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

A multicenter experience

Assaf Moore, MD\textsuperscript{a,b}, Yael Bar, MD, PhD\textsuperscript{b,c}, Corinne Maurice-Dror, MD\textsuperscript{d,e}, Inbar Finkel, MD\textsuperscript{a}, Hadar Goldvaser, MD\textsuperscript{b,g}, Elizabeth Dudnik, MD\textsuperscript{a,b}, Daniel A. Goldstein, MD\textsuperscript{a,b}, Noa Gordon, MSc, MPH\textsuperscript{d}, Salem Billan, MD\textsuperscript{d,e}, Orit Gutfeld, MD\textsuperscript{b,c}, Ido Wolf, MD\textsuperscript{b,c}, Aron Popovtzer, MD\textsuperscript{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 (CI\%95) 9.8–66.6] and 2 months (CI\%95 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 (CI\%95 89.7–111.4) months and was 77.8 months (CI\%95 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, PDCD = 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 = Swi/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

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\textsuperscript{a} Institute of Oncology, Davidoff Center, Rabin Medical Center – Bellinson Hospital, Petach Tikva, \textsuperscript{b} Sackler Faculty of Medicine, Tel Aviv University, \textsuperscript{c} Oncology Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, \textsuperscript{d} Institute of Oncology, Rambam Health Care Campus, \textsuperscript{e} Ruth & Bruce Reppaport, Faculty of Medicine, Technion Israel Institute of Technology, Haifa, \textsuperscript{f} Oncology Institute, Sheba Zedek Medical Center, \textsuperscript{g} The Hebrew University Faculty of Medicine, Jerusalem, Israel.

\textsuperscript{*} Correspondence: Aron Popovtzer, Davidoff Cancer Center, Rabin Medical Center – Bellinson Hospital, 39 Jabotinski St., Petach Tikva 491492, Israel (e-mail: aronpopovzer@yahoo.com).

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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. 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). The vast majority of patients with differentiated thyroid cancers are cured with surgery, radioiodine (RAI) when indicated, and thyroid hormone suppression. Although prognosis is excellent overall, about 5% of patients will develop distant metastases and become refractory to RAI. Some patients can have an indolent course; however, metastatic RAI-refractory thyroid cancer (IRTC) has an overall poor prognosis. Repeated RAI therapy is not recommended, as it does not result in clinical benefit and is associated with various adverse effects. Response rates (RRs) to cytotoxic chemotherapies have largely been low. Sorafenib was found to have a significantly longer median progression-free survival (PFS) compared with placebo. 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. These are currently the only approved systemic agents in the setting of IRTC. 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. 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%). BRAF-mutated PTC has a more aggressive course, a poor response to RAI, and a poorer prognosis. 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. 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. 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.

PDTC make up approximately 6% of thyroid cancers, and have a more aggressive clinical course and worse outcomes. Responses to RAI are minimal. Systemic treatment options are similar to those used in differentiated thyroid cancers. ATCs are RAI-refractory and have a very grim prognosis. Cytotoxic chemotherapy can be used in unresectable or metastatic disease. 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. A study of NGS found that the DNMT3A mutation was significantly enriched in PDTC and ATC, and may be associated with short life expectancy. 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.

