Precision oncology for RET-related tumors

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Aberrant activation of the RET proto-oncogene is implicated in a plethora of cancers. RET gain-of-function point mutations are driver events in multiple endocrine neoplasia 2 (MEN2) syndrome and in sporadic medullary thyroid cancer, while RET rearrangements are driver events in several non-medullary thyroid cancers. Drugs able to inhibit RET have been used to treat RET-mutated cancers. Multikinase inhibitors were initially used, though they showed modest efficacy and significant toxicity. However, new RET selective inhibitors, such as selpercatinib and pralsetinib, have recently been tested and have shown good efficacy and tolerability, even if no direct comparison is yet available between multikinase and selective inhibitors. The advent of high-throughput technology has identified cancers with rare RET alterations beyond point mutations and fusions, including RET deletions, raising questions about whether these alterations have a functional effect and can be targeted by RET inhibitors. In this mini review, we focus on tumors with RET deletions, including deletions/insertions (indels), and their response to RET inhibitors.

KEYWORDS
RET deletions, RET indels, acquired resistance, medullary thyroid cancer (MTC), RET-mutated cancers, pralsetinib, selpercatinib

Introduction

The RET proto-oncogene encodes for a transmembrane glycoprotein receptor with tyrosine kinase activity. It is involved in several cell processes during embryogenesis, including proliferation, differentiation, motility, and survival (1). RET gene mutations and fusions are known to be gain-of-function driver events in many cancer types (Figure 1).

RET germline gain-of-function mutations cause predisposition to multiple endocrine neoplasia 2 (MEN2) syndrome, while somatic RET mutations have been found in 40-65%
of sporadic medullary thyroid cancers (MTCs) (2–5). RET mutations carry out their oncogenic effect through two mechanisms. Mutations located in the extracellular cysteine-rich domain can lead to RET receptor constitutive dimerization and activation regardless of the presence of ligands, while mutations in the tyrosine kinase domains can cause a conformational change in the intracellular tyrosine kinase binding pocket, which allows constitutive kinase activation and altered substrate binding (1, 6–9) (Figure 1A).

Sanger sequencing is preferentially used for the detection of germline mutations in MEN2 syndrome; however, in evaluating the presence of somatic mutations, it may generate false negative results since it is not able to detect mutations below 15-20% of allele frequency. Alternatively, quantitative polymerase chain reaction (qPCR) or digital PCR can be used for the screening of somatic hotspot mutations, reaching a limit of detection of around 1% of allelic frequency or, in the case of digital PCR, less than 1% (10). The advent of high-throughput technologies, such as next-generation sequencing (NGS), has allowed hundreds to thousands of genes to be simultaneously analyzed, thus increasing the detection of novel or rare variants with a high sensitivity.

RET rearrangements occur in 10–20% of papillary thyroid carcinomas (PTCs), 1–2% of non-small cell lung cancers (NSCLCs), and in <1% of other cancers (e.g., colorectal cancer, breast cancer, chronic myelomonocytic leukemia, ovarian and salivary gland cancers, etc.) (11). RET rearrangements produce chimeras formed by the juxtaposition of an N-terminal partner to the RET C-terminal portion, including its catalytic domain. This leads to aberrant RET overexpression, ligand-independent dimerization, and kinase activation (Figure 1B). Notably, the RET fusion partner may influence the oncogenic potential of the chimeric RET protein affecting the intracellular location of the RET kinase (consequently the activated signaling pathways), and its expression levels (12). In addition, the altered function of the fusion partner may also have a role in the neoplastic transformation (13, 14).

Fluorescent in situ hybridization (FISH) is considered the standard used for the detection of RET rearrangements and has good sensitivity and specificity (10, 15). However, it may not be adequately informative regarding the specific RET fusion unless a specific fusion partner probe can be used (10, 15). Moreover, FISH results are difficult to interpret in many circumstances, such as in presence of pericentric fusions, deletions, and when possible partner genes are in close proximity to the RET gene (10). Reverse transcription-polymerase chain reaction (RT-PCR) can identify specific known RET fusion partners, but since it uses preselected primers, it is not able to detect novel fusion partners. Thus, it could underestimate the presence of RET rearrangements. Since this methodology is not ideal for the degraded and poor RNA quality isolated from formalin-fixed paraffin-embedded tissues, it is generally used together with other methodologies, such as immunohistochemistry (IHC) and FISH (10, 15). IHC is currently not indicated for the screening of RET alterations due to the high false positive and negative rates (15). Although DNA-based NGS can be designed for gene fusion detection, it doesn’t achieve high sensitivity and an RNA-based NGS is preferred (10).

