**RET Aberrations in Diverse Cancers: Next-Generation Sequencing of 4,871 Patients**

Shumei Kato¹, Vivek Subbiah², Erica Marchlik³, Sheryl K. Elkin³, Jennifer L. Carter³, and Razelle Kurzrock¹

**Abstract**

**Purpose:** Aberrations in genetic sequences encoding the tyrosine kinase receptor RET lead to oncogenic signaling that is targetable with anti-RET multitarget inhibitors. Understanding the comprehensive genomic landscape of RET aberrations across multiple cancers may facilitate clinical trial development targeting RET.

**Experimental Design:** We interrogated the molecular portfolio of 4,871 patients with diverse malignancies for the presence of RET aberrations using Clinical Laboratory Improvement Amendment–certified targeted next-generation sequencing of 182 or 236 gene panels.

**Results:** Among diverse cancers, RET aberrations were identified in 88 cases (1.8% (88/4,871)), with mutations being the most common alteration (38.6% [34/88]), followed by fusions (30.7% [27/88], including a novel SQSTM1-RET) and amplifications (25% [22/88]). Most patients had coexisting aberrations in addition to RET anomalies (81.8% [72/88]), with the most common being in TP53-associated genes (51.9% [52/88]), cell cycle–associated genes (39.8% [35/88]), the PI3K signaling pathway (30.7% [27/88]), MAPK effectors (22.7% [20/88]), or other tyrosine kinase families (21.6% [19/88]). RET fusions were mutually exclusive with MAPK signaling pathway alterations. All 72 patients harboring coaberrations had distinct genomic portfolios, and most (98.6% [71/72]) had potentially targetable coaberrations with either an FDA-approved or an investigational agent. Two cases with lung (KIF5B-RET) and medullary thyroid carcinoma (RET M918T) that responded to a vandetanib (multikinase RET inhibitor)-containing regimen are shown.

**Conclusions:** RET aberrations were seen in 1.8% of diverse cancers, with most cases harboring actionable, albeit distinct, coexisting alterations. The current report suggests that optimal targeting of patients with RET anomalies will require customized combination strategies.

**Introduction**

The RET proto-oncogene encodes a transmembrane receptor tyrosine kinase composed of an extracellular cadherin domain, cysteine-rich region, transmembrane domain, and an intracellular kinase domain (1, 2). It functions as the receptor for the growth factors of the glial cell line–derived neurotrophic factor family (3). Binding of ligand facilitates RET kinase activation, which leads to activation of multiple downstream effectors, including MAPK and PI3K pathways (3). Physiologically, RET is crucial for neural crest development (1, 2); loss-of-function mutations in RET are associated with aganglionic megacolon in Hirschsprung disease (4).

RET aberrations can result in gain of function via amplification or mutations and rearrangements that result in ligand-independent kinase activation. These alterations have been reported in different types of malignancies and in hereditary conditions.

Mutations in RET have been reported in patients with medullary thyroid carcinoma. They are seen in 43% to 71% of sporadic cases, with the most common mutation being RET M918T (5–8). Of note, germline mutations of RET are a hallmark of multiple endocrine neoplasia (MEN), including type 2A, 2B, and familial medullary thyroid carcinoma, with all three subtypes being associated with a high risk of developing a medullary thyroid carcinoma (70%–100% risk by age 70 years; ref. 9). Clinically, MEN 2A is also associated with pheochromocytoma and parathyroid hyperplasia, whereas MEN 2B is associated with mucosal neuromas, pheochromocytomas, intestinal ganglioneuromas and marfanoid habitus; familial medullary thyroid carcinoma is not associated with other conditions (9). Interestingly, different RET mutations are associated with distinct subtypes of MEN: (i) RET C634R, which leads to ligand-independent receptor dimerization, is most commonly associated with MEN 2A; (ii) RET M918T, which leads to decreased auto-inhibition and increased kinase activity, as well as ATP binding, is associated with MEN 2B; and (iii) various mutations at codons 609, 618, 620, 768, 804, and 891 are reported in both MEN 2A and familial medullary thyroid carcinoma (9, 10). These observations suggest that different RET-activating mutations have dissimilar oncogenic effects.
To facilitate the clinical trials targeting RET, a comprehensive understanding of RET aberrations among diverse cancer types is essential. Therefore, we examined the genomic landscape of RET alterations using targeted next-generation sequencing (NGS) in 4,871 patients with diverse malignancies, and we also show two illustrative cases of lung and medullary thyroid carcinoma with KIF5B-RET and RET M918T alterations, respectively, who both responded to vandetanib (multikinase RET inhibitor) containing regimen.

