Spotlight on Clinical Response

Medullary thyroid cancer: targeting the RET kinase pathway with sorafenib/tipifarnib

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Abstract

Medullary thyroid carcinoma (MTC) is an uncommon malignancy of hereditary and sporadic presentation. Mutations in the *RET* proto-oncogene are involved in the pathogenesis of familial MTC and >50% of the sporadic cases. Currently, there is no effective treatment for recurrent or metastatic MTC. We report here a rapid response to a sorafenib (RET and RAF kinase and vascular endothelial growth factor receptor inhibitor)–based regimen in a patient with sporadic MTC who had advanced, progressive disease and a novel RET kinase aberration at exon 11 shown in tumor tissue. [Mol Cancer Ther 2008;7(5):1001–6]

Introduction

Medullary thyroid carcinoma (MTC), an uncommon tumor of neuroendocrine origin, arises from parafollicular C cells, comprises 5% of thyroid cancers and presents in hereditary (25% of cases) or sporadic (75% of MTC cases) forms (1). The hereditary form is associated with multiple endocrine neoplasia type 2 (MEN2), including MEN2A (MTC ± pheochromocytoma and hyperparathyroidism), MEN2B (MTC and pheochromocytoma, mucosal neuromas, marfanoid habitus), and familial MTC. The *RET* proto-oncogene, located at chromosome 10q11.2, has been implicated in the pathogenesis of familial MTC and in as many as 50% of cases of sporadic MTC (2).

Surgical resection is the mainstay of treatment; however, once MTC becomes unresectable, neither radiotherapy nor systemic chemotherapeutic regimens have proved effective in prolonging survival. Therefore, investigational therapies directed at molecularly defined etiologies of MTC are being pursued (3). Here we describe the rapid response of a patient with spontaneous MTC, who had aggressive disease and had progressed on conventional therapies. The patient's tumor had an aberrant RET kinase gene and was treated with a combination of sorafenib (RET and RAF kinase and vascular endothelial growth factor receptor inhibitor) and tipifarnib (a farnesyltransferase inhibitor), targeted therapies that would disrupt RET-mediated signal transduction.

Materials and Methods

A phase I trial dose escalation combining two orally bioavailable agents, sorafenib, a multikinase inhibitor, and tipifarnib, a potent and selective inhibitor of the farnesyltransferase enzyme (which is critical for Ras activity), was initiated in advanced cancer patients (local protocol 2005-0363, National Cancer Institute protocol 7156). Patients took sorafenib twice daily for 28 d. During the first 21 d of this 28-d cycle, they also took tipifarnib twice daily. Restaging scans were done after every two cycles. Consent was obtained in accordance with M. D. Anderson Cancer Center Internal Review Board guidelines.

RET Sequencing

DNA was extracted from paraffin-embedded tumor tissues with the DNeasy Tissue Kit (Qiagen) according to the manufacturer's instructions. PCR was done to amplify exons 10, 11, 13, 14, 15, and 16 of the *RET* gene by LA-Taq (TaKaRa) and was carried out in a PTC-100 thermocycler (MJ Research). After being purified by the Exonuclease I-Shrimp Alkaline Phosphatase method (Roche), the products were directly sequenced in an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems). Mutations were cross-referenced with the Human Gene Mutation Database,[5] Entrez SNP,[6] and PubMed.[7]

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[5] http://www.hgmd.cf.ac.uk/ac/index.php
[6] http://www.ncbi.nlm.nih.gov/sites/entrez
[7] http://www.pubmed.org

Patient Report

A 37-y-old male patient with no family history of MEN syndrome, familial MTC, or sporadic MTC was diagnosed with spontaneous MTC in September 2004. The patient underwent total thyroidectomy, central neck dissection, and left modified radical neck dissection, revealing left lobe MTC with transcapsular extension and an 8-cm mass. Two lymph nodes resected from the central neck and five lymph nodes resected during modified radical neck dissection revealed metastasis with extranodal extension in all lymph nodes. Chest computed tomography and laparoscopy were negative. Twenty-four-hour urinary levels of metanephrine and catecholamine were normal, and the tumor was classified as $cT_4N_{1a}M_x$.

