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Comprehensive genomic profiling of clinically advanced medullary thyroid carcinoma

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Abstract

Objective—To determine the genomic alterations of cancer-related genes in advanced medullary thyroid carcinoma during the course of clinical care.

Methods—Hybrid-capture based comprehensive genomic profiling was performed on 34 consecutive medullary thyroid carcinoma cases to identify all four classes of genomic alterations, and outcome for an index patient was collected.

Results—*RET* was mutated in 88% (30/34) of cases, with *RET*M918T being responsible for 70% (21/30) of the *RET* alterations. The other *RET* alterations were *RET*E632_L633del, C634R, C620R, C618G/R/S, V804M, and *RET* amplification. Two of the four *RET* wild-type patients

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harbored mutations in *KRAS* or *HRAS* (1/34 each). The next most frequent genomic alterations were amplifications of *CCND1*, *FGF3*, *FGF19* and alterations in *CDKN2A* (3/34 each). One case with a *RET*M918T mutation developed acquired resistance to progressively dose-escalated vandetanib. When the mTOR inhibitor everolimus was added to continued vandetanib treatment, the patient achieved a second, 25% reduction of tumor volume (RECIST 1.1) for 8 months.

Conclusions—Comprehensive genomic profiling identified the full breadth of *RET* alterations in metastatic medullary thyroid carcinoma and possible co-operating oncogenic driver alterations. This approach may refine the use of targeted therapy for these patients.

Keywords

medullary thyroid carcinoma; RET; genomic profiling; vandetanib; cabozantinib; everolimus; precision medicine

Introduction

Patients with early-stage medullary thyroid carcinoma (MTC), a tumor derived from the neuroendocrine cells of the thyroid, have a favorable prognosis, as surgery is effective in this setting. For patients with metastatic or recurrent disease, however, the 10-year overall survival rate is only 40% regardless of whether the MTC arose in a familial setting or as a sporadic malignancy [1]. Chemotherapy and external beam radiotherapy have shown very little efficacy in patients with advanced MTC [2, 3]. Two targeted therapies are approved in the US and Europe to treat unresectable, locally advanced, or metastatic MTC, but have not been well linked to efficacy in the context of a particular genomic alteration (GA). Vandetanib (Caprelsa® 300 mg once daily) significantly prolonged median progression-free survival (PFS) compared with placebo (30.5 vs. 19.3 months) in a Phase 3 study of patients with advanced MTC [4]. Retrospective subgroup analysis of patients with sporadic MTC suggested a higher objective response rate (ORR) in *RET*M918T mutation-positive vs. –negative patients (54.5 vs. 30.2%), although *RET* status was unknown for a high fraction (45%) of patients. The approval of cabozantinib (Cometriq®) is based on a Phase 3 trial of patients with progressive metastatic MTC, in which cabozantinib (140 mg once daily) significantly improved median PFS compared with placebo (11.2 months vs 4.0 months) and achieved an ORR of 28% (0% for placebo) [5]. Responses were observed in patients both with and without any detectable *RET* mutations (ORR of 32% and 25%, respectively), while sporadic MTC with *RET*M918T had a numerically lower hazard ratio (HR) than *RET* M918T-negative cases in this study [5]. Median PFS on cabozantinib was longer for patients with *RET* mutation than for patients with wild-type *RET* (60 weeks vs. 25 weeks, $p = 0.0001$), and *RET*M918T associated with longer PFS than any other *RET* mutation (61 weeks vs. 36 weeks, $p = 0.009$), suggesting that *RET* mutation, which correlates with worse outcome [6], and *RET*M918T in particular confer a survival benefit from cabozantinib treatment [7]. The secondary endpoint of this Phase 3 study was improved overall survival (OS) and not met with median OS of 26.6 months for cabozantinib and 21.1 months for placebo (HR = 0.85, $p = 0.241$); however, patients with *RET*M918T mutations experienced significantly longer median OS with cabozantinib (44.3 months vs. 18.9 months with placebo, HR = 0.60, $p = 0.026$) [8].

Examining the results of these trials, the heterogeneity of responses suggests the possibility of *de novo* resistance to vandetanib or cabozantinib. The gatekeeper *RET*V804M/L mutation, present in 1-2% of MTC, has been characterized as resistant to vandetanib and less sensitive to cabozantinib [9, 11, 12]. Moreover, a Phase 2 study of vandetanib in *MEN2B*-associated MTC reported 3 patients who experienced disease progression after an initial decrease in tumor size, indicative of acquired resistance, despite high prevalence of the *RET* M918T driver mutation (>95%) in this population [13].

