, as observed in melanoma and NSCLC.[31]

This study has several strengths that support its results: the review by the same expert pathologist, the closely (monthly) radiological follow-up by the same expert radiologist, the long-term follow-up of ATC to assess OS and PFS, the PET–CT validation of CR, and two contemporary ATC groups that reflect the local practice. An important validation of these results is the significant increase in OS and PFS, previously obtained with DT compared with standard care, in the retrospective MD Anderson series published in 2022 (56 ATC treated with BRAF and MEK directed therapy had an OS of 26 months compared with an historical OS of <6 months) and the prospective ROAR clinical trial (36 ATC with an OS of 14.5 months and a median PFS of 6.7 months). This supports the idea that a fast infrastructure to diagnose and start treatment of *BRAF* positive ATC patients can change their natural history in a real-world setting (33% of the ATC series). Nevertheless, some limitations, such as the small ATC sample size and the small number of tissue samples at progression for molecular studies, are important to discuss. The first is explained by the recent use of DT in these cases (FDA approved in 2018) and the disease rarity, combined with the country's population of 10 million people (even considering the referral of up to 40% of the country's aggressive thyroid cancers to our institution). Regarding the second point, only four patients presented disease progression, and, among these, there was only access to post-DT samples in two cases (patients 3 and 4).

In conclusion, this study of closely followed DT-treated patients shows promising real-world results of the efficacy and safety of this therapy compared with standard care.

Acknowledgments

Authors are thankful to Teresa Pereira, to Ana Paula Cardoso, and to Dr. José Cabeçadas, from the Pathology Department of IPOLFG, Lisboa, Portugal, for providing all the staff and facilities for tumor samples preparation.

Authorship confirmation statement

T.N.S. was the attended physician of the patients, designed the project, performed the investigation, clinical data analysis, formal analysis, writing original draft, writing—review and editing; R.R. performed the investigation, genetic analysis, formal analysis, data analysis, writing original draft, writing

—review and editing; A.S. performed the genetic analysis, provided substantial contribution for the interpretation of the genetic data, writing—review and editing; C.P. performed the genetic analysis, writing—review and editing; M.R. reviewed the pathological slides and selected the tumor for molecular analysis, performed data analysis, writing—review and editing; M.H. performed and reviewed all CT scans; C.M. performed the genetic analysis, writing—review and editing; V.L. provided substantial contribution for the clinical data, performed the investigation, clinical data analysis, writing—review and editing; B.M.C. designed the project, provided supervision, formal analysis, interpretation of the genetic data, revised critically the manuscript, and provided funding for the project. All the mentioned authors approved the version to be publish and agreed to be accountable for all the aspects to this work in ensuring that the questions related to accuracy and integrity of this work were addressed.

Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

Funding

This work was funded by Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES, Portugal) through national funds to iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associated Laboratory LS4FUTURE (LA/P/0087/2020), by Associação de Endocrinologia Oncológica (AEO), and by Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG). Carolina Pires was granted with a PhD scholarship by FCT - 2020.07120.BD. Ricardo Rodrigues was granted with a PhD scholarship by iNOVA4Health Research Unit - UIDP/04462/2020; UI/BD/154256/2022.

Ethical statement

This research was conducted ethically in agreement with the World Medical Association Declaration of Helsinki.

Conflicts of interest: The authors declare that they have no conflicts of interest.

