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Targeting rearranged during transfection (RET) in Cancer: A perspective on small molecule inhibitors and their clinical development

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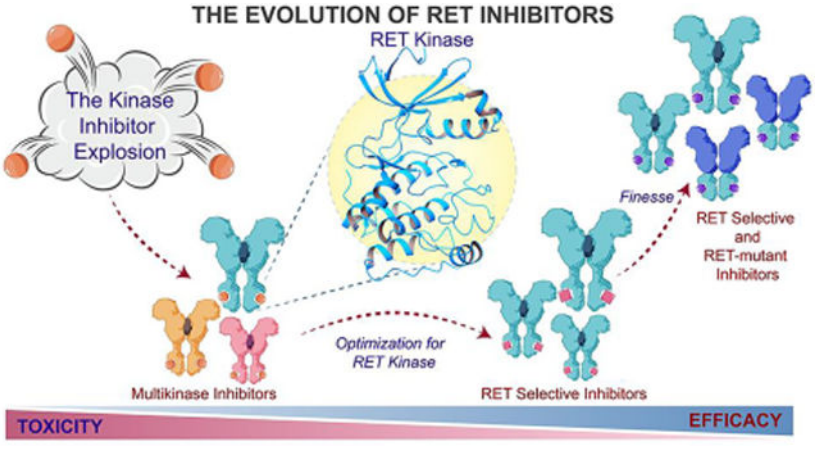
Abstract

REarranged during Transfection (RET) is a receptor tyrosine kinase essential for the normal development and maturation of a diverse range of tissues. Aberrant RET signaling in cancers, due to RET mutations, gene fusions, and over-expression, results in the activation of downstream pathways promoting survival, growth, and metastasis. Pharmacological manipulation of RET is effective in treating RET-driven cancers, and efforts towards developing RET specific therapies has increased over the last five years. In 2020, RET selective inhibitors pralsetinib and selpercatinib achieved clinical approval, which marked the first approvals for kinase inhibitors specifically developed to target the RET oncoprotein. This Perspective discusses current development and clinical applications for RET precision medicine by providing an overview of the incremental improvement of kinase inhibitors for use in RET-driven malignancies.

Graphical Abstract



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1. Introduction

The REarranged during Transfection (RET) gene was identified as a transmembrane receptor tyrosine kinase with proto-oncogenic properties by Takahashi *et al* in 1985, following the transfection of NIH/3T3 cells with human lymphoma DNA.[1–2] RET is essential for the normal development and maturation of a diverse range of tissues including kidney, central and peripheral nervous systems, thyroid, adrenal and pituitary glands, maturation of spermatogonia, and the survival and expansion of hematopoietic stem cells.[3–14] Following ligand binding to RET coreceptors, RET is recruited to the coreceptors-ligand complex and localized to lipid rafts, where it undergoes dimerization and autophosphorylation of the RET intracellular tyrosine kinase domain (Figure 1).[4, 15] Adaptor and signaling proteins then bind to the RET kinase domain resulting in the activation of multiple signaling pathways, which regulate proliferation, differentiation, and survival.[6, 16] RET is activated in numerous cancer types mainly through chromosomal rearrangements that generate fusion genes containing the active RET kinase domain. RET fusions occur in 10–20% of papillary thyroid carcinoma (PTC), 3% of spitzoid tumors, and 1–2% of non-small-cell lung carcinoma (NSCLC) and have also been identified in other cancers.[17–32] Gain-of-function mutations in RET cause multiple endocrine neoplasia 2 (MEN2), which is an autosomal dominant cancer characterized by high risk of developing early-onset medullary thyroid carcinoma (MTC).[33] Increased expression or activity of wildtype RET has also been shown to contribute to the pathogenesis of several cancer types including breast cancer, pancreatic ductal adenocarcinomas, acute myeloid leukemia, and prostate adenocarcinomas.[24, 34–52] Aberrant RET signaling enhances activation of downstream pathways that promote tumor growth, survival, and metastasis leading to poor prognosis in numerous cancers. RET is a targetable kinase and many studies have investigated the use of both multikinase inhibitors as well as RET specific inhibitors as therapeutic strategies. Our prior efforts and continuous interest in RET inhibitors prompted us to compile a detailed review of RET and RET inhibitors developed to pharmacologically modulate RET signaling in RET-driven malignancies.[53–56]

2. RET Biology and Signaling

RET receptor

RET is a receptor tyrosine kinase (RTK) containing an intracellular tyrosine kinase domain, a transmembrane domain, and a large extracellular domain (Figure 1A).[1] The RET extracellular domain contains four cadherin-like domains and a conserved cysteine-rich region important for ligand interactions and protein structure.[57–59] Following the transmembrane domain, a juxtamembrane segment marks the start of the intracellular portion of RET, which is followed by the kinase domain and an isoform specific C-terminus.[58–60] RET has three functionally distinct protein isoforms, which differ in their C-terminus due to alternative splicing. The three isoforms, RET9, RET43, and RET51, have high homology and are co-expressed in many tissues.[61–62] However, numerous studies have identified differences in temporal and spatial regulation of expression, cellular localization, trafficking, with the isoforms showing different contributions to both RET-mediated normal development and oncogenicity.[58, 60–64] RET51 may play a more prominent role in tumors,

with RET51 being more effective at promoting cell proliferation, migration, and anchorage-independent growth.[58, 60, 65] Transcripts of RET51 are more abundant than those of RET9 in some MEN2 tumors, and increased RET51 expression was seen in stage IIB pancreatic tumors.[49, 66]