The comprehensive genetic profiling of tumors made possible by novel detection technologies has resulted in the identification of multiple cancers with rare RET alterations beyond point mutations and fusions (16, 17), including a

![Figure 1](https://via.placeholder.com/150)

**FIGURE 1**
Molecular mechanisms of RET activation: mutations (Panel A) and fusions (Panel B). CRD, cysteine-rich domain; TKD, tyrosine kinase domain; sMTC, sporadic medullary thyroid cancer; PTC, papillary thyroid cancer; NSLC, non-small cell lung cancer; PDTC, poorly differentiated thyroid cancer; SCLC, small cell lung cancer.
nonnegligible number of RET deletions. In this review, RET deletions also include deletions/insertions (indels).

Ret deletions in cancers

RET deletions are not frequently found in MTC. They have been reported in 5% of all RET-mutated sporadic MTCs, as reported in the Catalogue of Somatic Mutations in Cancer (COSMIC database; https://cancer.sanger.ac.uk/cosmic, accessed June 2022), and they represent around 3.5% of all germlines RET alterations found in MEN2 patients, as reported in the ARUP database (https://arup.utah.edu/database/MEN2/MEN2_display.php accessed June 2022). In non-MTC cancers, their frequency is very low, ranging from 0.03% (COSMIC database) to 0.2% (cBioPortal for Cancer Genomics public databases; https://www.cbiportal.org/, accessed June 2022).

Ret in-frame deletions

Overall, 37 RET deletions have been described in MTC patients and almost all of them (36/37, 97%) are in-frame. Seven are germline deletions and are mainly located in the cysteine-rich domain, at exons 11 and 10 (18–24), and two are in the cadherin-like coding regions, at exons 6 and 7 (25, 26). Most RET deletions have been found in the tumor tissue of sporadic MTCs, mainly at exons 11 and 15 (3, 4, 25, 27–49), and to a lesser extent at exons 10 and 8 (30, 45, 49–51). Although only a few deletions have been reported in non-“hotspot” exons (i.e., exons 6 and 7), we cannot exclude that their frequency may be higher since those exons are not routinely studied. The prognostic role of the RET deletions (including indels) has not been clearly proved due to the few available data. However, in a recent paper, Elisei R. et al. observed that MTC harboring RET indels, show a more aggressive phenotype with a high prevalence of advanced cases at diagnosis (45).

RET in-frame deletions have also been described in other cancer types, as reported in the COSMIC and cBioPortal databases (52, 53). Fifteen in-frame RET deletions have been found in 30 oncologic patients. Interestingly, 6/30 patients (20%) are affected by pheochromocytoma (PHEO) and carry deletions in common with MTCs, mapping at RET exons 11 and 15. This is not surprising since both MTC and PHEO can be induced by activating RET alterations. The remaining in-frame RET deletions have been observed in breast, large intestine, gastric, pancreatic, kidney, and lung cancers.

Ret frameshift deletions

Only one RET frameshift deletion, p. Glu681Argfs*50, has been reported in an MTC patient. However, it was found in copresence with the RET p.A680T point mutation and its functional effect has not yet been demonstrated (36). Conversely, a greater number of RET frameshift deletions has been described in other cancers, as reported in COSMIC and cBioPortal public databases (52, 53). These deletions are spread out along the gene, including the hotspot exons.

Frameshift deletions are commonly loss-of-function alterations since they result in a shift of the reading frame used for protein translation, leading to a completely different sequence of the polypeptide. They often introduce an early stop codon resulting in a truncated protein. However, the major mechanism explaining the loss of function is nonsense-mediated mRNA decay, by which mutated mRNA is degraded (54).

