Systematic population-based identification of NTRK and RET fusion-positive thyroid cancers

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Abstract

Objective: The aim of the study was to identify patients with NTRK fusion-positive or RET fusion/mutation-positive thyroid cancers, who could benefit from neurotrophic tyrosine kinase receptor (NTRK) or receptor tyrosine kinase (RET) inhibitors.

Methods: Patients were identified in the Calgary prospective thyroid cancer database (N = 482). Patients were 'pre-screened' with clinically available MassARRAY® BRAF test, Colon Panel, Melanoma Panel, or ThyroSPEC™. Mutation-negative tumors were 'screened' for NTRK fusions and RET fusions/mutations with the Oncomine™ Comprehensive Assay v3 (OCAv3).

Results: A total of 86 patients were included in 1 of 2 separate analyses. Analysis A included 42 patients with radioactive iodine (RAI)-resistant distant metastases. After pre-screening, 20 BRAF and RAS mutation-negative patients underwent OCAv3 screening, resulting in the detection of 4 patients with NTRK fusions and 4 patients with RET fusions (8/20, 40% of analyzed patients). Analysis B included 44 patients, 42 with American Thyroid Association (ATA) high and intermediate risk of recurrence and 2 with medullary thyroid carcinoma. During pre-screening, 1 patient with an NTRK fusion, 1 patient with a RET fusion, and 30 patients with BRAF mutations were identified. The remaining 9 patients received OCAv3 screening, resulting in detection of 1 patient with an NTRK fusion and 1 with a RET fusion (4/11, 36% of analyzed patients).

Conclusions: Our findings indicate a higher rate of NTRK fusions and RET fusions in patients with thyroid cancer with RAI-resistant distant metastases and ATA high or intermediate risk of recurrence. This highlights the importance of early screening to enable intervention with a NTRK or RET inhibitor.
Introduction

The neurotrophic tyrosine kinase receptor (NTRK) inhibitors, larotrectinib and entrectinib, were approved by the United States Food & Drug Administration (FDA) for the treatment of NTRK fusion-positive solid tumors that are metastatic, where surgery would result in severe morbidity, or where no satisfactory alternative therapy is available. The receptor tyrosine kinase (RET) inhibitors, selpercatinib and pralsetinib, were approved by the FDA for advanced or metastatic RET mutation-positive medullary thyroid cancer (MTC) or RET gene fusion-positive thyroid cancer. The early approval of these drugs was based on phase 1/2 data (1, 2, 3, 4, 5). Patients with thyroid cancer were one of the larger patient groups in the larotrectinib basket trial, and the selpercatinib study was conducted entirely in patients with thyroid cancer (1, 3, 4, 5). Five patients with NTRK fusion-positive thyroid cancer were included in the entrectinib phase 1/2 trials and one had a response (6). Both larotrectinib and selpercatinib showed marked and durable overall response rates (1, 3, 4, 5). For the first time for thyroid cancer, complete response was demonstrated in 7% of the patients with NTRK fusion-positive, larotrectinib-treated, metastatic or locally advanced thyroid cancer, 9% of the patients with previously treated, RET mutation-positive, selpercatinib-treated MTC, and 5% of the patients with previously treated RET gene fusion-positive, selpercatinib-treated thyroid cancer (5, 7). Only 2% of selpercatinib- and larotrectinib-treated patients and 4% of entrectinib-treated patients discontinued the treatment because of treatment-related adverse events (4, 5, 6). This compares favorably with vandetanib, cabozantinib, or lenvatinib that showed drug discontinuation rates of 21, 8, and 20%, respectively (8). Further, TRK and RET inhibitors are currently being tested in clinical trials (4, 9). The very specific and selective activity profile of these drugs requires stratification of patients for these therapies based on detection of NTRK gene fusions and RET gene fusions/mutations, respectively.

The prevalence of NTRK fusion and RET fusion has been reported in several prior studies. In The Cancer Genome Atlas (TCGA) study, NTRK fusions and RET fusions were found in 1.2 and 6.8% of mostly non-metastatic thyroid cancers (10). Across 13 TCGA-analyzed tumor types, thyroid cancer displayed the highest percentage of tumors with druggable kinase fusions at 8.7% (11). In a Massachusetts General Hospital series of primary thyroid carcinoma, NTRK fusions were found in 3.1% of cases and 6/11 patients with NTRK fusions had distant metastases (12). In a series of papillary thyroid carcinomas (PTCs), NTRK fusions were found in 12.6% of patients and RET gene fusions in 14.3% (13).

