Next-generation sequencing in thyroid cancers: do targetable alterations lead to a therapeutic advantage?

A multicenter experience

Assaf Moore, MD[a,b], Yael Bar, MD, PhD[b,c], Corinne Maurice-Dror, MD[d,e], Inbar Finkel, MD[a], Hadar Goldvaser, MD[f,g], Elizabeth Dudnik, MD[a,b], Daniel A. Goldstein, MD[a,b], Noa Gordon, MSc, MPH[a], Salem Billan, MD[d,e], Orit Gutfeld, MD[b,c], Ido Wolf, MD[b,c], Aron Popovtzer, MD[a,b,*]

Abstract

Radioiodine-refractory thyroid cancers (IRTCs) are uncommon and have a poor prognosis. Treatment options for radioiodine-refractory and anaplastic tumors (ATCs) are limited. Although the genomic landscape of thyroid cancer has been studied, there is little evidence on whether next-generation sequencing (NGS) findings translate to tumor control.

We analyzed all patients with IRTC and ATC who underwent commercially available NGS in 3 cancer centers.

Twenty-two patients were identified, 16 patients with IRTCs and 6 patients with ATCs. Eighteen (82%) had targetable findings in NGS, nine patients were treated accordingly. Median progression-free survival for targeted treatment was 50 months [95% confidence interval (CI95%) 9.8–66.6] and 2 months (CI95% 0.2–16.5) for IRTC and ATC, respectively. Of 4 patients who achieved durable responses of 7 to 50 months, 2 are ongoing. The estimated median OS of IRTC receiving targeted treatment was not reached (CI95% 89.7–111.4 months) and was 77.8 months (CI95% 52.5–114.6) for patients treated conventionally (P=.3).

NGS may detect clinically significant genetic alterations and benefit patients with advanced thyroid cancers.

Abbreviations: ALK = Anaplastic lymphoma kinase, ATC = anaplastic thyroid carcinoma, FTC = follicular thyroid carcinoma, HER3 = human epidermal growth factor receptor 3, IRTC = radioiodine-refractory thyroid cancers, MAPK = mitogen-activated protein kinase, mTOR = mammalian target of rapamycin, NGS = next generation sequencing, NSCLC = non-small cell lung cancer, NTRK = neurotrophic tropomyosin receptor kinase, OS = overall survival, PARP = poly ADP ribose polymerase, PAX8–PPARG = Paired box gene 8 - Peroxisome proliferator activated receptor gamma, PCR = polymerase chain reaction, PDTC = poorly differentiated thyroid carcinomas, PFS = progression-free survival, PI3K = phosphatidylinositol 3-kinase, PTC = papillary thyroid carcinoma, RAI = radioiodine, RET = rearranged during transfection, RR = response rate, SWI/SNF = SWItch/Sucrose Non-Fermentable, TERT = telomerase reverse transcriptase, TMB = tumor mutational burden, TP53 = tumor protein P53.

Keywords: anaplastic thyroid cancer, differentiated thyroid cancer, next-generation sequencing, targeted treatment

1. Introduction

Thyroid cancer is relatively uncommon. An estimated 52,890 new cases of thyroid cancer will be diagnosed in 2020 in the US, and 2180 patients will die of this disease.[1] According to Surveillance, Epidemiology, and End Results (SEER) data, 89.9% of patients diagnosed with thyroid cancers have papillary thyroid carcinoma (PTC), 4.5% follicular thyroid carcinoma (FTC), 1.5% Hurthle-cell carcinoma, and 0.8% anaplastic thyroid carcinoma (ATC).[2]

The vast majority of patients with differentiated thyroid cancers are cured with surgery, radioiodine (RAI) when indicated, and thyroid hormone suppression.[3–5] Although prognosis is excellent overall, about 5% of patients will develop distant metastases and become refractory to RAI.[3] Some patients can have an indolent course; however, metastatic RAI-refractory thyroid cancer (IRTC) has an overall poor prognosis.[4–7] Repeated RAI therapy is not recommended, as it does not result in clinical benefit and is associated with various adverse effects.[3,4] Response rates (RRs) to cytotoxic chemotherapies have largely been low.[3,4,6–9] Sorafenib was found to have a significantly longer median progression-free survival (PFS) compared with placebo.[7] Lenvatinib was also found to have a significant advantage in median PFS over placebo and a RR of 64.8%. The median overall survival (OS) was not reached.[6] These are currently the only approved systemic agents in the setting of IRTC.[4–7] No standard treatment options exist for patients who have failed or cannot tolerate these agents.

The genomic landscape of differentiated thyroid cancer has been studied.[5,8,10–15] PTC has several tumor subtypes that harbor mutually exclusive mutations of the mitogen-activated protein kinase (MAPK) pathway, including BRAF V600E that accounts for 60% of cases, RAS in 15% and other chromosomal rearrangements such as Rearranged During Transfection (RET), Neurotrophic Tropomyosin Receptor Kinase (NTRK), and Anaplastic lymphoma kinase (ALK) (12%).[5] BRAF-mutated PTC has a more aggressive course, a poor response to RAI, and a poorer prognosis.[10,11] FTCs are associated with mutually exclusive mutations of RAS or of the Paired box gene 8 - Peroxisome Proliferator Activated Receptor Gamma (PAX8–PPARG) fusion oncogene.[12] Mutations in Telomerase Reverse Transcriptase (TERT) are very common in thyroid cancers, from 22% to 23% in PTC to over 50% in poorly differentiated thyroid carcinomas (PDTC) and ATCs.[13] In fact, there is clear cooccurrence of *TERT* mutations in advanced thyroid cancers harbouring *BRAF* or *RAS* alterations, as compared with those that are wild-type.[13]

PDTC make up approximately 6% of thyroid cancers, and have a more aggressive clinical course and worse outcomes.[5] Responses to RAI are minimal. Systemic treatment options are similar to those used in differentiated thyroid cancers.[5]

ATCs are RAI-refractory and have a very grim prognosis.[4,5,16] Cytotoxic chemotherapy can be used in unresectable or metastatic disease.[1,5,17] The genomic landscape of ATCs has also been studied. BRAF and RAS are the major oncogenic drivers. Other common alterations include mutations in Tumor Protein P53 (TP53), the TERT promoter, effectors of the phosphatidylinositol 3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) pathway, and genes involved in epigenetic regulation, including components of the SWItch/Sucrose Non-Fermentable (SWI/SNF) complex and histone methyltransferases.[16] A study of NGS found that the DNMT3A mutation was

significantly enriched in PDTC and ATC, and may be associated with short life expectancy.[18] In another study, ATC had significantly higher BRAF, TP53, TERT, and PIK3CA mutation rates than PDTC. Sixty-nine percent of ATC cases with PTC components harbored BRAF mutations. Twenty-two percent of PDTCs had an oncogenic fusion (including RET, NTRK1, and ALK), all had with PTC components. Concurrent TERT and PIK3CA mutations were associated with poor OS.[19]

Despite the elaborate work done with genetic characterization of thyroid cancers, there is very little evidence regarding whether these findings translate to meaningful tumor control or response. In this study, we aimed to explore the yield of performing NGS for all patients. As these tests are performed at the patients' expense, determining their contribution to patient care may have significant implications.