RET activation in normal conditions occurs *via* the binding of a coreceptor-ligand complex of glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs): GDNF, neurturin (NRTN), artemin (ARTN), or persephin (PSPN) and one of four cell surface glycosylphosphatidylinositol-linked GDNF family receptor-α (GFRα) (Figure 1A).[4, 6, 67] These GFLs bind to GDNF family receptor-α (GFRα) coreceptors, which recruit RET for dimerization inducing RET autophosphorylation.[15, 68] The RET-coreceptor-ligand complex is then incorporated into lipid rafts, where adaptor and signaling proteins bind to docking sites on the RET intracellular tyrosine kinase domain allowing for RET-mediated activation of multiple downstream pathways (Figure 1B).[4, 6, 16, 57, 66–67, 69–71] The intracellular domain of RET contains twelve autophosphorylation sites: Y687, Y752, Y806, Y809, Y826, Y900, Y905, Y928, Y981, Y1015, Y1062 and Y1062, which serve as docking sites for adapter proteins.[72] A primary docking site is the phosphorylated tyrosine, Y1062, which binds to several adapter proteins such as Src homology and collagen (SHC), insulin receptor substrate 1/2 (IRS1/2), fibroblast growth factor receptor substrate 2 (FRS2), protein kinase C (PKC), downstream of tyrosine kinase 1/4/5/6 (DOK1/4/5/6), and Enigma.[72–73] Other docking sites include Y687 and Y981, which bind to tyrosine phosphatases, Shp2, and *c*-Src kinase, respectively.[74–75] Y905 is the docking site for Grb7/10; Y752 and Y928 are STAT3 docking sites; Y1015 is involved in the activation of PKC signaling through binding of phospholipase Cγ (PLCγ), and Y1096 binds Grb2.[58, 72–73, 76–77] Activation of these adapter proteins leads to the activation of several signaling pathways such as RAS/extracellular signal-regulated kinase (ERK), RAS/mitogen activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/AKT, and *c*-Jun N-terminal kinase (JNK), which are mediators of cell motility, proliferation, differentiation, and survival.[78–84]

3. RET Implications in Cancer

RET Gene Fusions

RET fusions occur in a variety of malignancies and are most common in PTC, Spitzoid tumors (rare melanocytic lesions), and NSCLC (Figure 2).[17–18, 24–27, 84] RET fusions have also been identified in other cancer types following deep sequencing approaches: chronic myelomonocytic leukemia, colorectal, breast, ovarian, spitzoid melanomas, and head and neck tumors.[17, 19–23, 29–31, 59] These somatic RET fusions of the RET gene result from chromosomal rearrangements or inversions which juxtaposition the RET intracellular kinase domain with the N-terminal region of another gene that contains dimerization domains such as: coiled-coil motifs, Lis1 homology (LisH) domain, or a sterile α motif (SAM) domain.[18, 85–87] More than 30 genes are reported to form fusion genes with RET.[58] The most frequently occurring RET fusions contain either the coiled-coil domain containing 6 (CCDC6), the nuclear receptor co-activator 4 (NCOA4), or the kinesin family 5B (KIF5B).[18, 58, 88–89] RET fusions or rearrangements are thought to arise from errors that occur during the repair of double-stranded DNA breaks including nonhomologous end

joining, break-induced replication, and other complex rearrangements [58, 90–92] Many factors can lead to double-stranded DNA breaks, such as ionizing radiation and genotoxic chemicals or stress factors such as hypoxia and replication stress.[93–94] Patients with papillary thyroid cancer who were exposed to the atomic bomb in Japan or to the Chernobyl radioactive fallout had RET fusions in 50–80% of cases.[95–98] Fusion to the RET kinase domain can lead to ligand independent dimerization resulting in constitutive activation of the RET kinase.[28, 99] Altered localization from the plasma membrane to the cytosol results in a loss of RET kinase regulation and enhanced downstream signaling of growth and survival pathways.[100–101] The KIF5B-RET fusion has been shown to increase RET kinase expression by 2- to 30-fold in lung tissue.[102] The NCOA4-RET fusion is associated with more aggressive papillary thyroid cancer histology and promotes tumor cell migration and invasion *in vitro*.[18, 63] Clearly, RET gene fusions are implicated in the pathology of numerous cancer types and pharmacological modulation of these gene fusions is an important therapeutic strategy.[103]

Elevated RET expression

Beyond gene fusions, increased expression or activity of wildtype RET is a contributing factor for oncogenesis in many tumor types.[42] GFLs are released by some tumor cells and by the tumor microenvironment, promoting autocrine activation of RET, increasing tumor growth, and metastasis. In breast cancer, tumor specific expression of GDNF and ARTN promotes tumor growth and resistance to several endocrine therapy regimes.[34, 44–48] GDNF and ARTN, as well as soluble forms of GFRα1, are secreted by pancreatic ductal adenocarcinomas cells as well as pancreatic nerve cells.[50–51, 100, 104] This stimulates metastasis and invasion of RET-expressing tumor cells along nerve fibers within the central nervous system.[35–37, 49–51] In 60–70% of acute myeloid leukemias, increased RET activity was observed due to the secretion of NRTN or ARTN from stromal cells promoting cell viability.[39, 43] Increased RET expression in prostate adenocarcinomas is linked to perineural invasion and increased proliferation.[40–41] Elevated GFL secretion and RET expression are detected in numerous other cancers including: melanoma, glioma, neuroblastoma, seminoma, endometrial, head and neck cancers, and renal cell carcinomas.[105–111] In many cases, RET stimulates tumor cell migration and invasion, and is correlated with poor prognosis and reduced overall survival.[20, 106–107, 109–111] Targeting RET may be of greater therapeutic value than first realized and has the potential to be clinically relevant for a much broader group of human cancers.