The prevalence of fusions increases for metastatic/advanced patients’ WT of other aberrations. In radioactive iodine (RAI)-resistant metastatic thyroid cancers without BRAF, NRAS, HRAS, or KRAS mutations, gene fusions were previously reported in 12% of 60 samples (4 NTRK fusions, 2 RET fusions, 1 ALK fusion) (14). In patients with BRAF mutation-negative PTC and distant metastases, 67% had ALK, NTRK, or RET fusions (15). In advanced metastatic MTC, the prevalence of RET mutations was reported as 90% (16).

There are several recent reviews of the pros and cons for different detection methods (17, 18) and for the treatment of kinase fusion-positive cancers (19). However, appropriate and cost-effective screening algorithms to identify and stratify patients with kinase fusion-positive cancers still need to be developed for specific cancer types. A Canadian consensus for adult patients recommends NTRK1-3 gene fusion testing at diagnosis in unresectable or metastatic/advanced patients with all thyroid histologies and at recurrence after surgery ± RAI treatment if not already performed (20). However, since resensitization for RAI treatment (21) and treatment with the RET inhibitor, selpercatinib (5), are additional options for these patients, integrated molecular stratification strategies are required that allow stratification for all currently available treatment options.

We used a stepwise, recurrence risk-adapted, prioritization approach. This approach had a stepwise application of molecular screening tests, with increasing spectrum of mutation coverage to identify patients with thyroid cancer who could benefit from these new, highly selective, and effective drugs with low adverse event rates. We report the results of this approach in a systematic, integrated, population-based study of patients with thyroid cancer. Our aim was to identify patients with NTRK fusion-positive thyroid cancer and RET fusion/mutation-positive thyroid cancer who can benefit from treatment with NTRK and RET inhibitors.

Materials and methods

Alberta Health Services is a comprehensive, integrated, single-payer healthcare system with centralized laboratory, pathology, surgery, endocrinology, and oncology services. It has a single electronic medical record system for over 1.5 million inhabitants of the Calgary and Southern Alberta Healthcare regions. The patients for the NTRK fusion and RET fusion/mutation screening were identified in the Calgary
prospective thyroid cancer database, which contains ethics approved research-consented patients with a new diagnosis of thyroid cancer in the Calgary and Southern Alberta Healthcare regions since April 2017. As of December 2020, the Calgary prospective thyroid cancer database contained 482 patients, reflecting a consenting rate of 55.9% of 862 patients with thyroid cancer since April 2017 with American Thyroid Association (ATA) high (n = 87), ATA intermediate (n = 126), and ATA low (n = 256) recurrence risk and non-invasive follicular thyroid neoplasm with papillary-like nuclear features or MTC patients (n = 13) (230 new patients/year). All new patients with thyroid cancer in the Calgary and Southern Alberta Healthcare regions are prospectively assessed for their ATA recurrence risk, TNM (tumor-nodes-metastases)/MACIS (distant metastasis, patient age, completeness of resection, local invasion, and tumor size) staging, and their indication for RAI treatment, by the thyroid cancer triage group of the University of Calgary, Division of Endocrinology. The study was approved by the University of Calgary’s ethical committee. Consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used.

A total of 86 patients were included in 1 of 2 separate analyses we will refer to as Analysis A and Analysis B. Analysis A focused on the primary tumors of 42 patients with RAI-resistant distant metastases, identified in June 2020 (Fig. 1). These 42 patients comprise all patients with RAI-resistant distant metastases in the Calgary and Southern Alberta Healthcare regions diagnosed and either treated with Lenvatinib or observed for evidence of progression in June 2020 by the 2 medical oncologists and 2 endocrinologists treating patients with RAI-resistant distant metastases in the Calgary and Southern Alberta Healthcare regions. Sixteen patients with RAI-resistant distant metastases who were diagnosed with thyroid cancer since April 2017 were covered by the thyroid cancer database. Further 3 patients who were not consented until June 2020 and 23 patients who were diagnosed with RAI-resistant distant metastases in the Calgary and Southern Alberta Healthcare regions before April 2017 were identified by capturing all patients with thyroid cancer treated by the 2 medical oncologists and 2 endocrinologists treating patients with RAI-resistant distant metastases in the Calgary and Southern Alberta Healthcare regions.