In this study, comprehensive genomic profiling (CGP) was performed in the course of clinical care on 34 advanced MTC cases to assess opportunities for benefit from targeted therapy. An index case (patient #1) with acquired resistance to vandetanib monotherapy derived clinical benefit from the addition of everolimus to the regimen.

Patients and Methods

CGP was performed in a CLIA-certified, CAP-accredited, NYS regulated reference laboratory (Foundation Medicine, Inc.). At least 50 ng of DNA per specimen was extracted from 34 clinical formalin-fixed paraffin-embedded consecutively submitted MTC tumor samples and next-generation sequencing was performed on hybridization-captured, adaptor ligation based libraries to high, uniform coverage (>500×) for all coding exons of 315 cancer-related genes and 28 genes commonly rearranged in cancer (n = 22), 236 cancer-related genes plus 19 genes frequently rearranged in cancer (n = 8), or 182 genes and 14 genes frequently rearranged in cancer (n = 4), with the different number of sequenced genes representing different generations of FoundationOne®.

Base substitutions, short insertions and deletions (indels), focal gene amplifications, homozygous deletions and select rearrangements, were determined and reported for each patient sample. To maximize mutation detection sensitivity in heterogeneous tumor biopsies and resections, the test was validated to detect base substitutions at 10% mutant allele frequency with 99% sensitivity and indels at 20% mutant allele frequency with 95% sensitivity, with a false discovery rate of <1% [14]. Actionable alterations are defined as those whose effect is targetable using anticancer drugs currently on the market or in registered clinical trials. Local site permissions to use clinical samples were obtained for this study.

A computational method was used to predict somatic versus germline *RET* variant status for each of the *RET*-mutant specimens without a matched normal control as previously described [15].

We reviewed the medical records of a patient (case #1) with sporadic MTC who presented to the Department of Investigational Cancer Therapeutics at The University of Texas MD Anderson Cancer Center after failing standard of care therapy. Treatment and consent on investigational trial (NCT01582191) as well as data collection were conducted in accordance with the guidelines of the University of Texas MD Anderson Cancer Center Institutional Review Board (IRB). Tumor response was determined using RECIST (version 1.1) by CT

scans obtained about every six to eight weeks. Clinical evaluation and assessments were performed per protocol.

Results

Thirty four consecutively submitted MTC cases had CGP performed in the course of clinical care (Table 1). The median age of the patients was 53 years (range 21-85); 71% (23/34) patients were male and 29% (10/34) were female. MTC generally shows a slight female preponderance [1, 16], and we do not have an explanation for the relatively high frequency of male cases in this unselected dataset. At the time of profiling, 33 MTC were Stage IV and one MTC was Stage II. CGP was performed on tumor specimens from the primary tumor in 47% (16/34) cases, from lymph node metastases in 21% (7/34) or other metastatic sites in 32% (11/34) of cases.

All cases harbored at least one genomic alteration (GA), with 83 total GA identified for an average of 2.4 GA per tumor (range 1-11). There were 44 base substitutions and indels, 23 gene amplifications, 15 truncations, 1 homozygous deletion, and no rearrangements. 67% (56/83) of the genomic alterations were considered clinically relevant GA (CRGA) and could potentially guide decisions regarding targeted therapy, with an average of 1.6 CRGA per tumor (range 1-7).

RET was the most frequently altered gene, mutated in 88% (30/34) of cases (Table 1). *RET* M918T was the most common *RET* alteration, observed in 62% (21/34) of patients [17-19]. *RET* C618G/R/S was detected in three cases (9%), *RET* C634R and E632_L633del each in two cases (6%); *RET* V804M or C620R was each present in one tumor (3%) [9, 20-25]. One case harbored both a *RET* amplification and *RET* M918T [26], and no other cases had more than one *RET* alteration. A computational method was used to determine the *RET* variant status without a matched normal control [15] and predicted the *RET* GA to be somatic in the majority of the *RET*-positive cases (73%), consistent with the pathology reports that did not indicate any MEN2A or MEN2B cases. Although the two patients with predicted germline *RET* M918T (cases #21 and #25) could be MEN2B, their ages are more consistent with sporadic MTC and their pathology reports did not mention any known clinical history of MEN2B or pheochromocytoma.