2. Materials and methods

2.1. Patients

The registries of 3 major referral cancer centers in Israel were screened for all patients with advanced histologically confirmed IRTC and ATC who had undergone a commercially available NGS for recurrent or metastatic disease from January 2016 to January 2020. Medullary thyroid cancers were excluded from this analysis. Twenty-two patients were indentified and comprised the study group. NGS panels used included FoundationOne CDx (Foundation Medicine, Cambridge, MA), and Tempus (Tempus, Chicago, IL). Patients' demographics, clinico-pathological characteristics, treatment details, and survival outcomes were collected from electronic databases and medical records. NGS was performed at the discretion of the treating phisician, and not for the purpose of this study. The study was approved by the institutional ethics committees before any research procedures.

2.2. Statistics analysis

Data were analyzed using the Statistical Package for the Social Sciences 25.0 (SPSS) at a significance level of 0.05. Survival was estimated with the Kaplan–Meier method and compared using log-rank test. For the purpose of survival and PFS analysis, PDTCs were grouped with PTCs and FTCs.

3. Results

3.1. Clinicopathologic features

The median age at diagnosis was 61 years (range 26–78). Thirteen patients were females (59%). The histologic type was PTC in 9 patients (41%), FTC in 4 (18%), PDTC in 3 (14%), and ATC in 6 (27%). Fourteen patients (64%) were initially treated with curative intent. The median time from curative treatment to recurrence was 46 months (range, 2.1–245.0 months). All patients with differentiated thyroid cancers who were treated with targeted agents were previously treated with lenvatinib or sorafenib in the first-line setting.

3.2. NGS

In all cases, NGS was performed using a commercially available assay after disease progression by imaging studies. In 7 cases, NGS was performed before systemic therapy initiation, 2

(12.5%) patients with differentiated cancers, and all patients with ATC. In the remaining cases, NGS was performed after failure of conventional systemic therapy. NGS findings are summarized in Table 1. Eighteen patients (81.8%) had potentially targetable findings in NGS, 12 (75%) of differentiated cancers and all ATCs. Targetable alterations included 8 patients with BRAF V600E mutations, 5 patients with NRAS mutations, 3 patients with RET rearrangements, 3 patients with intermediate tumor mutational burden (TMB), 2 patients with NOTCH mutations, 1 patient with ALK rearrangement, and 1 patient with a BRCA2 mutation. Microsatellite instability (MSI) status was known in 19 patients and was stable in all cases. TMB was known in 19 patients, was low (fewer than or equal to 5 mutations /Mb) in 16 (84.2%) and intermediate (6–19 mutations /Mb) in 3 (15.8%). No patient had high TMB (greater than or equal to 20 mutations /Mb). TMB was known in 13 patients with differentiated cancers, was intermediate in 2 patients (15.4%), and low in the remainder (84.6%). TMB known in all ATC patients, was intermediate in 1 (16.7%), and low in 5 (83.3%).

3.3. Treatment outcomes

The median number of systemic treatment lines was 2 (range 0–4) and 1 (range 0–2) for differentiated cancers and ATCs, respectively. Nine (41%) patients were treated according to NGS findings. Four patients with ATC were treated as first-line therapy. Three and two patients with differentiated cancers were treated in the second- and third-line, respectively. NGS-guided therapies included 6 patients treated with BRAF/MEK inhibitors (3 patients with differentiated cancers and 3 ATCs) for a BRAF V600E mutation, 1 patient treated with a BRAF inhibitor for a BRAF V600E mutation, 1 patient treated with a MEK inhibitor for an NRAS mutation, and 1 ATC patient treated with a MEK inhibitor and immunotherapy for NRAS mutation and intermediate TMB. The median follow-up for all patients was 29.2 months (range, 1.6–108.6 months). Median PFS for targeted treatment was 50 months (CI95% 9.8–66.6 months) and 2 months (CI95% 0.2–16.5 months) for differentiated cancers and ATCs, respectively. Of 4 patients who achieved durable responses of 7 to 50 months, 2 are ongoing. Targeted therapies, responses and PFS are presented in Table 2 and Figure 1. An ongoing response to BRAF and MEK inhibitor combination is presented in Figure 2. At the time of data analysis, 10 patients had died; 5 (31.2%) of differentiated cancers and 4 (66.7%) of ATCs. The median OS was 108.6 months (CI95% 74.1–110.0) and 6.1 months (CI95% 3.3–16.7) for differentiated cancers and ATCs, respectively ($P < .001$). The estimated median OS of differentiated cancers receiving targeted treatment was not reached (CI95% 89.7–111.4) and was 77.8 months (CI95% 52.5–114.6) for patients treated conventionally ($P = .3$) (Fig. 3).

4. Discussion

The key question when offering NGS to unselected cancer patients is whether a substantial proportion of targetable alterations can be found. The greater bulk of literature is based on nonsmall cell lung cancer (NSCLC). In 1 report of 209 NSCLC patients, 46% of cases harbored potentially actionable mutations. Targeted therapy was instituted on the basis of NGS in 11% of patients.[20] In a report of 282 unselected newly diagnosed metastatic NSCLC patients, a guideline biomarker for targeted therapy was identified in 27.3% of cases.[21] In a

publication of NGS in 27 salivary gland cancers, 14 (51.8%) had targetable findings in NGS, 10 were treated accordingly and seemed to benefit clinically.[22] Our results compare favorably with these data, as over 80% of patients had targetable findings in NGS and 50% of them were treated accordingly. Therefore, 41% of patients were treated according to NGS results. NGS-guided therapy was relatively homogeneous, mainly BRAF - MEK inhibition-based, due to accessibility of these drugs.

As mentioned above, BRAF V600E mutations are very common in thyroid cancers, and indeed, 7 patients (31.8%) in our cohort were treated with targeted agents. The BRAF inhibitor vemurafenib was studied in IRTC in a nonrandomized phase 2 trial.[23] A relatively low RR was suggested to be due to activation of human epidermal growth factor receptor 3 (HER3) signaling.[5,23] One patient in our cohort was treated with vemurafenib and achieved a durable response. When the patient progressed, an NGS panel revealed a new NRAS mutation that conferred resistance to vemurafenib. This case was previously published,[24] and demonstrated the potential benefit of NGS panels for clinical decision-making with targeted therapy. Although combinations of BRAF and MEK inhibitors were shown to improve outcomes in advanced melanoma,[25,26] to the best of our knowledge, there is currently no data on this combination in BRAF-mutated PTC or FTC. In our study, 3 of 4 patients with differentiated cancers were treated with a BRAF and MEK inhibitor combination. Two achieved durable ongoing partial responses of 7 and 40 months at the time of data analysis. For 1 patient, response was yet to be assessed. Treatment with a dabrafenib and trametinib combination in ATC achieved an overall RR of 69%, and the median duration of response, PFS, and OS were not reached, with 12-month estimates of 90%, 79%, and 80%, respectively.[27] Neoadjuvant treatment with dabrafenib and trametinib was even able to render all 6 initially unresectable BRAF V600E-mutated ATCs operable in one study.[28] In our study, only 1 of 3 patients treated with this combination had a PR. As this cohort is of limited size, no clear conclusions can be drawn.