Materials and Methods

Patients

We investigated the RET gene status of patients with diverse malignancies that were referred for NGS from October 2011 to November 2013 (N = 4,871; Table 1 and Supplementary Tables S2 and S3; Fig. 1). The submitting physicians provided specification of tumor types. The database was deidentified with only diagnosis available. NGS data were collected and interpreted by N-of-One, Inc. The dataset of 4,871 sequenced tumors was queried for RET and coexisting gene alterations. Clinical impact was demonstrated by selected case studies. This study was performed in accordance with the guidelines of the UCSD and the MD Anderson Internal Review Board.

Tissue samples and mutational analysis

We collected sequencing information from 4,871 cancers whose formalin-fixed, paraffin-embedded (FFPE) tumor samples were submitted to a Clinical Laboratory Improvement Amendments–certified laboratory for genomic profiling (Foundation Medicine). Samples were required to have a surface area $\geq 25 \text{mm}^2$, volume $\geq 1 \text{mm}^3$, nucleated cellularity $\geq 80\%$, and tumor content $\geq 20\%$ (28). The methods used in this assay have been validated and reported previously (28–30). In short, 50 to 200 ng of genomic DNA was extracted and purified from the submitted FFPE tumor samples. This whole-genome DNA was subjected to shotgun library construction and hybridization-based capture before paired-end sequencing on the Illumina HiSeq2000 platform. Hybridization selection is performed using individually synthesized baits targeting the exons of 182 or 236 cancer-related genes and the introns of 14 or 19 genes frequently rearranged in cancer (Supplementary Table S4). Sequence data were processed using a customized analysis pipeline (28). Sequencing was performed with an average sequencing depth of coverage greater than 250X, with $>100X$ at $\geq99\%$ of exons. This method of sequencing allows for detection of copy number alterations, gene rearrangements, and somatic mutations with $99\%$ specificity and $>99\%$ sensitivity for base substitutions at $\geq 5$ mutant allele frequency and $>95\%$ sensitivity for copy number alterations. A threshold of $\geq8$ copies for gene amplification with $\geq6$ copies considered equivocal (except for ERRB2, which is considered equivocally amplified with $\geq5$ copies) was used.

cBio Cancer Genomics Portal data

For comparison purposes, we evaluated the RET alteration status using the cbio Cancer Genomics Portal data (cBioPortal; http://cbioportal.org, accessed May 2016; refs. 31, 32), which provides access to publicly available datasets with genomic information from a diverse array of cancer types (please refer to Supplementary Methods for additional information).
Results

Analysis of RET aberrations among diverse cancers (N = 4,871)

Among the 4,871 diverse cancer patients, the most common diagnosis was breast carcinoma [10.4% (506/4,871)], followed by lung adenocarcinoma [8.5% (412/4,871)], and sarcoma [7.1% (348/4,871); Table 1 and Supplementary Table S2]. Overall, RET aberrations were identified in 88 cases [1.8% (88/4,871)]. Among 88 cases with RET aberrations, 38.6% (34/88) were mutations, 30.7% (27/88) were fusions (defined as RET rearrangement with known fusion partner, e.g., KIF5B-RET), 25% (22/88) were amplifications, and 3.4% (3/88) were rearrangements without specific identified fusion partner (e.g., RET rearrangement, exon 11). In addition, RET duplication and loss were each observed in 1 of 88 cases. (Fig. 1).