RET Mutations

MEN2 is an autosomal dominant cancer syndrome characterized by high risk of developing early-onset MTC.[33] MEN2 can be classified into two subtypes, MEN2A and MEN2B, in which RET activating mutations are pathognomonic.[117–118] The most common subtype is MEN2A and occurs in ~95% of MEN2 cases.[58–59] Familial medullary thyroid carcinoma (FMTC) was previously considered a third MEN2 subtype but is now considered part of the MEN2A subtype.[59] MEN2A is characterized by MTC in all patients and may also be associated with pheochromocytoma, hyperparathyroidism, cutaneous lichen amyloidosis, and Hirschsprung disease.[117, 119–120] The MEN2B subtype is clinically more severe with an early onset of MTC, and makes up ~5% of MEN2 cases.[32, 121] MEN2 is caused by

mutations in the RET receptor; to date, more than 60 RET mutations are known, with most being gain-of-function mutations (Figure 2). The majority of these mutations occur as point mutations found in *RET* exons 5–16, which lead to constitutive RET kinase activity.[6, 122] Approximately 95% of MEN2A cases arise from substitutions of cysteine residues within the cysteine-rich domain of the RET extracellular domain (C609, C611, C618, C620, C634) and patients with the C634 mutation account for ~85% of cases.[58, 123–124] These mutations decrease the formation of intramolecular disulfide bonds promoting receptor dimerization resulting in constitutive activation of RET independent of ligand binding.[124–127] Less common MEN2A mutations include G533C in the RET extracellular domain and (E768, L790, V804, S891) in the RET intracellular domain—these mutations are associated with delayed onset or with MTC as the only disease characteristic.[6, 118, 128] In the less common MEN2B subtype, ~95% of cases are associated with the M918T mutation within the RET kinase domain resulting in altered phosphorylation kinetics, increased ATP-binding, and decreased auto inhibition. This leads to a dominant active kinase domain resulting in elevated downstream signaling.[33, 124, 128–130] A883F has also been identified in MEN2B patients and is located in the RET kinases domain leading to enhanced activation and signaling, however the A883F mutation is associated with a less aggressive phenotype compared to M918T.[131–133] Two dual mutations have also been identified in rare cases of MEN2B, V804M and Y806C; these act synergistically to enhance RET activity but are associated with a less aggressive phenotype compared to M918T.[133–134] In ~65% of sporadic MTCs, somatic MEN2B-type mutations have been identified and these are associated with a more aggressive phenotype.[115]

Next generation sequencing techniques in recent years have identified activating RET mutations in multiple cancer types including breast carcinoma (C634R), colorectal adenocarcinoma (V804M), GI stromal tumor (V804M), Merkel cell carcinoma (E511K), and paraganglioma (M918T). However, how these RET mutations contribute to cancers regarding cancer progression and prognosis require additional research.[30, 59] With most of the mutations leading to RET activation and increased downstream signaling, RET specific inhibitors to block activation of pro-survival pathways is a therapeutically valid approach.

Aberrant RET signaling occurs from several mechanisms including RET gene fusions, RET activating mutations, and over-expression of the RET kinase. Increased RET activity has been identified in many cancer types contributing to cell motility, proliferation, differentiation, and survival. RET is an actionable oncoprotein and pharmacological modulation of RET is effective in the treatment and management of many cancers. Initially, multikinase inhibitors with RET activity were investigated for RET-driven malignancies but were found therapeutically limited by off target effects. Current therapeutic investigation involves testing RET specific inhibitors with activity on numerous mutant forms of RET. Clinical investigation with these specific, RET mutant inhibitors is effective but promotes drug resistance via novel RET mutations. To counter this, a new generation of RET inhibitors is being developed to overcome novel, treatment-induced mutations. In the following perspective, we provide a comprehensive overview of RET inhibitors by discussing the incremental improvement of kinase inhibitors for use in RET-driven malignancies.

4. First generation Multikinase Inhibitors (MKIs) with RET activity

Sorafenib

Sorafenib (NEXAVAR®; Bayer Pharmaceuticals) was the first MKI brought to market in 2005 to obstruct Raf oncogenic signaling. It was later discovered that sorafenib inhibited the RET oncoprotein in an in-vitro kinase assay (IC_{50} = 5.9 nM) including the gatekeeper mutant RET^{V804M} (IC_{50} = 7.9 nM).[135–136] In the clinical setting, sorafenib is approved to treat renal cell and hepatocellular carcinomas. Clinical activity has also been documented in patients with metastatic radioiodine nonresponsive differentiated thyroid carcinomas, which may have a RET counterpart driving the malignancy.[137] Thyroid cancer patients receiving sorafenib achieved greater progression-free survival, but overall survival was similar to that of non-treated patients.[130]

In 1994, development of sorafenib was initiated by Bayer and Onyx with the intention of discovering therapies to interrupt the Ras–Raf–MEK–ERK pathway. The discovery platform relied on high-throughput screening of two million compounds that were tested for Raf1 kinase inhibitory activity.[135] From the enzymatic screen, 3-thienyl urea **1** was discovered as a hit compound (Raf1 IC_{50} = 17 μM).[138] The activity of the initial hit was improved ten-fold with the addition of a methyl substitution on the phenyl ring (compound **2**). Further, a library of bis-aryl urea analogues was synthesized and screened against Raf1 kinase in an effort to improve inhibition, and 3-amino-isoxazole (compound **3**) exhibited a Raf1 kinase IC_{50} of 1.1 μM.[139–140] Further scaffold refinement was completed by modifying the distal ring system, which furnished the 4-pyridyl analog **4.** Additional modification of the distal pyridine ring system, while maintaining the diphenylurea moiety, led to the identification of sorafenib.[135, 141–142]