The patient remained disease-free until March 2005, when a palpable left jugular chain lymph node was found during physical examination. A fine needle aspiration biopsy was positive for metastatic MTC. Another left modified radical neck dissection for high jugular chain lymph node involvement and a diagnostic laparoscopy to rule out studding of the liver revealed 2 of 24 resected midjugular positive nodes. Postoperative calcitonin level was 1,771 pg/mL (normal, <8.4 pg/mL), and carcinoembryonic antigen level was 45.2 ng/mL (normal, <3.0 ng/mL).

The patient was referred to our institution in June 2005. Full restaging studies were done and imaging tests showed residual and/or recurrent metastatic adenopathy in the left supraclavicular region, superior mediastinum, and right paratracheal midneck region. Ultrasound-guided biopsy of a paratracheal node confirmed metastatic MTC. The patient underwent right and left comprehensive neck dissection (levels II through V), complete thyroidectomy, extensive microdissection of the right recurrent laryngeal nerve, and transoral excision of the left lateral retropharyngeal lymph node. In addition, repeat analysis including germ-line RET sequencing in the blood of exons 10, 11, 13, 14, 15, and 16 showed no germ-line mutations.

Six months later, computerized tomographic scanning showed disease recurrence in mediastinal lymph nodes and the lateral left tracheal wall. Due to disease progression,