Sixteen of the 42 patients were on treatment with lenvatinib. One patient in whom lenvatinib was discontinued was subsequently identified as NTRK fusion-positive. To enrich the Oncomine® Comprehensive Assay v3 (OCAv3) screening for NTRK fusions and RET fusions/mutations, the primary tumors were first ‘pre-screened’ with the successively clinically available MassARRAY® tests, BRAF test, Colon Panel, Melanoma Panel, or ThyroSPEC™ test, with increasing the mutation coverage respectively in addition to BRAF to identify BRAF mutation-negative patients. The mutation spectrum analyzed by these MassARRAY tests in addition to BRAF is given in Supplementary Table 1 (see section on supplementary materials given at the end of this article).

Analysis B targeted the primary tumors of separate 44 patients out of the 482 patients in the Calgary...
prospective thyroid cancer database (Fig. 1). These 44 patients included 39/87 ATA high and 3/126 ATA intermediate recurrence risk patients whose primary tumors had previously been investigated with one of the MassARRAY tests. Performance of MassARRAY for the ATA intermediate- and ATA high-risk patients was dependant on the assessment by the six members of the thyroid cancer triage group of the University of Calgary, Division of Endocrinology treating nearly all patients with thyroid cancer in the Calgary and Southern Alberta Healthcare regions. The criteria for the use of MassARRAY assessment evolved based on the progressive availability of the four MassArray tests described in Supplementary Table 1, case discussions during monthly interdisciplinary thyroid cancer rounds and thyroid cancer treatment pathway evolutions and updates discussed during the meetings of the interdisciplinary provincial endocrine tumor team.

In addition, 2 patients with metastatic MTC were included in these 44 patients.

**Oncomine comprehensive assay analysis**

DNA and RNA were extracted from formalin-fixed paraffin-embedded (FFPE) tissues using the RecoverAll Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific) according to the manufacturer’s protocol. DNA and RNA were quantified using Qubit™ DNA HS assay and Qubit RNA HS assay, respectively. Samples were sequenced using the OCAv3 Chef kit (Thermo Fisher Scientific). Briefly, amplicon libraries were prepared on the Ion Chef instrument using 20 ng DNA input and 22 ng RNA input. RNA was reverse transcribed using SuperScript VILO IV (Thermo Fisher Scientific) according to the manufacturer’s protocol. DNA and RNA fusion libraries were combined at an 80:20 ratio (DNA:RNA) and then diluted to a final concentration of 50 pM. Sample libraries were then templated and amplified on ion sphere particles and loaded on to 540 chips using the Ion S40 Chef Kit. Each 540 chip multiplexed seven samples and one no template control. Samples were sequenced on the Ion S5 XL sequencer. Data were analyzed using Ion Torrent Suite software v5.14, and variant calling was performed with Ion Reporter v5.16 Oncomine comprehensive v3 workflow 4.1. All variant calls were manually reviewed using Integrated Genomics Viewer.

**BRAF, Colon Panel, Melanoma Panel, and ThyroSPEC assay analysis**

DNA and RNA were extracted from FFPE tissues using the AllPrep DNA/RNA FFPE kit (Qiagen), and RNA was reverse transcribed using Qiagen’s miScript II kit. Mutation and fusion profiling was performed with Agena Bioscience (San Diego, CA, USA) using iPLEX® Pro (ThyroSPEC, Calgary, Alberta, Canada), iPLEX® HS Colon, or iPLEX® HS Melanoma chemistry on the Agena Bioscience MassARRAY system, with all variant calls reviewed manually using Typer software. All kits were used according to the manufacturer's protocol.

The genotyping process was performed after PCR amplification using a single-base extension technique and mass spectrometry on the MassARRAY instrument. The sensitivity of these assays is 5%, although a conservative 10% minimum frequency threshold is used for ThyroSPEC.


**Results**

Analysis A included 42 thyroid cancer patients with RAI-resistant distant metastases. Pre-screening of these 42 patients led to exclusion of 22 patients from further OCAv3 analysis (Fig. 1). Of these 22 patients, 18 primary tumors were BRAF mutation-positive, 1 had an HRAS mutation and a TERT mutation, and 3 patients were excluded because of clinical reasons (Fig. 1 and Supplementary Table 2).