References

1. Simões-Pereira J, Capitão R, Limbert E, Leite V. Anaplastic thyroid cancer: clinical picture of the last two decades at a single oncology referral centre and novel therapeutic options. *Cancers (Basel)*. 2019;11(8):1188. https://doi.org/10.3390/cancers11081188
2. Molinaro E, Romei C, Biagini A, *et al.* Anaplastic thyroid carcinoma: from clinicopathology to genetics and advanced therapies. *Nat Rev Endocrinol*. 2017;13(11):644-660. https://doi.org/10.1038/nrendo.2017.76
3. Jannin A, Escande A, Al Ghuzlan A, *et al.* Anaplastic thyroid carcinoma: an update. *Cancers (Basel)*. 2022;14(4):1061. https://doi.org/10.3390/cancers14041061
4. Landa I, Ibrahimpasic T, Boucai L, *et al.* Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. *J Clin Invest*. 2016;126(3):1052-1066. https://doi.org/10.1172/JCI85271
5. Xu B, Fuchs T, Dogan S, *et al.* Dissecting anaplastic thyroid carcinoma: a comprehensive clinical, histologic, immunophenotypic, and molecular study of 360 cases. *Thyroid*. 2020;30(10):1505-1517. https://doi.org/10.1089/thy.2020.0086
6. Pozdeyev N, Gay LM, Sokol ES, *et al.* Genetic analysis of 779 advanced differentiated and anaplastic thyroid cancers. *Clin Cancer Res*. 2018;24(13):3059-3068. https://doi.org/10.1158/1078-0432.CCR-18-0373
7. Prasongsook N, Kumar A, Chintakuntlawar AV, *et al.* Survival in response to multimodal therapy in anaplastic thyroid cancer. *J Clin Endocrinol Metab*. 2017;102(12):4506-4514. https://doi.org/10.1210/jc.2017-01180
8. Wendler J, Kroiss M, Gast K, *et al.* Clinical presentation, treatment and outcome of anaplastic thyroid carcinoma: results of a multicentre study in Germany. *Eur J Endocrinol*. 2016;175(6):521-529. https://doi.org/10.1530/EJE-16-0574
9. Bible KC, Kebebew E, Brierley J, *et al.* 2021 American Thyroid Association Guidelines for management of patients with anaplastic thyroid cancer. *Thyroid*. 2021;31(3):337-386. https://doi.org/10.1089/thy.2020.0944
10. Bible KC, Suman VJ, Menefee ME, *et al.* A multiinstitutional phase 2 trial of pazopanib monotherapy in advanced anaplastic thyroid cancer. *J Clin Endocrinol Metab*. 2012;97(9):3179-3184. https://doi.org/10.1210/jc.2012-1520
11. Tahara M, Kiyota N, Yamazaki T, *et al.* Lenvatinib for anaplastic thyroid cancer. *Front Oncol*. 2017;7:25. https://doi.org/10.3389/fonc.2017.00025
12. Wirth LJ, Brose MS, Sherman EJ, *et al.* Open-label, single-arm, multicenter, phase II trial of lenvatinib for the treatment of patients with anaplastic thyroid cancer. *J Clin Oncol*. 2021;39(21):2359-2366. https://doi.org/10.1200/JCO.20.03093
13. Hyman DM, Puzanov I, Subbiah V, *et al.* Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *N Engl J Med*. 2015;373(8):726-736. https://doi.org/10.1056/NEJMoa1502309
14. Saini S, Tulla K, Maker AV, Burman KD, Prabhakar BS. Therapeutic advances in anaplastic thyroid cancer: a current perspective. *Mol Cancer*. 2018;17(1):154. https://doi.org/10.1186/s12943-018-0903-0
15. De Leo S, Trevisan M, Fugazzola L. Recent advances in the management of anaplastic thyroid cancer. *Thyroid Res* 2020; 13(1): 17. https://doi.org/10.1186/s13044-020-00091-w
16. Cabanillas ME, Drilon A, Farago AF, *et al.* 1916P Larotrectinib treatment of advanced TRK fusion thyroid cancer. *Ann Oncol*. 2020;31(suppl_4):S1086. https://doi.org/10.1016/j.annonc.2020.08.1404
17. Wirth LJ, Sherman E, Robinson B, *et al.* Efficacy of selpercatinib in *RET*-altered thyroid cancers. *N Engl J Med*. 2020;383(9):825-835. https://doi.org/10.1056/NEJMoa2005651
18. Cabanillas ME, Ryder M, Jimenez C. Targeted therapy for advanced thyroid cancer: kinase inhibitors and beyond. *Endocr Rev*. 2019;40(6):1573-1604. https://doi.org/10.1210/er.2019-00007
19. Subbiah V, Kreitman RJ, Wainberg ZA, *et al.* Dabrafenib and trametinib treatment in patients with locally advanced or metastatic BRAF V600-mutant anaplastic thyroid cancer. *J Clin Oncol*. 2018;36(1):7-13. https://doi.org/10.1200/JCO.2017.73.6785
20. Subbiah V, Kreitman RJ, Wainberg ZA, *et al.* Dabrafenib plus trametinib in patients with BRAF V600E-mutant anaplastic thyroid cancer: updated analysis from the phase II ROAR basket study. *Ann Oncol*. 2022;33(4):406-415. https://doi.org/10.1016/j.annonc.2021.12.014
21. Porter A, Wong DJ. Perspectives on the treatment of advanced thyroid cancer: approved therapies, resistance mechanisms, and future directions. *Front Oncol*. 2021;10:592202. https://doi.org/10.3389/fonc.2020.592202
22. Tangella LP, Clark ME, Gray ES. Resistance mechanisms to targeted therapy in BRAF-mutant melanoma—a mini review. *Biochim Biophys Acta Gen Subj*. 2021;1865(1):129736. https://doi.org/10.1016/j.bbagen.2020.129736
23. Tian Y, Guo WA. Review of the molecular pathways involved in resistance to BRAF inhibitors in patients with advanced-stage

