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Kinase Fusion–Related Thyroid Carcinomas: Towards Predictive Models for Advanced Actionable Diagnostics

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Abstract

The past decade has brought significant advances in our understanding of the molecular mechanisms of thyroid carcinogenesis. Among thyroid carcinomas, the most successful class of targeted therapeutics appears to be selective kinase inhibitors. Actionable kinase fusions arise in around 10–15% of cases of thyroid cancer, a significant subset. A cohort of molecular testing platforms, both commercial and laboratory-derived, has been introduced into clinical practice to identify patients with targetable tumors, requiring pathologists to develop an integrative approach that utilizes traditional diagnostic cytopathology and histopathology, immunohistochemistry, and cutting-edge molecular assays for optimal diagnostic, prognostic, and therapeutic efficiency. Furthermore, there has been increasing scrutiny of the clinical behavior of kinase fusion–driven thyroid carcinoma (KFTC), still regarded as papillary thyroid carcinomas, and in characterizing molecular predictors of kinase inhibitor resistance with an aim to establish standardized, evidence-based treatment regimens. This review presents an overview of the current literature on the clinicopathologic and molecular features of KFTC as well as the latest investigational progress and encountered challenges for this unique subset of thyroid neoplasias.

Keywords

Kinase fusion; *NTRK* ; *RET* ; Inhibitor; Resistance; Thyroid cancer

Introduction

The thyroid gland is a frequent site for human cancer, particularly in women, and differentiated thyroid carcinomas, most commonly papillary thyroid carcinomas (PTCs) followed by follicular thyroid carcinomas (FTCs), account for nearly all cases. Although

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PTC and FTC are usually cured by surgery alone, lesions that are large display “high-risk” histology and have residual disease, and locoregional or distant metastasis may require additional treatment, the most traditional adjuvant therapeutic being radioactive iodine (RAI). Furthermore, de novo and acquired RAI resistance may develop as a harbinger of therapeutic failure and poor outcomes, with merely 10% of these patients reaching 10-year survival [1]. High-grade thyroid carcinomas, such as poorly differentiated thyroid carcinomas, differentiated high-grade thyroid carcinomas, and anaplastic thyroid carcinomas, are generally RAI-refractory with unfavorable 5-year disease-specific survival (50–60% and nearly none, respectively [2, 3]). Fortunately, recent pharmaceutical developments have brought a growing armamentarium of molecularly targeted therapy, mainly selective kinase inhibitors (Table 1) [4–13], which has revolutionized the clinical landscape of RAI-refractory thyroid cancer.

The molecular pathogenesis of most thyroid carcinomas involves derangements of the mitogen-activated protein kinase (MAPK) and PI3K/Akt/mTOR pathways (Fig. 1). Both pathways can be activated by receptor tyrosine kinases (RTKs) on the cell surface, such as RET, NTRK, ALK, MET, and ROS1, through RAS activation (Fig. 1). *BRAF* encodes an intracytoplasmic serine-threonine kinase mediator of the MAPK pathway, in which an activating point mutation, p.V600E, accounts for over 50% of adult PTC occurrences [14]. In *BRAF* p.V600E-negative PTC, rearrangements of various kinase genes, mainly *RET* (around 28% and 14% in pediatric and adult PTC, respectively), *NTRK* (15% and 8%), *ALK* (4–6% and 3%), *BRAF* (1–19% and 3%), *MET*, and *ROS1* (rare), serve as important tumorigenic drivers (Table 2) [15–38]. Kinase rearrangements have been identified in 9–20% of poorly differentiated thyroid carcinomas (PDTC) [15, 30, 39–41] and 1–6% of anaplastic thyroid carcinomas (ATC) [15, 33, 39, 42]. Follicular-derived carcinomas that lack *BRAF* p.V600E mutation and kinase rearrangements, such as the majority of FTC, follicular-patterned PTC, PDTC, and ATC, may arise from *N/H/KRAS* mutations, non-p.V600E *BRAF* mutations, the *PAX8::PPARG* fusion, and various alterations of the PI3K/Akt/mTOR pathway [43]. Furthermore, additional fusions have been noted in salivary type carcinomas (*CRTC1*::*MAML*) [44] and NUT carcinomas (*NSD3*::*NUTM1*) [45, 46]. Changes in the SWI/SNF complexes, histone-modifying enzymes, and mismatch repair proteins are thought to underlie the de novo development or secondary transformation towards high-grade neoplasms (PDTC, DHGTC, and ATC) [43]. Medullary thyroid carcinomas (MTC), derived from parafollicular cells, are mainly driven by *RET* and *RAS* mutations but have been found to carry kinase fusions in exceptionally rare cases [47–50].

Kinase rearrangements produce valuable therapeutic targets by causing the fusion of the 3′ adenosine triphosphate (ATP)-binding kinase domain to a 5′ partner sequence that causes deregulated kinase activation through ligand-independent dimerization or loss of autoinhibition. Most partner genes involved in RTK rearrangement contain dimerization domains, such as the coiled-coil domains in *NCOA4*, *TPR*, *PPL*, *CCDC6*, *TPM3*, the WD domain in *EML4*, the PB1 domains in *TFG* and *SQSTM1*, the PNT domain in *ETV6*, and the RNA recognition motif in *RBPMS*, which allows the fusion oncoprotein to undergo dimerization and transactivation without the kinase’s physiologic ligand (Fig. 2A). Unlike RTK fusions, *BRAF* fusions may (e.g., *AKAP9*::*BRAF*) or may not (e.g., *AGK*::*BRAF*) contain partner-derived dimerization domains. The key oncogenic mechanism in *BRAF*

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fusions is believed to be the loss of 5′ autoinhibitory domains when replaced by a partner sequence (Fig. 2B). Regardless of the activation mechanisms, it is the retained active kinase domain that renders fusion oncoproteins vulnerable to inhibition by small molecules that obstruct ATP binding through ATP-competitive and non-competitive approaches [51]. These kinasetargeting agents have significantly improved the clinical outlook for patients with RAI-refractory thyroid cancers (Table 1) [4–13]. Timely and cost-effective detection of actionable kinase rearrangements has thus become a life-saving task entrusted to resourced pathology laboratories.

Since then, the clinical management of kinase fusion–related thyroid cancer (KFTC) has met with several challenges. As diagnostic techniques have evolved from the use of fluorescence in situ hybridization (FISH) and reverse transcriptionpolymerase chain reaction (RT-PCR) toward a growing dependence on next-generation sequencing (NGS) analysis, this improved molecular resolution is still relatively costly [52]. This has triggered recent research interest in the histologic and immunohistochemical correlates of KFTC that may inform strategic testing algorithms [15, 28, 38]. Furthermore, the literature has been notably heterogeneous on the prevalence and clinical behavior of KFTC (Tables 2 and 3), hampering the development of an evidence-based treatment standard. The decision to test tumors only in the face of advanced thyroid cancer (pre-operatively for unresectable tumors or post-operatively for incompletely resected tumors or those with distant disease) or with complete excision in order to bank genomic data from the outset remains controversial in regard to resource allocation, but arguable considering the pre-operative standard of care has become molecular testing for diagnostically indeterminate thyroid nodules, the majority of which are benign [53]. Lastly, secondary resistance-mediating mutations have emerged in KFTC patients treated with kinase inhibitors and are critical to incorporate into future therapeutic planning. This review presents recent advances and ongoing challenges in exploiting the molecular actionability of KFTC.