This pre-screening led to the identification of 20 BRAF mutation-negative patients with RAI-resistant distant metastases (Fig. 1 and Supplementary Table 3) who were further analyzed using the OCAv3 panel. We detected 4 RET gene fusions (20%) and 4 NTRK fusions (20%) in the 20 BRAF mutation-negative patients with RAI-resistant distant metastasis in Analysis A (Table 1). Of the 42 patients with RAI-resistant distant metastases, actionable fusions were ultimately identified in 19% of patient tumors (Fig. 2).

Twelve of the 87 ATA high recurrence risk patients in the database had distant metastases and were included in the Analysis A of patients with RAI-resistant distant metastases. Comparison of the 42 pre-screened patients with ATA high recurrence risk with the 36 not pre-screened ATA high recurrence risk patients (87 ATA high-risk...
patients in database) – 12 (with distant metastases) – 39 (who were pre-screened) = 36 (ATA high risk recurrence, not pre-screened) showed no significant differences for the percentage of excellent response, and the percentages of indeterminate response, structural incomplete response, and biochemical incomplete response, or the mean and median follow-up time.

Analysis B included 44 patients distinct from those included in Analysis A; 42 of these patients were ATA high and intermediate recurrence risk and 2 of these patients had metastatic MTC. Thirty-five of the 44 patients were included in Analysis A; 42 of these patients were ATA high recurrence risk patients with indeterminate response to treatment, and 2 other patients with metastatic MTCs (Fig. 1 and Supplementary Table 4).

The primary tumors of the remaining nine patients in Analysis B were selected for further OCAv3 analysis (Supplementary Table 5).

Out of the nine patients who received OCAv3 screening in Analysis B, we detected one NTRK fusion (11%) and one RET fusion (11%) (Table 2). Of the 42 ATA high- and intermediate-risk patients and 2 patients with MTC, RET mutations, RET/PTC fusions, or NTRK fusions were ultimately detected in 14% (Fig. 3).

All the OCAv3-detected RET and NTRK fusions detected in tumors that were pre-screened with the successively clinically available MassARRAY® tests, BRAF test, Colon Panel, and Melanoma Panel test, are covered by the ThyroSPEC assay and were subsequently confirmed with the clinically validated ThyroSPEC assay after their detection by OCAv3 analysis. One fusion-positive OCAv3-analyzed patient with RAI-resistant distant metastases was found to be ThyroSPEC mutation-negative in the pre-screening of a >15-year-old FFPE sample. Repeat ThyroSPEC testing with a more recent sample confirmed the ETV6-NTRK3 fusion detected by OCAv3.

### Discussion

The two most important results of this systematic approach to identify patients with NTRK fusion and RET fusion-positive thyroid cancers can be summarized as follows:

1. For 20 BRAF mutation-negative patients, among 42 patients with RAI-resistant metastatic thyroid cancer, the OCAv3 analysis detected 4 NTRK fusions and 4 RET fusions. This 40% prevalence of NTRK fusion or RET fusion positivity for BRAF mutation-negative RAI-resistant metastatic thyroid cancers significantly contrasts with the previously reported 12% prevalence (4 NTRK fusions, 2 RET fusions, and 1 ALK fusion) for 60 BRAF mutation-negative patients (Pearson’s Chi-squared test, \( P = 0.013 \)).

### Table 1: Analysis A: Result of first OCAv3 analysis for 20 BRAF mutation-negative patients with RAI-resistant distant metastasis.