Sorafenib is a multikinase RET inhibitor and several other molecular targets of sorafenib contribute to its broad-spectrum inhibitory activity against various human cancers (listed in Tables 1 & 2).[137] Beyond RET, molecular targets include wild-type BRAF and oncogenic $BRAF^{V600E}$ serine/threonine kinases, pro-angiogenic RTKs such as vascular endothelial growth factor receptors (VEGFRs) 1/2/3, platelet-derived growth factor receptor-β (PDGFRβ) and fibroblast growth factor receptor 1 (FGFR1), and RTKs involved in tumorigenesis (*c*-Kit and Flt-3).[138, 141]

Wilhelm *et al.* demonstrated that sorafenib inhibited VEGF- and PDGFβ-stimulated phosphorylation of VEGFR2 and PDGFRβ RTKs in human cells, respectively.[137] It was also found that sorafenib induced complete tumor stasis in colon and breast carcinoma xenograft models. In addition, sorafenib inhibited the growth of a number of human xenografts, including ovarian (SK-OV-3, EGFR+ and HER2/neu+), pancreatic (Mia PaCa 2, KRAS+), melanoma (LOX, UACC 903 and 1205 Lu containing B-RAF V600E) and thyroid (RET+).[138]

Many research groups became particularly interested in sorafenib because of the ability for sorafenib to inhibit RET activity.[141] Plaza-Menacho *et al.* investigated the mechanism of sorafenib inhibition of RET and studied structural aspects of the binding of sorafenib to

RET.[135] To gain insight into the binding pose of sorafenib in RET, it was modelled in a DFG-out (inactive fold) homology model as previously described (Figure 3B).[56, 138]

It was also found that sorafenib induced degradation of RET and, to further study this, a lysosome or proteasome inhibitor was co-administered with sorafenib. In both RET^{C634R} and RET^{M918T} transfected HEK293 cells, sorafenib-induced RET degradation was rescued by the lysosome inhibitor concanamycin A. Sorafenib was also found to inhibit the gatekeeper mutation RET^{V804M}.[135] The RET^{V804M} gatekeeper mutation desensitizes kinase inhibition of other RET inhibitors including PP1, PP2, and vandetanib. By examining the RET-PP1 crystal structure, a mechanism for resistance has been proposed (Figure 3A). The RET^{V804M} gatekeeper mutant contains a bulky methionine residue in place of valine that sterically hinders binding of PP1 to the kinase.[129] When examining the RET-sorafenib complex (Figure 3B), inhibitory potency is maintained as the binding of sorafenib shifts to accommodate the methionine residue. This is explained by compensatory conformational changes in the RET binding site, inducing a shift in the DFG (aspartic acid, phenylalanine, glycine) motif to adopt a DFG-out conformation when bound to sorafenib.[56, 129, 135, 141]

Due to its activity against B-RAF, VEGFR2, and RET, sorafenib was clinically investigated for the treatment of advanced renal cell carcinoma (RCC), unresectable hepatocellular carcinomas (HCC) and locally advanced, metastatic, or locally recurrent thyroid cancer.[56, 143–146] Sorafenib was also investigated as an adjuvant to radioiodine therapy in MTC (NCT00095693). A phase II trial of sorafenib against medullary thyroid carcinoma (MTC), a cancer that commonly harbors a RET oncogene, found that sorafenib is tolerated in advanced MTC with extended clinical benefits if adverse events are recognized and managed via reduction or discontinuation of treatment.[142, 147] Common adverse events include diarrhea, hand-foot-skin reaction, rash, hypertension, and, less common, death. Severity of the adverse events likely stems from the multikinase profile of sorafenib. Although sorafenib can effectively inhibit the RET kinase at a therapeutic dose, the multikinase activity becomes dose-limiting, which restricts therapeutic benefits.[137, 142] Sorafenib was also investigated in combination with tipifarnib, a farnesyltransferase inhibitor, and provided a clinical response in spontaneous MTC with an aberrantly activated RET kinase.[148] A reduction in tumor volume was confirmed by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria to be 36% by 8 weeks and 46% by 10 months.[148]

The clinical investigation of sorafenib supported the hypothesis that inhibiting RET in RET driven cancers can provide a therapeutic benefit. However, clinical investigation also suggested that selectivity of the RET-targeted agent was important to consider to reduce adverse events that lead to dose reduction or discontinuation of therapy. A summary of all clinically investigated RET inhibitors can be found in Table 6.

Regorafenib

Regorafenib (BAY 73–4506, STIVARGA®) is a multikinase RET inhibitor approved for the treatment of metastatic colorectal cancer (mCRC).[149–150] Regorafenib was discovered during the development of sorafenib *via* a traditional medicinal chemistry analoging approach. Regorafenib is active against several oncogenic RTKs, including RET, angiogenic RTKs (VEGFR-1, VEGFR-2, VEGFR-3, TIE-2), stromal RTKs (PDGFR-B, FGFR1), and

intracellular signaling kinases (*c*-RAF/RAF-1, BRAF, BRAFV600E). The biochemical enzymatic inhibition of regorafenib is listed in Table 2.[149] In contrast to sorafenib, regorafenib contains a fluorine in the center phenyl ring. This additional structural modification results in a similar, but distinct, therapeutic profile to that of sorafenib.[151]

It was found that regorafenib binds to the RET kinase domain like sorafenib (Figure 4A). Regorafenib is metabolized into two active metabolites, **M-1** (BAY 75–7495) and **M-2** (BAY 81–8752).[152] Kinase profiling of regorafenib and the two active metabolites revealed that regorafenib and the active metabolites have higher affinity for RET compared to angiogenic and stromal RTKs. The active metabolites also exhibited more pronounced inhibitory activity compared to regorafenib.[152] Regorafenib and metabolites also display dose-dependent inhibition of tumor growth in CRC xenograft models.[152]