Other clinically relevant alterations occurred in *CCND1* (9%), *CDKN2A* (9%), *FGF19* (9%), *KRAS* (6%), *VHL* (6%), and for one case each (3%) in *ATM*, *CCND2*, *CDK4*, *CDK6*, *CDKN2B*, *CDKN2C*, *DNMT3A*, *ERBB3*, *ERBB4*, *GLI1*, *HRAS*, *MDM2*, *MYC*, *NOTCH2*, and *PTEN*.

A 42-year old male (case #1) presented with a neck mass, and metabolic imaging suggested a neoplastic thyroid lesion. The patient underwent total thyroidectomy with lymph node dissection. Surgical pathology revealed multifocal MTC with the largest lesion of 2.2 cm in the right lobe. Metastases were detected in 8 out of 8 central nodes and multiple foci indicated extranodal disease. Hot-spot testing identified the *RET* M918T mutation. Five months later, right thoracotomy was performed and mediastinal metastases were resected.

Abdominal CT imaging indicated hepatic metastases and the patient received external beam radiation to treat right choroidal orbital metastases 8 months after the thyroidectomy.

CGP of the thyroidectomy specimen demonstrated the tumor harbored *RET*M918T (32% mutant allele frequency, MAF) and revealed the *ATM* truncations L804fs*4 (30% MAF) and S978fs*12 (28% MAF). The patient was treated with vandetanib monotherapy, which was dosed progressively (100 mg once daily, 3 weeks; 200 mg once daily, 8.5 months; 300 mg once daily, 2 months). Electrocardiographic QTc interval prolongation from 405 milliseconds at baseline to 442 milliseconds was the main side effect. Circulating calcitonin eventually increased, however, and vandetanib was discontinued due to disease progression after one year. The patient presented in the Phase 1 clinic at MD Anderson and was enrolled on the clinical trial (NCT01582191) combining vandetanib (300 mg once daily) and everolimus (10 mg once daily) at the defined maximum tolerated dose. After 2 cycles of therapy, the patient had a 25 % reduction in greatest unidimensional tumor measurement per RECIST 1.1 (Figure 2), designated as stable disease, while just under the partial response threshold. In addition, the patient reported that the nodes in the neck were less congested. The main toxicities included grade 2 diarrhea, likely from vandetanib, which was controlled by diphenoxylate and atropine, and grade 2 cough. However, the hepatic metastases in the liver were progressing after an initial mixed response. The patient received particulate trans-arterial embolization for the hepatic metastases and continued on systemic therapy. The stable disease in the neck area lasted for at least 8 months, but the patient was taken off the protocol because of progressive disease in the liver.

The patient underwent hepatic radiotherapy with trans-arterial Y⁹⁰ SIR-Spheres®, which lead to decrease in the volume of the liver disease, and a subsequent bilateral adrenalectomy for refractory ACTH-induced Cushing syndrome. He started on cabozantinib (100 mg once daily) and experienced painful keratotic rash on feet and intermittent diarrhea, which was managed with dose interruption and reduction. Given the symptomatic progressive disease, the patient underwent extended and complicated right parotidectomy with preservation of the seventh cranial nerve, right neck dissection, wide excision of the entire cervical skin, and reconstruction of the neck and upper chest. Pathology demonstrated MTC, and local hot-spot testing identified only *RET*M918T mutation. Cabozantinib was increased to 120 mg daily and then to 140 mg daily. The patient experienced transient symptomatic benefit but had a declining performance status and passed away 20 months after initiation of treatment with vandetanib plus everolimus.

Discussion

Medullary thyroid carcinoma (MTC) is an aggressive tumor of neuroendocrine origin arising from the calcitonin-producing parafollicular C cells of the thyroid gland and accounts for approximately 5% of thyroid cancers, which translates to an incidence of nearly 3000 new cases in the US. 75% of MTC cases are dubbed sporadic, and the remainder are part of the inherited autosomal dominant syndromes multiple endocrine neoplasia 2A (MEN2A), MEN2B, or familial. Germline mutations in *RET* are found in almost all patients with inherited MTC [17, 20, 21, 27] and *RET* somatic mutations have been observed in 40-65% of patients with sporadic MTC [6, 28]. The activating *RET*M918T mutation is the most

frequent somatic and MEN2B germline alteration. Mutations in the *RAS* pathway have been identified in patients with *RET* wild-type sporadic MTC [29-31]. Retrospective next-generation sequencing studies of the exomes or select cancer genes in 17 and 20 sporadic MTC cases, respectively, confirmed mutant *RET*, *HRAS*, and *KRAS* as principal drivers. These studies reported few additional somatic alterations and recurrent mutations only in the gene *MDC1*, which was not profiled in this study [32, 33].

