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# Genomics Review of Selective RET Inhibitors Sensitivity in Thyroid Cancer Clinical Trials

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## ABSTRACT

*RET* gene is a driver of thyroid cancer (TC) tumorigenesis. The incidence of TC has increased worldwide in the last few decades, both in medullary and follicular-derived subtypes. Several drugs, including multikinase and selective inhibitors, have been explored. Selpercatinib and pralsetinib are selective RET inhibitors that have shown clear clinical benefits for patients in the LIBRETTO and ARROW trials, respectively. Currently, their development and application in clinical practice are ongoing. However, its efficacy in different *RET* pathogenic variants has not yet been well established. Although selpercatinib and pralsetinib achieved a high ORR, no data are available regarding the differences in tumor responses of both TC groups according to *RET* pathogenic variants. Clinical trials and literature have analyzed the efficacy of selective RET inhibitors with a special interest in the most common variants. A review of LIBRETTO and ARROW trials was made regarding the change in tumor size depending on the pathogenic variants. M918T pathogenic variant resulted in a higher complete response rate. Patients who underwent fusion had the highest ORR (objective response rate). MKi-treated patients did not exhibit significant differences from untreated patients. Different *RET* pathogenic variants are not biomarkers of RETi response in TC. Selpercatinib showed a tendency to achieve a complete response. All patients with *RET* pathogenic variants should receive treatment with selpercatinib or pralsetinib at any moment of the therapeutic schedule owing to off-target inhibition and toxicity. Therefore, new targets for drug sensitivity and resistance should be explored.

## 1 | Introduction

### 1.1 | Thyroid Cancers

Thyroid cancer (TC) is the most prevalent malignant neoplasm of the endocrine system and its incidence has increased over the last decades (Cabanillas, McFadden, and Durante 2016). However, advances in the identification of genetic biomarkers and the development of targeted drug therapies are being made. However, aggressive TC mortality has not decreased. Radioactive iodine treatment after surgery improves the overall survival of differentiated thyroid cancer (DTC) patients with a high risk of recurrence (Boucai, Zafereo, and Cabanillas 2024). Nevertheless, virtually all patients with metastasis eventually progress after systemic treatment.

Depending on their cellular origin, TC can be classified as follicular and C cell-derived cancers. Follicular-derived tumors were further classified according to their histological degree of dedifferentiation. Follicular cell-derived (FC-TC) malignancies include

differentiated (DTCs), poorly differentiated (PDTC), and anaplastic thyroid carcinomas (ATCs) (Juhlin, Mete, and Baloch 2023) (Table 1). The most common driver of the disease is the *BRAF*$^{V600E}$ pathogenic variant. Other drivers have been reported to harbor pathogenic variants in the *RAS* isoforms and *RET* rearrangements. In recent years, the survival rate of DTCs has notably improved because of the use of multikinase inhibitors (MKi) (Boucai, Zafereo, and Cabanillas 2024; Fagin, Krishnamoorthy, and Landa 2023).

Medullary thyroid carcinoma (MTC) arises from C cells, with 25% of cases being related to familial or hereditary syndromes. *RET* pathogenic variants are the main driver of this neoplasm, followed by *RAS*. The survival rate for these tumors in 5 years is 65% (Stamatakos et al. 2011). MKis has resulted in an impressive improvement in the survival of these patients (Carling and Udelsman 2014).

### 1.2 | REarranged During Transfection

In 1985, a new oncogene, *RET* (REarranged during Transfection), was discovered. *RET* is located on chromosome 10 (10q.11.2), and the encoded protein is a transmembrane tyrosine kinase receptor (RTK) (Takahashi, Ritz, and Cooper 1985; Salvatore, Santoro, and Schlumberger 2021), predominantly found in parafollicular thyroid C cells. RET protein is not constitutively expressed in follicular cells compared with parafollicular cells.

RET receptor activation involves the binding of the glial cell-derived neurotrophic growth factor (GDNF) family of ligands to a glycosylphosphatidylinositol-linked co-receptor on the cell surface, called the GDNF receptor (GFRα). As a result of the interaction between RET kinase and the GDNF family of ligands, the receptor dimerizes, leading to the phosphorylation of specific tyrosine residues within the receptor tyrosine kinase domain, which in turn promotes receptor activation (Goodman et al. 2014). Consequently, activating RTK and RET triggers downstream pathways that promote cell growth, proliferation, survival, and differentiation, including the MAPK and PI3K signaling pathways.

Upon homodimerization of RET kinase, its intracellular domain undergoes phosphorylation at several tyrosine residues that are involved in signal transduction and activation of downstream kinases (Figure 1A). Numerous pathogenic variants in *RET*, including point mutations and rearrangements, have been shown to trigger constitutive ligand-independent oncogenic activation (Goodman et al. 2014; Regua, Najjar, and Lo 2022). Point mutations are a feature of MTCs, particularly in codons 634, 804, and 918. In contrast, oncogenic activation

**TABLE 1** | Incidence and prevalence of different alterations in thyroid cancer (San Román Gil et al. 2020; Hu et al. 2021; Ibrahimpasic et al. 2019; Stamatakos et al. 2011).

