**RET-Rearranged Lung Cancer**

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**Abstract**

In recent years, we have witnessed the discovery of several oncogenic driver mutations as well as the emergence of specific inhibitors with high response rates and few treatment-related adverse events. RET-rearranged lung cancers represent a small subset of lung cancer, most commonly encountered in patients with adenocarcinoma and minimal or no exposure to tobacco. Several multikinase inhibitors have been tested with high “off-target” toxicity and low RET inhibition activity. Early-phase clinical trials with more selective inhibitors are awaited. Here, we review the main aspects of the biology of RET, the challenges of RET inhibition in lung cancer, and some future perspectives.

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**Introduction**

Genomic analysis of lung cancer has shown that these tumors contain distinct genetic alterations. The discovery of the EGFR mutation and its sensitivity to EGFR tyrosine kinase inhibitors (TKIs) revolutionized the treatment of lung cancers. At that time, we were mainly focused on small genetic modifications. Notwithstanding, in 2007, a chromosomal rearrangement involving ALK was discovered, which was followed by publication of the activity of the ALK inhibitor crizotinib, with high response rates. The evolution of genomic analysis led to the discovery of novel oncogenic fusion genes such as ROS1 and RET.

In 1985, Takahashi and colleagues first described a new transforming gene that appeared to be activated by the recombination of 2 unlinked human DNA segments, possibly by co-integration during transfection of NIH 3T3 cells with human lymphoma DNA, which was designated RET (rearranged during transfection).

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**The Biology of RET**

RET is a proto-oncogene localized in the pericentromeric region of chromosome 10q11.2, which encodes the protein RET, a receptor tyrosine kinase (RTK). RET undergoes alternative splicing of 3’ exons to generate 3 protein isoforms: RET9, RET32, and RET51, which differ at their carboxy terminal amino acids number. RET has 3 domains: a large extracellular domain, a transmembrane region, and an intracellular kinase domain. It is the only RTK with 4 cadherin-like domains in its extracellular region. RET is the signaling receptor for the glial cell-derived neurotrophic factor (GDNF) family of ligands (GFLs): GDNF, neurturin, persephin, and artemin. Unlike other RTKs, downstream signaling requires co-receptors that are tethered at the lipid rafts (cholesterol-rich membrane subdomains). Although there can be some crosstalk, each GFL interacts primarily through its specific co-receptor, represented by 4 GDNF family receptor-alpha (GFR-α) 1-4. Upon binding of GFLs to GFR-α, RET dimerization and autophosphorylation stimulate multiple downstream pathways, including RAS-MAPK, PI3K-AKT, and STAT3. These signs play a key role in kidney and nervous system development, neuronal survival and differentiation, and maintenance of spermatogonial stem cells.

RET receptor is expressed in several neural and neuroendocrine cell lineages, such as the thyroid C cells and adrenal chromaffin cells. RET loss-of-function mutations give rise to Hirschsprung disease and congenital abnormalities of the kidney and urinary tract, while RET gain-of-function mutations result in aberrant activation of the receptor; they are pathognomonic in patients with multiple endocrine neoplasia type 2 (MEN2). Both germline and somatic RET mutations represent an important step of medullary thyroid carcinoma oncogenesis. At the same time, somatically occurring RET rearrangements occur in 20% to 40% of papillary thyroid carcinoma. The increasing use of new techniques,
such as genomic sequencing and transcriptome analysis, has led to the identification of chromosomal rearrangements in other cancers. Chromosomal rearrangements involving RET are frequently found in irradiation-induced papillary thyroid carcinoma.\(^5\)

**RET and Lung Cancers**

In 2012, Ju and colleagues\(^6\) first reported on a 33-year-old never-smoker patient with lung adenocarcinoma with a novel fusion gene between KIF5B and the RET proto-oncogene caused by a pericentric inversion of 10p11.22 – q11.21. KIF5B contains a coiled-coil domain functioning as a dimerization unit, which activates the oncogenic tyrosine kinase domain of RET by autophosphorylation after homodimerization. The RET kinase domain portion is preserved in all kinase fusions, despite the breakpoint leaving downstream intracellular kinase activity intact.