Histologic Features

The past decades have seen a growing number of histologicmolecular correlation research in search of histologic predictors of therapeutic targetability in thyroid cancer. *RET* is the most commonly rearranged kinase gene and has been associated with diffuse sclerosing papillary thyroid carcinoma (DSPTC). DSPTC is characterized by prominent lymphatic invasion (intra and extrathyroidal), stromal sclerosis, lymphocytic inflammation (commonly Hashimoto thyroiditis), squamous metaplasia, and numerous psammomatous calcifications, often clusters of smaller-sized psammomatous calcifications. DSPTC is not specific to *RET*-rearranged tumors but can also occur on occasion with *BRAF* V600E mutation in 24% of cases [54] and with *ALK*, *BRAF*, and *NTRK* fusions [15, 31, 34, 36]. *RET* rearrangements in PTC have also been linked the solid PTC [55], which has also been reported with *BRAF*, *NTRK*, and *ROS1* fusions [15, 34]. Tall cell PTC, although primarily associated with the *BRAF* V600E mutation, may rarely be seen with *ALK*, *BRAF*, *NTRK*, and *RET* fusions [15, 29, 31, 56]. In addition to the reported associations with unusual subtypes of PTC, fusion-driven PTC commonly present with classical, follicular, and mixed papillary-follicular architectures [15, 34, 57], which, just like fusion-driven PDTC, ATC, and MTC, overlap morphologically with fusion-negative counterparts. Fortunately,

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recent KFTC studies have observed several distinct features, including multinodularity [15, 38, 57], lymphovascular spread [15, 38, 41], and intratumoral fibrosis [15, 38], which are present in the majority of KFTC (Fig. 3) and, when concurrent with negativity for *BRAF* p.V600E in follicular-derived carcinomas by immunostaining or molecular assays, encourages consideration of fusion testing if clinically indicated [15]. Of late, artificial intelligence has shown great promise in identifying kinase fusions on morphologic grounds in lung cancers [58] and may apply to thyroid tumors in the near future, although, for now, a simple *BRAF* test and pathologist architectural review of a hematoxylin and eosin-stained tumor slide are great predictors.

Primary thyroid secretory carcinoma (SC) is a newly established entity in the 5th edition of the World Health Organization classification for endocrine tumors. Similar to its salivary gland counterpart, thyroid SC is characterized by the presence of *ETV6::NTRK3* fusion, which has been consistently identified in the 13 cases reported thus far [15, 59, 60]. SC tend to present at advanced clinical stage with large size and cervical lymph node involvement at the initial diagnosis [60]. Histologically, microcystic to papillary growth, densely fibrotic stroma, and eosinophilic secretion are typically noted (Fig. 3). The tumor cells have moderate cytoplasm and vesicular nuclei with conspicuous nucleoli and frequent nuclear grooves. Although papillary growth and nuclear grooves are reminiscent of PTC, SC show a distinct immunophenotype with negativity for thyroglobulin (thus insensitive to RAI therapy) and TTF-1 while being positive for S100, GATA3, and mammaglobin.

Molecular Diagnosis

When evaluating the KFTC literature, it is important to understand the capabilities and limitations of various molecular platforms so that a fusion-positive-versus-fusion-negative comparison would not be biased by the putative “fusion-negative” group potentially containing fusions that are outside the scope of the employed methodology. Immunohistochemistry (IHC) and FISH offer fast turnaround time but lack molecular resolution. By molecular approaches, kinase gene rearrangements can be detected at the breakpoint (i.e., demonstrating a hybrid sequence formed by the partner genes) or through 3′-to-5′ expression imbalance (EI). 3′-to-5′ EI occurs when an overexpressed fusion product contains only the 3′ region of the queried gene, such as where the kinase domain is located in most RTK and BRAF. Fusion breakpoint and EI can each be characterized by direct RNA hybridization–based transcript enumeration (e.g., NanoString nCounter®), reverse transcription quantitative RTqPCR, digital PCR, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), or NGS [61] (Table 2). The most commonly applied clinical platforms are reviewed here (Fig. 4).

Immunohistochemistry

Commercial IHC antibodies are currently available for querying rearrangements of *NTRK* (pan-TRK) [62, 63], RET [64], *ALK* [31, 32], and *ROS1* [33]. With its unique edge in allowing fast turnaround time and in situ visualization of protein expression within cellular context, kinase-based IHC has gained wide clinical applications. However, the performance of kinase-based IHC appears to be heterogeneous and partner gene-dependent. Pan-TRK

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IHC has a reported sensitivity and specificity of 81.1% and 99.9%, respectively [63]. The sensitivity is high in *NTRK1* (96%) and reasonably good in *NTRK3* (79%) fusion tumors; *NTRK2* fusions are rare but appear to be consistently labeled (100%) [63, 65]. RETbased IHC showed a sensitivity of 100% for *KIF5B::RET*, 88.9% for *CCDC6::RET*, and 50% for *NCOA4::RET*, with a specificity of around 82% [64]. ALK IHC has the most reliable performance, with nearly 100% sensitivity and specificity for ALK fusions [32]. In contrast, ROS1 IHC may often be compromised by nonspecific staining [33]. The use of multiplex fusion target immunohistochemistry as a screening tool has not been employed, but given the variable sensitivities of individual IHC markers, it seems to be of limited utility relative to NGS testing.

However, it is noteworthy that *BRAF* p.V600E-specific IHC is a valuable tool in identifying KFTC as the *BRAF* p.V600E mutation is mutually exclusive with kinase fusions in the pre-treatment setting. The commonly employed VE1 clone has a sensitivity of 89–100% and a specificity of 62–100% [66]. The VE1 antibody, however, does not label *BRAF* fusions and non-p.V600E mutations.

Fluorescence In Situ Hybridization

FISH has been the conventional gold standard for detecting gene rearrangements. The break-apart probe design waives the need for partner gene identity and breakpoint localization. However, FISH has limited multiplexing capability and may not be able to discern biologically nonproductive fusions such as those that are out-of-frame or lack therapeutically relevant structures such as the kinase domain in kinase fusions, leading to false positivity and treatment failure [67]. On the other hand, sources of false negativity include fusions that derive from short-segment inversions, such as the *NCOA4::RET* fusion, which often fail to produce visually apparent split signals [64].