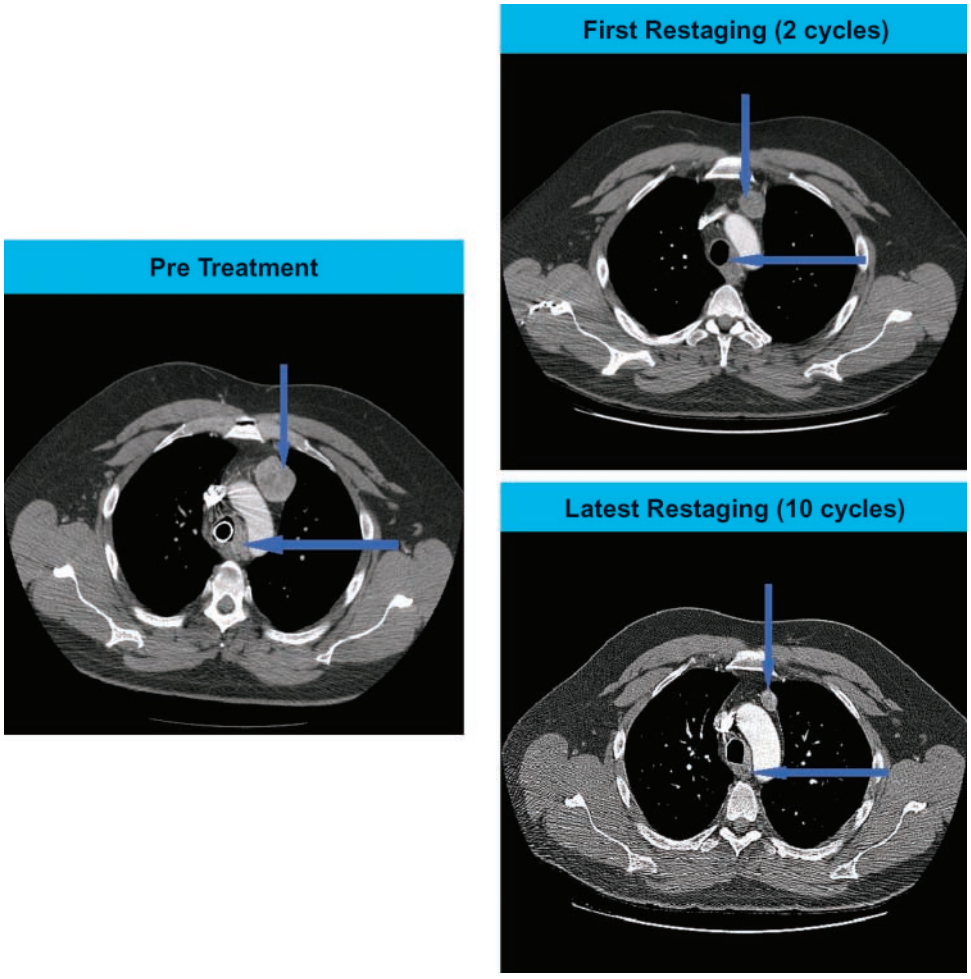

Figure 1. Both carcinoembryonic antigen (*CEA*; ng/mL) and calcitonin (pg/mL) were measured before dosing and at cycle 2 and at each cycle thereafter. The graphs show an initial marked decline after two cycles (and then plateau) in both carcinoembryonic antigen (693 – 217 ng/mL) and calcitonin (17,388 – 4178 pg/mL).