| Classification of thyroid tumors | | Thyroid cell origin | Incidence | Mutations | 5-year survival |
|---|---|---|---|---|---|
| **DTC** Differentiated Thyroid Cancer | **PTC** Papillary Thyroid Carcinoma | **FC** Follicular cells | 80% | ***RET* rearrangements**<br>*BRAF*<br>*RAS* | 98% |
| | **FTC** Follicular Thyroid Carcinoma | | 10% | *RAS* | 95% |
| **PDTC** Poorly Differentiated Thyroid Carcinoma | | | 2-15% | *BRAF* *EIF1AX*<br>*RAS* *TERT*<br>***RET* rearrangements** | 66% |
| **ATC** Anaplastic Thyroid Carcinoma | | | 1% | *BRAF* *TP53*<br>*RAS* *TERT*<br>***RET* rearrangements** | 12% |
| **MTC** Medullary Thyroid Carcinoma | | **C cells** | 2-3% | ***RET* mutations** | 65% |

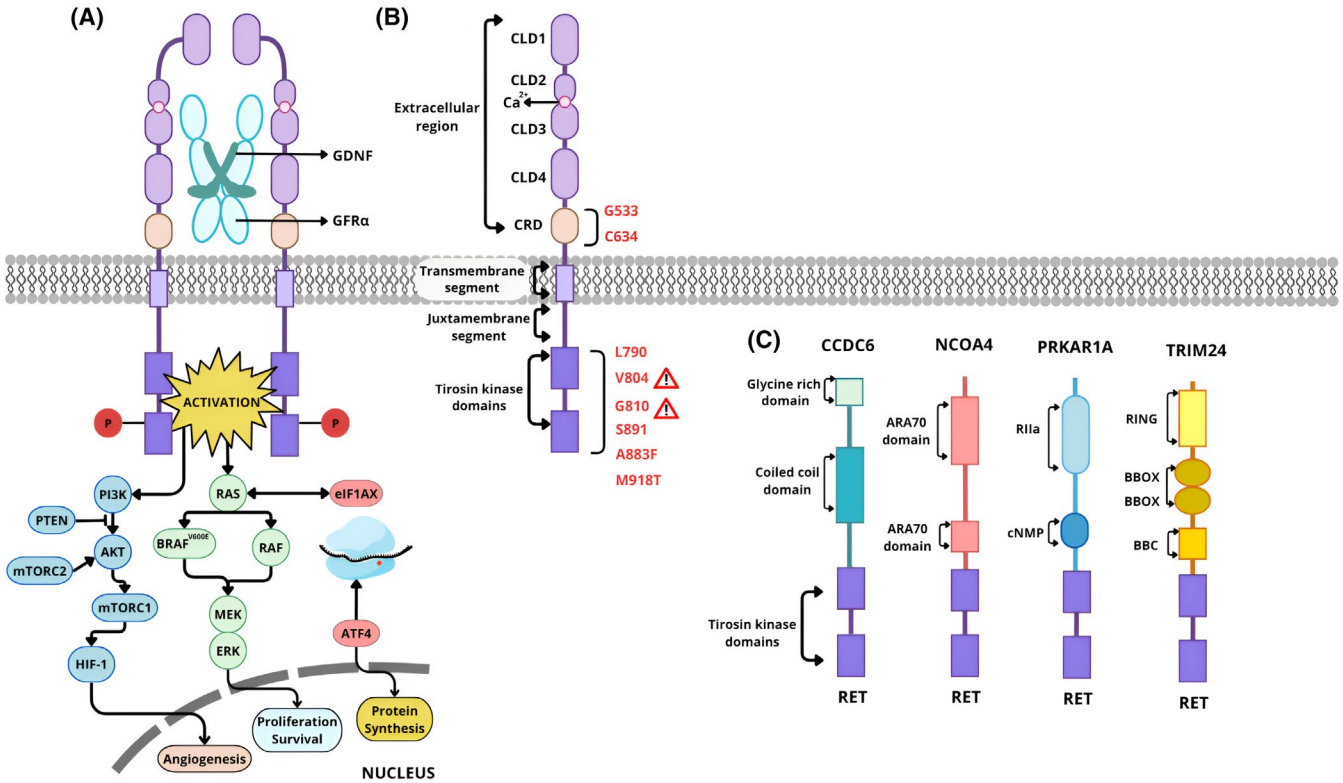


**FIGURE 1** | (A) RET pathway. (B) The most prevalent pathogenic variant of RET in MTCs, including V804, a resistance amino acid change for RET-selective inhibitors. (C) The most prevalent rearrangements in FC-TC.

of *RET* in DTCs is achieved via fusion with gene partners such as *CCDC6*, *NCOA4*, and *KIF5B* (Salvatore, Santoro, and Schlumberger 2021).

## 1.3 | RET Genomics in Thyroid Cancer

*RET* pathogenic variants are classified as point mutations (hereditary or sporadic), proper MTC disease, or rearrangements present in FC-TC (Figure 1).

### 1.3.1 | Hereditary RET Point Mutations

Point mutations are the most common germline *RET* pathogenic variants. Different types of syndromes can arise depending on the *RET* pathogenic variant detected.

MEN (Multiple Endocrine Neoplasia) 2 germline *RET* pathogenic variants result in gain-of-function mutations, in contrast to other hereditary predispositions to cancer syndromes caused by loss-of-function pathogenic germline variants (Santoro et al. 1995).