Hybrid Capture NGS

Hybrid capture NGS employs probes that hybridize with the genomic region of interest and are biotinylated to allow subsequent capture by streptavidin-labeled beads. Hybrid sequences (split reads) at fusion breakpoint can be captured by kinase gene-targeting probes without requiring partner gene identity, thus allowing detection of novel fusions. Gene fusions often occur at intronic locations, where repetitive sequences may create considerable sequencing and bioinformatic difficulties. At the DNA level, breakpoint characterization requires intronic probe tiling (Fig. 4A), which can be costly, particularly when large introns are encountered. In contrast, when hybrid capture is performed in complementary DNA (cDNA) derived from tumor RNA, the introns are spliced out, and the probe design can focus on exonic regions and circumvent the cost and bioinformatic challenges of intronic sequencing (Fig. 4B). Furthermore, analysis at the RNA level reflects the transcriptional activity and splicing outcome of gene fusions. In samples with low tumor content, the overexpression of fusion sequences can be beneficial for increasing assay sensitivity. The main limitation of cDNA sequencing is its dependence on tumor RNA quality. Meticulous examination of quality metrics is essential for ensuring result validity.

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RT-PCR and Bidirectional Amplicon-Based NGS

Fusion breakpoints can also be queried using bi-directional primer sets that target the rearranged genes (Fig. 4C). As primer design requires a focused interest in certain fusion partners and hotspot exons, non-targeted fusions may not be detectable. As listed in Table 2, earlier KFTC studies often employed multiplex RT-PCR that amplified the most frequent types of *RET* and *NTRK* rearrangements [21, 24]. The newest multiplex RT-PCR fusion assays have introduced microfluidic devices that automatically perform nucleic extraction and fusion detection with minimal personnel dependence, allowing ultra-rapid clinical testing [61]. Recently, the advent of amplicon-based NGS has significantly boosted the multiplexing capability by allowing hundreds of amplicons to be concurrently sequenced. Compared to hybrid capture NGS, amplicon-based NGS is advantageous for simple workflow, fast turnaround time, and relative tolerance for low-tumor samples, but, similar to RTPCR, is dependent on the starting probe design in terms of uncommon/novel fusion coverage.

Anchored Multiplex PCR-Based cDNA NGS

The anchored multiplex PCR (AMP) technology is a power platform invented by the Massachusetts General Hospital in Boston, MA, and commercially supplied by ArcherDx, Inc., that enables gene fusion detection in an amplicon-based and yet partner-agnostic manner [68]. This is achieved by deploying gene-specific primers (GSP) and adapter-complementary primers (Fig. 4D) that amplify fusion transcripts without preceding knowledge of partner gene identity. Multiple studies have utilized large AMP NGS panels with comprehensive coverage of oncogenic fusions in thyroid cancer [15, 16, 18], allowing more accurate assessment of KFTC prevalence. AMP assay specificity has been further improved by a twostep amplification protocol using two nested GSP pools for a given region [68].

3′ to 5′ Expression Imbalance

In KFTC, oncogenic fusion products are highly expressed and contain the 3′ kinase domain-encoding sequence but not the 5′ region of the kinase gene. As a result, when quantified separately, KFTC carry more transcripts of 3′ sequence than of 5′ sequence, leading to EI (i.e. 3′ region overexpression, Figure 4E). EI can be demonstrated using various methods such as direct RNA hybridization-based transcript enumeration (e.g., NanoString nCounter® [36]), quantitative RT-PCR [61], digital PCR, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), and NGS such as the Oncomine Focus Assay [69]. Although EI allows for fusion detection without knowing partner identity, the observed sensitivity may be suboptimal across different platforms [64]. The magnitude of EI is affected by endogenous tissue expression of the queried gene, tumor purity, and RNA quality [64]. A cutoff value for making positive fusion calls can be challenging to determine, as exemplified by the low sensitivity of *ROS1* EI (29%) in the pulmonary setting due to high background expression [61]. To complement this limitation, most assays that employ EI have co-operating fusion-specific detection mechanisms that cover the prevalent fusion types to achieve an overall acceptable sensitivity [36, 61]. Another downside of EI is inability to identify fusion partner and breakpoint location.

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Clinical Features

The reported prevalence of kinase fusions in thyroid tumors have been variable among the published series (Table 2) [15–38], depending on patient demographics, risk factors, tumor histology, and the analytic methods employed. KFTC has a well-established association with pediatric and radiation-associated thyroid cancers, traditionally classified as PTC, particularly those arising in post-Chernobyl radiation exposure victims. Histologically, PTC is the tumor type that shows the strongest association with kinase fusions, although whether or not many of these tumors truly have features of PTC, or are unique on their own, is a phenomenon continuing to evolve. It is noteworthy that studies which used IHC, FISH, and RT-PCR for fusion detection often focused on one to three kinase genes and may not be able to detect uncommon kinase fusions such as those of *BRAF*, *MET*, and *ROS1*. Coverage of partner genes may also be limited when using methods with low multiplexing capability. Since *RET* is the most frequently rearranged kinase in the thyroid, studies that did not cover *RET* tended to report lower KFTC prevalence. When drawing clinicopathologic comparison of KFTC against fusion-negative tumors, it is important to understand the scope and limitations of the source studies to ensure comparability of patient groups. Even when using NGS with comprehensive analysis of actionable genes, it is crucial to review panel details (e.g., tiled introns, DNA versus RNA sequencing, traditional amplicon versus AMP etc.) to properly interpret sequencing results.

For kinase-driven PTC, while most researchers have reported a predilection for early lymph node involvement, the observed distribution of primary tumor stage and the frequency of distant metastasis have each spanned a wide range in the literature (Table 3). To understand the cause of such variability, in addition to examining the analytic scope of the studies as discussed above, cohort identification approach is also important to consider. While most researchers have evaluated consecutive cohorts that included all available PTC patients from their chosen time period, some studies relied on data-mining of historical fusion testing results [15] and may have preferentially included patients who had more aggressive disease that triggered clinical testing, thus creating selection bias. Overall, the current literature on KFTC behavior is relatively scarce and notably heterogeneous. There is an ongoing need for additional clinical data, particularly those based on comprehensive molecular profiling, for more objective assessment of KFTC behavior.

Treatment and Acquired Resistance

The 2022 National Comprehensive Cancer Network (NCCN) guidelines recommend pursuing actionable molecular marker testing including *RET*, *NTRK*, and *ALK* rearrangements for advanced thyroid carcinoma to facilitate personalized utilization of kinase inhibitor therapy [70] (Table 1). The kinase domain of most RTK is composed of N-terminal and C-terminal lobes connected by a hinge region that is known as the ATP-binding site (Fig. 5A) [71]. The ATP-binding site is highly conserved among RTK, formed by the phosphate-binding loop, the catalytic loop, and the activation loop that contain the Asp-PheGly (DFG) and the Ala-Pro-Glu (APE) motifs (Fig. 5A). In the active state, the DFG motif assumes a “DFG-in” conformation that allows the aspartate to interact with the magnesium cofactor. In the inactive state, the DFG motif rotates the aspartate

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residue outward into a “DFG-out” conformation. On the back side behind the adenine ring binding site is a hydrophobic cleft. The access to this cleft is controlled by gatekeeper residues on the hinge. For TKI that bind to the kinase by passing through this gate, such as most multi-kinase inhibitors (MKIs), gatekeeper mutations may lead to steric hindrance and therapeutic resistance (Fig. 5A) [72]. Resistance may also result from other acquired mutations that conformationally alter the kinase domain such as at the solvent front (Fig. 5A) or through activation of bypassing signaling pathways (Fig. 5B). Recent advances in TKI therapy in thyroid cancer are reviewed here.