According to cBioPortal, RET aberrations have been reported in 3.0% (181/6,011) of diverse cancers (Supplementary Table S5; Supplementary Fig. S1). In the current report, most RET aberrations were activating alterations [71.6% (63/88)] and one inactivating RET alteration was observed (RET loss). The functional significance of 27.3% (24/88) of RET aberrations, including RET R163Q, M255I, R525Q, V706M, A756V, M1109I, and SQSTM1-RET, fusion was unknown (Supplementary Table S3).

Overview of cancer diagnoses and RET aberrations

RET aberrations were most commonly seen in patients with medullary thyroid carcinoma [80% (4/5)], followed by anaplastic thyroid carcinoma [16.7% (2/12)], lung carcinomasoma [16.7% (1/6)], and ureter urothelial carcinoma [16.7% (1/6)]; however, these cancer diagnoses were not reported in cBioPortal, and thus, direct comparisons were not feasible (Table 1 and Supplementary Table S5; Fig. 2 and Supplementary Fig. S1). Although there was only one patient each for hemangiopericytoma and pheochromocytoma, they both harbored RET aberrations (Table 1 and Supplementary Table S3). In some cancer diagnoses, including cholangiocarcinoma (n = 159), neuroendocrine carcinoma (n = 97), renal cell carcinoma (n = 92), and glioblastoma (n = 84), we did not observe RET aberrations (Supplementary Table S2). In contrast, data from cBioPortal showed rare RET alterations in cholangiocarcinoma.

Table 1. RET aberrations and associated cancer diagnosis (N = 88)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Any aberrations n (%)</th>
<th>Fusion* n (%)</th>
<th>Mutation* n (%)</th>
<th>Rearrangement* n (%)</th>
<th>Amplification n (%)</th>
<th>Duplication n (%)</th>
<th>Loss n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemangiopericytoma (n = 1)</td>
<td>1 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phaeochromocytoma (n = 1)</td>
<td>1 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medullary thyroid carcinoma (n = 5)</td>
<td>4 (80.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Paraganglioma (n = 4)</td>
<td>1 (25.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anaplastic thyroid carcinoma (n = 12)</td>
<td>2 (16.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uterine carcinosarcoma (n = 6)</td>
<td>1 (16.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Papillary thyroid carcinoma (n = 25)</td>
<td>3 (12.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adrenal carcinoma (n = 10)</td>
<td>1 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prostate adenocarcinoma (n = 12)</td>
<td>1 (8.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ureter urothelial carcinoma (n = 31)</td>
<td>2 (6.5)</td>
<td>1 (3.2)</td>
<td>0</td>
<td>1 (3.2)</td>
<td>1 (3.2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lung adenocarcinoma (n = 42)</td>
<td>23 (5.6)</td>
<td>16 (3.9)</td>
<td>3 (0.7)</td>
<td>2 (0.5)</td>
<td>2 (0.5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Meningioma (n = 18)</td>
<td>1 (5.6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duodenal adenocarcinoma (n = 20)</td>
<td>1 (5.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cervical adenocarcinoma (n = 24)</td>
<td>1 (4.2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adrenal carcinoma (n = 27)</td>
<td>1 (3.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gastroesophageal junction carcinoma (n = 29)</td>
<td>1 (3.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GIST (n = 30)</td>
<td>1 (3.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-small cell lung carcinoma (n = 125)</td>
<td>4 (3.2)</td>
<td>4 (3.2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cutaneous squamous cell carcinoma (n = 36)</td>
<td>1 (2.8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hepatocellular carcinoma (n = 44)</td>
<td>1 (2.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pancreatic ductal adenocarcinoma (n = 160)</td>
<td>3 (1.9)</td>
<td>1 (0.6)</td>
<td>1 (0.6)</td>
<td>1 (0.6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prostate adenocarcinoma (n = 64)</td>
<td>1 (1.6)</td>
<td>0</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Melanoma (n = 136)</td>
<td>2 (1.5)</td>
<td>1 (0.7)</td>
<td>0</td>
<td>1 (0.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endometrial adenocarcinoma (n = 79)</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovarian serous carcinoma (n = 169)</td>
<td>2 (1.2)</td>
<td>0</td>
<td>0</td>
<td>2 (1.2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoma unknown primary (n = 270)</td>
<td>3 (1.1)</td>
<td>2 (0.7)</td>
<td>0</td>
<td>1 (0.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bladder urothelial (transitional cell) carcinoma (n = 91)</td>
<td>1 (1.1)</td>
<td>0</td>
<td>1 (1.1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Colorectal adenocarcinoma (n = 500)</td>
<td>3 (0.6)</td>
<td>0</td>
<td>0</td>
<td>2 (0.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HNSCC (n = 108)</td>
<td>1 (0.9)</td>
<td>0</td>
<td>0</td>
<td>1 (0.9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoma (n = 548)</td>
<td>3 (0.9)</td>
<td>1 (0.5)</td>
<td>0</td>
<td>2 (0.6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gastric adenocarcinoma (n = 134)</td>
<td>1 (0.7)</td>
<td>0</td>
<td>1 (0.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Breast carcinoma (n = 506)</td>
<td>3 (0.6)</td>
<td>0</td>
<td>1 (0.2)</td>
<td>2 (0.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: HNSCC, head and neck squamous cell carcinoma.