MEN2A is the most common hereditary type of MTCs. Pathogenic variants in the 634 codon of *RET* exon 11 have been observed in most MEN2A cases. Other alterations have been reported in codons 609, 611, 618, and 620 in exon 10 (Mathiesen et al. 2022). All of these pathogenic variants are in the *RET* exon that codified the extracellular domain and are enriched with cysteine residues (Figure 1B). MEN2A patients usually develop MTC, pheochromocytoma, and hyperparathyroidism. Cutaneous lichen amyloidosis is ligated to variants of codon 634 and Hirschsprung's disease with alterations in the other aforementioned extracellular regions.

Regarding MEN2B syndrome, the most common pathogenic variant is M918T, followed by A883F (Mathiesen et al. 2017). The clinical manifestations of MEN2B include MTC, pheochromocytoma, and extra-endocrine factors, including ganglioneuromatosis of the aerodigestive tract (Mathiesen et al. 2022).

In patients with Familial MTC (FMTC) syndrome, the most prevalent alterations are located in codons 533, 768, and 804 in the extracellular and intracellular domains (Mathiesen et al. 2022). In patients with early FMTC, it is difficult to distinguish it from the MEN2A syndrome. The main difference is the follow-up period, in which subjects with FMTC syndrome did not develop pheochromocytoma or primary hyperparathyroidism (Wells et al. 2015).

### 1.3.2 | Sporadic RET Point Mutations

Somatic *RET* point mutations are also found in sporadic MTCs, with M918T being the most common pathogenic variant. Indels have been also described (Ciampi et al. 2019; Elisei et al. 2022).

### 1.3.3 | RET Fusions

*RET* fusions are related to DTCs and reported in 10%–20% of PTCs (Fagin, Krishnamoorthy, and Landa 2023; Cancer Genome Atlas

Research Network 2014). The most prevalent rearrangement occurred in the *RET* intron 11. Coiled-coil domain-containing 6 (*CCDC6*) *RET* (called RET–PTC1) and nuclear receptor co-activator 4 (*NCOA4*) *RET* (known as RET–PTC3) are the most frequent *RET* fusion partners in PTCs. These alterations have been predominantly reported in pediatric (Franco et al. 2022) cases and radiation-induced (Morton et al. 2021) TC. Lymph node and distant metastases were more common in patients with RET-PTC3 (Pekova et al. 2023).

Fusions are mutually exclusive with each other and with other settled pathogenic variants in follicular thyroid carcinogenesis (e.g., *RAS* and *BRAF*). Younger age is a significant factor in the development of positive *RET* fusions in PTCs. Other fusions reported in the literature include *KIF5B*, *PRKAR1A, KTN1*, or *TRIM24* gene (Salvatore, Santoro, and Schlumberger 2021).

### 1.4 | RET Inhibitors in TC

Advances in biomarker detection and genomic sequencing have led to the development of novel targeted drugs. MKi with an anti-angiogenic profile significantly improves progression-free survival (PFS) in patients. Over the years, they have been on the front line of advanced DTC and MTC treatment (Gild et al. 2011). Notable examples of DTC treatment in a radioiodine-refractory setting include sorafenib (Brose et al. 2014), lenvatinib (Schlumberger et al. 2015), and cabozantinib (Brose et al. 2022), all of which mainly target the VEGFR. All Phase 3 trials showed an impact on PFS compared with placebo and significant overall response rates (ORRs).

MKi used in MTC settings includes vandetanib (Wells et al. 2012) and cabozantinib (Elisei et al. 2013). Vandetanib is effective against pathogenic variants in VEGFR2, RET, and EGFR proteins, whereas cabozantinib acts in the same manner as VEGFR2, RET, and MET kinases. Clinical trials have shown a remarkable enhancement in PFS and ORR compared with placebo (Wells et al. 2012; Elisei et al. 2013).

Despite the clinically meaningful impact of these drugs, MKi disadvantages include limited efficacy and high rates of adverse events reported with implications for quality of life, mainly asthenia and hypertension (Liu et al. 2016; Højer Wang et al. 2023). MKi can inhibit *RET* pathogenic variants, but only the M918T at nanomolar concentration (Seoane and Capdevila 2018). Furthermore, these drugs were ineffective against the *RET* V804 gatekeeper mutations (Carlomagno and Santoro 2004; Nakaoku et al. 2018; Dagogo-Jack et al. 2018; Wirth et al. 2019).

Selective RET inhibitors (RETi) have been used to treat *RET*-mutant cancers. Selpercatinib (LOXO-292) is an ATP-competitive, selective RET kinase inhibitor. Its antitumor activity is strong in human cancer cell lines and xenografts (Subbiah, Velcheti et al. 2018). The clinical trial LIBRETTO 001 (NCT03157128) demonstrated impressive results across three cohorts, including previously treated and treatment-naïve patients with *RET* pathogenic variants (subjects with MTC), as well as *RET* fusions (patients with PTCs); ORR and 1y-PFS were 69%/82%, 73%/92%, and 79%/64%, respectively (Wirth et al. 2020). In the Phase 3 LIBRETTO-531 trial, selpercatinib exhibited superior efficacy compared with MKi (vandetanib or cabozantinib) in treatment-naïve MTC patients, with 69 versus 38% ORR and 86 versus 65% 1y-PFS, respectively (Hadoux et al. 2023).