Multi-Kinase Inhibitors (MKIs)

Sorafenib and lenvatinib are currently the first-line TKI therapy for clinically significant RAI-refractory differentiated thyroid carcinoma. Their anti-tumoral effects mainly originate through the inhibition of endothelial growth factor receptors (VEGFR) signaling and suppressing angiogenesis. In the SELECT trial that included 392 randomized subjects, the median progression-free survival (PFS) was significantly better in the lenvatinib group compared to placebo (18.3 months versus 3.6 months) [4]. In the DECISION trial for sorafenib, the median PFS was improved to 10.8 months compared to 5.8 months in the placebo group [5]. Many MKI also demonstrate inhibitory activity against RET. However, their molecular non-selectivity leads to frequent toxicities and inferior pharmacokinetics which motivated subsequent development of selective RET inhibitors. One key difference between MKI and selective RET inhibitor is that MKI bind to the RET kinase domain by passing through the aforementioned structural gate and are therefore subjected to gatekeeper mutationmediated resistance, such as p.V804L/M [73]. Unlike MKI, selective RET inhibitors access the back cleft by wrapping around the gate wall without passing through it [72], thus remaining active against V804 mutants.

Selective RET Inhibitors

Selpercatinib and pralsetinib are selective RET inhibitors with improved efficacy and safety compared to MKI. In September 2022, Selpercatinib received United State Food and Drug Administration (FDA) accelerated approval for histology-agnostic treatment of advanced or metastatic solid tumors driven by *RET* rearrangements based on the LIBRETTO-001 trial (Table 1) [10]. The trial evaluated a total of 19 non-medullary thyroid carcinoma patients, achieving an objective response rate (ORR) of 79%, including 1 complete response and 14 partial responses, with the remaining patients experiencing stable disease [10]. The median PFS was 20.1 months [10]. Around 30% of the subjects required dose reductions and 2% terminated treatment due to side effects including abnormal liver function and hypersensitivity [10]. Pralsetinib received FDA approval in 2020 for thyroid cancer based on the ARROW trial (Table 1) that evaluated 11 RET fusion-positive thyroid cancer patients with an ORR of 89% [11]. Acquired resistance to selpercatinib has been reported through both on-target and bypassing mechanisms. We recently observed a *ERC1::RET* fusion ATC that developed *EGFR* amplification causing selpercatinib resistance [15]. In two lung carcinomas harboring *KIF5B::RET* and *CCDC6::RET* fusions, an MTC driven by *RET* p.M918T and p.V804 M/L mutations and 39 selpercatinib-resistant cell lines, two research groups found p.G810 C/S/R at the solvent front, p.Y806 C/N in the hinge region, and p.V738A at the β2 strand to confer resistance to both selpercatinib and pralsetinib [72,

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74]. Second-generation agent TPX-0046 is in development to tackle solvent front p.G810 mutations but may be subjected to other structural hindrance based on in silico predictions [75].

NTRK Inhibitors

Larotrectinib and entrectinib are FDA-approved TRK inhibitors for the histology-agnostic treatment of adult and pediatric solid tumors driven by *NTRK* rearrangements. *NTRK1/2/3* rearrangements are uncommon drivers of thyroid, lung, breast, pancreatic, and colonic carcinomas, sarcomas, melanomas, and gliomas, while being the defining genetic feature for several rare neoplasms such as secretory carcinomas and congenital mesoblastic nephromas. Combining three phase 1/2 trials that included 159 patients with a wide spectrum of tumor types, larotrectinib achieved an ORR of 79% with complete response seen in 16% [76]. Entrectinib, an inhibitor of not only TRK but also ALK and ROS1, showed an ORR of 57% in three phase 1/2 trials [13]. Both larotrectinib and entrectinib were well tolerated with <5% toxicity-related treatment termination. Dose reduction was documented in 8% (larotrectinib) and 30% (entrectinib) due to anemia, abnormal renal, hepatic or pancreatic function, and fatigue [13, 76]. Acquired resistance-mediating mutations have been identified at gatekeeper residues (*NTRK1* p.F589L and *NTRK3* p.F617L), the solvent front (*NTRK1* p.G595R and *NTRK3* p.G623R), and in the activation loop (*NTRK1* p.G667C/S, *NTRK3* p.G696A) in tumors of various organs [77–79]. Next-generation NTRK inhibitors selitrectinib and repotrectinib are being developed with demonstrated efficacy against solvent front and activation loop mutants [80].

ALK Inhibitors

ALK fusions are rare in thyroid cancer, with small numbers of reports in PTC, PDTC, ATC, and MTC (Table 2) [48, 81, 82]. Crizotinib is a first-generation ALK, ROS1, and MET inhibitor that received FDA approval in 2011 for *ALK*-rearranged lung cancer. Since the discovery of *ALK*-driven malignancies in virtually every organ, crizotinib has shown systemic therapeutic success including in the thyroid. However, most patients developed resistance in 1 to 2 years due to acquired kinase domain mutations and activation of bypassing signaling caused by mutations or amplifications of *EGFR*, *KRAS*, and *MET* [83, 84]. In scattered case reports, therapeutic response could sometimes be restored by switching to brigatinib and alectinib with some cases demonstrating lasting response (Table 4) [15, 48, 81, 85, 86].

Summary

Despite the significant recent advances in targeted therapy for thyroid cancer, several unsolved clinical needs remain. Although *ALK*, *MET*, and *ROS1* fusions are well documented in thyroid tumors with commercially available inhibitors, these agents currently do not have FDA-approved thyroid indications due to scarcity of data. Furthermore, although most KFTC studies reported high frequencies of early lymph node spread of KFTC, other aspects of clinical behavior, including long-term survival, remain poorly understood due to inconsistent study designs and findings among the published clinical series and/or a lack of solid evidence due to case rarity and test selection bias. Fortunately,

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as comprehensive genomic profiling becomes increasingly accessible and affordable in both the pre-treatment and post-treatment settings, one can expect more high-quality evidence to arrive in the near future and to provide novel diagnostic, prognostic, and treatment resistance predictors that support effective tumor behavior modeling and management planning for optimal patient outcomes.

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Availability of Data and Materials

This is a review article of published data accessible ad hoc by request.