ALK, RET, and NTRK are also potential driver mutations for targeted therapy. All 5 patients with TRK fusion positive thyroid cancers treated with larotrectinib responded in 1 study.[29] In a pooled analysis of 7 patients with TRK fusion positive thyroid cancers, the overall RR was 75%.[30] Although we did not have any cases with TRK fusion in our cohort, this is a promising target for therapy. RET activation leads to initiation of pathways that signal cell proliferation and survival, and has been found in several malignancies, including thyroid, lung, and salivary gland cancers.[31–33] The FDA approved selpercatinib for RET fusion-positive IRTC.[34] This drug achieved an overall RR of 79% in previously treated patients and 87% of responses lasted 6 months or longer. All patients treated in the first-line setting responded and 75% had responses lasting 6 months or longer.[34] In our cohort, 2 patients with differentiated cancers had RET fusions (CCDC6-RET and NCOA4-RET fusion) as well as 1 patient with ATC (ERC1-RET fusion). None of our patients were eventually treated with a RET targeted agent, but based on data presented, this finding may be clinically significant. One patient in our cohort was found to have an ALK translocation, however, was treated conventionally since. There have been anecdotal reports of ALK inhibitors in ATC,[35] suggesting this may be an actionable alteration in this malignancy. To the best of our knowledge, no trials have been published.

One patient in our cohort was found to have a pathogenic BRCA2 mutation (R2787H).[36] The BRCA2 gene is mutated in

Table 1

NGS results by histology.

		Genetic alterations				
Pt. no.	Histology	With potential therapeutic implications	Without therapeutic implications	Variants of uncertain significance	MSI status	TMB
1	PTC	NRAS Q61R	TERT promoter -124C>T	CSF1R (E920D), RAD54L (S318R), ROS1 (L691S), TNFAIP3 (T647P)	Stable	Low; 3 Muts/Mb
2	PTC	BRAF V600E	-	ARID1A (N1313S), CSF1R (K707Q), JAK3 (V217M), MLL2 (T1246M)	N/A	N/A
3	PTC	BRAF V600E	NOTCH2 (S2115G), TERT promoter -124C>T	AXL (T637I), ERBB3 (T96A), GLI1 (Y593C), GNAS (P239R), MLH1 (P603R), MLL2 (K4848R), SMO (R398*,V54M), SNCAIP (SNCAIP)	Stable	Low; 2 Muts/Mb
4	PTC	RET CCDC6-RET fusion	EGFR L838R, TERT promoter -124C>T	FGFR1 (E338*), IRS2 (A699_A701del), MED12 (Q2119_Q2120insHQQQ, Q2120H), MYC (S362F), NF1 (T1295A), TAF1 (R1660Q)	Stable	Low; 5 Muts/Mb
5	PTC	NRAS (Q61R)	, RBM10 (R680*), TERT promoter -124C>T	BRCA2 (P655R), HSD3B1 (E60G), IRS2 (N28_H29insN), MED12 (Q2076_Y2077insQ and Q2119_Q2120insHQQQ), PDGFRB (V316M), SETD2 (G933V), TSC1 (T360N), TSC2 (S1774G)	Stable	Intermediate; 8 Muts/Mb
6	PTC	BRAF V600E	TERT promoter -124C>T	ARFRP1 (S143L), BRAF (A33_A34insGA), CDK12 (R1333H), EGFR (D1084H), FANCA (V372F), GATA6 (H333del), NOTCH2 (G2449V), SETD2 (M737T)	Stable	Low; 5 Muts/Mb
7	PTC	BRAF V600E, NOTCH1 (H1564P)	ATM (E343fs*2), BCOR (V379fs*62 - subclonal), PBRM1 (S1518fs*73), TERT promoter -146C>T	ATM (S99G), ATRX (E1464del), CALR (Y57C), FAM123B (E385_E387del), MSH6 (I1054F), NKX2-1 (R10Q), PTCH1 (R603H), RNF43 (P192L), RPTOR (V1228M)	Stable	Low; 0 Muts/Mb
8	PTC	-	PTEN (G132fs*5), HGFamplification, BAP1 (A415fs*3), SETD2 (S2470fs*14), TP53 (H193R)	FANCD2 (Q26H), FLT4 (E955Q), GRM3 amplification, KDM6A (S114C), MAGI2 amplification, NKX2-1 (R10Q), NOTCH3 (R113Q), NUP93 (I583M), PRSS8 (R25W), SETD2 (I1398T)	Stable	Low; 3 Muts/Mb
9	PTC	BRAF V 600E	CDKN2A, CDKN2C, RB1, TERT		Stable	Low; 2 Mut/Mb
10	FTC	RET NCOA4-RET fusion, BRCA2 R2787H, NOTCH1 A1640T	CDKN2A/B p16INK4a R58* and p14ARF P72L, TERT promoter -124C>T, WHSC1 (MMSET) E1099K		Stable	Low; 1 Muts/Mb
11	FTC	-	APC rearrangement exon 16, TERT promoter -124C>T	GNAS (A272V)	N/A	N/A
12	FTC	-	HRAS Q16R, BCORL1 S936fs*10, TERT promoter-124C>T	C11ORF30 (H989R), CREBBP (A1907T), EPHA3 (E827D), KLHL6 (V39F), MED12 (Q2119_Q2120insHQQQ), NOTCH3 (P2201A), RNF43 (V627A), TET2 (Q317H)	Stable	Low; 5 Mut/Mb
13	FTC	NRAS Q61K	RBM10 W756fs*37	ARID1B (G246S), BRCA2 (P655R), C11orf30 (A896G), CREBBP (R742P), DNMT3A (L411P), IDH1 (F32V), INPP4B (V594A), PBRM1 (P1476S), PDGFRB (V316M), POLE (F931S), RBM10 (W756G), SDHA amplification, TSC2 (F1510del)	Stable	Low; 5 Mut/Mb
14	PDTC	NRAS Q61R	TERT promoter -124C>T	ALK (R412C), APC (N1118D), ARAF (Q562fs*?), CALR (Y57C), ETV4 rearrangement, FANCC (V60I),	Stable	Low; 1 Muts/Mb

Table 1

(continued).