It has yet to be proven whether RET in-frame and frameshift deletions in non-MTC and non-PHEO cancers are pathogenic.

Ret-targeted therapies

The identification of key driver oncogenes as targetable activated kinases has allowed clinicians to explore new treatment options. Therefore, multikinase inhibitors (MKIs) that target multiple tyrosine kinase receptors, including RET and those involved in angiogenesis, such as VEGFRs and PDGFRs, were initially used to treat advanced RET-mutated MTC and subsequently other RET-altered cancers (55, 56). Given their multi-target inhibition, it is not clear whether their observed antitumor activity is due to RET inhibition or the inhibition of other kinase targets (57, 58) (Table 1).

Cabozantinib and vandetanib have been approved for first-line treatment in MTC regardless of RET mutational status, even if the presence of RET mutations, particularly the RET p.M918T mutation, seems to be associated with a better response to cabozantinib in terms of overall response rate and progression-free survival (62, 63). Similarly, M918T mutation-positive patients also showed a higher response to cabozantinib (64). Vandetanib showed a higher median progression-free survival (mPFS) than placebo (30.5 vs 19.3 months) in the ZETA trial (64), as had cabozantinib in the EXAM trial (11.2 vs 4.0 months) (65). The clinical effectiveness of vandetanib and cabozantinib in advanced MTC patients was also confirmed from real-world data, showing a mPFS up to 47 months for vandetanib (66–68) and up to 4 months for cabozantinib (66). The median overall survival (OS) for vandetanib and cabozantinib was 53 months and 24 months, respectively, in the German real-world multicenter cohort (66).

MKI treatment of RET-rearranged NSCLC showed a modest clinical benefit that was lower than that observed with EGFR, ALK, and ROS1 inhibitors (61) (Table 1). Moreover, MKI response can differ depending on the fusion partner. For
| MTC-targeting agents                                | IC50 (nM) for RET (11) | Targets                                                      | Study phase | Mutations          | ORR  | mPFS* | mOS* | NCT         |
|---------------------------------------------------|------------------------|--------------------------------------------------------------|-------------|--------------------|------|-------|------|-------------|
| **Multitarget kinase inhibitors**                 |                        |                                                              |             |                    |      |       |      |             |
| Vandetanib                                        | 0.13                   | VEGFR2-3, EGF(R, RET                                        | III         | RET+RAS+unknown    | 45   | 30.5  | NR   | NCT00410761|
| Cabozantinib                                      | 5.2                    | VEGFR2, KIT, FLT-3, RET, MET                                | III         | RET+RAS+unknown    | 28   | 11.2  | 26.6 | NCT00704730|
| Sorafenib                                         | 5.9                    | BRAF, KIT, FLT-3, VEGFR2, PDGFR                             | II          | Not assessed       | 25   | NR    | NR   | NCT02114658|
| Lenvatinib                                        | 1.5                    | VEGFR1-3, FGFR1-4, PDGFRa, KIT, RET                         | II          | RET+RAS+          | 36   | 9     | 16.6 | NCT00784303|
| Anlotinib                                         |                        | VEGFR1-3, FGFR1-4, KIT                                      | II          | Not assessed       | 48.4 | 22.4  | 50.4 | NCT02586350|
| Sunitinib                                         | 5                      | PDGFR, KIT, VEGFR1-3, FLT-3, RET                            | II          | Not assessed       | 38.5 | 16.5  | 29.4 | NCT00510640|
| **Investigational**                              |                        |                                                              |             |                    |      |       |      |             |
| Regorafenib                                       | 1.5                    | BRAF, VEGFR1-3, PDGFRa, RET                                  | II          | -                  | -    | -     | -    | NCT02657551|
| **Selective RET-targeting inhibitors**            |                        |                                                              |             |                    |      |       |      |             |
| Pralsetinib                                       | 0.4                    | RET, VEGFR2                                                 | I/II        | RET/previous TKI   | 60   | NR    | NR   | NCT03037385|
| Selpercatinib                                     | 0.4                    | RET, VEGFR2                                                 | I/II        | RET/TKI Naive      | 71   | NR    | NR   | NCT03157128|
| **Investigational**                              |                        |                                                              |             |                    |      |       |      |             |
| TPX-0046                                          |                        | RET                                                          | I/II        | RET alterations    | -    | -     | -    | NCT04161391|
| TAS95953/HM06                                     |                        | RET                                                          | I/II        | RET alterations    | -    | -     | -    | NCT04683250|
| BOS172738                                         |                        | RET                                                          | I           | RET alterations    | -    | -     | -    | NCT03780517|
| SL-1001#                                         |                        | RET                                                          |              | -                  | -    | -     | -    |             |
| **RET-mutated NSCLC-targeting agents**             |                        |                                                              |             |                    |      |       |      |             |
| Selpercatinib (first line)                        | 0.4                    | RET, VEGFR2                                                 | I/II        | RET fusion-positive| 85   | NR    | -    | NCT03157128|
| Selpercatinib (previously received at least platinum-based chemotherapy) |                    | RET                                                          | I/II        | RET fusion-positive| 64   | 16.5  | -    | NCT03157128|
| Selpercatinib vs. carboplatin/ cisplatin + pemetrexed ± pembrolizumab |                    | RET                                                          | III         | RET fusion-positive| -    | -     | -    | NCT04194944|
| Pralsetinib (first line)                          | 0.4                    | RET, VEGFR2                                                 | I/II        | RET fusion-positive| 70   | 9.1   | NR   | NCT03037385|
| Pralsetinib (previously received platinum-based chemotherapy) |                    | RET                                                          | I/II        | RET fusion-positive| 61   | 17.1  | NR   | NCT03037385|
| Pralsetinib vs. carboplatin/ cisplatin + pemetrexed ± pembrolizumab or |                    | RET                                                          | III         | RET fusion-positive| -    | -     | -    | NCT04222972|