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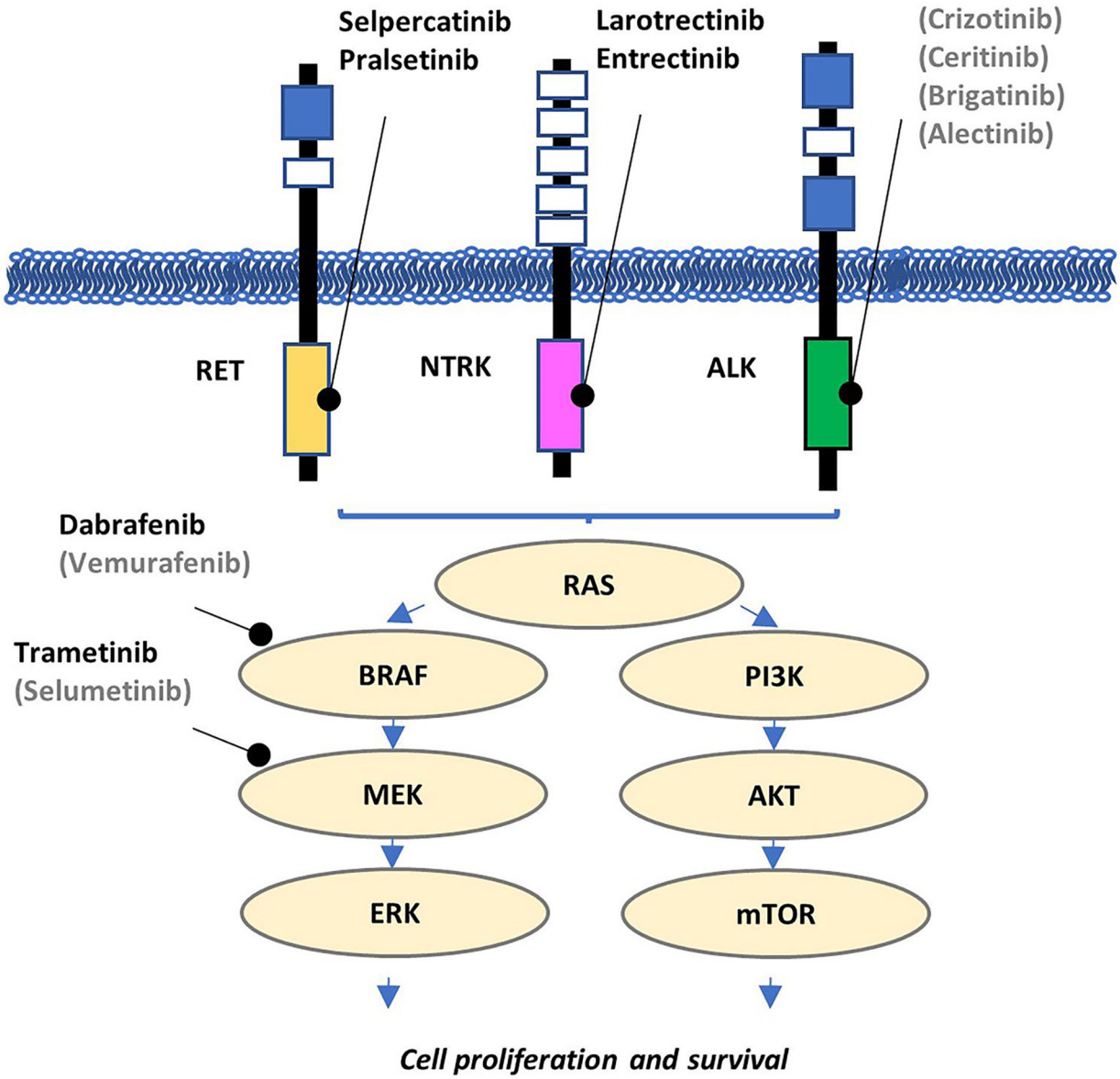

Fig. 1.

Oncogenic signaling pathways in thyroid carcinogenesis. The mitogen-activated protein kinase (MAPK) and PI3K pathways play a central role in thyroid oncogenesis and harbor the most common targetable molecular drivers. Targeted inhibitors listed in gray and in parentheses have yet to receive FDA approval

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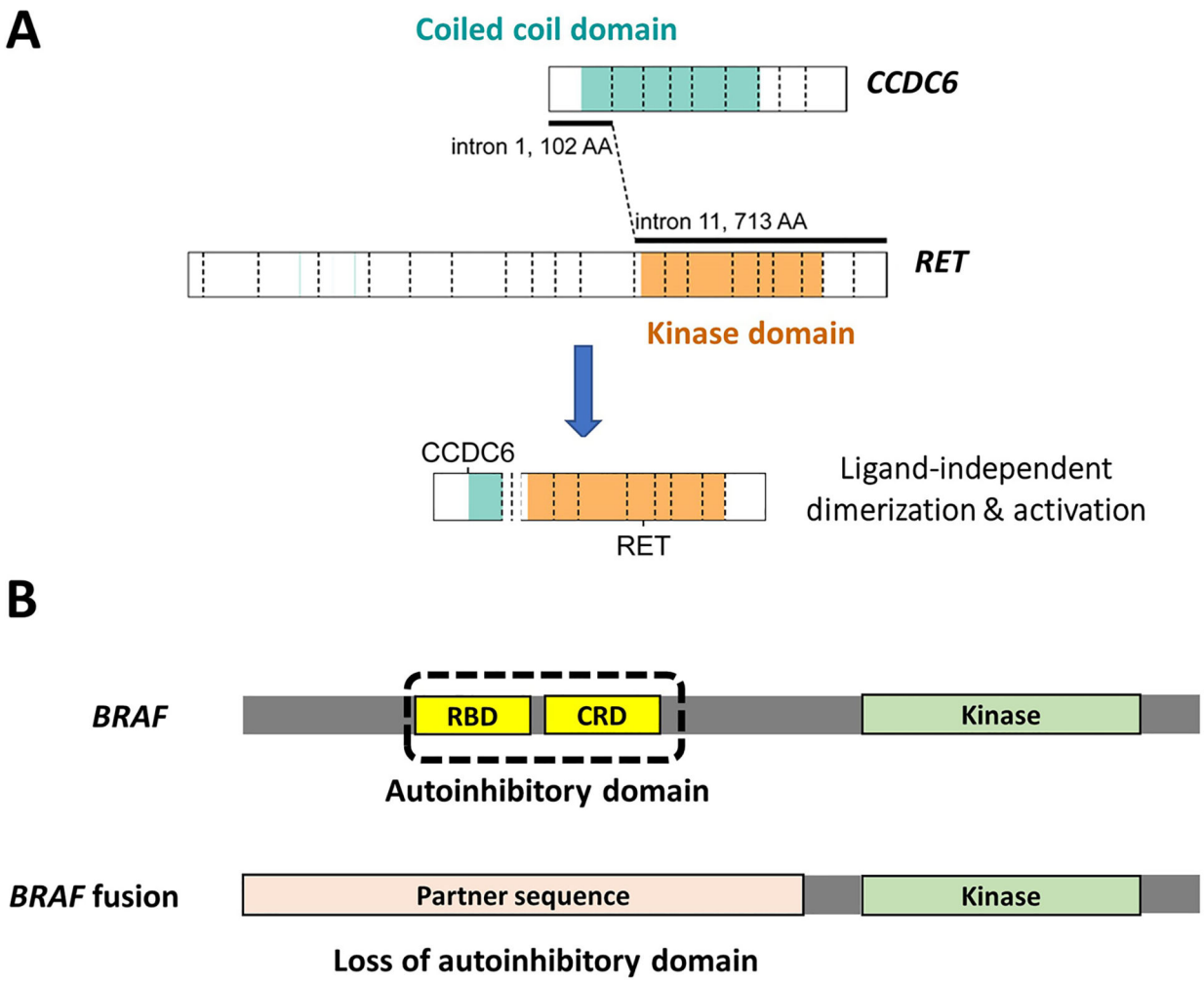

Fig. 2.