melanoma. *Med Sci Monit*. 2020;26:e920957. https://doi.org/10.12659/MSM.920957
24. Mao M, Tian F, Mariadason JM, *et al*. Resistance to BRAF inhibition in BRAF-mutant colon cancer can be overcome with PI3K inhibition or demethylating agents. *Clin Cancer Res*. 2013;19(3):657-667. https://doi.org/10.1158/1078-0432.CCR-11-1446
25. Corcoran RB, Atreya CE, Falchook GS, *et al*. Combined BRAF and MEK inhibition with dabrafenib and trametinib in BRAF V600-mutant colorectal cancer. *J Clin Oncol*. 2015;33(34):4023-4031. https://doi.org/10.1200/JCO.2015.63.2471
26. Facchinetti F, Lacroix L, Mezquita L, *et al*. Molecular mechanisms of resistance to BRAF and MEK inhibitors in $BRAF^{V600E}$ non-small cell lung cancer. *Eur J Cancer*. 2020;132:211-223. https://doi.org/10.1016/j.ejca.2020.03.025
27. Manzano JL, Layos L, Bugés C, *et al*. Resistant mechanisms to BRAF inhibitors in melanoma. *Ann Transl Med*. 2016;4(12):237. https://doi.org/10.21037/atm.2016.06.07
28. Proietti I, Skroza N, Bernardini N, *et al*. Mechanisms of acquired BRAF inhibitor resistance in melanoma: a systematic review. *Cancers (Basel)*. 2020;12(10):2801. https://doi.org/10.3390/cancers12102801
29. Emery CM, Vijayendran KG, Zipser MC, *et al*. MEK1 Mutations confer resistance to MEK and B-RAF inhibition. *Proc Natl Acad Sci U S A*. 2009;106(48):20411-6. https://doi.org/10.1073/pnas.0905833106
30. Wu PK, Park JI. MEK1/2 Inhibitors: molecular activity and resistance mechanisms. *Semin Oncol*. 2015;42(6):849-862. https://doi.org/10.1053/j.seminoncol.2015.09.023
31. Khaliq M, Fallahi-Sichani M. Epigenetic mechanisms of escape from BRAF oncogene dependency. *Cancers (Basel)*. 2019;11(10):1480. https://doi.org/10.3390/cancers11101480
32. Johnson DB, Menzies AM, Zimmer L, *et al*. Acquired BRAF inhibitor resistance: a multicenter meta-analysis of the spectrum and frequencies, clinical behaviour, and phenotypic associations of resistance mechanisms. *Eur J Cancer*. 2015;51(18):2792-2799. https://doi.org/10.1016/j.ejca.2015.08.022
33. Cabanillas ME, Dadu R, Iyer P, *et al*. Acquired secondary RAS mutation in $BRAF^{V600E}$-mutated thyroid cancer patients treated with BRAF inhibitors. *Thyroid*. 2020;30(9):1288-1296. https://doi.org/10.1089/thy.2019.0514
34. Bagheri-Yarmand R, Busaidy NL, McBeath E, *et al*. RAC1 Alterations induce acquired dabrafenib resistance in association with anaplastic transformation in a papillary thyroid cancer patient. *Cancers (Basel)*. 2021;13(19):4950. https://doi.org/10.3390/cancers13194950