Distribution studies revealed that regorafenib and its metabolites concentrate at high levels in mammary alveolar cells, which presents a risk of neonatal exposure. Despite this, regorafenib was progressed into clinical trials to assess safety, pharmacokinetics, pharmacodynamics, and efficiency in patients with advanced solid tumors. Large, multinational Phase III and IV studies were completed to assess regorafenib efficacy in mCRC patients that progressed after treatment with standard therapy. In this patient class, regorafenib was approved for the treatment of mCRC in 2012.[153]

Although rare, 0.2% of mCRC patients have a RET oncogenic fusion, which can occur as NCOA4-RET, CCDC6-RET, TRIM24-RET, TNIP1-RET and SNRNP70-RET.[20] In a mCRC patient harboring a CCDC6-RET fusion oncogene, a reduced regorafenib dose compared to the starting mCRC dose produced a therapeutic response.[22, 151] Further investigation is required to confirm the efficacy of regorafenib in mCRC patients that harbor a RET fusion oncogene. Beyond mCRC, regorafenib can inhibit the RET-mediated PI3K/AKT/mTOR pathway in neuroblastoma.[154] This suggests regorafenib penetrates the blood brain barrier and could be utilized to treat central nervous system cancers or metastases driven by a RET oncogene.

Sunitinib

Sunitinib (SU11248, SUTENT®; Pfizer, Inc.) is a multitargeted kinase inhibitor that inhibits RET, VEGFRs (1, 2, and 3), PDGFRs α and β, KIT, FLT3, and CSF1R.[155] Sunitinib was approved in 2006 for the treatment of advanced RCC and gastrointestinal stromal tumors (GISTs).[156] The discovery of sunitinib was initiated at Sugen Inc. with the identification of three indolin-2-one cores with inhibitory properties against various RTKs. Both **1** and **3**, with a *Z*-configuration, were found to be potent and selective inhibitors of VEGFR, whereas **2**, an *E*-configuration, was found to inhibit RTKs non selectively.[157]

The E/Z configuration was determined by the nature of substitutions at the C - 3 position of the indolin-2-ones. The potency was found to be dependent on adopting a *Z*-isomeric form. This is supported by co-crystal studies using **SU5402** bound to the active sites of FGFR1 and VEGFR2.[156, 158] Co-crystallized structures of **SU5402** with both FGFR1 and VEGFR2 demonstrate that **SU5402** coordinates to a conserved asparagine residue (Asn568) through its C-3’ propioninc acid substituent on the pyrrole ring. The asparagine residue is not

conserved in PGDFRβ, which contains an aspartic acid in the corresponding position, and efforts to improve PGDFRβ affinity while maintaining VEGFR2 affinity were completed.[158] This led to the discovery of **SU6668**, which maintained both PDGFRβ and VEGFR2 inhibitory activity.[159] Binding of **SU6668** in the active site of FGFR1 revealed that the C-4′ position on the pyrrole ring orients to the solvent front, and thus substitution at this position was completed to improve pharmaceutical properties of the indolin-2- ones.[159] Various basic amine side chains were introduced at the C-4′ position among which sunitinib (**SU11248**) was identified and exhibited the most optimal profile.[156] Initial kinome profiling of sunitinib demonstrated selectivity for class III and V RTKs, which included RET, VEGFRs 1–3, PDGFRs α and β, KIT, FLT3, and CSF-1R (Table 2).[156]

To identify the individual roles of RTK targets, sunitinib was compared to selective RTK inhibitors.[157, 160] This identified that the reduction of micro vessel density and antitumor efficacy of an indolin-2-one analog **SU10944** combined with imatinib was similar to that of single-agent sunitinib and was superior to that of each compound. Together, these data suggested that inhibition of VEGFR, PDGFR, and KIT synergistically contribute to the antitumor and antiangiogenic profile of sunitinib.[157]

Sunitinib was designed with a fluoro substitution at the C-5 position to prevent aromatic hydroxylation by CYP.[157, 161] The major metabolite **M-3** is the *N*-dealkylation product of sunitinib, **SU12662**, which exhibited comparable *in vitro* and *in vivo* properties. Sunitinib was found to exhibit desired pharmacokinetic properties (i.e., oral bioavailability, solubility, stability) and tumor regression was observed in tumor xenografts.[157, 161]

Tumor growth inhibition and pharmacodynamic modulation of RTKs was evaluated, which indicated a plasma level ≥ 50 ng/mL per day was required to efficiently block targeted RTKs. Sunitinib exhibited direct antiproliferative activity against a subset of tumor cells including the acute myeloid leukemia cell line MV4–11, presumably from activity on FLT3.[162] It was also reported that sunitinib had a benefit in lung adenocarcinoma patients harboring a KIF5B-RET fusion.[163] Despite activity on RET, sunitinib is not approved to treat RET-driven disease but is approved for other malignancies.[164]

Vandetanib

Vandetanib (ZD6474, CALPRESA®, Genzyme) is a heteroaromatic-substituted anilinoquinazoline developed by Astra-Zeneca to inhibit VEGFR with inhibitory effects on RET and epidermal growth factor receptor (EGFR) kinases.[167] Vandetanib inhibits cancer cell-proliferation *in vitro* and impairs tumor growth in xenograft models of prostate, lung, breast, ovarian, vulvar and colorectal cancers, and in syngenic murine models of lung cancer and melanoma.[167]

Due to its multitargeted nature (enzymatic inhibitory activities are listed in Table 2), vandetanib exhibits anti-angiogenic, anti-tumorigenic, and anti-metastatic properties, and exhibits efficacy in orthotopic murine models of lung, gastric, pancreatic, and renal cancers.[165]