Oncogenic mechanisms of kinase fusions. Most tyrosine kinase fusions contain partner-derived dimerization domains that enable ligand-independent activation (**A**). *BRAF* fusions, however, may or may not have partner-derived dimerization domains and thought to be activated by loss of autoinhibition (**B**)

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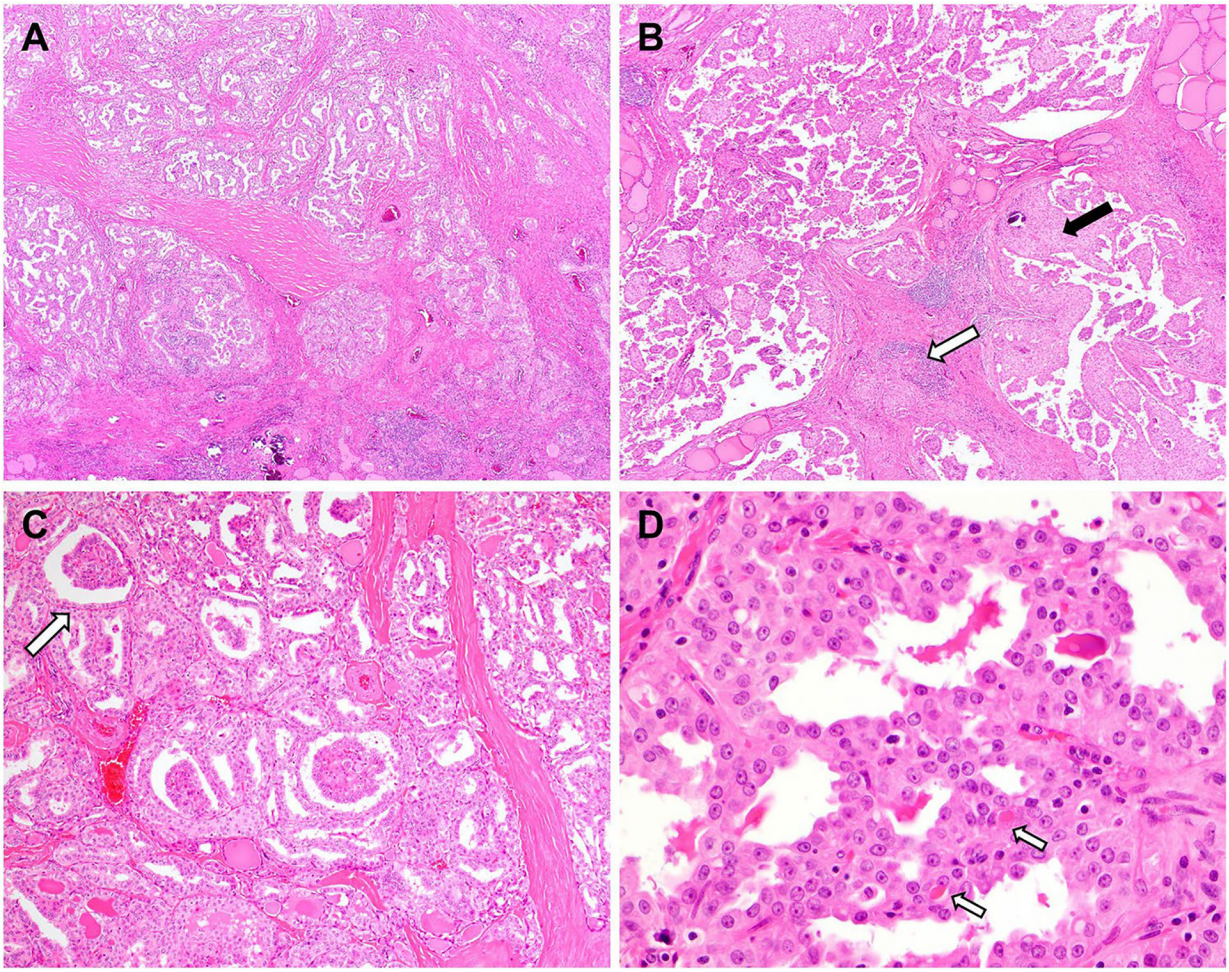

Fig. 3.

Histologic features of KFTC include multinodularity (**A**), lymphovascular spread (not shown) and prominent intratumoral fibrosis (**A**) that have been noted in several series. *RET* fusions are well-known to be associated with the diffuse sclerosing PTC (**B**) characterized by chronic lymphocytic inflammation (white arrow) and squamous metaplasia (black arrow) in addition to stromal fibrosis/sclerosis. *NTRK* rearranged tumors may show intriguing glomeruloid architectural formations (**C**, arrow). Primary secretory carcinomas are histologically reminiscent of its salivary counterpart with microcystic architecture (**D**) and eosinophilic secretions (arrows). The nuclei are vesicular with conspicuous nucleoli

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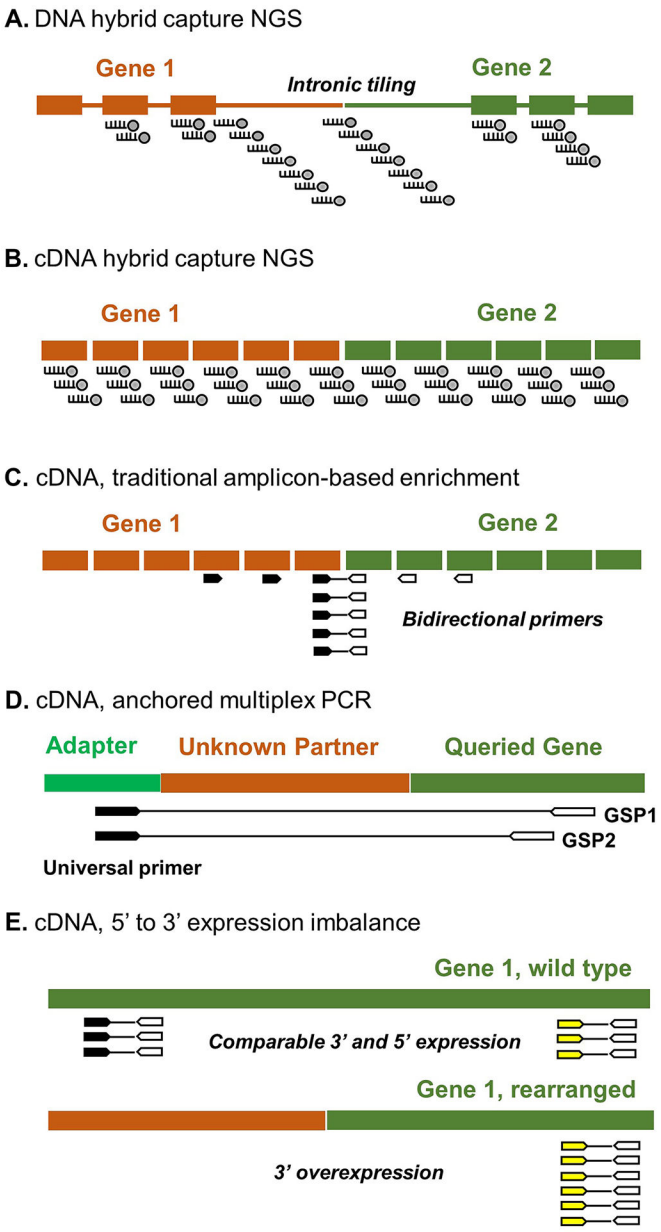

Fig. 4.