Pt. no.	Histology	Genetic alterations		Variants of uncertain significance	MSI status	TMB
		With potential therapeutic implications	Without therapeutic implications			
15	PDTC	-	PTEN splice site 209+2T>G	MKNK1 (R93Q), NOTCH1 (N104S), NTRK1 (S190R), PDK1 (N176H), TGFBR2 (S553T) APC (Q203E and R283Q), CUL4A (T246S), FAM123B (T625A), PARP2 (E412K), POLE (P370T), SPEN (C766Y), SYK (SYK), TET2 (H1881R), VHL (Y112F)	N/A	N/A
16	PDTC	ALK rearrangement intron 15	TERT promoter -124C>T	ERBB3 (N963S), FAM123B (T625A), IRS2 (N28_H29insN), MAP3K1 (S939C), NTRK3 (H622D), POLE (E1240K), RAD54L (N60S), SDHA (V425G), TSC2 (D162N)	Stable	Intermediate; 6 Muts/Mb
17	ATC	BRAF V600E	AKT2 (E17K), IDH2 (R140Q), TERT promoter -124C>T, TP53 (R282W)	ABL2 (T769S), ARID1B (Q129_Q131del), FOXL2 (P205R), HSP90AA1 (P13S), MCL1 (MCL1), MLL (A67del), MLL2 (T1246M), PLCG2 (A308V), RPTOR (V1228M), SPEN (S2139T)	Stable	Low; 3 Muts/Mb
18	ATC	BRAF (p.K601E)	TP53 (p.R273H), TERT promoter -124C>T, CCNE1 Copy number gain, MAPK1 Copy number gain	TSC1 (p.R689H)	Stable	Low; 1 Muts/Mb
19	ATC	BRAF V600E	–	MAP3K1 (S939C), RET (Y791F), SDHA (SDHA)	Stable	Low; 0 Muts/Mb
20	ATC	RET ERC1-RET fusion	DNMT3A (W313*), NF2 (W41*), TERT promoter -124C>T, TP53 (S241F)	ATRX (I435V), CSF1R (V32G), FLT1 (S733del)	Stable	Low; 1 Muts/Mb
21	ATC	BRAF V600E	–	BRIP1 (V193I), CHEK2 (Y424H), DAXX (I663M), EED (T50P), IRF2 (T20S), MAP3K1 (S939C), MSH3 (L911W), MTOR (I637V), P2RY8 (V358A), PRDM1 amplification, PTPN11 (I309V), TBX3 (S435L), TGFBR2 amplification	Stable	Low; 1 Muts/Mb
22	ATC	NRAS (Q61R)	NF2 (S87*), BRD4 (E1113del), CDKN2A (p16INK4a D108Y and p14ARF R122L EP300 Q965*), MAP3K1 (S924*), TERT promoter -124C>T, TP53 (E285K)	AR (E898D), ARID1B (A767T), ARID2 amplification, ASXL1 (M168L, P1259L), ATM (L2307F), BRCA2 (P655R), EP300 (P955T), ERBB4 (S522L), FAT1 (A636T), GATA2 (P161A), MDM2 (E396Q, E421Q), MLL2 (P3665A), MYST3 (M482K), PAK3 (M121I), PDGFRB (Q31*), TSC1 (T360N)	Stable	Intermediate; 7 Muts/Mb

ATC = anaplastic thyroid carcinoma, FTC = follicular thyroid carcinoma, MSI = microsatellite instability, Muts/Mb = Mutations per megabase, PDTC = poorly differentiated thyroid carcinoma, PTC = papillary thyroid carcinoma, TMB = tumor mutational burden.

under 1% of thyroid cancers.[37] Whether BRCA mutations confer a higher risk for thyroid cancer and whether these cancers have different prognoses remains undetermined.[38–40] To the best of our knowledge, there are no data on the use of targeted agents such as poly ADP ribose polymerase (PARP) inhibitors, nor is there proof of increased efficacy of platinum compounds for this indication. Important to note, a recent publication demonstrated responses to olaparib in certain somatic mutations in breast cancer.[41] This patient has been treated with lenvatinib since October 2018. Response is ongoing.

Two patients in our cohort had mutation in the NOTCH gene. NOTCH1 can have oncogene or tumor suppressor qualities, varying between types of malignancies. Its expression in thyroid cancer was found to be associated with poor prognostic markers such as large tumor size, lymph node metastasis, capsular invasion, and extrathyroidal extension.[42] Specific and pan-NOTCH inhibitors have been studied and are being developed in solid and hematologic malignancies.[43–46] To the best of our knowledge, there are no data on the use of NOTCH inhibitors in thyroid cancers.

Table 2

NGS-guided treatment outcomes.

Histologic type	Alteration	Therapy	Best response	Duration of response, mo
PTC	NRAS Q61R	cobimetinib	progression of disease	3
PTC	BRAF V600E	vemurafenib + cobimetinib	partial response	6, ongoing
PTC	BRAF V600E	vemurafenib + cobimetinib	partial response	27, ongoing
PTC	BRAF V600E	vemurafenib + cobimetinib	Not yet assessed	Not yet assessed
PTC	BRAF V600E	vemurafenib	partial response	50
ATC	BRAF V600E	dabrafenib + trametinib	Stable disease	8
ATC	BRAF V600E	dabrafenib + trametinib	progression of disease	2
ATC	BRAF V600E	dabrafenib + trametinib	progression of disease	2
ATC	NRAS Q61R, Intermediate TMB	cobimetinib + nivolumab	partial response	20, ongoing

ATC = anaplastic thyroid carcinoma, PTC = papillary thyroid carcinoma, TMB = tumor mutational burden.