*The term fusion was used when RET was rearranged with known fusion partner (e.g., KIF5B-RET). On the other hand, the term rearrangement was used when there was no specific identified fusion partner (e.g., RET rearrangement, exon 11).
patients also had cogenetic aberrations along with aberrations and possible cognate targeted therapies (Supplementary Table S3). Among these 72 cases, a total of both infrequently associated with tyrosine kinase families and MAPK signaling pathway were aberrations (Supplementary Table S3). Those include coaberrations with RET rearrangement, exon 11), 11% (1/88) were duplication, and 11% (1/88) were loss.

Overview of cancer diagnosis and specific RET aberrations
As mentioned, the most common type of RET aberrations were mutations [38.6% (34/88)], followed by fusions [30.7% (27/88)] and amplifications [25.0% (22/88); Fig. 1]. This observation was similar to cbioPortal data in relative frequencies, although the actual percentages for cbioPortal mutations, fusions, and amplifications differed a bit from our data [60.2% (109/181), 15.5% (28/181), and 12.7% (23/181), respectively; Supplementary Table S3; Supplementary Fig. S1]. In the current report, RET mutations were most commonly seen in patients with medullary thyroid carcinoma [80% (4/5)], followed by paraganglioma [25% (1/4)], anaplastic thyroid carcinoma [16.7% (2/12)], and uterine urothelial carcinoma [16.7% (1/6); Table 1; Fig. 2]. RET fusions were seen in patients with lung carcinomas [16.7% (1/6)], followed by papillary thyroid carcinoma [8.7% (2/23)] and lung adenocarcinoma [3.9% (16/412)]. RET amplifications were detected in patients with fallopian tube adenocarcinoma [8.3% (1/12)], uterine carcinosarcoma [5.3% (1/19)], and duodenal adenocarcinoma [5.0% (1/20); Table 1; Fig. 2].

Coaberrant oncogenic pathways associated with RET aberrations
Among 88 patients with RET aberrations, 72 also harbored coaberrations (Supplementary Table S3). Those include coaberrations with TP53-associated genes [59.1% (52/88)], cell cycle-associated genes [39.8% (35/88)], aberrations in the PI3K signaling pathway [30.7% (27/88)], MAPK effectors [22.7% (20/88)], and other tyrosine kinases families [21.6% (19/88); Fig. 3; Supplementary Table S5]. Coaberrant oncogenic pathways were all readily observed (greater than 20%) among RET mutations, amplifications, and fusions, except the coaberrations with tyrosine kinase families and MAPK signaling pathway were both infrequently associated with RET fusions [7.4% (2/27) and 0% (0/27), respectively; Supplementary Table S5].

Number of cogenetic aberrations associated with RET aberrations and possible cognate targeted therapies
As mentioned, among 88 cases with RET aberrations, 72 patients also had cogenetic aberrations along with RET aberrations (Supplementary Table S3). Among these 72 cases, a total of 292 coaberrations were identified. Among 292 coaberrations, 80.8% (236/292) were potentially targetable with FDA-approved agents as off-label use, and an additional 8.2% (24/292) were theoretically targetable with therapies that are currently in clinical trials. Altogether, among all coaberrations, 89.0% (260/292) were potentially actionable either with therapies that are approved by the FDA (albeit off label) or with therapies that are in clinical trials (Supplementary Tables S3 and S7).