<table>
<thead>
<tr>
<th>OCAv3 test results</th>
<th>Gene (number of mutations) (histology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET fusions (n = 4)</td>
<td>RET fusion only (3)</td>
</tr>
<tr>
<td></td>
<td>CDC6-RET (dsPTC)</td>
</tr>
<tr>
<td></td>
<td>NCOA4-RET (PTC not specified)</td>
</tr>
<tr>
<td></td>
<td>NCOA4-RET (dsPTC)</td>
</tr>
<tr>
<td></td>
<td>NCOA4-RET &amp; TERT c.1-124C&gt;T (1) (PTC solid variant)</td>
</tr>
<tr>
<td>NTRK fusions (n = 4)</td>
<td>NTRK fusion only (3)</td>
</tr>
<tr>
<td></td>
<td>SQSTM1-NTRK1 (PTC classic)</td>
</tr>
<tr>
<td></td>
<td>EML4-NTRK3 (mixed histology (classic and follicular variant PTC))</td>
</tr>
<tr>
<td></td>
<td>ETV6-NTRK3 (PTC clear cell variant)</td>
</tr>
<tr>
<td>Other mutations (n = 11)</td>
<td>RAS only (2) (FTC minimally invasive, PTC tall cell variant)</td>
</tr>
<tr>
<td></td>
<td>RAS &amp; PTEN only (1) (PTC poorly differentiated)</td>
</tr>
<tr>
<td></td>
<td>TERT only (1) (PTC oncogenic variant)</td>
</tr>
<tr>
<td></td>
<td>PTEN &amp; TERT (1) (Hurlte cell carcinoma)</td>
</tr>
<tr>
<td></td>
<td>MAP2K1 &amp; TERT (1) (PTC not specified)</td>
</tr>
<tr>
<td></td>
<td>RAS &amp; PIK3CA (1) (FTC widened invasive)</td>
</tr>
<tr>
<td></td>
<td>PIK3CA &amp; EGFR &amp; RAS (1) (PTC follicular variant)</td>
</tr>
<tr>
<td></td>
<td>RAS &amp; AKT1 &amp; TERT (1) (PTC poorly differentiated)</td>
</tr>
<tr>
<td>No mutation (n = 1)</td>
<td>FTC widely invasive</td>
</tr>
</tbody>
</table>

*Oncomine Comprehensive Assay v3; **Diffuse sclerosing papillary thyroid cancer; ***Follicular thyroid cancer.
Figure 2
(A) Mutations and fusions detected in 42 patients with radioiodine-resistant distant metastasis. (B) Mutations and fusions detected in 42 American Thyroid Association (ATA) high and intermediate risk patients and 2 patients with medullary thyroid cancer (MTC).

Table 2
Analysis B: Result of OCAv3 analysis for nine patients with ATA high or intermediate recurrence risk and no BRAF mutation, RET mutation, NTRK fusion, or RET fusion in the pre-screening.

<table>
<thead>
<tr>
<th>RET fusion (n = 1)</th>
<th>RET fusion only (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTRK fusion (n = 1)</td>
<td>NTRK fusion only (1)</td>
</tr>
<tr>
<td>Other mutations (n = 3)</td>
<td>ATRX only (1) (PTC poorly differentiated)</td>
</tr>
<tr>
<td></td>
<td>MSH2 &amp; CDKN1B (1) (PTC poorly differentiated)</td>
</tr>
<tr>
<td></td>
<td>ATM &amp; TERT (1) (PTC poorly differentiated)</td>
</tr>
<tr>
<td>No mutation (n = 4)</td>
<td>Hurthle cell carcinoma (1)</td>
</tr>
<tr>
<td></td>
<td>PTC tall cell (1)</td>
</tr>
<tr>
<td></td>
<td>PTC classic (1)</td>
</tr>
<tr>
<td></td>
<td>PTC follicular variant (1)</td>
</tr>
</tbody>
</table>

*Oncomine Comprehensive Assay v3; °Diffuse sclerosing papillary thyroid cancer.
2. In 11 *BRAF* mutation-negative patients among 42 patients with ATA high and intermediate recurrence risk follicular thyroid cancers, the OCAv3 and ThyroSPEC analyses detected 2 *NTRK* fusions and 2 *RET* fusions (36%) (2 identified during pre-screening with ThyroSPEC and 2 identified during subsequent OCAv3 analysis).

The results of our systematic screening strategy suggest a much higher rate of *NTRK* fusions and *RET* fusions in our pre-selected patient groups than previously reported in the TCGA study with mostly non-metastatic thyroid cancers or for RAI-resistant metastatic thyroid cancers without *BRAF*, *NRAS*, *HRAS*, or *KRAS* mutations (10, 14). Our results align closer to the more recent series of patients from Lan and colleagues, where 67% of *BRAF* mutation-negative patients with PTC with distant metastases were found to have gene fusions (15).

Most important, these results suggest that all *BRAF* mutation-negative patients with RAI-resistant metastatic thyroid cancer should be screened for *NTRK* fusions and *RET* fusions to stratify these patients early for treatment with the NTRK and RET inhibitors larotrectinib, entrectinib, and selpercatinib, pralsetinib. If *NTRK* fusions and *RET* fusions are identified, these patients could benefit from higher and more sustained response rates and a much lower burden of adverse events than current standard of care.