(Continued)
example, vandetanib showed a greater effect in CCDC6-RET fusion tumors compared with KIF5B-RET (57). However, the adverse effects of non-selective RET inhibitors observed in all treated tumors due to their off-target side effects are responsible for high discontinuation and dose reduction rates (e.g., 12% and 35% for vandetanib and 16% and 79% for cabozantinib when used as thyroid cancer treatments, respectively) (57).

In the last years, small and highly selective RET inhibitors have been designed to overcome the treatment-related toxicities of non-selective RET inhibitors and acquired resistance to them (57). The new selective RET inhibitors pralsetinib (LOXO-292) and selpercatinib (BLU-667) have demonstrated both good efficacy and tolerability: in phase I/II trials, the mPFS was not reached, and the overall response rate was 71% and 73% (first line treatment), and 60% and 69% (second line treatment), respectively (Table 1). Currently, both drugs have been approved by the Food and Drug Administration (FDA) for the treatment of patients more than 12 years of age with: i) advanced or metastatic RET-mutant MTC; ii) RET fusion-positive metastatic NSCLC, and iii) advanced or metastatic RET fusion-positive thyroid cancer patients who require systemic therapy and who are radioactive iodine refractory. These drugs also show robust activity in other RET alteration-positive solid tumors (69).

Specific mutations and acquired resistance

Some specific mutations are expected to cause acquired resistance to MKI treatments (12). Preclinical studies have shown that acquired gatekeeper mutation V804L is associated with MKI resistance (12). The emergence of a V804M mutation was reported in a patient with RET-mutant, sporadic MTC treated previously with multiple MKIs (74). Emergent V804L and S904F mutations were reported in patients with RET fusion-
positive NSCLC during treatment with vandetanib (75, 76). The frequency, prognostic role, and clinical actionability of these mutations are not entirely clear (75). Some preclinical models identified other resistance mutations, including the V804E, G810A/S/R, I788N, 730I, E732K, V871I, V738A, A807V, F998V and Y806N (13, 77, 78).

Selpercatinib has a specific binding modality: both front and back pockets of RET are occupied without being affected by V804 mutations (unlike other tyrosine kinase inhibitors) (11). Selpercatinib was developed to be effective in RETV804L and RETV804M gatekeeper mutations and was found to be 60–1300 fold more effective than multitarget inhibitors against cell lines engineered with KIF5B-RETV804L/M gatekeeper mutations (74).