Several preclinical studies suggests that vandetanib inhibits two key pathways: (1) indirect tumor growth arrest *via* inhibition of VEGF-dependent tumor angiogenesis and VEGF-dependent endothelial cell survival, and (2) direct tumor growth arrest *via* inhibition of oncogene-dependent tumor cell proliferation and survival. Vandetanib exhibits broad-spectrum antitumor activity in preclinical xenograft models of lung, prostate, breast, ovarian, colon, and vulvar.[165, 167]

Vandetanib treatment was studied in MTC, which is commonly driven by a RET oncoprotein in approximately 10–30% of cases.[157–158, 169–170] It was hypothesized that vandetanib inhibited the growth of MTC by the blockade of both RET and VEGFR pathways. Carlomagno *et al* investigated the inhibitory profile of vandetanib against various oncogenic RET kinases.[171] It was found that vandetanib could block *in vivo* phosphorylation and signaling of the RET/PTC3 and RET/MEN2B oncoproteins and the EGF-activated EGF-receptor/RET chimeric receptor.[172] Vandetanib prevented the growth of two human PTC cell lines that carry RET/PTC1 oncogene rearrangements.[171] Also, vandetanib blocked anchorage-independent growth of RET/PTC3-transformed NIH3T3 fibroblasts and *in vivo* formation of RET/PTC3 driven tumors in nude mice.[172] Therefore, although vandetanib is a multikinase RET inhibitor, the multikinase profile appears advantageous in RET-driven carcinomas.

Co-crystal studies of vandetanib bound to RET illustrates that the molecule occupies the ATP-binding site of RET by displacing the nucleotide-binding loop.[128] Vandetanib binds to the hinge region through a hydrogen bond between the quinazoline core and Ala807. The bromofluorophenyl group of vandetanib occupies a hydrophobic pocket at the back of the ATP site, which is gated by VAL804 (Figure 4B). The gatekeeper VAL804 cannot form hydrogen bonds with vandetanib, and the size of the amino acid side chain at this position controls access to the pocket. This explains why VAL804 mutants, with more bulky amino acid side chains, confer resistance to vandetanib.

Vandetanib is metabolized by CYP3A4 and hepatic flavin-containing mono-oxygenases, which generates the metabolites *N*-desmethyl vandetanib and vandetanib *N*-oxide, respectively. Investigation of the *in vitro* activity of these metabolites has shown that *N*-desmethyl vandetanib is able to inhibit VEGFR and RET and contributes to the overall pharmacological profile of vandetanib. The *N*-oxide metabolite does not retain pharmacological activity.[173]

Vandetanib was approved in April 2011 for advanced or metastatic MTC.[173–174] In a Phase III trial, 89% of patients in the vandetanib arm developed a rash, and 13% of patients had a photosensitivity reaction. Clinical efforts were launched at 16 different European medical centers to expand the therapeutic profile of vandetanib, but QT prolongation was a major dose-limiting adverse event that blunted therapeutic development.[173]

The clinical effects of vandetanib stem from other kinase targets beyond RET, such as VEGFR2, which can impair VEGF-dependent tumor angiogenesis and VEGF-dependent endothelial cell survival. However, this activity also leads to discontinuation and dose-limiting toxicities as excessive VEGFR2 inhibition is linked to cardiotoxicity and the EGFR

inhibitory component of vandetanib is likely the culprit of dermatological toxicities.[175] Further, vandetanib does not retain inhibition of clinically relevant RET point mutations that have been shown to drive drug resistance.[171, 173] The discovery and clinical development of vandetanib highlight that a lack of target specificity for RET may increase adverse events and discontinuation rates. Also, the clinical utility of vandetanib is limited since the drug does not retain activity on RET mutations that drive drug resistance. Although clinically effective for MTC, adverse drug events blunt clinical utility.

Lenvatinib

Lenvatinib (LENVIMA®) is a quinoline based multikinase inhibitor developed by Eisai in 2015.[168, 176] Lenvatinib targets RET, VEGFR 1–3, FGFR 1–4, mast/stem factor receptor kit (SCFR) or *c*-Kit, and PDGFR*β*.[168] (Biochemical IC_{50}s are listed in Table 2) Lenvatinib elicits antitumor effects by interfering in pro-angiogenic and oncogenic-pathways in a similar fashion to vandetanib.[168, 176] Due to activity on the RET oncogene, lenvatinib can inhibit proliferation of RET-driven malignancies.[166] Lenvatinib was discovered by screening a compound library against an angiogenic-factor-induced tube formation assay, which identified an active quinoline skeleton.[177] The quinoline underwent optimization to improve the VEGFR inhibitory profile.[159] From drug development efforts, lenvatinib was identified and found to simultaneously inhibit VEGF-induced proliferation (IC_{50} = 3.4 nM) and tube formation of HUVECs (IC_{50} = 2.7 nM) and FGF-induced angiogenesis (IC_{50} = 7.3 nM).[178]

Binding kinetics of lenvatinib with VEGFR2 demonstrate the compound is 14–16 times more potent than sunitinib and sorafenib, respectively. The X-ray cocrystal structure of the lenvatinib-VEGFR2 complex (Figure 4C) reveal that lenvatinib binds to the active (DFG-in) conformation of VEGFR2.[168] The nitrogen in the quinoline ring binds to the hinge residue CYS919 and the cyclopropane ring uniquely interacts in the allosteric pocket of the kinase. Typically, kinase inhibitors that interact in the allosteric pocket of a kinase induce a DFG-out conformation (type II/III kinase inhibitors). However, lenvatinib does not induce a DFG-out conformational change but still interacts in the allosteric pocket. This type of binding interaction is unique to lenvatinib and is classified as a ‘Type V’ inhibitor.[168] In comparison with other types of kinase inhibitors, Type V is distinguished by rapid binding and generally greater affinity.[179]