Common molecular platforms for clinical fusion testing. See text for details

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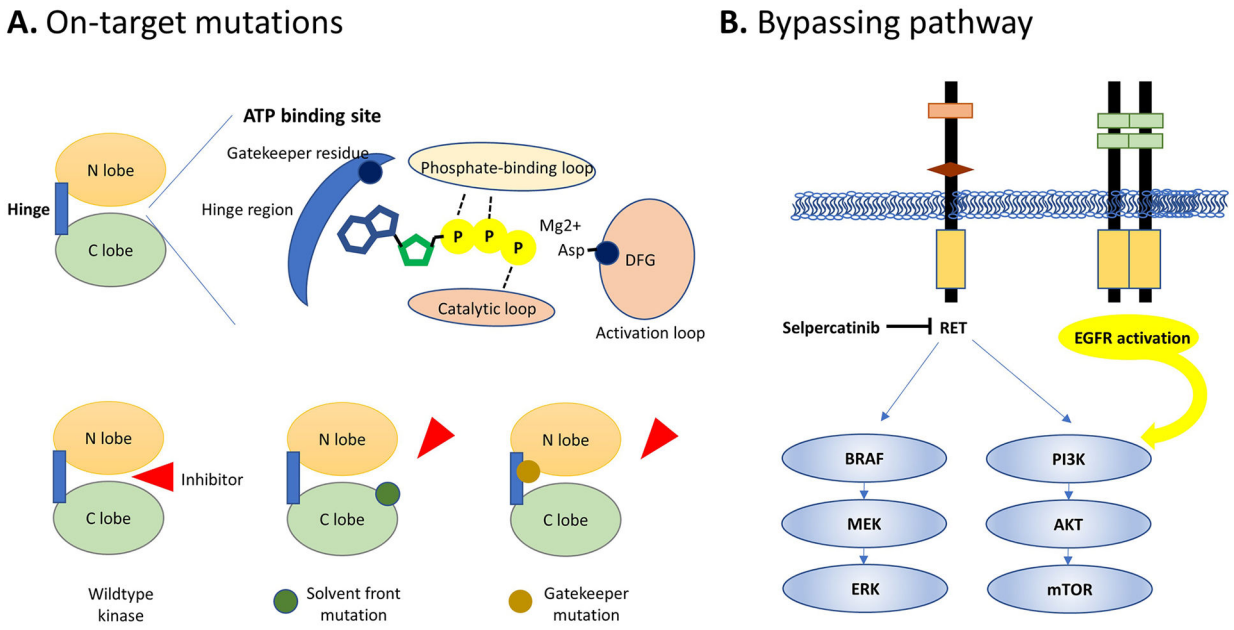

Fig. 5.

Mechanisms of acquired resistance to kinase inhibitor therapy. On-target resistance is mediated by acquired mutations at various locations in the kinase domain (**A**). Alternative pathway activation, such as another receptor tyrosine kinase that drives downstream MAPK and PI3K signaling, bypasses the inhibition of RET in this example

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Table 1

FDA-approved targeted therapy for thyroid cancer

Agent	Target(s)	Thyroid indication(s)	Trial registration (reference)	Design	Histology (case no.)	ORR	Median PFS
Lenvatinib	VEGFR1/2/3, FGFR1/2/3/4, PDGFRα, RET, KIT	RAI-refractory thyroid cancer	SELECT (4)	Phase III	PTC (200), FTC (75), OTC (70), PDTC (47)	64.8%	18.3 months (lenvatinib) vs. 3.6 months (placebo)
Sorafenib	VEGFR1/2/3, RET, RAF, PDGFRβ, KIT	RAI-refractory thyroid cancer	DECISION (5)	Phase III	PTC (237), FTC (106), PDTC (40), WDC (3)	12.2%	10.8 months (sorafenib) vs. 5.8 months (placebo)
Cabozantinib	VEGFR1/2/3, RET, MET, FLT3, KIT	DTC failing prior anti-VEGF therapy	COSMIC-311 (6)	Phase III	PTC (102), FTC (90)	15%	Not reached (cabozantinib) vs. 1.9 months (placebo)
		MTC	EXAM (7)	Phase III	MTC (330)	28%	11.2 months (cabozantinib) vs. 4.0 months (placebo)
Vandetanib	VEGFR1/2/3, RET, EGFR	MTC	ZETA (8)	Phase III	MTC (331)	45%	Not reached (vandetanib) vs. 19.3 months (placebo)
Dabrafenib, trametinib	BRAF (dabrafenib), MEK (trametinib)	*BRAF* V600E-mutant solid tumors **	ROAR/ BRF117019 (9)	Phase II	ATC (36)	56%	6.7 months
Selpercatinib	RET	*RET* fusion+ solid tumors **	LIBRETTO-001 (10)	Phase I/II	PTC (13), PDTC (3), ATC (2), OTC (1)	79%	20.1 months
		RET-mutant MTC	LIBRETTO-001 (10)	Phase I/II	MTC (55 with and 88 without prior MKI treatment)	69%, 73%	Not reached (previously treated with MKI); 23.6 months (MKI-naïve)
Pralsetinib	RET	*RET* fusion+ thyroid cancer	ARROW (11)	Phase I/II	*RET* fusion-positive thyroid cancer (11)	89%	Not reached
		RET-mutant MTC	ARROW (11)	Phase I/II	55 with and 21 without prior MKI treatment	60%, 71%	Not reached
Larotrectinib	NTRK1/2/3	*NTRK* fusion+ solid tumors **	NAVIGATE, SCOUT, LOXO-TRK-14001 (12)	Phase I/II	PTC (20), ATC (7), FTC (2)	71%	2.2 months for ATC; not reached for other histologic types
Entrectinib	NTRK1/2/3, ROS1, ALK	*NTRK* fusion+ solid tumors **	ALKA-372–001, STARTRK-1, STARTRK-2 (13)	Phase I/II	*NTRK* fusion-positive thyroid cancer (5)	20%	-

ORR objective response rate, PFS progression-free survival, RAI radioactive iodine, DTC diferentiated thyroid carcinoma, PTC papillary thyroid carcinoma, FTC follicular thyroid carcinoma, PDTC poorly diferentiated thyroid carcinoma, WDC well-diferentiated carcinoma, OTC oncocytic thyroid carcinoma, ATC anaplastic thyroid carcinoma, MTC medullary thyroid carcinoma, MKI multi-kinase inhibitors vandetanib and cabozantinib

** Histology-agnostic approval

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Table 2

Summary of testing methodologies and kinase gene coverage in published series of KFTC [15–42]