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, clinicopathological characteristics, treatment details, and survival outcomes were collected from electronic databases and medical records. NGS was performed at the discretion of the treating physician, 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 tumoral 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 30 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.[3,23] 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 (ERCI-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
| Pt. no. | Histology | Genetic alterations | MSI status | TMB |
|---------|-----------|---------------------|------------|-----|
| 1       | PTC       | NRAS Q61R, TERT promoter -124C>T | CSF1R (E920D), RAD51L (S318R), ROS1 (L691S), TNFAP3 (T647P), ARD1A (N1313S), CSF1R (K707Q), JAK3 (V217M), MLL2 (T1246M) | Stable | Low; 3 Muts/Mb |
| 2       | PTC       | BRAF V600E | - | N/A | N/A |
| 3       | PTC       | BRAF V600E | NOTCH2 (S2115G), TERT promoter -124C>T | AXL (T637I), ERBB3 (T696A, G111 (Y593C), GNAS (P239R), MLH1 (P603R), MLL2 (K484B), SMO (R398* V54M), SNCAP (SNCAP) | Stable | Low; 2 Muts/Mb |
| 4       | PTC       | RET CDDO-RET fusion | EGR L833R, TERT promoter -124C>T | FGRF1 (E338*), IRS2 (A699_A701del), MED12 (Q2119 Q2120insHQQQ, Q2120H), MYC (S362F), NF1 (T1295A), TAFI (R1660Q) | Stable | Low; 5 Muts/Mb |
| 5       | PTC       | NRAS (Q61R) | RBM10 (R680*), TERT promoter -124C>T | BRCA2 (P655R), HSD3B1 (E60G), IRS2 (N28 H29insN), MED12 (Q2197 Y2077insQ and Q2119 Q2120insHQQQ), PDGFRB (V316M), SETD2 (G2449M), TSC1 (T360N), TSC2 (S1774G) | Stable | Intermediate; 8 Muts/Mb |
| 6       | PTC       | BRAF V600E | TERT promoter -124C>T | ARFRP1 (S143L), BRAF (A33 A34insGA), CDK12 (R1333H), EGF (D1084H), FANCA (V372F), GATA6 (I333del), NOTCH2 (G2449M), SETD2 (M737T) | Stable | Low; 5 Muts/Mb |
| 7       | PTC       | BRAF V600E, NOTCH1 (H1564P) | ATM (E343fs*), BCCOR (V379fs*62 - subclonal), PB1M1 (S1518s*73), TERT promoter -146C>T | ATM (S99Q), ATRX (E1464del), CALR (V57C), FAM123B (E385 E387del), GATA6 (I333del), NOTCH2 (G2449M), SETD2 (M737T) | Stable | Low; 0 Muts/Mb |
| 8       | PTC       | - | PTEN (G132fs*5), HGF amplification, BAP1 (A419fs*3), SETD2 (S2470fs*14), TPS3 (I193R) | FANCDD2 (Q269H), FLT4 (E955Q), GRM3 amplification, KDM6A (S114C), MAG2 amplification, NXX2-1 (R100Q), NOTCH3 (R1875Q), NUPT3 (S63M), PRSS8 (R25W), SETD2 (I1398T) | Stable | Low; 3 Muts/Mb |
| 9       | PTC       | BRAF V 600E | CDKNA2A, CDKNA2C, RB1, TERT | GNAS (A272V) | N/A | N/A |
| 10      | FTC       | RET NCOA4-RET fusion, BRCA2 R2787H, NOTCH1 A1640T | CDKNA2A/P16NKN4a R58* and p14ARF P72L, TERT promoter -124C>T, WHSC1 (MMSET) E1099K | Stable | Low; 2 Muts/Mb |
| 11      | FTC       | - | APC rearrangement exon 16, TERT promoter -124C>T | C110RF30 (H889R), CREBBP (A1907T), EPH4 (E827D), KIPL6 (V39F), MED12 (Q2119 Q2120insHQQQ), NOTCH3 (P2201A), RNF43 (V627A), TET2 (Q317H) | Stable | Low; 1 Muts/Mb |
| 12      | FTC       | - | HRAS G16R, BCR/1 S936fs*10, TERT promoter-124C>T | C110RF30 (H889R), CREBBP (A1907T), EPH4 (E827D), KIPL6 (V39F), MED12 (Q2119 Q2120insHQQQ), NOTCH3 (P2201A), RNF43 (V627A), TET2 (Q317H) | Stable | Low; 5 Muts/Mb |
| 13      | FTC       | NRAS Q61K | RBM10 W756fs*37 | ARD1B (G2465), BRCA2 (P655R), C11or50 (A986G), CREBBP (R742P), DMNT3A (L411P), IDH1 (F32V), INPP4B (V594A), PBRM1 (P1476S), PDGFRB (V316M), POLE (P303S), RBM10 (W756), SDHA amplification, TSC2 (F1510del) | Stable | Low; 5 Muts/Mb |
| 14      | PDTC      | NRAS Q61R | TERT promoter -124C>T | ALX (K412C), APC (N1118D), ARAF (Q562fs*), CALR (Y57C), ETF4 rearrangement, FANCN (V600), | Stable | Low; 1 Muts/Mb |

(continued)
Whether BRCA mutations confer a higher risk for thyroid cancer and whether these cancers have different prognoses remains undetermined. 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. This patient has been treated with lenvatinib since October 2018. Response is ongoing.

Table 1 (continued).

| Pt. no. | Histology    | With potential therapeutic implications | Without therapeutic implications | Variants of uncertain significance | MSI status | TMB |
|---------|--------------|------------------------------------------|----------------------------------|-----------------------------------|------------|-----|
| 15      | PTC          | -                                        | PTEN splice site 209+2T>G        | MKNK1 (R93Q), NOTCH1 (N104S), NTRK1 (S190R), PDK1 (N176R), TGFBR2 (S535T) | N/A        |     |
| 16      | PTC          | ALK rearrangement intron 15              | TERT promoter -124C>T          | APC (G203E and R283Q), CUL4A (T246S), FAM12B (T625A), PAPR2 (E412K), POLE (P370T), SPEN (C766Y), SYK (S94K), TET2 (H188I), VHL (Y112R) | 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 ERCC1-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 (T205S), MAP3K1 (S939C), MSH3 (L911W), MTR (I637V), P2RY8 (V358A), PRDM1 amplification, PTPN11 (300V), TBX3 (S453L), TGFBR2 amplification | Stable Low; 1 Muts/Mb |     |
| 22      | ATC          | NRAS (Q61R)                              | NF2 (S87*), BRD4 (E1113del), C89N2A (p16INK4a D108Y and p1A4AF R122L EP300 G965*), MAP3K1 (S924*), TERT promoter -124C>T, TP53 (E285K) | AR (E898D), ARID1B (A767T), ARID2 amplification, ASXL1 (M168L), P1259L, ATM (L2307F), BRC2 (H665R), EP300 (P955T), ERBB4 (S522L), FAT1 (A363T), GATA2 (P161T), MDM2 (E296Q), E421D), MLL2 (P3665A), MYST3 (M482K), PAK3 (M121T), POGFRB (Q31*), TSC1 (T360N) | Stable Intermediate; 7 Muts/Mb |     |

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

under 1% of thyroid cancers. Whether BRCA mutations confer a higher risk for thyroid cancer and whether these cancers have different prognoses remains undetermined. 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. 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 oncogenic 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. Specific and pan-NOTCH inhibitors have been studied and are being developed in solid and hematologic malignancies. To the best of our knowledge, there are no data on the use of NOTCH inhibitors in thyroid cancers.
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.

### 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.

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![Figure 1. Swimmer plot. NGS-guided treatment. PD = progressive disease, PR = partial response.](image-url)
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 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.
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.

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