Pralsetinib (formerly BLU-667) is a highly selective small RETi. It demonstrated impressive outcomes compared with MKi in in vivo and in vitro models (Subbiah, Gainor et al. 2018). The ARROW (NCT03037385) Phase 1 trial explored its efficacy in three different cohorts. The pretreated *RET* mutant had an ORR of 60% and a 1y-PFS of 75%. In *RET* mutation-naïve patients, ORR was 71% and 81%, respectively. *RET* fusions resulted in an ORR of 89% and PFS of 81% (Subbiah, Hu et al. 2021). These efficacy data were maintained for further trial (Subbiah, Hu et al. 2024).

The toxicity profiles of both drugs were better than those of MKi. LIBRETTO 531 reported a better toxicity profile with selpercatinib than with the standard therapy. RETi may cause nonconventional adverse events (such as chylous effusion and gastrointestinal side effects during selpercatinib treatment) that are not observed with MKi administration (Hadoux et al. 2023). Both RET-selective inhibitors can target *RET* pathogenic variants, including the V804 gatekeeper mutations related to resistance to MKi. However, new potential resistance *RET* pathogenic variants have been described as resistance (Elisei et al. 2013) mechanisms for RETi, such as 806 and 810 RET aminoacids (Subbiah, Shen et al. 2021). New-generation RETi are currently under development for Phase I trials.

In this review, we analyzed the most common *RET* pathogenic variants in TC and their potential implications for the efficacy of RETi.

## 2 | Methods

The pathogenicity of *RET* variants was contrasted with the literature in the databases OncoKB (Chakravarty et al. 2017; Suehnholz et al. 2024), and COSMIC (Tate et al. 2019; COSMIC n.d.).

The criteria for including clinical trials in the review were RET-selective inhibitor trials that received FDA or EMA approval for clinical use in TC since 2020. These were only the trials for selpercatinib and pralsetinib. Clinical trials that did not obtain approval were excluded.

Data from the waterfall plots of the figures reported in ARROW (Subbiah, Hu et al. 2024) (clinical trial for pralsetinib) and LIBRETTO (Wirth et al. 2020) (clinical trial for selpercatinib) were extracted to study the maximum change in tumor size. Three cut-offs were used: 30%, the rate established by Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1. protocol (Eisenhauer et al. 2009), 80%, and 100% (complete response by RECIST v.1.1).

*RET* pathogenic variants were independently studied based on histological subgroups.

Variables (pathogenic variants, drug administrated, and the achievement of the different cut-offs) codified as qualitative features were analyzed using a two-tailed Fisher's exact test in

IBM SPSS Statistics v29.0.1.0. Statistical significance was set at $p \leq 0.05$. A tendency of correlation was assumed between *p*-values of 0.05 and 0.170. The *p-values* between 0.170 and 0.250 were marked in the tables as weak association trends.

## 3 | *RET* Pathogenic Variants in TC Published Cohorts

### 3.1 | Hereditary RET Point Mutations

Characterization of large hereditary MEN2 MTC cohorts (Maciel et al. 2019; Romei et al. 2010; Machens et al. 2013; Lebeault et al. 2017) (Figure S1) revealed that the most prevalent pathogenic variants in all studies were the ones that imply a change in the amino acid site C634. Some differences have been reported among different casuistics and origins. The second prevalent pathogenic variant depends on the survey was M918T or V804. G533 amino acid had one of the highest prevalence rates in a Brazilian cohort. Changes in the amino acid site L790 were more frequent in French and German studies than in V804. Italian reports have also shown differences with a high prevalence of S891 amino acid changes.

### 3.2 | Sporadic RET Point Mutations

Regarding one of the largest sporadic MTCs cohort (Ciampi et al. 2019), the results of 148 patients showed that the most prevalent point mutation was M918T (40.5%). This pathogenic variant coexisted with others in six cases (three with *RET* and three with *RAS* variants). The second most prevalent pathogenic variants were the changes in the amino acid C643 (12.2%).

*RET* indels were found in 14 (9.5%) patients. Few studies have investigated *RET* indels. Elisei et al. reported that these pathogenic variants are related to aggressive behavior. The efficacy of selpercatinib was detailed for two patients, who reported a meaningful tumor response (Elisei et al. 2022).

The pathogenic variants specifically studied in LIBRETTO and ARROW were M918T and the changes in the amino acid site V804, respectively. Two other variant groups were analyzed: those that affected the cysteine-rich extracellular domain (EC) and other that were not previously named.

### 3.3 | RET Fusions

Analyzing the cBioportal v.6.0.2 database, in which only three studies of FC-TC are available, 807 samples (500 PTCs of TCGA, (Cancer Genome Atlas Research Network 2014), 117 samples of the Landa et al. (2016) study one PDTCs and ATCs, and 190 ATCs from the GATCI initiative (Zeng et al. 2024)) were studied, and 8% showed a *RET* pathogenic variant. Of the PTC cohort (Cancer Genome Atlas Research Network 2014), 7% showed pathogenic variants, one case reported a point mutation V945M, 35 harbored a structural variant, and two patients also had a homozygous deletion. The most prevalent rearrangement was *CCDC6-RET* (17/35), followed by the *NCOA4-RET* fusion (5/35). Note that both subjects with homozygous deletions also harbored an *NCOA4-RET* fusion. In the article by Landa et al. (2016), 4% (5/117) of the cases showed *RET* rearrangements. All the subjects with altered *RET* in this cohort were PDTCs. Of the five cases, three reported RET-PTC1 fusion and the other reported RET-PTC3 fusion. Nonetheless, regarding the GATCI article (Zeng et al. 2024), 13% of the ATCs reported pathogenic variants in *RET*. Surprisingly, fusion was not observed. Ten patients had amplifications and 8 homozygous deletions. Three patients harbored *RET* pathogenic variants in the extracellular region.