Study	Testing approach	Method	Cohort Size	No. with kinase fusions								% fusion-positive
				ALK	*BRAF*	*MET*	*NTRK1*	*NTRK2*	*NTRK3*	*RET*	*ROS1*	
Adult papillary thyroid carcinoma												
Musholt et al.	Consecutive	RT-PCR	119	-	-	-	15	-	-	17	-	27%
Brzezińska et al.	Consecutive	RT-PCR	33	-	-	-	4	-	-	7	-	33%
Chou et al.	Consecutive	IHC, FISH	498	14	-	-	-	-	-	-	-	3%
Park et al.	Consecutive	IHC, FISH, NanoString	341	4	-	-	-	-	-	-	-	1%
Lu et al.	Consecutive	NGS	138	0	1	-	1	-	2	7	0	8%
Lee et al.	Consecutive	IHC, FISH, RT-PCR	769	0	-	-	3	-	-	16	-	2%
Bastos et al.	Consecutive	RT-PCR	116	4	0	-	-	-	6	-	-	9%
Liang et al.	Consecutive	NGS	355	1	1	0	3	0	9	30	0	12%
Panebianco et al.	Selective	NGS	na	27	-	-	-	-	-	-	-	-
Chu et al.	Selective	NGS	212	2	6	2	8	0	10	28	1	27%
Lee et al.	Consecutive	IHC, FISH, NGS	525	-	-	-	2	0	10	-	-	2%
Nozaki et al.	Consecutive	FISH	307	1	-	-	2	-	1	-	0	1%
Kong et al.	Consecutive	IHC, FISH	315	-	-	-	3	0	16	-	-	6%
Pediatric papillary thyroid carcinoma												
Fenton et al.	Consecutive	RT-PCR	33	-	-	-	-	-	-	15	-	45%
Prasad et al.	Consecutive	NGS	27	0	0	0	1	-	6	6	-	48%
Cordioli et al.	Consecutive	RT-PCR	35	-	4	-	-	-	3	13	-	57%
Sisdelli et al.	Consecutive	RT-PCR, FISH	80	-	15	-	-	-	-	-	-	19%
Alzahrani et al.	Consecutive	NGS	48	1	0	0	1	0	5	14	0	44%
Pekova et al.	Consecutive	NGS	93	6	2	1	3	0	14	26	0	56%
Lee et al.	Consecutive	NGS, IHC, FISH	106	6	0	0	2	0	2	20	0	28%
Macerola et al.	Consecutive	NanoString	163	6	0	-	5	-	18	17	-	28%
Rogounovitch et al.	Consecutive	RT-PCR	34	-	0	-	-	-	6	12	-	53%
Franco et al.	Consecutive	NGS	131	0	1	2	4	0	5	34	0	35%
Ricarte-Filho et al.	Selective	NGS	144	-	-	-	7	0	13	-	-	14%
Radiation-associated papillary thyroid carcinoma												

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Study	Testing approach	Method	Cohort Size	No. with kinase fusions								% fusion-positive
				ALK	*BRAF*	*MET*	*NTRK1*	*NTRK2*	*NTRK3*	*RET*	*ROS1*	
Bounacer et al.	Consecutive	RT-PCR	15	-	-	-	2	-	-	-	-	13%
Rabes et al.	Consecutive	RT-PCR	191	-	-	-	2	-	-	38	-	21%
Dinets et al.	Consecutive	RT-PCR	70	-	-	-	-	-	-	24	-	34%
Efanov et al.	Consecutive	NGS	65	5	7	0	2	0	7	22	0	66%
Poorly differentiated thyroid carcinoma												
Landa et al.	Selective	NGS	84	3	0	0	0	0	0	5	0	10%
Panebianco et al.	Selective	NGS	na	5	-	-	-	-	-	-	-	-
Duan et al.	Consecutive	NGS	41	1	0	-	1	-	-	6	-	20%
Chu et al.	Selective	NGS	23	1	0	0	0	0	0	1	0	9%
Pekova et al.	Consecutive	NGS	10	-	-	-	1	0	1	-	-	20%
Anaplastic thyroid carcinoma												
Duan et al.	Consecutive	NGS	25	0	0	-	1	-	-	0	-	4%
Chu et al.	Selective	NGS	39	0	0	0	0	0	0	2	0	5%
Nozaki et al.	Consecutive	FISH	16	0	-	-	1	-	0	-	0	6%
Xu et al.	Consecutive	NGS	360	0	0	0	1	1	0	2	0	1%

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Table 3

Clinical features of kinase fusion-related papillary thyroid carcinomas in published series

Study	Queried kinases	%T1-T2	%T3-T4	%N1	%M1
Adult					
Chu et al.	*ALK, BRAF, MET, NTRK1/2/3, RET, ROS1*	37	61	79	6
Chou et al.	*ALK*	45	55	27	0
Kong et al.	*NTRK1/2/3*	53	47	83	11
Nozaki et al.	*ALK, NTRK1/3, ROS1*	75	25	75	0
Panebianco et al.	*ALK*	89	11	30	0
Lee et al.	*NTRK1/2/3*	92	8	42	8
Park et al.	*ALK*	100	0	50	0
Pediatric					
Cordioli et al.	*BRAF, NTRK3, RET*	35	65	88	35
Pekova et al.	*ALK, BRAF, MET, NTRK1/2/3, RET, ROS1*	44	56	81	17
Franco et al.	*ALK, BRAF, MET, NTRK1/2/3, RET, ROS1*	47	49	93	40
Ricarte-Filho et al.	*NTRK1/2/3*	55	45	80	45
Prasad et al.	*ALK, BRAF, MET, NTRK1/3, RET*	77	23	69	0
Rogounovitch et al.	*BRAF, NTRK3, RET*	78	22	83	6
Alzahrani et al.	*ALK, BRAF, MET, NTRK1/2/3, RET, ROS1*	75	15	85	15

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Table 4

Reported cases of thyroid carcinomas treated with ALK inhibitor forgings (mm)

Study	Histology	*ALK* Fusion	ALK Inhibitor	Best Response, Duration
Demeure et al. [85]	PTC	*EML4*::*ALK*	Crizotinib	SD, over 6 months to the end of study
de Salins et al. [86]	OC	*ALK* fusion with unknown partner	Crizotinib	CR, 6 months
Chu et al. [15]	PDTC	*STRN*::*ALK*	Crizotinib	SD, 11 months
Leroy et al. [81]	ATC	*STRN*::*ALK*	Crizotinib, switched to ceritinib and then brigatinib for resistance	CR, 36 months (crizotinib); PR, 16 months (ceritinib); PR, 8 months (brigatinib)
Hillier et al. [48]	MTC	*CCDC6*::*ALK*	Crizotinib, switched to alectinib for tolerance	PR, over 280 days to the end of the study

PTC papillary thyroid carcinoma, *OC* oncocytic carcinoma, *PDTC* poorly differentiated thyroid carcinoma, *ATC* anaplastic thyroid carcinoma, *MTC* medullary thyroid carcinoma, *SD* stable disease, *CR* complete response, *PR* partial response

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