Immunotherapy has also been explored in thyroid cancers. In KEYNOTE-028, 22 patients with advanced PTC or FTC with PD-L1 expression in ≥ 1% were treated with pembrolizumab. Two patients (9.1%) had a PR and 12 patients achieved SD (54.5%). The 6-month PFS rate was 58.7%.[47] Immunotherapy has also been studied in ATC. In 1 trial, 42 patients were treated with the PD-1 inhibitor spartalizumab. The overall RR was 19%, including 3 patients with CRs and 5 with PRs. The RR was higher in PD-L1–positive (8/28; 29%) versus PD-L1–negative (0/12; 0%) patients. Responses occurred in BRAF-mutated as well as nonmutant tumors.[48] All patients in our cohort were MSI-stable. Two patients with differentiated cancers and one patient with ATC had intermediate TMB, and could be potential candidates for immunotherapy. We did not test PD-L1 in our patients. Whether PD-L1 or TMB is the optimal biomarker for response to immunotherapy in thyroid cancers is currently unknown. One patient with intermediate TMB and NRAS mutation has been treated with a nivolumab - cobimetinib combination since July 2018 and achieved a durable PR that is ongoing. Immunotherapy – TKI combos have been explored in several malignancies and are being developed in others. A lenvatinib - pembrolizumab combination has had promising results in one report of PD-L1 positive (>1%) ATCs and PDTCs.[49] NRAS Q61R mutation causes activation of RAS signaling by activating the RAF-MAPK-ERK, PI3K, and other pathways. Targeting NRAS has been a challenging task. MEK inhibition has shown clinical benefit in advanced NRAS-mutant melanoma.[50] STK19 inhibition may be also be a therapeutic strategy for that indication.[51] A phase II trial is assessing whether the MEK inhibitor trametinib given with RI in RAS-mutated thyroid cancers enhances RAI uptake.[52] Another study is investigating the combination of the PD-L1 inhibitor atezolizumab with cobimetinib in patients with various solid tumors.[53] Whether our patient is benefiting from immunotherapy, MEK inhibition or the combination is unclear. Another patient in our cohort with PTC was treated with cobimetinib as a single agent and quickly progressed. Clear conclusions to the efficacy of these agents for this indication cannot be drawn.
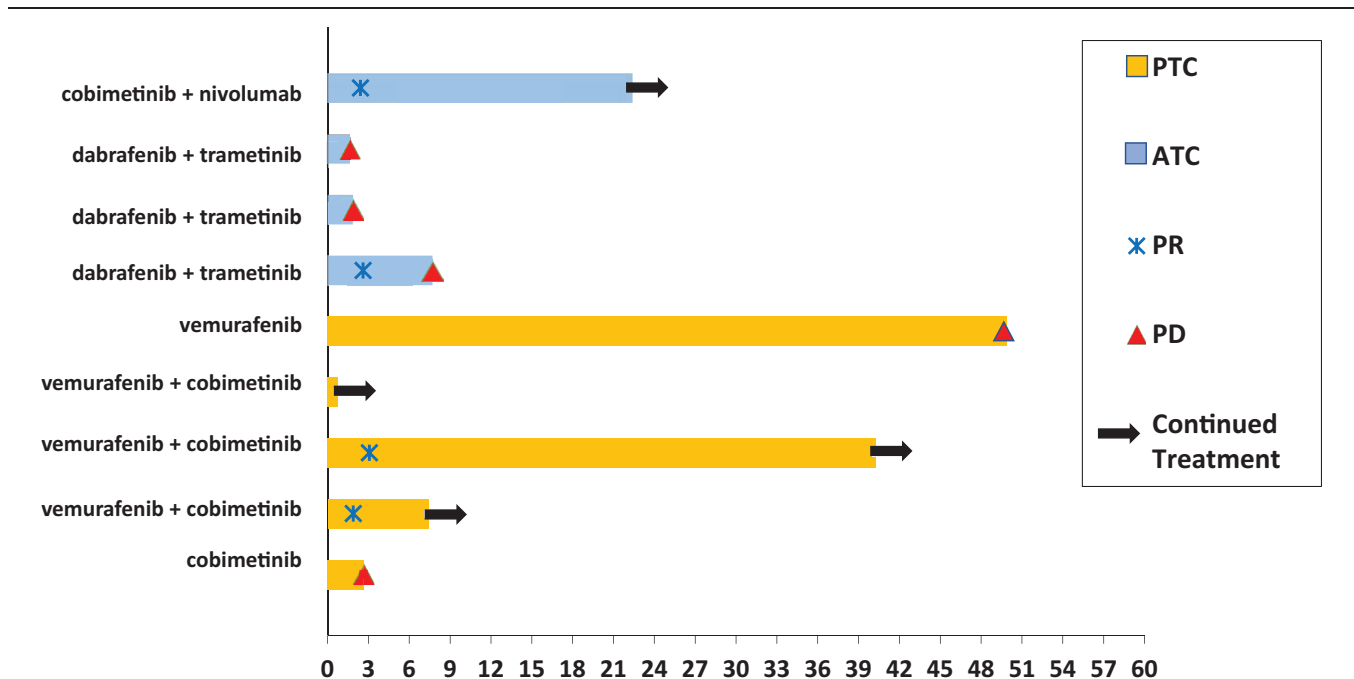

Figure 1. Swimmer plot. NGS-guided treatment. PD = progressive disease, PR = partial response.
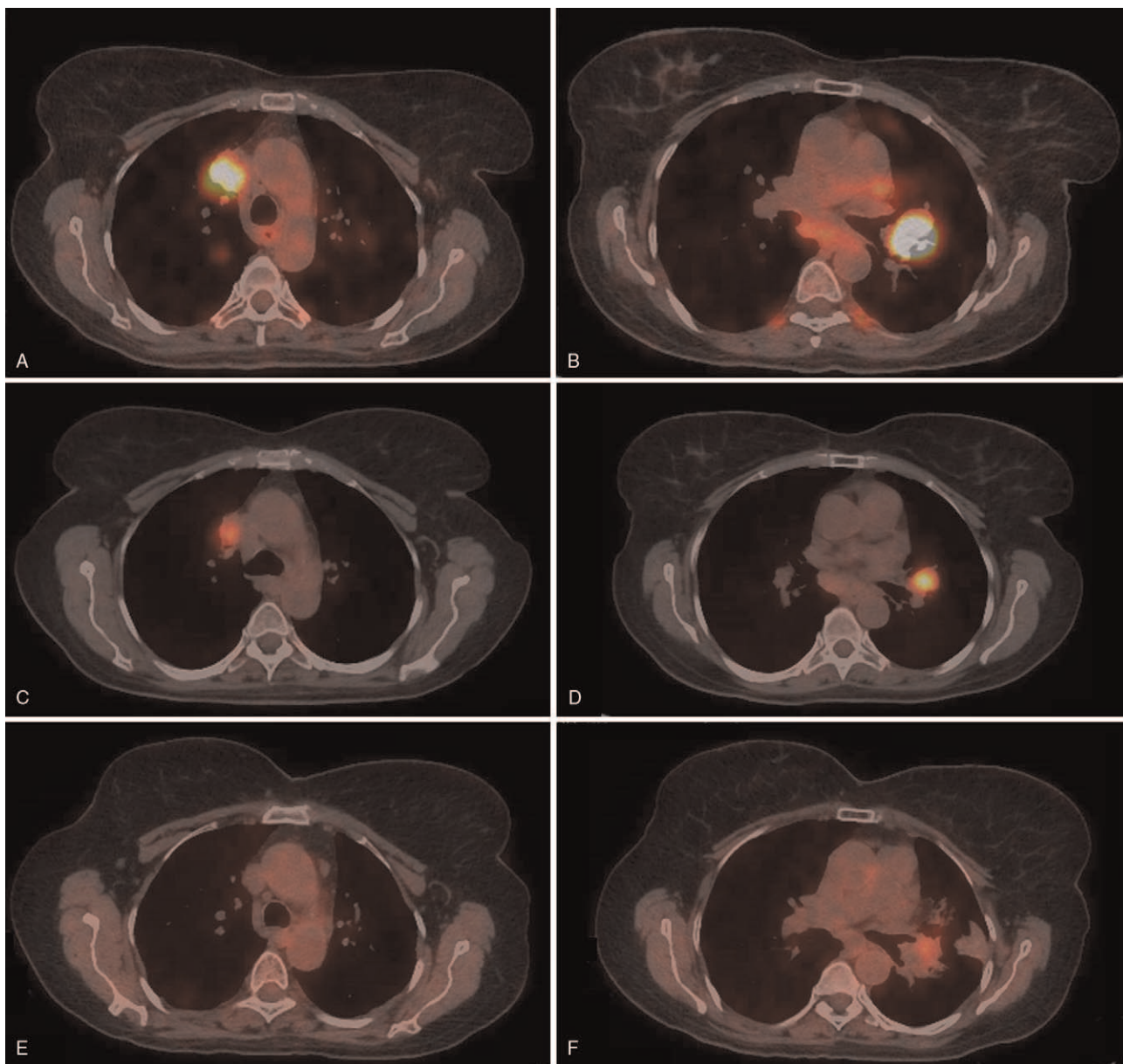
Figure 2. An ongoing response to a vemurafenib/cobimetinib combination in a patient with BRAF V600E mutated papillary thyroid cancer. (A) A baseline PET-CT scan (12/2016) demonstrating a right upper lobe (RUL_ mass extending into the mediastinum. (B) A baseline PET-CT scan (12/2016) demonstrating a left upper lobe (LUL) mass. (C) A PET-CT scan (4/2017) demonstrating a partial response in the RUL mass. (D) A PET-CT scan (4/2017) demonstrating a partial response in the LUL mass. (E) A PET-CT scan (4/2020) demonstrating an ongoing response in the RUL mass. (F) A PET-CT scan (4/2020) demonstrating an ongoing response in the LUL mass.