Based on this data, the most prevalent fusions were represented in the ARROW analysis for pralsetinib (Subbiah, Hu et al. 2021, 2024) (*CCDC6-RET* and *NCOA4-RET*). Other fusions were included in the same group of analyses. Despite *RET* fusions, patients were also included in the clinical trial LIBRETTO, and response rates were reported for specific rearrangements. Nevertheless, other studies have demonstrated that selpercatinib responds significantly to *RET*-fusion-altered tumors (Dias-Santagata et al. 2020; Drilon et al. 2023).

## 4 | Sensitivity of Alterations for Selective RETi

Different *RET* pathogenic variants have shown specific efficacy outcomes for selective RETi in patients with MTC and FC-TC. The complete datasets of ORR and PFS for specific pathogenic variants have not been reported in published clinical trials. Both studies (LIBRETTO and ARROW) demonstrated differences in ORR and PFS among the three groups analyzed (Table 2). LIBRETTO results revealed that the highest ORR and 12-month PFS among patients with MTC were obtained in naïve cases, which occurred in ARROW.

Patients with FC-TC exhibited the highest ORR in both the clinical trials. However, although more than half of the patients obtain a 12-months PFS with LIBRETTO and ARROW, there was a difference of 64% and 87%, respectively.

The maximum change in tumor size in the LIBRETTO-001 group of patients with MTC is given in Table 3. Among the treated cases, 71.4% (20/28) with the M918T pathogenic variant achieved 30% reduction. A weak inverse tendency was observed for this association ($p = 0.160$). It is worth mentioning that just five patients in this cohort achieved a total response, and 80% of them (4/5) reported the M918T pathogenic variant.

Regarding naïve patients, the association of M918T patients with 30% baseline showed a trend ($p = 0.159$).

Among the EC-mutated cases, 67% showed a 30% response rate, which resulted in an inverse tendency of association ($p = 0.094$).

Only seven of the 79 naïve patients showed a complete response. Strikingly, 71.4% (5/7) of the patients exhibited the M918T pathogenic variant. *RET* fusion cohort results were not reported considering the percentage of tumor size reduction in LIBRETTO-001.

Considering all MTC patients described in LIBRETTO-001 ($n = 127$), no tendency or significant association was observed

**TABLE 2** | PFS (progression-free survival) and ORR (objective response rate) for ARROW and LIBRETTO.

| | LIBRETTO 001[38] | LIBRETTO 531[39] | ARROW (2021)[41] | ARROW (2024)[42] |
|---|---|---|---|---|
| ***RET* mutant treated (MTC)** | n=55<br>ORR=69%<br>PFS=82% | | n=61<br>ORR=60%<br>PFS=82% | n=67<br>ORR=52%<br>PFS=74% |
| ***RET* mutant naïve (MTC)** | n=88<br>ORR=73%<br>PFS=92% | n=291<br>ORR=69%<br>PFS=86% | n=23<br>ORR=71%<br>PFS=81% | n=67<br>ORR=72%<br>PFS=85% |
| ***RET* fusions (FC-TC)** | n=19<br>ORR=79%<br>PFS=64% | | n=11<br>ORR=89%<br>PFS=81% | n=25<br>ORR=84%<br>PFS=87% |

*Note:* PFS was calculated as 12 months' rate, % [95% CI].
Abbreviations: FC-TC, follicular cell derived-thyroid cancer; MTC, medullary thyroid cancer.

**TABLE 3** | Associations between tumor size reduction and specific pathogenic variants in LIBRETTO-001 patients treated with selpercatinib.

| | NAÏVE n=79 | | | | | | TREATED n=48 | | | | | | TOTAL n=127 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30% | | 80% | | 100% | | 30% | | 80% | | 100% | | 30% | | 80% | | 100% | |
| | p-value | n (%) | p-value | n (%) | p-value | n (%) | p-value | n (%) | p-value | n (%) | p-value | n (%) | p-value | n (%) | p-value | n (%) | p-value | n (%) |
| M918T | 0.159 | 39/45 (87) | 0.246 | 11/45 (24) | 0.692 | 5/45 (11) | 0.160* | 20/28 (71) | 0.737 | 8/28 (29) | 0.385 | 4/28 (14) | 1 | 59/73 (81) | 0.188 | 19/73 (26) | 0.234 | 9/73 (12) |
| V804 | 0.239* | 3/5 (60) | 1 | 1/5 (20) | 1 | 0/5 (0) | 1 | 4/5 (80) | 1 | 1/5 (20) | 1 | 0/5 (0) | 0.412 | 7/10 (70) | 1 | 2/10 (20) | 0.596 | 0/10 (0) |
| EC | 0.094* | 12/18 (67) | 0.499 | 2/18 (11) | 1 | 1/18 (6) | 0.320 | 6/6 (100) | 1 | 1/6 (17) | 1 | 0/6 (0) | 0.568 | 18/24 (75) | 0.405 | 3/24 (13) | 0.461 | 1/24 (4) |
| OTHER MUTATIONS | 0.680 | 10/11 (91) | 0.680 | 1/11 (9) | 1 | 1/11 (9) | 0.661 | 8/9 (89) | 1 | 2/9 (22) | 1 | 1/9 (11) | 0.360 | 18/20 (90) | 0.563 | 3/20 (15) | 1 | 2/20 (10) |
| TREATED PATIENTS | | | | | | | | | | | | | 0.821 | 38/48 (79) | 0.504 | 12/48 (25) | 0.764 | 5/48 (10) |

significant association | tendency | weak trend | no trends

*Note:* All patients were diagnosed with MTC (Medullary thyroid cancer). Numbers with * indicate inverse relationships.
Abbreviation: EC, extracellular domain.