The transformation potential of RET fusions has been reported in Ba/F3 cells and LC-2/ad (human adenocarcinoma cell-line), while anchorage-independent cell proliferation has also been shown in NIH3T3 cells.\(^10\) The mutually exclusive nature of the RET fusions and other oncogenic alterations suggests that the KIF5B-RET fusion is a driver mutation. However, Kim and colleagues\(^11\) reported the co-occurrence of EGFR or KRAS mutations in KIF5B-RET rearranged lung adenocarcinoma, and RET rearrangement was also reported in patients with EGFR-mutated lung adenocarcinoma who had progressed on TKI therapy.\(^12\)

A variety of breakage points have been identified within the KIF5B locus, which is the most common fusion partner gene. More importantly, several other RET fusion partner genes have been identified: CCDC6 (coiled-coil domain containing 6), CUX1 (cutlike-homeobox 1), TRIM33 (tripartite-motif containing 33), NCOA4 (nuclear-receptor coactivator 4), KIAA1468, KIAA1217, CLIP1 (CAP-Gly domain containing linker protein 1), ERCl (ELKS/Rab6-interacting/CAST family member 1), and MYOS3 (myosin 5C), among others. Importantly, all of these fusion partners contain coiled-coil domains that are believed to mediate ligand-independent dimerization and constitutive activation of RET.\(^10,13-16\)

To date, several cancer genome sequencing studies have discovered RET fusions in 1% to 2% of unselected lung cancers, which might be higher in the pan-negative population (negative for all known oncogenic driver mutations).\(^14,17\) Several studies have tried to elucidate the clinicopathological characteristics of RET-rearranged lung cancers. Most of the tumors are adenocarcinoma, but some cases involve other histological types, such as adenosquamous carcinoma. The tumors were significantly more common in younger patients and tended to occur in never-smokers and light smokers. The RET-rearranged lung adenocarcinomas are mostly well or moderately differentiated cancers and are thyroid transcription factor 1 (TTF-1) positive; the predominant growth pattern is very heterogeneous.\(^18,21\) Interestingly, Lee and colleagues\(^22\) reported that the mucinous cribriform pattern was more frequent with CCD6-RET- positive tumors (4/5, 80%), whereas the solid signet-ring cell pattern was present in 3 of 6 (50%) of the KIF5B-RET- positive tumors.

Takeuchi and colleagues\(^23\) showed results similar to the aforementioned ones: that the frequency of mucinous cribriform carcinoma was significantly higher in the kinase-fusion–positive group (ALK, ROS1 and RET) of tumors than in the fusion-negative adenocarcinomas. Conversely, the mucinous cribriform pattern was infrequently observed (13.6%) in a Japanese cohort of 22 cases selected from resected specimens at the National Cancer Center, Tokyo.\(^24\) Unlike in non–small cell lung cancer (NSCLC), there are some reports of RET gain-of-function point mutations in small cell lung cancer (SCLC). Dabir and colleagues\(^25\) identified an activating M918T RET somatic mutation in a metastatic small–cell lung cancer (SCLC) tumor specimen, which is among the most highly transforming RET mutations in vitro and leads to a severe clinical MEN2B phenotype.

It is interesting that RET rearrangements develop with a large prevalence in radiation- induced thyroid cancers. Furthermore, exposure to radon is a major risk factor for developing lung cancers. Thus, RET fusions may represent a genetic mechanism of radiation-induced lung adenocarcinoma, but further studies are needed.\(^25\)

There is no gold-standard technique to detect RET gene fusions, and most studies use multiple techniques, such as whole-genome and transcriptome sequencing, RNA sequencing, reverse transcription polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH), and immunohistochemistry (IHC). Although normal lung tissue shows low RET expression, IHC is not a reliable method to detect overexpressed RET because staining can vary and the immunoreactivity of available antibodies is weak. Overall, a combined strategy of RT-PCR and FISH, with dual color break-apart probe, is an effective tool for detection of RET chromosomal rearrangements. Reverse transcription polymerase chain reaction alone is usually insufficient to detect new partners or isoforms; therefore, FISH may be better in terms of sensitivity.\(^26\) More recently, broad hybrid, capture-based, next-generation
sequencing (NGS) was able to identify genomic alterations in 65% of tumors from never- or light-smokers with lung cancers that had previously been deemed free of genomic alterations by the aforementioned types of non-NGS testing. Therefore, NGS should be considered, if feasible.27