The recent clinical trials for MTC with targeted therapy demonstrated clinical benefit for only some MTC cases, suggesting the other cases may be *de novo* resistant to targeted therapy. Several observations from the genomic profiles in this series may explain *de novo* resistance. We identified a range of *RET* mutations that occurred mutually exclusively to *RET*M918T, specifically *RET*C618G/S/R, C634R, E632_L633del, C620R, and *RET*V804M. Various *RET* mutations may be differentially sensitive to targeted therapy, as preclinical data suggests for *RET*V804M, but this remains a hypothesis for now as clinical evidence linking specific *RET* alterations other than *RET*M918T to therapeutic efficacy is lacking. The potential value of *RET* mutations as predictive markers of therapy response hinges on the ability of the deployed assay to identify *RET* alterations with high sensitivity. Both large Phase 3 studies of MTC with targeted therapy reported unknown *RET* mutation status for 30-40% of cases [4, 5], increasing the sample size required to correlate *RET* mutation status with treatment outcome, although many cases with unknown *RET* status in the cabozantinib trial had no tumor sample available at the time of analysis [5]. Furthermore, both vandetanib and cabozantinib are anti-angiogenic and inhibit other tyrosine kinases in addition to RET, with vandetanib also targeting VEGFR2 and EGFR and cabozantinib also targeting VEGFR2 and MET [4, 5]. Predicting responses to these therapies solely on the basis of *RET* status thus likely misses other mechanisms of benefit and may be improved by correlating responses with comprehensive genomic profiles of MTC.

De novo resistance may also be linked to alterations in genes other than *RET*. Activated RAS acts downstream of RET and is associated with resistance to therapies targeting other receptor protein tyrosine kinases; however, in the cabozantinib Phase 3 trial, patients with *RAS* mutation experienced similar response rate and PFS as patients with *RET* mutation (31% vs. 32% and 47 weeks vs. 60 weeks, respectively)[7]. Alterations in *CCND1*, *CCND2*, *CDK4*, *CDK6*, *CDKN2A/B* or *CDKN2C* occurred in 21% of specimens and may lead to hyperactivation of the cyclin D-dependent kinases CDK4 and CDK6. Future clinical studies need to address whether *CCND1* or *CDK4* amplification may associate with sensitivity to CDK4/6 inhibitors, as preliminary clinical data suggest for other tumor types [34, 35]. *CDKN2C* has previously been observed to be inactivated in MTC and *CDKN2C* loss cooperated with oncogenic *RET* to promote MTC development in mice [36, 37]. Alterations in cell cycle genes co-existent with *RET* mutations may therefore contribute to resistance to RET targeting. The current study sensitively assesses alterations throughout the entire *RET* and *RAS* genes as well as most known cancer genes, and thus is a better foundation for illuminating potential mechanisms of resistance to the approved therapies.

Everolimus demonstrated modest activity against metastatic MTC, as a Phase 2 study of everolimus reported durable stable disease (at least 24 weeks) for 7 out of 9 cases, with 5 patients experiencing tumor shrinkage [38]. Case studies describe one patient who achieved

stable disease for at least one year on everolimus [39] and two patients with biochemical responses on everolimus plus octreotide [40]. These clinical data are supported by preclinical studies that validate mTOR signaling as potential target in MTC [41, 42]. However, as the efficacy of single-agent everolimus in MTC has been limited and may result in even less clinical benefit in the second-line setting [43], mTOR inhibitors may be most useful in targeted therapy combinations to overcome resistance to RET targeting. A case report describes systemic activity of vandetanib plus everolimus against *RET*-rearranged lung cancer [44]. Addition of everolimus to HER2-targeted therapy has shown efficacy against trastuzumab-resistant breast cancer [45], and persistent mTOR activation correlates with resistance to other targeted therapies [46]. Although a specimen of the progressive vandetanib-resistant disease was not available for CGP in the case described here, CGP can potentially identify genomic alterations in the mTOR pathway and point to benefit from added everolimus [45]. Vandetanib combined with everolimus may hold merit in the second-line setting for metastatic MTC, and could be most rationally deployed with the use of genomic profiling.