Among 292 coexisting aberrations, 200 were molecularly distinct alterations, occurring either in separate genes or distinct alterations within the same gene. However, there were 16 cases of CDKN2A/B loss, and those were considered as single alteration. Among these molecularly distinct aberrations, 80.0% (160/200) were targetable by an FDA-approved drug, and an additional 7.5% (15/200) were targetable by drugs that are under investigation in clinical trials (Supplementary Tables S3 and S7).

Among 88 patients with RET aberrations, the median number of coaberrations per patient was three (range, 0–16; excluding RET alterations). The median number of coaberrations was similar among patients who were tested with the 182-gene panel (n = 16 patients, median of 3 coaberrations, range 0–6) and 236-gene panel (n = 72 patients, median of 3 coaberrations, range 0–16; Supplementary Table S4 for list of genes). The median number of potentially targetable coaberrations per patient was two (range, 0–16; Fig. 4). Among all 88 patients with RET aberrations, 78.4% (69/88) of patients had theoretically actionable coaberrations by an FDA-approved agent, and an additional 2.3% (2/88) patients had coaberrations targetable with investigational agents in clinical trial. Altogether, 80.6% (71/88) patients had actionable coaberrations either with FDA-approved or with investigational agents. However, if we only focus on 72 patients who had coaberrations along with RET aberrations, almost all patients had actionable coaberrations [98.6% (71/72)] either with FDA-approved or with investigational agents (Fig. 4; Supplementary Tables S3 and S7).

Distinctness of genomic aberrations among 88 patients with RET aberrations
Among 88 patients with RET aberrations, 12 patients had identical genomic portfolios [RET C634R (ID #1 and #2), RET M918T (patient ID #6, #8, #9, #11, and #12), RET-NCOA4 fusion (ID #37 and #38), and RET-CCDC6 fusion (ID #57, #58, and #60); Supplementary Table S3]. However, among 72 patients...
harboring coaberrations along with RET aberrations, there were no two patients with identical genomic portfolios (Supplemental Table S3). If we consider the genetic alterations at the level of the gene (and not the specific molecular aberration), then five patients had coaberrations identical to at least one other patient. Those include patient ID #32 and #87 with KRAS and TP53 and ID #39, #41, and #45 with RB1, STK11, and TP53 coaberrations (Supplementary Table S3).

Clinical impact of multikinase inhibitors with anti-RET activity in patients with RET aberrations

To demonstrate the impact of therapies with anti-RET activity in cancer patients harboring RET aberrations, we report two patients with RET alterations who were treated with multikinase inhibitors that possess anti-RET activity. The first is a 43-year-old woman with adenocarcinoma of the lung and a KIF5B-RET fusion, refractory to multiple lines of therapies including the multikinase RET/MET/VEGFR2 inhibitor cabozantinib. Treatment with another multikinase inhibitor, vandetanib (RET/EGFR/VEGFR2 inhibitor) in combination with everolimus (an mTOR inhibitor), led to a major response (Fig. 5A); the second patient was a 35-year-old man with sporadic medullary thyroid carcinoma and a RET M918T mutation as well as ATM L804fs/C3 and ATM S978fs/C3 alterations. Additional tumor evaluation with an IHC panel also showed strong positivity of phospho-AKT. The patient was initially treated with single-agent vandetanib with prolonged stable disease; however, the addition of everolimus led to significant tumor shrinkage (Fig. 5B).

Discussion

We report a comprehensive landscape of RET aberrations among 4,871 patients with diverse cancers. RET aberrations were identified in 1.8% (88/4,871) of tumors, with mutations being the most frequent aberration [38.6% (34/88)], followed by fusions [e.g., KIF5B-RET; 30.7% (27/88)], amplifications [25.0% (22/88)], rearrangements without specific identified fusion partner [e.g., RET rearrangement, exon 11; 3.4% (3/88)] and n = 1 each of duplication and loss (Fig. 1). The overall frequency of RET aberrations in this current report is similar to the
frequency reported in the cBioPortal dataset 3.0% (181/6,011; Supplementary Table S5; Supplementary Fig. S1).