Conversely, RET mutations at the C-lobe solvent front (RET p.G810C/S/R), hinge (RETY p.806C/N), and b2 strand (RET p.V738A, only identified in cell lines) cause acquired resistance to selpercatinib (61, 79–81). Structural modeling showed that selpercatinib binding to the kinase ATP/selpercatinib binding site can be hindered if the glycine residue at position 810 in the RET solvent front is substituted with charged or polar residues (79, 80). In vitro experiments using BaF3/KIF5B-RET cells showed that pralsetinib and selpercatinib bind to RET in a similar mode and both are resistant to the same mutations (80), although some mutations (i.e., L730V/I) seem to be resistant only to pralsetinib (82).

New selective inhibitors in clinical development

New selective RET inhibitors are under development (Table 1). TPX-0046 is a dual RET/SRC kinase inhibitor, with activity in drug-resistant and naïve RET-driven cancer models. It is in phase I/II clinical trials for advanced solid tumors harboring RET fusions or mutations (NCT04161391). TAS0953 (HM06) is undergoing a phase I/II study in patients with advanced solid tumors with RET gene abnormalities (NCT04683250). SYHA1815 has an approximately 20-fold selectivity for RET over VEGFR2 and is being studied in a phase 1 trial in China (83). Other potential drug compounds, such as LOX-18228, LOX-19260, BOSI72738 (DS-5010), and SL-1001 (84–86), are still in the preclinical stage. There are also research efforts to obtain mutant-selective inhibitors that may offer clinical advantages.

Clinical response in patients with RET deletions

Despite efforts to develop super-selective inhibitors, data available on the response of cancers harboring RET deletions to selective RET inhibitors are scarce and concern only MTCs. A RET p.D378_G385delinsE MTC was treated with selpercatinib and achieved partial response, with a maximum tumor reduction of 86% (87). The treatment of two RET p.L629_D631delinsH MTCs, one with cabozantinib and the other with a combination of sorafenib and tipifarnib, showed a partial response, with a tumor reduction of 48% and 46%, respectively (43, 44). Two MTCs with RET p.E632_636del and p.L633_A639del were treated with vandetanib and cabozantinib, respectively, showing stable disease (25, 43). In one case, disease progression was observed after seven months of treatment (22). Recently, two MTC patients with the p.E632_L633del and p.D631_L633delinsS RET deletion, respectively, who were previously treated with cabozantinib and/or vandetanib, experienced a treatment benefit with selpercatinib, with a rapid biochemical response. In particular, the first patient showed a partial response in the target lesions and stable disease in non-target lesions, while the second patient showed stable disease and a partial response in target and non-target lesions, respectively (45). An in vitro study provided evidence that the p.G630del RET alteration is sensitive to pralsetinib (23). A RET p.D898_E901del MTC was treated with cabozantinib, showing stable disease (43). Lastly, Zhao et al. used mutant-transformed Ba/F3 cells to demonstrate that p.D898_E901del is sensitive to selpercatinib and pralsetinib (88).

Discussion

The advancement of sequencing technologies has allowed comprehensive genetic profiling of tumors and the identification of new RET alterations, including deletions. Although the reported tumors with RET deletions are few, we cannot exclude that their real prevalence may be higher. Indeed, in clinical practice, RET deletions are usually not investigated through the gene.

Data on RET deletions as driver alterations in cancer are still scarce. In MTC and PHEO, only RET in-frame deletions have been reported, supporting their possible gain-of-function role. For a few of them, their oncogenic potential has been demonstrated through in vitro experiments (21, 23, 26, 49, 88–90). Conversely, frameshift deletions have been observed in a wide range of tumor types, except MTC and PHEO, though their functional role as driver alterations has not yet been demonstrated.

To date, limited information about the response of tumors with RET deletions to RET inhibitors is available and only concerns MTC patients. In those patients, treatment efficacy seems to be comparable to MTCs with RET point mutations. Considering the potential benefit of treating tumors with RET inhibitors, it is crucial to understand the real impact of these
deletions in cancer development and progression and their response to RET targeted therapies.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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