(Table 3). 81% of M918T cases had a rate of at least 30%. Interestingly, of the patients who achieved total tumor reduction, 75% (9/12) were M918T mutant cases. There was no significant difference between naïve and MKi pretreated patients in selpercatinib tumor response, considering the change in tumor size.

The ARROW clinical trial was analyzed according to the size of tumor reduction using three previously described baselines.

Table 4A presents the results of the ARROW separated into naïve and treated patients with MTC. Notably, all patients (4/4) who reported total tumor shrinkage exhibited the M918T pathogenic variant. Three ARROW-treated patients harbored the M918T variant, coexisting with the change in the amino acid site V804. These patients were identified as M918T patients in this study.

A cohort of naïve patients was evaluated using the same criteria. In particular, the only subject that reached a complete reduction

**TABLE 4** | Associations between reduction in tumor size and specific pathogenic variants in ARROW 2024 patients treated with pralsetinib.

A)

| | NAÏVE n=61 | | | | | | TREATED n=59 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30% | | 80% | | 100% | | 30% | | 80% | | 100% | |
| | p-value | n (%) | p-value | n (%) | p-value | n (%) | p-value | n (%) | p-value | n (%) | p-value | n (%) |
| M918T | 0.738 | 29/34 (85) | 0.647 | 2/34 (6) | 0.443 | 0/34 (0) | 1 | 7/41 (17) | 1 | 7/41 (17) | 0.303 | 4.41 (10) |
| V804 | 0.164* | 0/1 (0) | 1 | 0/1 (0) | 1 | 0/1 (0) | 1 | 0/2 (0) | 1 | 0/2 (0) | 1 | 0/2 (0) |
| EC | 0.729 | 20/23 (87) | 0.356 | 3/23 (13) | 0.377 | 1/23 (4) | 0.479 | 1/12 (8) | 0.670 | 1/12 (8) | 0.572 | 0/12 (0) |
| OTHER MUTATIONS | 0.421 | 2/3 (67) | 1 | 0/3 (0) | 1 | 0/3 (0) | 0.564 | 2/4 (50) | 0.130 | 2/4 (50) | 1 | 0/4 (0) |

B)

| | FUSIONS n=22 | | | | | |
|---|---|---|---|---|---|---|
| | 30% | | 80% | | 100% | |
| | p-value | n (%) | p-value | n (%) | p-value | n (%) |
| CCDC6 | 1 | 12/13 (92) | 1 | 4/13 (31) | 0.409 | 0/13 (0) |
| NCOA4 | 1 | 5/5(100) | 1 | 2/5 (40) | 0.227 | 1/5 (20) |
| OTHER FUSIONS | 1 | 4/4 (100) | 1 | 1/4 (25) | 1 | 0/4 (0) |

C)

| | TOTAL n=120 | | | | | |
|---|---|---|---|---|---|---|
| | 30% | | 80% | | 100% | |
| | p-value | n (%) | p-value | n (%) | p-value | n (%) |
| M918T | 1 | 59/75 (79) | 1 | 9/75 (12) | 0.649 | 4/75 (5) |
| V804 | 0.507 | 2/3 (67) | 1 | 0/3 (0) | 1 | 0/3 (0) |
| EC | 1 | 28/35 (80) | 1 | 4/35 (11) | 1 | 1/35 (3) |
| OTHER MUTATIONS | 1 | 6/7 (86) | 0.212 | 2/7 (9) | 1 | 0/7 (0) |
| TREATED PATIENTS | 0.265* | 44/59 (75) | 0.175 | 10/59 (17) | 0.203 | 4/59 (7) |

significant association | tendency | weak trend | no trends

*Note:* The numbers with * indicate an inverse relationship. (A) Patients with RET-point mutations (MTC cases). (B) RET-fusion patients (FC-TC cases). (C) Naïve and treated RET-mutated groups joined (MTC cases).
Abbreviations: EC, extracellular domain; MTC, medullary thyroid cancer.

among the naïve mutant cases in ARROW harbored an EC pathogenic variant.

Moreover, only one patient harbored the change in the amino acid V804. An inverse tendency of V804 and an association of achieving a tumor response of 30% were observed ($p = 0.164$).

In the FC-TC cohort, we found differences in the availability of *RET* fusion information between ARROW and LIBRETTO-001. Twenty-two patients with *RET* fusions, previously treated with systematic therapies, were studied using ARROW (Table 4B). Specific fusions used were *CCDC6-RET* and *NCOA4-RET*. The remaining fusions were included in other groups. *CCDC6-RET* cases had a 30% rate in 92.3% (12/13) of cases. At the second baseline, this percentage was reduced to 30.8% (4/13).