Furthermore, this study demonstrates for the first time that all *BRAF* mutation-negative patients with progressive structural incomplete response to total thyroidectomy and RAI treatment and ATA high- or intermediate-recurrence risk patients with progressive structural incomplete response should be screened for *NTRK* fusions and *RET* fusions to enable early intervention. These patients require early stratification for cutting-edge treatment options, since 56–72 and 21–34% of patients with high or intermediate risk of recurrence patients, respectively, have persistent/recurrent structural disease a median of 4–10 years after total thyroidectomy and RAI (22, 23, 24, 25). Revision surgery and/or a second RAI treatment is largely unsuccessful and will only lead to remission in up to 51% of these patients (26, 27, 28).

Since all the OCAv3-detected *RET* fusions and *NTRK* fusions were subsequently confirmed with the ThyroSPEC Panel, future primary molecular stratifications of progressive RAI-resistant metastatic and ATA high recurrence risk thyroid cancers with structural incomplete response in the Alberta Healthcare regions will be performed with the ThyroSPEC Panel. Aberration-negative samples should still be further investigated with broader tests like OCAv3 or Archer...
FusionPlex Thyroid and Lung to account for the rarer fusions with DIAH1, EML4, IRE2BP2, NFASC, and PPL, which were NTRK partners reported in the larotrectinib phase 1 and 2 trials and ETV6, EML4, RBPMS, SQSTM1, TPM3, IRE2BP2, SQSTM1, and TPR detected in a recent NTRK rearrangement detection study that are not included in the ThyroSPEC Panel and CCDC186, ERC1, KTN1, and RUFY3, which were RET partners reported in the selpercatinib trial not included in the ThyroSPEC Panel (4, 5, 29, 30). This approach will stratify BRAF mutation-positive patients for re-expression of the iodine symporter with dabrafenib and trametinib to re-enable RAI treatment. Moreover, this approach will simultaneously identify most patients eligible for treatment with larotrectinib, entrectinib and selpercatinib, pralsetinib. Thus, very few patients will require analysis with more comprehensive mutation and gene fusion panels to provide access to currently available drugs (Fig. 3).

A recent international expert consensus recommends that all patients with advanced (unresectable or metastatic) solid tumors without actionable driver gene mutations/fusions/amplifications should be tested for NTRK fusions, especially ETV6-NTRK3 fusions in tumors with a high incidence of NTRK fusions (19). Also, patients with locally advanced tumors with a high incidence of NTRK fusions should be tested when considering neoadjuvant therapy before resection (19). This testing should be performed before or during the standard treatment of advanced solid tumors (19). Our results support these recommendations and add further detail to these recommendations for thyroid cancer. The Canadian consensus for adult patients recommends NTRK1-3 fusion testing at diagnosis in unresectable or metastatic/advanced patients with all thyroid histologies and at recurrence after surgery ± RAI if not already performed (20). While these recommendations focused on NTRK inhibitors, our integrated molecular strategy allows stratification for all currently available treatment options and includes resensitization for radiiodine treatment or treatment with the RET inhibitor selpercatinib (5, 21).

Patients with metastatic dsPTC (2 of 20 OCAv3-screened patients within Analysis B) show a very high yield for RET fusions (2/3, 66%). This is in line with a previous report by Joung and colleagues, which demonstrated for 37 cases of dsPTC, 17 were positive for RET/PTC1 (46%) and 6 for RET/PTC3 (16%) (31). The OCAv3 analysis also detected further mutations summarized in Table 3 with potential relevance for off-label therapy.

### Table 3 Mutations detected with OCAv3 with potential relevance for off-label drug use based on current clinical trials.