Lenvatinib was studied in thyroid cancer because of its VEGFR 1–3 anti-angiogenic activity and inhibition of oncoproteins including RET.[179–181] Lenvatinib demonstrated anti-tumor activity in xenograft mouse models of thyroid cancer including differentiated thyroid cancer (DTC), MTC, and anaplastic thyroid cancer (ATC). However, *in vitro* cancer cell proliferation was inhibited in only two cell lines: RO82-W-1 (FGFR1 overexpression) and TT cells (RET point mutation).[182] It is important to note that inhibition of VEGF-mediated pathways in cell culture does not reduce proliferation as cell culture lacks a vascularized microenvironment.[181] Lenvatinib was also found to inhibit autophosphorylation of three RET gene fusions (KIF5B-RET, CCDC6-RET, and NCOA4-RET) and exhibited antitumor activity in RET gene fusion tumor models.[177, 182]

Lenvatinib, in combination with everolimus, was examined as a treatment for RCC as VEGF-promoted angiogenesis and overactivity of the mTOR pathway are characteristics of this malignancy. The combination of lenvatinib and everolimus displayed synergy by suppressing mTOR–S6K–S6 signaling *via* VEGFR and FGFR and angiogenesis via VEGFR.[183]

Clinical studies of lenvatinib for thyroid cancer was first evaluated in phase I dose-escalation trials in patients with solid tumors and clinical benefits were observed in 55% of patients.[184–185] A single-arm phase 2 trial was initiated with 58 patients that had radioiodine-refractory differentiated thyroid cancer (RR-DTC) and were then enrolled and treated with lenvatinib. After a follow-up of 14 months, the objective response rate (ORR) was 50%. Out of all patients that received prior VEGFR-targeted therapy the observed ORR was similar to patients who had not received such therapy (59% vs 46%, respectively).[186] A phase 3 randomized, double-blind, placebo-controlled study of lenvatinib was completed in patients that had differentiated thyroid cancer (SELECT).[187] In total, 392 eligible patients were recruited in a 2:1 ratio to receive oral lenvatinib once daily (261 patients) or placebo (131 patients). Patients were further categorized based on age, geographic region, and receipt or non-receipt of prior TKI treatment.[187] Lenvatinib prolonged progression free survival compared to placebo (18.3 months vs 3.6 months), and there was a marked improvement in response rate (64.8% lenvatinib vs 1.5% placebo). The overall survival in patients >65 years of age showed a significant improvement (vs placebo) in comparison to patients ≤ 65. This suggests that lenvatinib produces a more favorable clinical response in the elderly.[187–188]

A phase II study (59 patients) of lenvatinib in progressive MTC obtained a high objective response rate and disease control rate. However, no significant tumor shrinkage in RET positive tumors was identified. This suggests tumor shrinkage is not a necessary outcome to achieve disease control in RET-driven malignancies.[189] Clinical investigation of lenvatinib illustrated the advantage of the pharmacological impairment of VEGF-stimulated angiogenesis while also blocking the RET oncogene within the tumor.

Cabozantinib

Cabozantinib (Cometriq®, XL-184), developed by Exelixis, is a VEGFR2 selective inhibitor with additional activities against RET, MET, FLT3, *c*-KIT, AXL, and Tie-2 (*in-vitro* kinase inhibition profile is demonstrated in Table 2).[160] Cabozantinib was originally developed as dual inhibitor of VEGFR2 and MET and was approved by the FDA for the treatment of MTC in 2012.

To understand the RET inhibitory mechanism of action, cabozantinib was docked into the RET kinase domain and was found to exhibit a similar binding pose to that of vandetanib. The major difference is that cabozantinib binds to RET in the DFG-out fold, which is an inactive conformation of the RET kinase. The quinoline moiety adopts a similar H-bond interaction with the RET hinge residue ALA807, which is a key interaction for many RET kinase inhibitors. (Figure 4D)

Studies of cabozantinib in MTC displayed a reduction in MET phosphorylation with drug treatment. Xenograft studies utilizing cabozantinib exhibited reduced cell proliferation, reduced vascular density, and increased apoptosis.[160] In phase I and II trials evaluating cabozantinib for MTC, ten patients out of thirty-five showed a partial response and twenty-five exhibited tumor shrinkage.[190] Three patients that had confirmed responses received previous treatment with vandetanib and sorafenib. Genotyping of each tumor showed twenty-five of thirty-five patients had an active RET mutation. A Phase III EXAM (Efficacy of XL184 in Advanced Medullary Thyroid Cancer) trial evaluating cabozantinib for MTC identified an overall response rate of 28% in the cabozantinib group versus 0% in the placebo group. The duration of response was 14.6 months, which was similar in both RET-positive and RET-negative patients suggesting that VEGFR2 inhibition is a major contributing component to efficacy.[191] Drilon *et al.* reported clinical efficacy of cabozantinib in advanced NSCLC with a KIF5B-RET gene fusion.[192] In phase II trials, three patients with RET fusion-positive NSCLCs were treated with cabozantinib, out of which two patients had confirmed partial responses and the third patient had prolonged stable disease for 8 months.[190]

Clinical development of cabozantinib suggests VEGFR2 inhibition is integral for the efficacy in the treatment of RET-driven malignancies, regardless of RET mutation status.[193] This is supported since RET-positive and RET-negative MTC patients exhibit a similar response to cabozantinib treatment. It is unclear from the development of cabozantinib the importance of inhibiting the RET oncoprotein compared to inhibiting tumor angiogenesis *via* VEGFR2. Cabozantinib does not retain clinical activity against drug-resistant RET point mutations, which is a flaw shared with both vandetanib and lenvatinib.[193] The lack of activity on drug-resistant RET mutations may represent a clinical shortcoming among first-generation RET inhibitors. Although VEGFR2 inhibition appears robust regardless of RET status, as a RET-positive patient progresses, additional RET mutations are identified that confer resistance to treatment.[183] Therefore, a contributing factor in the progression of RET-positive patients may be the selection of drug-resistant clones that are resistant to RET inhibition. This hypothesis shifted the effort of RET drug development to focus on identifying agents with RET mutant profiles capable of blocking common mutations resistant to vandetanib, lenvatinib, and cabozantinib.