RET mutations are a hallmark of medullary thyroid cancer (both sporadic and familial cases); they are reported in 43% to 71% of sporadic medullary thyroid carcinomas (5–8). RET M918T is the most common mutation reported in sporadic disease (5, 6, 8), which is consistent with the current report, wherein four of five medullary thyroid cancers

Figure 3.
Coaberrant oncogenic pathways associated with RET aberrations. Among 88 patients with RET aberrations, some patients also harbored coaberrations that can lead to tumorigenesis. Those coaberrations include TP53-associated genes [e.g., MDM2, ATM, or TP53; 59.1% (52/88)], cell-cycle–associated genes [e.g., CDKN2A/B, CDK6, or RB1; 39.8% (35/88)], PI3K signaling pathway [e.g., PIK3CA, PTEN, AKT, or RPTOR; 30.7% (27/88)], MAPK effectors [e.g., KRAS, NF1, or BRAF; 22.7% (20/88)], and other tyrosine kinase families [e.g., FGFR, EGFR, ERBB2, ALK, or KIT; 21.6% (19/88)]. Please see Supplementary Tables S3 and S5 for a complete list of co-occurring aberrations associated with RET aberrations.

Figure 4.
Number of all reported coaberrations and possibly actionable coaberrations per patient. Among 88 patients with RET aberrations, there was a median of 3 coaberrations per patient (range, 0–16) and a median of 2 (range, 0–16) possibly actionable coaberrations per patient. Please see Supplementary Table S6 for a complete list of co-occurring aberrations and rationale for possible targeted therapies.
harbored RET mutations [M918T (n = 3) and C634R (N = 1); Table 1 and Supplementary Table S3; Fig. 2]. Activating RET mutations lead to enhanced downstream signaling with multiple effectors, including those in the MAPK and PI3K pathways, resulting in increased cell proliferation and anchorage-independent cell growth (33–35). Clinically, RET mutations are associated with poor clinical outcomes, including larger tumor size, metastasis, and poorer survival, when compared with RET wild-type cases among patients with medullary thyroid carcinoma (7).

Various FDA-approved multikinase inhibitors that possess anti-RET activity have recently become available: vandetanib, cabozantinib, lenvatinib, ponatinib, sunitinib, regorafenib, and sorafenib (19, 20). Among these agents, one of the earliest studies that demonstrated clinical activity against RET-mutated tumors was a phase I trial with cabozantinib (RET/MET/VEGFR2 inhibitor), which enrolled 37 patients with medullary thyroid carcinoma (8). In that study, 81% (25/31) of analyzed tumors harbored activating RET mutations (including 3 patients with germ-line RET mutations). Stable disease of at least 6 months or a partial...
response (PR) was observed in 68% (25/37) of patients [PR, 25.9% (17/67)]. Tumor regression was also observed among medullary thyroid carcinomas without identified RET mutations, which could be due to anti-VEGFR2 and anti-MET activities (2 patients had MET amplification) of cabozantinib or because of other unknown aberrations in the RET pathway (8). Cabozantinib was further studied in a double-blind, phase III trial in patients with advanced medullary thyroid carcinoma and demonstrated statistically significant progression-free survival (PFS) when compared with placebo (PFS, 11.2 months versus 4.5 months; HR, 0.28; P = 0.001), with a subgroup analysis demonstrating statistically significant HR only seen among the RET mutation-positive group (22). On the basis of these studies, cabozantinib is currently approved for patients with advanced medullary thyroid carcinoma. In this current report, RET-activating mutations were also found in diverse cancers other than medullary thyroid carcinoma with variable frequencies: anaplastic thyroid (16.7% (2/12)), Merkel cell (10% (1/10)), GIST (3.3% (1/30)), hepatocellular (2.3% (1/44)), endometrial (1.3% (1/79)), colorectal (0.7% (2/300)), and breast (0.2% (1/506)) carcinomas (Table 1 and Supplementary Table S3; Fig. 2). Additional clinical trials targeting diverse cancer types with RET mutations in so-called basket trials may yield insights as to the relevance of histology in the presence of RET mutations (Supplementary Table S1). It is important to also note that medullary thyroid carcinoma patients being targeted with multikinase inhibitors can have frequent initial declines in tumor measurements and markers (calcitonin and carcinoembryonic antigen), followed by short-lived cycling patterns that include an increase in tumor measurements more than 20% above nadir values (which is defined as progressive disease per RECIST version 1.1) with or without increases in tumor markers (36). However, we have previously demonstrated that these fluctuations can be transient, and continued RET inhibition was associated with durable tumor regression (36).