In conclusion, CGP of MTC has the potential to better delineate potential markers of response to approved targeted therapies, while suggesting routes to overcome resistance to these therapies. Genomic profiles of MTC need to be further investigated in the course of clinical care for patients with advanced MTC to inform the optimal management of these patients.

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Figure 1.
Tile plot of genomic alterations identified by genomic profiling in 34 cases of advanced medullary thyroid carcinoma.

Figure 2.

(A) Computed tomography (CT) scans of the index patient (case #1) before treatment shows a right mass measuring 2.2×1.9 cm. (B) CT scan of the same patient was taken 2 months after beginning of treatment with vandetanib plus everolimus and shows a mass of 1.5×1.5 cm. White arrows point to the target lesion.

Table 1
Genomic alterations (GA) identified in 34 cases of medullary thyroid carcinoma

Case	Age	Gender	Specimen	RET GA	Predicted RET GA status	GAs in non-RET genes
1	42	M	thyroid	RET M918T	ambiguous	ATM L804fs*4, S978fs*12
2	50	M	metastasis	RET M918T	somatic	
3	45	M	thyroid			HRAS Q61R, CDKN2C G38*
4	85	F	thyroid	RET C634R	somatic	
5	37	F	thyroid	RET M918T	ambiguous	TP53 R283C
6	31	F	metastasis	RET M918T	somatic	DDR2 R806Q
7	50	M	metastasis	RET E632_L633del	somatic	
8	35	F	thyroid	RET M918T	somatic	
9	21	M	thyroid	RET M918T	ambiguous	
10	41	F	thyroid			KRAS amp, CCND1 amp, CCND2 amp, CDKN2A p16INK4a D108H and p14ARF R122P, FGF19 amp, FGF23 amp, FGF3 amp, FGF4 amp, FGF6 amp, SMARCA4 K1219*, TP53 E294*
11	46	M	metastasis	RET M918T	somatic	BCOR K454fs*2
12	58	M	metastasis	RET C618G	germline	
13	49	M	thyroid	RET E632_L633del	somatic	VHL L198fs*4
14	44	M	thyroid	RET M918T	somatic	CDKN2A splice site 151-2A>T, RAD50 R1077*, VHL V130F
15	60	M	metastasis			EPHA5 E279K
16	57	M	metastasis	RET C634R	somatic	DNMT3A R736C
17	62	M	metastasis	RET M918T	somatic	
18	45	M	thyroid	RET M918T	somatic	GRIN2A T1223S
19	67	F	thyroid	RET C620R	somatic	KRAS Q61H, LRP1B N2330fs*2, MYC amp, RAD50 R193W, RBM10 E451*
20	67	M	metastasis	RET M918T	somatic	ARID1A splice site 5125-1G>A, CDKN2A/B loss
21	62	M	thyroid	RET M918T	germline	SF3B1 K700E
22	74	F	thyroid	RET M918T	somatic	LZTR1 K89fs*12
23	71	F	metastasis			CCND1 amp, CDK4 amp, EMSY amp, ERBB3 amp, ERBB4 amp, FGF19 amp, FGF3 amp, GLI1 amp, MDM2 amp, RBM10 F175fs*88
24	78	M	metastasis	RET V804M	germline	
25	50	M	metastasis	RET M918T	germline	
26	45	M	thyroid	RET M918T	somatic	
27	57	M	metastasis	RET M918T	somatic	

Case	Age	Gender	Specimen	RET GA	Predicted RET GA status	GAs in non-RET genes
28	46	M	thyroid	RET M918T	somatic	
29	82	F	thyroid	RET amp. M918T	ambiguous	CCND1 amp, FGF19 amp, FGF3 amp
30	73	M	metastasis	RET C618S	somatic	
31	66	F	metastasis	RET M918T	somatic	PTEN V16fs*14, CDK6 amp
32	79	M	metastasis	RET M918T	somatic	MAP3K1 E1242*, MUTYH Y165C
33	73	M	metastasis	RET C618R	somatic	
34	39	M	metastasis	RET M918T	somatic	NOTCH2 R1410H

M, male; F, female; amp, amplification; RET GA status (somatic, germline, or ambiguous) was predicted with a computational method [15].