Targeting RET
Several commercially available multikinase inhibitors, such as vandetanib (Caprelsa), cabozantinib (Cabometyx), sorafenib (Nexavar), sunitinib (Sutent), lenvatinib (Lenvima), ponatinib (Iclusis), dovitinitib (TKI-258), and alectinib (Alecensa), have activity against the RET kinase. In 2013, Drilon et al28 first reported the response to a RET inhibitor, cabozantinib, in patients on a prospective, molecularly enriched trial for RET-positive lung cancers, and in 2016, they published the first stage of a phase II study with 25 cases29 (Table). The most common grade 3 treatment-related adverse events (TRAEs) were lipase elevation, increased levels of alanine aminotransferase and aspartate aminotransferase, decreased platelet count, and hypophosphatemia. Seventy-three percent of patients required cabozantinib dose reduction, most commonly due to palmar-plantar erythrodysesthesis, fatigue, and diarrhea.

Subsequent reports from 2 phase II trials testing the effect of vandetanib on RET-positive lung cancers showed discordant results, which may be explained by differences in patient selection and choice of assay30,31 (Table). The most common AEs with vandetanib were hypertension, diarrhea, rash acneiform, dry skin, prolonged QT corrected interval, anorexia, and increased creatinine.

Twenty-one percent of patients required vandetanib discontinuation, most commonly due to rash and pneumonitis, and 81% required dose reductions due to rash and hypertension.

Lenvatinib showed clinical benefits in patients with RET-rearranged lung adenocarcinomas, with a disease control rate of 76%, according to a phase II study presented at the 2016 European Society for Medical Oncology Congress. The most commonly reported trAEs were hypertension, nausea, anorexia, diarrhea, and proteinuria.

All of the aforementioned drugs are multikinase inhibitors with activity against advanced RET-rearranged lung cancers. The objective response rates (ORRs) were modest, but greater than with single-drug chemotherapy or single-drug immunotherapy, after progression on initial platinum doublet treatment in unselected patients with advanced NSCLC. Although clinically meaningful benefit was seen (Table), their activity was lower than that shown with EGFR and ALK inhibitors. These multikinase inhibitors are much more effective at inhibiting VEGFR, EGFR, and KIT than RET, which explains the high rate of off-target dose-limiting toxicities leading to frequent dose reductions and drug discontinuations. Hypertension and proteinuria, both commonly reported, can be related to VEGFR inhibition, while rash acneiform and diarrhea can be due to EGFR inhibition, and skin hypopigmentation and marrow suppression are related to KIT inhibition.

Alectinib, a known inhibitor of ALK, was shown to inhibit RET kinase activity (IC50 = 4.8 nmol/L) and the growth of RET fusion–positive cells by suppressing RET phosphorylation.32 In addition, alectinib showed kinase inhibitory activity against RET gatekeeper mutations (RET V804L and V804M). Lin and colleagues13 described 4 patients with advanced RET-rearranged lung cancers who were treated with alectinib. In total, 2 of 4 patients had overall responses, with durations of therapy of 6 months and more than 5 months. Given its more favorable safety profile, alectinib may be dosed more effectively to target RET, and it can represent an alternative to multikinase inhibitors.

More-specific RET inhibitors, with improved potency

<table>
<thead>
<tr>
<th>Drug</th>
<th>Kinase inhibition</th>
<th>Author</th>
<th>N</th>
<th>ORR</th>
<th>mPFS</th>
<th>mOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabozantinib</td>
<td>RET, ROS1, MET, VEGFR2, AXL, TIE2, KIT</td>
<td>Drilon et al28</td>
<td>26</td>
<td>28% (95% CI, 12%-49%)</td>
<td>5.5 mos (95% CI, 3.8-8.4)</td>
<td>9.9 mos (95% CI, 8.1-NR)</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>RET, EGFR, HER2, VEGFR</td>
<td>Yoh et al (LURET)29</td>
<td>17</td>
<td>53% (95% CI, 28%-77%)</td>
<td>4.7 mos (95% CI, 2.8-8.5)</td>
<td>11.1 mos (95% CI, 9.4-NR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lee et al30</td>
<td>18</td>
<td>18%</td>
<td>4.5 mos</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Velchetti et al31</td>
<td>25</td>
<td>16%</td>
<td>7.3 mos (95% CI, 3.6-10.2)</td>
<td>NR (95% CI, 5.8-NR)</td>
</tr>
</tbody>
</table>