RET fusions were the second most common RET aberrations identified (30.7% (27/88); Table 1 and Supplementary Table S3; Fig. 1). Most fusion partners contain coiled-coil or leucine zipper domains that drive the dimerization or oligomerization of the fusion kinase and lead to ligand-independent RET activation (20). RET fusions have been described in approximately 20% to 40% of papillary thyroid carcinomas (2, 11), with even higher frequency when associated with radioiodine exposure (60%; ref. 12). More than 10 RET fusion partners have been reported (2). In our study, 8.7% of papillary thyroid carcinomas (2/23) harbored RET fusions (Table 1; Fig. 2). The differences in frequency between previous reports and the current study may be due to small sample size and/or differing detection methods. Recent studies also demonstrated RET fusions in about 1% to 2% of patients with NSCLC, with several of the fusion partners overlapping with those identified in papillary thyroid carcinoma (e.g., CCDC6, KIF5B, NCOA4, and TRIM33; refs. 13–18, 24), which is consistent with findings in this current report (Table 1 and Supplementary Table S3; Fig. 2). Studies analyzing case series have reported that individual patients with NSCLC harboring a CCDC6-RET fusion treated with vandetanib (n = 1; ref. 21) as well as NSCLC bearing TRIM33-RET (n = 1) or KIF5B-RET (n = 1) treated with cabozantinib have achieved PRs (15). Herein, we also show a patient with lung adenocarcinoma and a KIF5B-RET fusion, refractory to multiple lines of therapy, who attained a major response (76% regression) with vandetanib (RET/EGFR/VEGFR2-inhibitor)-based therapy (Fig. 5A). Along with these promising reports (15, 21, 24), there are multiple ongoing phase I and II trials targeting RET fusions in patients with NSCLC and other advanced solid tumors (Supplementary Table S1).

Of note, we have identified a patient (papillary thyroid; case #61, Supplementary Table S3) with a sequestosome 1 (SQSTM1)-RET fusion, which, to our knowledge, has not been previously reported (2, 12, 18, 20). The functional and clinical relevance of SQSTM1-RET is worth investigating as the SQSTM1 fusion with other partners, such as ALK (37), showed transforming activity in vitro, and there is a case report of a patient with NSCLC and a SQSTM1-NTRK1 fusion demonstrating a durable response to entrectinib (a multikinase inhibitor with targets including TrkA, B, and C; ref. 38).

We have also evaluated aberrations that cooccurred with RET. Indeed, 81.8% (72/88) of cases harboring RET aberrations had additional alterations. The most common pathways altered involved TP53-associated genes [59.1% (52/88)], followed by cell cycle–associated genes [39.8% (35/88)], the PI3K signaling pathway [30.7% (27/88)], MAPK effectors [22.7% (20/88)], and other tyrosine kinase families [21.6% (19/88); Fig. 3]. In fact, among 292 coexisting aberrations detected in this report, 80.8% (236/292) were potentially targetable with FDA-approved agents (albeit off label) and an additional 8.2% (24/292) with experimental agents that are under investigation in clinical trials (Supplementary Tables S3 and S7). Moreover, almost all patients [98.6% (71/72)] harboring coaberrations with RET had potentially actionable coaberrations with either FDA-approved or investigational agents (Supplementary Tables S3 and S7). Although targeting RET aberrations has been reported to be successful, especially when RET was the only identifiable target (15, 21), the current report suggests that therapeutic combination approaches may be necessary to move beyond PRs with limited durability. Several such trials have been suggested or described previously (39–42). Consistent with this notion, we show a patient with sporadic medullary thyroid carcinoma harboring a RET M918T alteration, who had prolonged stable disease on vandetanib (~12 months) and had strong positivity for phospho-AKT by IHC. The patient’s tumor demonstrated 25% reduction with the addition of everolimus (an mTOR inhibitor; Fig. 5B).