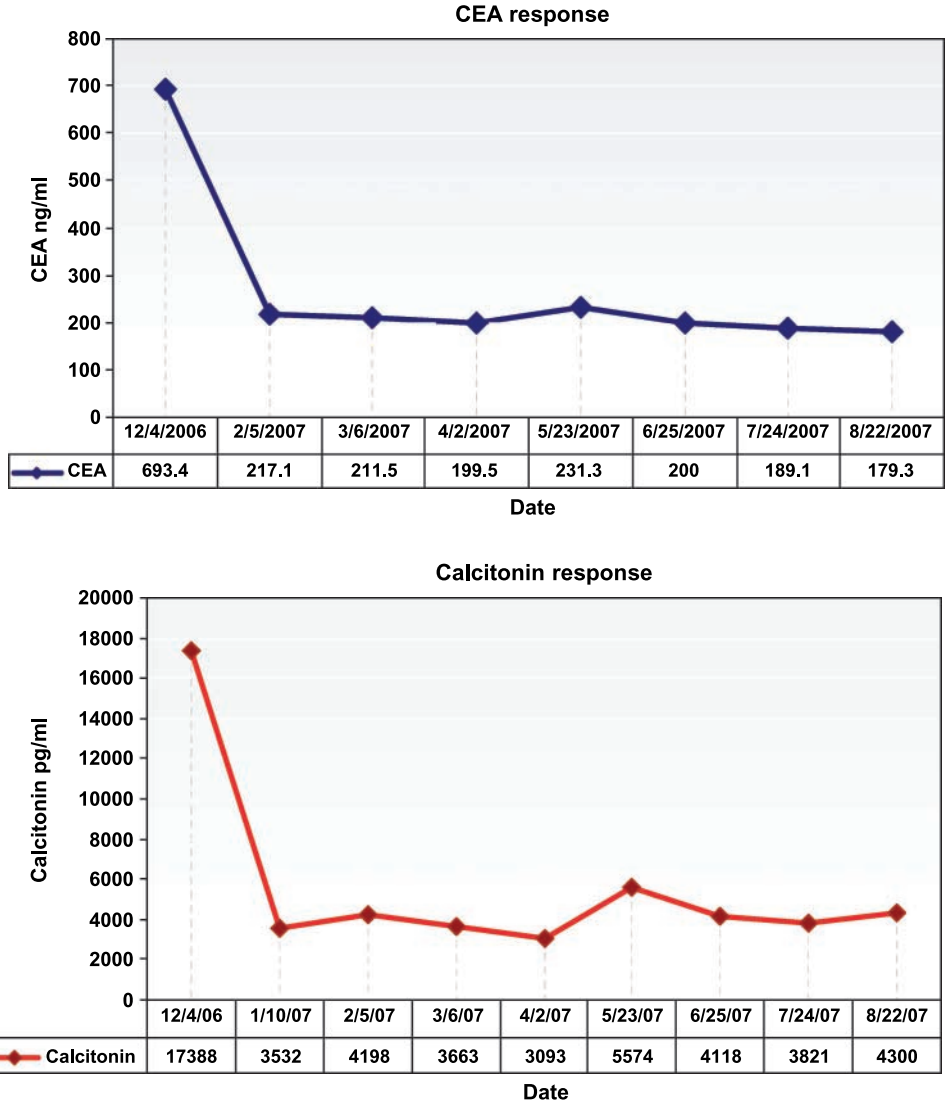

Figure 2. The 37-y-old spontaneous MTC patient enrolled in dose level 4 [sorafenib 400 mg each morning and 200 mg each evening (p.o.), tipifarnib 200 mg p.o. twice daily] achieved a confirmed partial response (36% decrease by RECIST) after 2 mo and a 46% decrease by RECIST by 10 mo. Patient remains on study with no significant adverse events. Figure shows computed tomography scans of the chest with bulky paratracheal disease and a tracheal stent (that was needed because of extrinsic tracheal compression, endoluminal growth and a 50% fixed airway obstruction; pretreatment). At 3 wk after starting treatment, the tracheal stent dislodged because of rapid tumor shrinkage. It was removed and was no longer seen in the scans at first restaging. *Arrows,* areas of significant tumor shrinkage used for RECIST assessment.

the patient was treated in a phase I trial using a direct Ras inhibitor. After 4 mo (four cycles) of therapy, restaging showed 13% increased disease by Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The patient elected to terminate his participation in the study and underwent a required 1-mo washout period. At this point, circumferential, bulky paratracheal disease was noted with extrinsic compression and endoluminal growth within his trachea, causing a 50% fixed airway obstruction. Rigid bronchoscopy was done to resect the endoluminal component and a silicone stent was placed.

On his postoperative recovery, the patient was deemed eligible for a phase I study of tipifarnib and sorafenib in patients with advanced cancer. Baseline imaging studies showed extensive lymph node disease in the superior and intrathoracic mediastinum. The patient started at dose level 4 (400 mg sorafenib every morning and 200 mg every evening, p.o., for 28 d, along with 200 mg tipifarnib p.o. twice a day for 21 d) and reported no specific toxic effects. At week 3, because of rapid tumor shrinkage confirmed by outside imaging, his silicone stent dislodged, obstructed his vocal cords, and was removed. By week 8, his calcitonin level decreased from the original level of 17,388 to 4,178 pg/mL, and carcinoembryonic antigen from 693 to 217 ng/mL (Fig. 1). The patient was again restaged at week 8 (end of cycle 2), showing (by RECIST criteria) a confirmed partial response (Fig. 2). Since then, the patient has stayed on the protocol and has reached cycle 10 of the combination. His last restaging after cycle 8 showed a sustained response with tumor volume decreased by 46% (RECIST), stabilized calcitonin of 3,327 pg/mL, but without any significant adverse events.

Analysis for RET Kinase Aberration

Macrodissected paraffin-embedded tissue from the level II through V neck dissection was analyzed for somatic RET mutations. Examination of the frequent mutation regions