All patients with *NCOA4-RET* achieved 30% tumor reduction. Forty percent of the subjects achieved 80%, and only one achieved a total reduction. Notably, only one *RET* fusion gene achieved a 100% response. This harbored an *NCOA4-RET* rearrangement ($p = 0.227$).

ARROW reported on 142 patients for whom the tumor response rate was studied. However, due to the different histotypes analyzed (MTCs and FC-TC) and their disparities in prognosis, there could be a bias in analyzing the tumor reduction size together. Taking all these data together, no associations or tendencies were found when *RET*-mutated patients with ARROW were analyzed (MTC cohort, Table 4C). A weak trend was observed between the MKi-treated patients and a better response than that of the naïve patients.

The prevalence of achieving a 30% cutoff for pathogenic variants was 79% (95/120). The number of patients in the 80% group decreased to 15 (13%). This reduction was also reported in the fusion group, in which almost 96% (21/22) of patients reached 30%. Nevertheless, only 32% (7/22) achieved 80% size reduction, and 4.5% (1/22) achieved a complete response.

A total of 269 pooled MTC patients were analyzed in both trials. To elucidate the different alterations in response to drugs, no trends were established regarding tumor size reduction in patients with *RET* pathogenic variants ($n = 247$). M918T mutated patients showed a weak trend in the total response ($p = 0.201$) and were the most prevalent variant in the achievement of cutoffs (80%, 12%, and 9%).

The changes in the amino acid site V804 showed the lowest prevalence among all baselines (69%, 0%, and 0%).

Regarding all *RET* mutant patients described in clinical trials, 80% achieved a 30% tumor size reduction. However, only 11% achieved an 80% response and 7% achieved a complete reduction.

No significant differences were observed in response efficacy regarding the use of previous MKi.

**TABLE 5** | Associations between reduction in tumor size and specific mutations in ARROW 2024 and LIBRETO-001.

| | TOTAL n=247 | | | | | |
|---|---|---|---|---|---|---|
| | 30% | | 80% | | 100% | |
| | p-value | n (%) | p-value | n (%) | p-value | n (%) |
| M918T | 1 | 118/148(80) | 0.535 | 18/148 (12) | 0.201 | 13/148 (9) |
| V804 | 0.305* | 9/13 (69) | 0.371* | 0/13 (0) | 0.608 | 0/13 (0) |
| EC | 0.712 | 46/59 (78) | 0.635 | 5/59 (9) | 0.375* | 2/59 (3) |
| OTHER MUTATIONS | 0.310 | 24/27 (89) | 0.512 | 4/27 (15) | 1 | 2/27 (7) |
| TREATED PATIENTS | 0.338 | 82/107 (77) | 0.217 | 15/107 (14) | 0.453 | 9/107 (7) |
| RETi | 0.875 | 102/127(80) | 0.542 | 12/127 (9) | 0.132 | 12/127 (9) |

significant association  tendency  weak trend  no trends

*Note:* Numbers with * indicate inverse relationships.
Abbreviations: EC, extracellular domain; RETi, RET inhibitors.

In total, 77% of treated patients and 82% of untreated patients achieved a 30% rate. This indicated a weak trend in patients previously treated with MKi and an 80% response rate ($p = 0.217$).

Comparing selpercatinib and pralsetinib responses in the 247 *RET*-mutated subjects, no tendencies arose with the association of drug usage and the reduction of 30% and 80% in tumor size.

Nonetheless, the complete response showed a tendency toward the use of selpercatinib ($p = 0.132$). LIBRETTO-001 reported a total reduction in 9.4% of the patients, whereas in ARROW of 4.2% (Table 5).

## 5 | Discussion

ARROW and LIBRETTO have been the most relevant clinical trials for selective RETi, and both have marked significant milestones in TC treatment.

From a genomic point of view, the ORR of LIBRETTO-001 and ARROW (2021 and 2024) were higher in the *RET* fusion group (79% and 89%–84%, respectively) than in *RET*-mutated patients. These results are not unpredictable, and several studies have demonstrated that fusions respond better to drugs than point mutations do (Nikanjam et al. 2020). In contrast, among the MTC cases, *RET*-mutated patients previously treated with MKi had the lowest ORR in both studies.

In MTC patients with LIBRETTO-001, EC pathogenic variants without MKi treatment had the lowest ORR ($p = 0.094$), closely followed by V804. Similar results for V804 have been reported in ARROW, where no patient exceeded the second cut-off. Gatekeeper mutations have been a hot topic in *RET* research owing to MKi resistance (Subbiah, Velcheti et al. 2018). Better results for V804 were observed in MKi-treated patients in both trials.

The specific analysis in the clinical trials for the changes in the amino acid sites Y806 and G810 would have been interesting to shed light on RETi resistance. Solvent front mutations in *RET* arise in a critical region of the RET protein that directly interacts with kinase inhibitors, typically proximal to the ATP-binding site. These pathogenic variants induce conformational alterations in the protein structure, which disrupts the binding affinity of inhibitors, thereby contributing to therapeutic resistance in RET-targeted treatments (Subbiah, Shen et al. 2021; Subbiah, Gouda et al. 2024). Research on other pathogenic variants, such as L730V/I, could be of interest to determine the accuracy of selpercatinib treatment in contrast to pralsetinib. Shen et al. demonstrated that L730V/I *RET* pathogenic variants are resistant to pralsetinib, but not to selpercatinib. These point mutations differ from the changes in the amino acid site G810 in the roof of the solvent front region. Although pralsetinib did not inhibit the growth of xenograft tumors, selpercatinib inhibited these tumors in an animal model (Shen et al. 2021).