mOS indicates median overall survival; m, months; mPFS, median progression-free survival; NR, not reached; ORR, objective response rate.
and reduced toxicity, are currently being investigated in the clinical and preclinical settings. Early-phase clinical trials of RXDX-105, a RET and BRAF inhibitor, which spares VEGFR2/KDR and VEGFR1/FLT, have been launched. A patient with advanced RET-rearranged lung cancer had a rapid and sustained response to RXDX-105 in both intracranial and extracranial disease.24 Other RET-specific inhibitors in development include LOXO-292 and BLU-667, which are both potent VEGFR-sparing RET inhibitors with specificity for RET and predicted resistant mutants. Of note, different sensitivities to RET inhibitors among different RET fusion forms are still unknown and need further study.

As in the case of other oncogene-driven lung cancers, resistance to RET inhibition is likely to emerge. We speculate that resistance to RET inhibition from the available multikinase inhibitors may be mediated more frequently by bypass signaling mechanisms than by RET-resistant mutations, because lower activity against RET exerts less selective pressure over the RET pathway. Also, RET-rearranged lung cancers might rely on alternative signaling pathways, and combination treatment may represent an alternative in the future.15,36

As with ALK- and ROSI-rearranged lung cancers, durable benefits with pemetrexed-based therapies in RET-rearranged lung cancers were seen. Drilon and colleagues37 retrospectively evaluated 104 patients with RET-rearranged lung cancers who received treatment with pemetrexed alone or in combination. Patients had a median PFS of 19 months (95% CI, 12-not reached) and an ORR of 45%. One might expect lower response rates to immunotherapy in RET-positive lung cancers, in accordance with other oncogene-driven lung cancers. A recent meta-analysis to assess the role of immune checkpoint inhibitors as second-line therapy in EGFR-mutant, advanced NSCLC showed that immunotherapy does not improve OS over docetaxel in this population. Gainor and colleagues38 observed a low ORR in a cohort of 58 patients with EGFR-mutant and ALK-positive lung cancer treated with a PD-1/PD-L1 inhibitor. Also, poor results with checkpoint blockade in patients with MET exon 14-mutant lung cancer were presented at the 2017 American Society of Clinical Oncology Annual Meeting. While PD-L1 expression was found in RET-rearranged lung cancers, the potential efficacy of checkpoint blockade in this population has not been tested so far.

Conclusions

RET-rearranged lung cancers represent a small subset of lung adenocarcinomas with clinicopathological features similar to those of other rearrangement-driven lung cancers. Given the low frequency of these cancers, collaboration among various international research centers can generate meaningful knowledge about them. A global, multicenter network of thoracic oncologists (RET registry) identified 165 patients with RET-rearranged lung cancers and has recently published the resultant data.39

Several multikinase inhibitors have shown activity and clinical benefit with RET-rearranged lung adenocarcinomas, which raises the question of whether this activity might be related to VEGF inhibition solely, as these drugs have shown increased response rates with unselected lung cancers after platinum-based chemotherapy.40,41 Dose reductions, likely related to off-target toxicities due to concomitant inhibition of non-RET kinases, prevent the delivery of optimal dosage. In addition, RET-rearranged lung cancer may also harbor concomitant genetic alterations that can decrease the likelihood of response to available RET inhibitors.

We eagerly await the new specific RET inhibitors, which, encouragingly, have less off-target toxicity and more potency. Recent advances in diagnostics should facilitate the identification of patients who will potentially benefit. Unbiased approaches using next-generation sequencing, including whole-genome sequencing, sequencing after capture of selected regions of RNA or DNA encompassing the relevant breakpoints in RET, or transcriptome sequencing of RNA, may be the best methodologies for the detection of RET chromosomal rearrangements in lung adenocarcinoma.42 This approach supports the conduct of “basket trials,” early-phase studies of novel targeted therapies specifically in patients whose tumors harbor the putative oncogenic target.

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