<table>
<thead>
<tr>
<th>Mutations</th>
<th>ClinVar interpretation</th>
<th>OncoKB interpretation</th>
<th>OncoKB therapeutic effect</th>
<th>Clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN R130L (27%)</td>
<td>Pathogenic</td>
<td>Oncogenic</td>
<td>Compelling biological evidence supports the biomarker as being predictive of response to a drug but neither biomarker nor drug is standard of care</td>
<td>NCT02465060</td>
</tr>
<tr>
<td>PIK3CA H1047R (23%)</td>
<td>Pathogenic</td>
<td>Oncogenic</td>
<td>FDA-recognized biomarker predictive of response to an FDA-approved drug in this indication (breast cancer: fulvestrant + alpelisib)</td>
<td>NCT02465060</td>
</tr>
<tr>
<td>EGFR P848L (52%)</td>
<td>Likely benign/unknown significance</td>
<td>Likely neutral</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MSH2 Q510* (73%)</td>
<td>Pathogenic</td>
<td>Unknown oncogenic effect</td>
<td>No FDA-approved or NCCN compendium listed treatments specifically for patients with thyroid cancer with MSH2-truncating mutations.</td>
<td>–</td>
</tr>
<tr>
<td>CDKN1B S7* (8%)</td>
<td>Uncertain significance</td>
<td>Unknown oncogenic effect</td>
<td>No FDA-approved or NCCN compendium listed treatments specifically for patients with CDKN1B S7 mutant thyroid cancer.</td>
<td>–</td>
</tr>
<tr>
<td>ATM S515* (85%)</td>
<td>Uncertain significance</td>
<td>Unknown oncogenic effect</td>
<td>No FDA-approved or NCCN compendium listed treatments specifically for patients with ATM S515 mutant thyroid cancer.</td>
<td>28 ongoing studies for patients with ATM-mutated solid tumors NCT03718091</td>
</tr>
<tr>
<td>ATRX E680* (42%)</td>
<td>No entry</td>
<td>Unknown oncogenic effect</td>
<td>No FDA-approved or NCCN compendium listed treatments specifically for patients with ATRX E680 mutant thyroid cancer.</td>
<td>–</td>
</tr>
</tbody>
</table>

*Oncomine Comprehensive Assay v3; \(^b\)Variant allele frequency; \(^c\)Food & Drug Administration; \(^d\)National Comprehensive Cancer Network.
drug use based on current clinical trials (clinicaltrials.gov) (32). The future treatment and follow-up of the respective patients and availability of new drugs will determine if patients with negative results for pre-screening tests like ThyroSPEC should undergo more extended mutation screening as currently done for lung cancers in the Lung Cancer Master Protocol (Lung-MAP) (33, 34) or multiple myeloma in the Bellini study (35) and other programs.

Possible limitations of this study include the lack of RNAseq as a comprehensive gold standard for rearrangement detection and the past report of concomitant RAS and BRAF or RET/PTC and BRAF mutations in 11 of 88 mostly advanced stage PTCs and in 14 of 72 conventional PTCs (36, 37). Similarly, Yoshihara and colleagues found targetable kinase mutations in 4.9% of patients with thyroid cancer and RET fusions, 1% of patients with thyroid cancer with NTRK1 fusions, and 1.8% of patients with thyroid cancer with NTRK3 fusions (11). Therefore, further data are required to decide if BRAF- and RAS-positive patients should be excluded in future screening strategies for RET/PTC or NTRK fusions. However, pre-screening with ThyroSPEC covering the most frequent BRAF, RAS, and RET point mutations, as well as the most frequent RET/PTC and NTRK fusions, may effectively address this question.

In summary, our results demonstrate a high frequency of NTRK fusions and RET fusions/mutations in patients with thyroid cancer with RAI-resistant distant metastases, ATA high, or ATA intermediate risk of recurrence, whose tumors are negative for other mutations/fusions. Our data emphasize the need for genomic testing in these patient populations to identify those who may benefit from effective and well-tolerated therapies, larotrectinib, entrectinib, and selpercatinib.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/ETJ-21-0061.

Declaration of interest
A B reports: Advisory board honoraria from AstraZeneca and Bayer. M H reports: Advisory board and teaching honoraria from Bayer. D R reports: Advisory board honoraria from Eisai, Ipsen, Novartis, Pfizer. R P reports: Grants from Bayer, and advisory board honoraria from Bayer and Eisai. The other authors have nothing to disclose.

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Author contribution statement
M E: study outline and organization, data collection, verification of underlying data and discussion of data, and writing of the manuscript. P S: data collection, verification of underlying data and discussion of data, and writing of the manuscript. J B M: performed the OCAv3 analysis. A B: responsible for melanoma and Colon Panel and ThyroSPEC analysis. M K: performed histology review. M H: performed histology review. K K: performed histology review. D R: contributed patients with metastatic thyroid cancer. J W: data collection, verification of underlying data, and statistical analysis. R P: study outline and organization, data collection, verification of underlying data and discussion of data, and writing of the manuscript.

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References


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Thyroid Society risk of recurrence classification systems. Thyroid 2013 23 1401–1407. (https://doi.org/10.1089/thy.2013.0011)

29 Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawak MA, Schlimburger M, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid 2016 26 1–133. (https://doi.org/10.1089/thy.2015.0020)