35. Roque L, Rodrigues R, Pinto A, Moura-Nunes V, Soares J. Chromosome imbalances in thyroid follicular neoplasms: a comparison between follicular adenomas and carcinomas. *Genes Chromosomes Cancer*. 2003;36(3):292-302. https://doi.org/10.1002/gcc.10146
36. Wang JR, Montierth M, Xu L, *et al*. Impact of somatic mutations on survival outcomes in patients with anaplastic thyroid carcinoma. *JCO Precis Oncol*. 2022;6(6):e2100504. https://doi.org/10.1200/PO.21.00504
37. Maniakas A, Dadu R, Busaidy NL, *et al*. Evaluation of overall survival in patients with anaplastic thyroid carcinoma, 2000-2019. *JAMA Oncol*. 2020;6(9):1397-1404. https://doi.org/10.1001/jamaoncol.2020.3362
38. Park J, Jung HA, Shim JH, *et al*. Multimodal treatments and outcomes for anaplastic thyroid cancer before and after tyrosine kinase inhibitor therapy: a real-world experience. *Eur J Endocrinol*. 2021;184(6):837-845. https://doi.org/10.1530/EJE-20-1482
39. Wang JR, Zafereo ME, Dadu R, *et al*. Complete surgical resection following neoadjuvant dabrafenib plus trametinib in BRAFV600E-mutated anaplastic thyroid carcinoma. *Thyroid*. 2019;29(8):1036-1043. https://doi.org/10.1089/thy.2019.0133
40. Melo M, da Rocha AG, Vinagre J, *et al*. TERT Promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. *J Clin Endocrinol Metab*. 2014;99(5):E754-E765. https://doi.org/10.1210/jc.2013-3734
41. Liu X, Bishop J, Shan Y, *et al*. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. *Endocr Relat Cancer*. 2013;20(4):603-610. https://doi.org/10.1530/ERC-13-0210
42. Panebianco F, Nikitski AV, Nikiforova MN, Nikiforov YE. Spectrum of TERT promoter mutations and mechanisms of activation in thyroid cancer. *Cancer Med*. 2019;8(13):5831-5839. https://doi.org/10.1002/cam4.2467
43. Tan J, Liu R, Zhu G, Umbricht CB, Xing M. *TERT* Promoter mutation determines apoptotic and therapeutic responses of *BRAF*-mutant cancers to BRAF and MEK inhibitors: Achilles heel. *Proc Natl Acad Sci U S A*. 2020;117(27):15846-15851. https://doi.org/10.1073/pnas.2004707117
44. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nat Rev Endocrinol*. 2011;7(10):569-580. https://doi.org/10.1038/nrendo.2011.142
45. Irvine M, Stewart A, Pedersen B, Boyd S, Kefford R, Rizos H. Oncogenic PI3K/AKT promotes the step-wise evolution of combination BRAF/MEK inhibitor resistance in melanoma. *Oncogenesis*. 2018;7(9):72. https://doi.org/10.1038/s41389-018-0081-3
46. Tsubaki M, Takeda T, Noguchi M, *et al*. Overactivation of AKT contributes to MEK inhibitor primary and acquired resistance in colorectal cancer cells. *Cancers (Basel)*. 2019;11(12):1866. https://doi.org/10.3390/cancers11121866