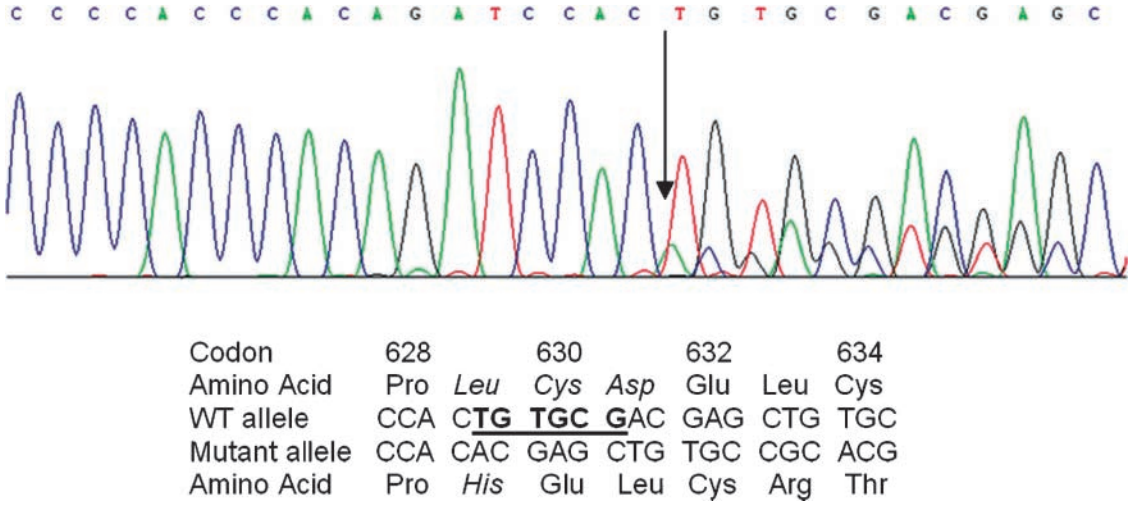

Figure 3. Paraffin-embedded tissues from the level II through V neck dissection were analyzed for somatic RET mutations. Direct sequencing of the hotspots, exons 10, 11, 13, 14, 15, and 16, revealed a novel 6-bp deletion mutation (TGTGCG) in exon 11 (2076–2081 del), seen as a double peak following the arrow. This deletion alters Leu, Cys, and Asp at codons 629 to 631 into His.

of RET (exons 10, 11, 13, 14, 15, and 16) revealed a novel 6-bp deletion mutation (TGTGCG) in exon 11, seen as double peaks after codon 628 in sequencing analysis. This deletion alters Leu, Cys, and Asp at codons 629 to 631 into His (Fig. 3). Mutations were cross-referenced with the Human Gene Mutation Database,[8] Entrez SNP,[9] and PubMed[10] and confirmed as a novel mutation of RET.

Discussion

The *RET* proto-oncogene mutations are believed to be the Achilles heel of MTC, particularly the hereditary form. The *RET* proto-oncogene is composed of 21 exons (4) that encode a receptor tyrosine kinase that binds to a glial cell line–derived neurotrophic factor, the ligand for RET, and leads to RET receptor dimerization. Subsequent autophosphorylation of COOH tyrosine residues, specifically Tyr^{1062}, causes adaptor and effector proteins to be recruited to the RET receptor, including Shc, ShcC, FRS2, IRIS1/2, Dok1, Dok4/5, Dok6, and Enigma. A cascade of downstream signal transduction pathways are activated, including Janus-activated kinase/signal transducers and activators of transcription, extracellular signal–regulated kinase 5, p38mitogen-activated protein kinase (MAPK), c-jun NH_2-terminal kinase, Ras/Raf/MAPK/extracellular signal–regulated kinase kinase/extracellular signal–regulated kinase, and phosphatidylinositol 3-kinase/AKT (5–7).

RET gene germ-line mutations of MEN2A, typically found in codons 634 and 618, result in ligand-independent homodimerization by covalent bonds of the cysteine-rich regions of the RET extracellular domain. MEN2B mutations, most commonly identified at codon 918, seemingly activate the RET receptor in the monomeric state by increasing Tyr^{1062} phosphorylation (8–11). Mutations associated with familial MTC affect the cysteine-rich extracellular domain and the tyrosine domain (12). Somatic mutations of the *RET* proto-oncogene have been estimated to be involved in 23% to 70% of cases of sporadic MTC (13, 14). A MEN2B-like M918T mutation in exon 16 accounts for the largest number of cases, a portent of a poor prognosis and more aggressive disease (15).

Several RET inhibitors have been investigated in phase I and phase II clinical trials. ZD6474, an oral vascular endothelial growth factor receptor and epidermal growth factor receptor inhibitor that also targets RET kinase, has shown success in phase II testing for hereditary MTC (3). However, preclinical studies showed that mutations of Val804 within the *RET* proto-oncogene may confer resistance to ZD6474. Other candidates affecting RET kinase include imatinib mesylate, a weak RET inhibitor; 17-*N*-allylamino-17-demethoxygeldanamycin, a heat shock protein 90 inhibitor and indirect inhibitor of RET; and AMG 706, a multikinase inhibitor of the vascular endothelial growth factor receptor and the *RET* proto-oncogene (16); and XL184, a MET, RET, and vascular endothelial growth factor receptor inhibitor.