Although NGS-guided therapy has been shown to improve survival outcomes in patients with advanced NSCLC,[54,55] the open-label, randomized, controlled phase 2 SHIVA trial has shown that targeted agents outside their indications do not improve PFS in heavily pretreated patients.[56] In our cohort, patients who received NGS-guided therapy had better survival outcomes, although not statistically significant, potentially due to the small sample size. Overall, 4 of 9 patients receiving targeted therapy responded and 1 patient achieved durable SD, comprising a RR of 44% and disease control rate of 55.5%. Although a small cohort, these findings compare favorably with previously published reports.[55–58]

Our study has several major limitations. The retrospective design and lack of a control arm are generally associated with methodological biases and difficulties in results interpretation. Also, as thyroid cancers are very diverse, especially in terms of prognosis, survival outcomes may be significantly biased by the more favorable indolent clinical course of PTCs. The most concerning bias in our study is clearly associated with patient selection. As NGS is not routinely recommended in advanced thyroid cancers and the test is not reimbursed, funding remains a substantial obstacle in its widespread use. NGS-guided therapies are also controversial, as evidence is scarce and costs are considerable. However, as the cost of sequencing is steadily
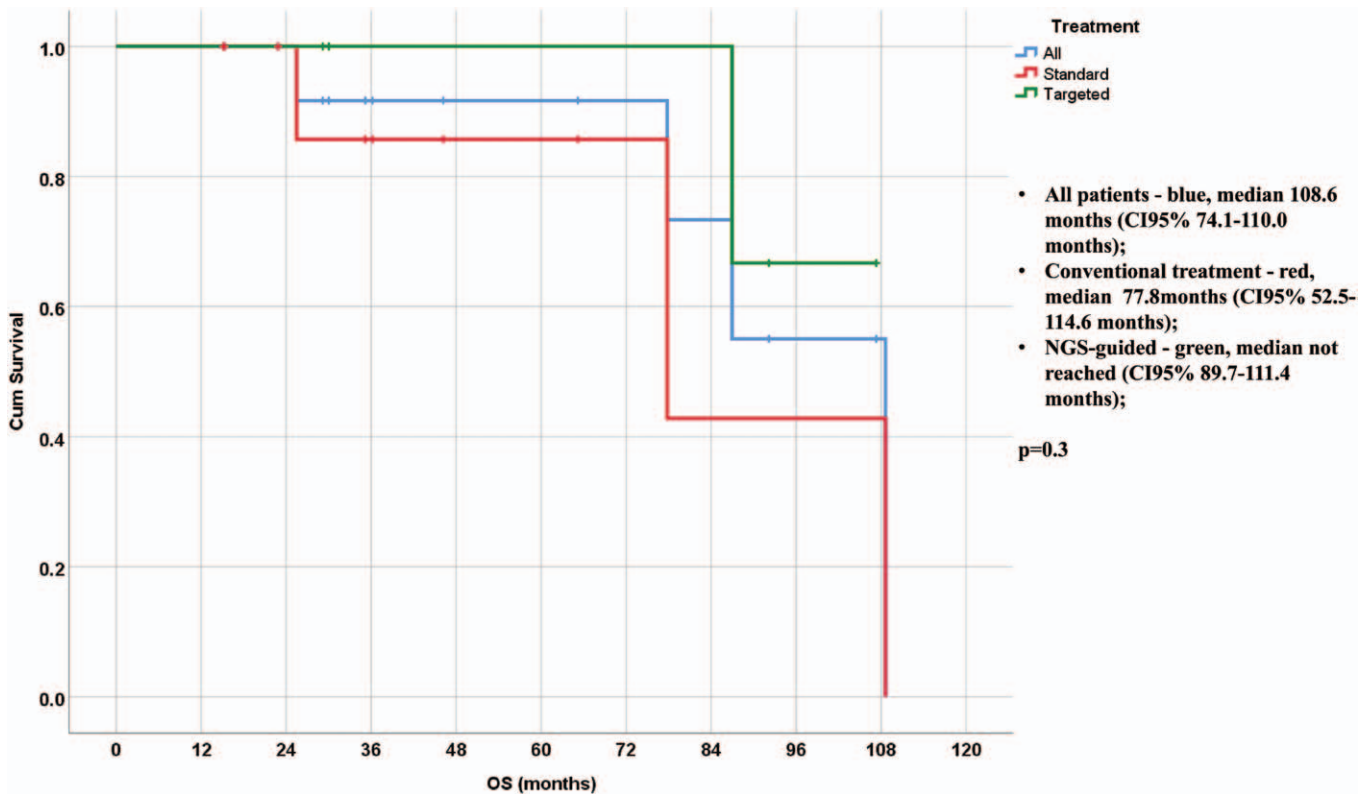
Figure 3. Overall survival of patients with radioiodine-refractory thyroid cancer. All patients - blue, median 108.6 months (CI95% 74.1–110.0); NGS-guided - green, median not reached (CI95% 89.7–111.4); conventional treatment - red, median 77.8 months (CI95% 52.5–114.6); pP = .3.

declining, tumor genome sequencing in everyday clinical decision making is expected to become more common. BRAF-targeted agents are the most used in this experience. However, detecting BRAF mutations can be done with polymerase chain reaction (PCR), and does not require NGS. Nonetheless, even after excluding BRAF mutations, 8 (36%) patients had potentially targetable alterations. One clear benefit of NGS is time saved by running a comprehensive panel over testing for oncogenic drivers sequentially. This could be of particular significance in ATC.