When both studies were analyzed regarding the pathogenic variant outcomes for patients with MTC ($n = 247$), M918T had the best tumor reduction rates, showing a weak trend with a complete response ($p = 0.201$). Previous studies have reported that the pathogenic variant M918T requires the lowest half maximal inhibitory concentration ($IC_{50}$) of pralsetinib (Luo et al. 2021) and selpercatinib (Seoane and Capdevila 2018) compared with other pathogenic variants and *RET* wild-type (WT). This result also supports the conclusion of another study, which demonstrated that different treatments could be more effective depending on the specific *RET*

pathogenic variant present in TC (Rodríguez-Antona et al. 2013). Patients with changes in the amino acid C634 of *RET* had higher expression of VEGFR3, PDGFRB, and KIT, and could benefit from drugs that target these molecules. However, for M918T *RET*-mutant cases, drugs targeting RET (such as selpercatinib or pralsetinib), among others, will be more effective (Rodríguez-Antona et al. 2013). Our results demonstrate that the pathogenic variants harbored in the tumor were not significantly different in response to selpercatinib or pralsetinib in patients with MTC.

Considering the FC-TC patient data in the ARROW, 95.5% of patients achieved at least 30% tumor reduction. Nevertheless, only one patient got a complete response. Only 22 patients with *RET* fusions were included in the analysis. Analyses of larger cohorts that can evaluate the efficacy of different fusions are recommended.

Considering the limitations of the present review, information regarding *RET* pathogenic variants has not been reported as germline or somatic. Strikingly, LIBRETTO did not show results of size reduction for the fusions, and ARROW analyzed DTCs and ATCs, the prognoses of which also had a large disparity. However, different pathogenic variants have been analyzed in ARROW and LIBRETTO (Wirth et al. 2020; Subbiah, Hu et al. 2024), which represent the most prevalent *RET* variants in the literature (Maciel et al. 2019; Romei et al. 2010; Machens et al. 2013; Lebeault et al. 2017).

Selpercatinib and pralsetinib did not show significant differences in tumor size reduction. However, there was a tendency to obtain a complete tumor response with selpercatinib compared with that with pralsetinib.

Currently, several RETi are in the early phases of clinical trials and the preclinical stages. Zelentinib (Boston Pharmaceuticals 2023) (BOS172738, NCT03780517) has demonstrated strong nanomolar potency against WT *RET* and *RET* pathogenic variants, including gatekeeper mutations. Phase I of the study was completed and the ORR was 44% for MTC patients (Schoffski et al. 2021). Vepafesintinib (TAS0953/HM06) is another selective RETi undergoing Phase I/II (Helsinn Healthcare SA 2023) (NCT04683250) with promising results because of its activity against not only the previously mentioned pathogenic variants but also against solvent-front mutations (Miyazaki et al. 2023). Other RETi are now in the first development stages, such as SY5007 (Shouyao Holdings [Beijing] Co. LTD 2023) or APS03118 (Applied Pharmaceutical Science Inc 2023), paving the way for next-generation RETi. There is an unmet need to describe the mechanisms of resistance to RETi to develop new strategies for this population of TC patients.

## 6 | Conclusions

RETi selpercatinib and pralsetinib are active against all *RET* pathogenic variants, with high efficacy in both fusions and point mutations, resulting in a clinical response in MTCs and FC-TC tumors. Despite some trends in ORR and tumor reduction percentage, all patients with *RET* pathogenic variants showed clinical benefits. Currently, specific *RET* point mutations and fusions are predictive biomarkers for RETi therapy in TC but do not allow the establishment of effective subgroups. Individuals exhibiting *RET* pathogenic variants should be administered a selective RETi at any stage of the therapeutic protocol. Therefore, novel biomarkers for the assessment of sensitivity and resistance require further investigation.

### Author Contributions

**Sara Gil-Bernabé:** conceptualization, methodology, formal analysis, and writing – original Draft. **Lucía García-DeLaFuente:** validation and visualization. **Alejandro García-Álvarez:** writing – review and editing. **Ginesa García-Rostán:** writing – review and editing and supervision. **Jaume Capdevila:** writing – review and editing and supervision. **Jorge Hernando:** conceptualization, validation, writing – review and editing, and supervision.


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### Conflicts of Interest

Jaume Capdevila: *Personal conflicts of interest*—Scientific consultancy role (speaker and advisory roles) from Novartis, Pfizer, Ipsen, Exelixis, Bayer, Eisai, Advanced Accelerator Applications, Amgen, Sanofi, Roche, Lilly, Huchmed, ITM, Merck Serono, Advanz, Esteve. *Research support*—Research grants from Novartis, Pfizer, Astrazeneca, Advanced Accelerator Applications, Eisai, Amgen, Bayer, Gilead, Roche, Ipsen, ITM. Jorge Hernando: *Speakers' bureau and expert opinion*—Eisai, Ipsen, Novartis, Bayer, Lilly, Adacap, Angelini, and Leo Pharma. Alejandro García Álvarez: *Personal conflict of interest*—ADACAP (Novartis), Advanz, EISAI, Ipsen. The remaining authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this study.

### Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.