Sorafenib (BAY 43-9006) was initially developed to target the Raf family of kinases mainly B-Raf and C-Raf, but was also found to inhibit other kinases including vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor β, c-Kit, FLT3, and RET. Sorafenib is also a known potent inhibitor of RET kinase. The drug has recently been approved in the United States to treat renal cell cancer. Carlomagno et al. (17) showed that 20 to 50 nmol/L sorafenib inhibits 50% (IC_{50}) of NIH 3T3 fibroblasts expressing one of three oncogenic versions of *RET* (RET/PTC3, RET/C634R, or RET/M918T) and

[8] http://www.hgmd.cf.ac.uk/ac/index.php
[9] http://www.ncbi.nlm.nih.gov/sites/entrez
[10] http://www.pubmed.org

showed almost complete inhibition with 100 nmol/L. Cells expressing both RET/V804L and RET/V804M, which render resistance to ZD6474, are sensitive to sorafenib at 110 and 147 nmol/L, respectively (17).

Tipifarnib (R115777) is a member of a novel class of chemotherapeutic agents developed to inhibit the farnesylation of Ras and other proteins. Tipifarnib shows antiproliferative effects against many human tumor cell lines, among which 75% were sensitive (IC_{50} ≤100 nmol/L) irrespective of *Ras* mutation status. To the best of our knowledge, tipifarnib has not shown direct activity against RET kinase, but it can inhibit RET signaling through the MAPK pathway. Tipifarnib is also active in myelodysplastic syndrome and in acute myelogenous leukemia where remissions have been achieved (18, 19).

We hypothesize that our patient responded because his tumor had an aberrantly activated RET kinase. Initial genetic screening of the patient's blood sample showed no evidence of a germ-line mutation. However, the discovery of a novel deletion in exon 11 of the *RET* gene in the tumor indicates a somatic mutation, which affects one of the six cysteine residues in the cysteine-rich region of the extracellular domain, specifically cysteine 630, and would be expected to cause ligand-independent homodimerization and constitutive activation of the RET kinase (20). Further experiments to confirm that the exon 11 deletion is activating are ongoing.

Another possibility includes direct inhibition of diverse pathogenic pathways that may be downstream of RET. One candidate is the Ras/Raf/MAPK pathway. Aberrant signaling through the Ras pathway has been implicated in many types of tumors. Ras mutations occur in >20% of tumors. Activation of the Ras/Raf pathway has been shown to lead to cell cycle arrest through secretion of an autocrine-paracrine factor, leukemia inhibitory factor, with subsequent activation of the Janus-activated kinase/signal transducer and activator of transcription 3 pathway (21). Contrarily, others have implicated the Ras/Raf/MAPK pathway as important to RET activation. Ludwig et al. (22) showed constitutive activation of nuclear factor κB in MTC, its requirement for RET-induced transformation, and RET signaling through Ras and Raf to activate IκB kinase B and nuclear factor κB. Carlomagno et al. (17) noted that Shc and p44/42MAPK phosphorylation, Ras/Raf/MAPK pathway components, were significantly inhibited by sorafenib. It is also conceivable in our case that tipifarnib and sorafenib combined targeted multiple points in the RET/Ras/Raf/IκB kinase B/nuclear factor κB cascade, abrogating signaling. Although papillary thyroid cancer shows activating BRAF mutations, no data currently suggest that familial or spontaneous MTC is associated with activating Ras or Raf mutations, and no Ras or Raf mutation analysis was done in our patient (23).

In summary, our patient with sporadic MTC and a novel, somatic deletion in exon 11 of the RET kinase gene showed a rapid and marked response to a regimen incorporating the RET kinase inhibitor sorafenib. Moreover, tipifarnib, which can effect downstream signaling of RET kinase, may have synergistically contributed to the profound clinical response. Further studies of this combination are warranted in patients with MTC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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