5. Conclusion

This study demonstrates a potential effect of NGS on the management and outcomes of the general population of patients with recurrent and metastatic RI-refractory thyroid cancers and ATCs through the detection of actionable alterations and mechanisms of resistance. Although these results are retrospective and limited by cohort size, they are hypothesis generating. Prospective validation of these findings is needed.

Author contributions

Conceptualization: Assaf Moore, Orit Gutfeld, aron popovtzer.
Data curation: Assaf Moore, Yael Bar, Corinne Maurice-Dror, Inbar Finkel, Hadar Goldvaser, Elizabeth Dudnik, Daniel A Goldstein, Noa Gordon, Salem Billan, Orit Gutfeld, Ido Wolf, aron popovtzer.
Formal analysis: Assaf Moore, Noa Gordon.
Funding acquisition: Assaf Moore.
Investigation: Assaf Moore, Yael Bar, Corinne Maurice-Dror, Inbar Finkel, Hadar Goldvaser, Elizabeth Dudnik, Daniel A Goldstein, Noa Gordon, Salem Billan, Orit Gutfeld, Ido Wolf, aron popovtzer.
Methodology: Assaf Moore.
Software: Noa Gordon.
Supervision: aron popovtzer.
Writing – original draft: Assaf Moore, Yael Bar, Corinne Maurice-Dror, Inbar Finkel, Hadar Goldvaser, Elizabeth Dudnik, Daniel A Goldstein, Noa Gordon, Salem Billan, Orit Gutfeld, Ido Wolf, aron popovtzer.
Writing – review & editing: Assaf Moore, aron popovtzer.

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7–30.
[2] Cancer of the Thyroid (Invasive) Trends in SEER Incidenceab and U.S. Mortalityc Using the Joinpoint Regression Program, 1975–2015 With up to Five Joinpoints, 1992–2015 With up to Four Joinpoints, Both Sexes by Race/Ethnicity. Available at: https://seer.cancer.gov/archive/csr/1975_2015/results_merged/sect_26_thyroid.pdf.
[3] Busaidy NL, Cabanillas ME. Differentiated thyroid cancer: management of patients with radioiodine nonresponsive disease. J Thyroid Res. 2012;2012:618985.
[4] Weber T, Schilling T, Büchler MW. Thyroid carcinoma. Curr Opin Oncol 2006;18:30–5.
[5] Fagin JA, Wells SAJ. Biologic and clinical perspectives on thyroid cancer. N Engl J Med 2016;375:1054–67.
[6] Schlumberger M, Tahara M, Wirth LJ, et al. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. N Engl J Med 2015;372:621–30.
[7] Brose MS, Nutting CM, Jarzab B, et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a

randomised, double-blind, phase 3 trial. Lancet (London, England) 2014;384:319–28.

[8] Haugen BR. Management of the patient with progressive radioiodine non-responsive disease. Semin Surg Oncol 1999;16:34–41.

[9] Gottlieb JA, Hill CS. Chemotherapy of thyroid cancer with adriamycin. N Engl J Med 1974;290:193–7.

[10] Chakravarty D, Santos E, Ryder M, et al. Small-molecule MAPK inhibitors restore radioiodine incorporation in mouse thyroid cancers with conditional BRAF activation. J Clin Invest 2011;121:4700–11.

[11] Xing M, Westra WH, Tufano RP, et al. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metab 2005;90:6373–9.

[12] Nikiforova M, Lynch R, Biddinger P, et al. RAS point mutations and PAX8-PPAR(Rearrangement in Thyroid Tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab 2003;88:2318–26.

[13] Landa I, Ganly I, Chan TA, et al. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab 2013;98:E1562–6.

[14] Chen H, Luthra R, Routbort MJ, et al. Molecular profile of advanced thyroid carcinomas by next-generation sequencing: characterizing tumors beyond diagnosis for targeted therapy. Mol Cancer Ther 2018;17:1575–84.

[15] Lu Z, Zhang Y, Feng D, et al. Targeted next generation sequencing identifies somatic mutations and gene fusions in papillary thyroid carcinoma. Oncotarget 2017;8:45784–92.

[16] Landa I, Ibrahimpasic T, Boucai L, et al. Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. J Clin Invest 2016;126:1052–66.

[17] Smallridge RC, Ain KB, Asa SL, et al. American Thyroid Association guidelines for management of patients with anaplastic thyroid cancer. Thyroid 2012;22:1104–39.

[18] Guo L-C, Zhu W-D, Ma X-Y, et al. Mutations of genes including DNMT3A detected by next-generation sequencing in thyroid cancer. Cancer Biol Ther 2019;20:240–6.

[19] Duan H, Li Y, Hu P, et al. Mutational profiling of poorly differentiated and anaplastic thyroid carcinoma by the use of targeted next-generation sequencing. Histopathology 2019;75:890–9.

[20] Hagemann IS, Devarakonda S, Lockwood CM, et al. Clinical next-generation sequencing in patients with non-small cell lung cancer. Cancer 2015;121:631–9.

[21] 4460 - Clinical utility of comprehensive cell-free DNA (cfDNA) analysis to identify genomic biomarkers in newly diagnosed metastatic non-small cell lung cancer (mNSCLC). Available at: https://www.abstractsonline.com/pp8/#!/6812/presentation/4770.

[22] Moore A, Bar Y, Maurice-Dror C, et al. Next-generation sequencing in salivary gland carcinoma: targetable alterations lead to a therapeutic advantage-Multicenter experience. Head Neck 2020;42:599–607.

[23] Brose MS, Cabanillas ME, Cohen EEW, et al. Vemurafenib in patients with BRAF(V600E)-positive metastatic or unresectable papillary thyroid cancer refractory to radioactive iodine: a non-randomised, multicentre, open-label, phase 2 trial. Lancet Oncol 2016;17:1272–82.

[24] Ofir Dovrat T, Sokol E, Frampton G, et al. Unusually long-term responses to vemurafenib in BRAF V600E mutated colon and thyroid cancers followed by the development of rare RAS activating mutations. Cancer Biol Ther 2018;19:871–4.

[25] Ascierto PA, McArthur GA, Dréno B, et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. Lancet Oncol 2016;17:1248–60.

[26] Long GV, Stroyakovskiy D, Gogas H, et al. Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. Lancet (London, England) 2015;386:444–51.