Interestingly, RET fusions were mutually exclusive with MAPK signaling pathway (which includes NF1, KRAS, NRAS, and BRAF that were seen with RET mutations/amplifications) and infrequently associated with aberrations in other tyrosine kinase families [7.4% (2/27); Fig. 3; Supplementary Table S6], which is consistent with a previous report (15). However, coaberrations in effectors of MAPK signaling or other tyrosine kinases were readily (greater than 20%) seen among tumors with either RET mutations or amplifications (Fig. 3; Supplementary Table S6). The extent to which this holds true in larger groups of patients merits investigation.

There are several limitations to the current data. First, correlations with overall disease outcome were not feasible, as the data were not clinically annotated. Second, the impact of germline mutations [especially important for medullary thyroid carcinoma and pheochromocytoma that can be associated with MEN (9, 10)] was not evaluable. Third, the possibility of sample size bias exists as the number of cases in each
malignancy relied on the number of specimens submitted by physicians for NGS. Finally, the diagnosis was determined on the basis of the submitting physician’s designation. However, despite these limitations, the current report provides a large and comprehensive analysis of RET aberrations in diverse cancers.

In conclusion, we have evaluated 4,871 patients with diverse cancers and shown that alterations in RET are found in 1.8% (88/4,871) of cases. We have also identified a novel SQSTM1-RET fusion (n = 1) in a papillary thyroid tumor. Most patients [81.8% (72/88)] had coexisting genomic aberrations that accompanied their RET alterations. In the vast majority of individuals [98.6% (71/72)], at least one of the coalterations was pharmacologically tractable. Although various trials targeting RET aberrations are ongoing (Supplementary Table S1), the current report suggests that individualized cotargeting of multiple aberrant genes along with RET inhibition may be required for optimal clinical outcome.

Disclosure of Potential Conflicts of Interest
V. Subbiah reports receiving commercial research grants from Novartis. S.K. Elkin and J.L. Carter have ownership interest (including patents) in N-of-One. R. Kurzrock is an employee of and has ownership interests (including patents) in Novena Inc. and CaremaRx, Inc. is a consultant/advisory board member for Actuate Therapeutics, Squenom, and Xbiotech; and reports receiving commercial research grants from Foundation Medicine, Genentech, Guardant, Merck, Pfizer, and Squenom. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: S. Kato, E. Marchlik, S.K. Elkin, J.L. Carter, R. Kurzrock
Development of methodology: S. Kato, E. Marchlik, S.K. Elkin
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): V. Subbiah, E. Marchlik, S.K. Elkin
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Kato, V. Subbiah, E. Marchlik, S.K. Elkin, R. Kurzrock
Writing, review, and/or revision of the manuscript: S. Kato, V. Subbiah, E. Marchlik, S.K. Elkin, J.L. Carter, R. Kurzrock
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): V. Subbiah
Study supervision: R. Kurzrock

Grant Support
This study was funded in part by the Joan and Irwin Jacobs fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 7, 2016; revised August 22, 2016; accepted September 4, 2016; published OnlineFirst September 28, 2016.

References


**Clinical Cancer Research**

*RET Aberrations in Diverse Cancers: Next-Generation Sequencing of 4,871 Patients*

Shumei Kato, Vivek Subbiah, Erica Marchlik, et al.


---

Updated version

Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-16-1679

Supplementary Material

Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2016/09/28/1078-0432.CCR-16-1679.DC1

---

Cited articles

This article cites 42 articles, 21 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/23/8/1988.full.html#ref-list-1

---

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.