[27] 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;36:7–13.

[28] Wang JR, Zafereo ME, Dadu R, et al. Complete surgical resection following neoadjuvant dabrafenib plus trametinib in BRAF(V600E)-mutated anaplastic thyroid carcinoma. Thyroid 2019;29:1036–43.

[29] Drilon A, Laetsch TW, Kummar S, et al. Efficacy of larotrectinib in TRK fusion–positive cancers in adults and children. N Engl J Med 2018;378:731–9.

[30] Brose MS, Albert CM, Waguespack SG, et al. Activity of larotrectinib in patients with advanced TRK fusion thyroid cancer. Thyroid 2018;28 (Suppl 1):A4–14.

[31] Mendoza L. Clinical development of RET inhibitors in RET-rearranged non-small cell lung cancer: update. Oncol Rev 2018;12:352.

[32] Ross JS, Gay LM, Wang K, et al. Comprehensive genomic profiles of metastatic and relapsed salivary gland carcinomas are associated with tumor type and reveal new routes to targeted therapies. Ann Oncol 2017;28:2539–46.

[33] Wang K, Russell JS, McDermott JD, et al. Profiling of 149 salivary duct carcinomas, carcinoma ex pleomorphic adenomas, and adenocarcinomas, not otherwise specified reveals actionable genomic alterations. Clin Cancer Res 2016;22:6061–8.

[34] FDA approves selpercatinib for lung and thyroid cancers with RET gene mutations or fusions. Available at: https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-selpercatinib-lung-and-thyroid-cancers-ret-gene-mutations-or-fusions.

[35] Godbert Y, Henriques de Figueiredo B, Bonichon F, et al. Remarkable response to crizotinib in woman with anaplastic lymphoma kinase-rearranged anaplastic thyroid carcinoma. J Clin Oncol 2015;33:e84–7.

[36] COSMIC; BRCA A, p.R2787H. Available at: https://cancer.sanger.ac.uk/cosmic/mutation/overview?id=171366772.

[37] Catalogue Of Somatic Mutations In Cancer (COSMIC). Available at: https://cancer.sanger.ac.uk/.

[38] Mersch J, Jackson MA, Park M, et al. Cancers associated with BRCA1 and BRCA2 mutations other than breast and ovarian. Cancer 2015;121:269–75.

[39] Xu L, Doan PC, Wei Q, et al. Association of BRCA1 functional single nucleotide polymorphisms with risk of differentiated thyroid carcinoma. Thyroid 2012;22:35–43.

[40] Shih HA, Nathanson KL, Seal S, et al. BRCA1 and BRCA2 mutations in breast cancer families with multiple primary cancers. Clin Cancer Res 2000;6:4259–64.

[41] Tung NM, Robson ME, Ventz S, et al. TBCRC 048: a phase II study of olaparib monotherapy in metastatic breast cancer patients with germline or somatic mutations in DNA damage response (DDR) pathway genes (Olaparib Expanded). J Clin Oncol 2020;38(15_suppl):1002.

[42] Park HS, Jung C-K, Lee SH, et al. Notch1 receptor as a marker of lymph node metastases in papillary thyroid cancer. Cancer Sci 2012;103:305–9.

[43] Even C, Lassen U, Merchan J, et al. Safety and clinical activity of the Notch inhibitor, crenigacestat (LY3039478), in an open-label phase I trial expansion cohort of advanced or metastatic adenoid cystic carcinoma. Invest New Drugs 2020;38:402–9.

[44] Gavai AV, Quesnelle C, Norris D, et al. Discovery of clinical candidate BMS-906024: a potent Pan-Notch inhibitor for the treatment of leukemia and solid tumors. ACS Med Chem Lett 2015;6:523–7.

[45] Wu CX, Xu A, Zhang CC, et al. Notch inhibitor PF-03084014 inhibits hepatocellular carcinoma growth and metastasis via suppression of cancer stemness due to reduced activation of Notch1-Stat3. Mol Cancer Ther 2017;16:1531–43.

[46] Knoechel B, Bhatt A, Pan L, et al. Complete hematologic response of early T-cell progenitor acute lymphoblastic leukemia to the (-secretase inhibitor BMS-906024: genetic and epigenetic findings in an outlier case. Cold Spring Harb Mol case Stud 2015;1:a000539.

[47] Mehnert JM, Varga A, Brose M, et al. Pembrolizumab for advanced papillary or follicular thyroid cancer: preliminary results from the phase 1b KEYNOTE-028 study. J Clin Oncol 2016;34(15_suppl):6091.

[48] Capdevila J, Wirth LJ, Ernst T, et al. PD-1 blockade in anaplastic thyroid carcinoma. J Clin Oncol 2020;38:2620–7.

[49] Dierks C, Miething C, Thomusch O, et al. Lenvatinib and pembrolizumab as save and effective combination treatment in 8 patients with metastasized anaplastic (ATC) or poorly differentiated thyroid carcinoma (PDTC). Ann Oncol 2018;29:viii646.

[50] Dummer R, Schadendorf D, Ascierto PA, et al. Binimetinib versus dacarbazine in patients with advanced NRAS-mutant melanoma (NEMO): a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol 2017;18:435–45.

[51] Yin CQ, Zhu B, Zhang T, et al. Pharmacological targeting of STK19 inhibits oncogenic NRAS-driven melanomagenesis. Cancer Discov 2019;9:OF8L

[52] A phase II study of trametinib to enhance radioactive iodine (RAI) uptake in patients with RAI-resistant recurrent or metastatic thyroid cancer. Available at: https://www.mskcc.org/cancer-care/clinical-trials/13-157.

[53] A study to investigate the efficacy and safety of cobimetinib plus atezolizumab in participants with solid tumors. Available at: https://clinicaltrials.gov/ct2/show/NCT03264066.

[54] Geva S, Rozenblum AB, Roisman LC, et al. 145P Impact of next-generation sequencing on survival in lung cancer. J Thorac Oncol 2018;13:S86–7.

[55] Schwaederle M, Zhao M, Lee JJ, et al. Impact of precision medicine in diverse cancers: a meta-analysis of phase II clinical trials. J Clin Oncol 2015;33:3817–25.

[56] Le Tourneau C, Delord J-P, Goncalves A, et al. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. Lancet Oncol 2015;16:1324–34.

[57] Hainsworth JD, Meric-Bernstam F, Swanton C, et al. Targeted therapy for advanced solid tumors on the basis of molecular profiles: results from MyPathway, an open-label, phase IIa Multiple Basket Study. J Clin Oncol 2018;36:536–42.

[58] Targeting specific genomic mutation in breast cancer improves outcomes, first study shows [ESMO 2018 Press Release]. Available at: https://www.esmo.org/Press-Office/Press-Releases/SOLAR-aplelisib-fulvestrant-breast-cancer-Andre.