5. First generation MKIs with RET mutant activity

As of September 2020, seventy-five drugs targeting protein kinases have been clinically approved. Out of these seventy-five drugs, numerous possess activity on RET mutations, which helped progress the development of RET mutant inhibitors (Scheme 7).

Ponatinib

Ponatinib (AP24534) is a imdazopyridazine based multikinase inhibitor, which exhibits inhibitory activities against RET (observed in thyroid cancer cells, IC_{50} = 25.8 nM), BCR-ABL (observed in Ba/F3 Cellular proliferation assays, IC_{50} = 0.5 nM), SRC (observed in hematologic cells, IC_{50} = 5.4 nM), FLT3 (observed in Hematologic cells, IC_{50} = 0.3–2 nM), KIT (observed in hematologic and gastrointestinal stromal tumor cells, IC_{50} = 8–20 nM),

FGFR (IC_{50} = 2.2 nM), VEGFR (observed in hematologic cells, IC_{50} = 1.5 nM), PDFGR (observed in hematologic cells, IC_{50} 1.1 nM) and others.[194–195] Ponatinib was approved for clinical use in chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) (NCT01207440) in 2012.[196]

Ponatinib was developed by ARIAD Pharmaceuticals using computational and structure-based drug design approaches by first screening an in-house library, which identified compound **10** as a lead candidate.[195] Template morphing and linker modification to target the T315I gatekeeper mutation within the kinase domain of BCR-ABL generated **11**. To improve pharmacokinetic properties, the amine/acetamide group at C8 was removed to furnish **12** (Scheme 8). Alternate hinge-region heterocycles were explored to improve pharmacokinetic and pharmacodynamic properties.[195]

SAR exploration and modification to improve pharmacokinetics led to the discovery of ponatinib. The co-crystal structure of ponatinib with ABL-T315I revealed the acetylene-linker helps extend the inhibitor around the T315I gatekeeper mutation to retain inhibition of the kinase.[197] This can be explained as the acetylene-linker forms favorable van der Waals' interactions with gatekeeper ILE315 and PHE382 of the DFG motif. The crystal structure of ponatinib bound to RET kinase shows the molecule binds to the DFG-out conformation and is classified as a Type II inhibitor (Figure 5A).

Ponatinib inhibits RET with an IC_{50} of 25.8 nM and the RET gatekeeper mutation, RET^{V804M}, with an IC_{50} of 33.9 nM. De Falco *et al.* reported a reduction in tumor volume of MTC cells harboring a RET^{C634W} mutation receiving ponatinib treatment.[198] It was found that ponatinib could inhibit $RETV^{804M/L}$ gatekeeper mutations, which are resistant to multikinase inhibitors including cabozantinib, vandetanib, and levantinib.[198] A phase II clinical trial of ponatinib for NSCLC was conducted in patients with RET mutations (NCT01813734). Investigation of the drug was suspended by the FDA because of safety concerns from an increase in serious vascular occlusion events, including blood clots and severe narrowing of blood vessels. Although ponatinib did not receive approval for a RET-driven malignancy, ponatinib was the first agent that exhibited broad activity on RET point mutations. This set a new precedent for the discovery and development of RET inhibitors by focusing on the development of RET inhibitors with activity on clinically significant RET mutations.

Alectinib

Alectinib is a second generation ALK inhibitor (IC_{50} = 1.9 nM) bearing a naphtha-[2,3-*b*]benzofuran-11(6*H*)-one framework.[199] Chugai, a subsidiary of Roche, developed alectinib using a high throughput screening platform.[200–201] Beyond ALK, alectinib has weak or no inhibition for other protein kinases.[202] When subjected to Ambit's kinase profiling screen, only three other kinases (GAK, LTK, and RET) showed more than 50% of inhibition at 10 nM.[203] Replacement of the benzofuran fragment with an indole moiety, followed by optimization at the solvent front and the ATP binding region, generated alectinib.[200] Alectinib inhibited ALK with an IC_{50} of 1.9 nM and the ALK gatekeeper mutation L1196M with an IC_{50} of 1.56 nM. In ALK-positive cell lines, KARPAS-299 (lymphoma), NB-1 (neuroblastoma), and NCIH2228 (lung cancer), alectinib inhibited cell proliferation with

IC_{50} values of 3, 4.5, and 53 nM, respectively.[203] Alectinib is an ATP-competitive ALK inhibitor, and inhibits EML4-ALK positive NCI-H2228 xenografts in a dose-dependent manner. Kodama *et al.* showed that alectinib inhibits RET kinase activity and RET gatekeeper mutations (RET, IC_{50} = 4.8 nM; RET^{V804L}, IC_{50} = 32 nM; RET^{V804M}, IC_{50} = 53 nM).[202] Alectinib was also shown to inhibit other clinically relevant RET mutations (RET^{G691S}, IC_{50} = 9.5 nM; RET^{Y719F}, IC_{50} = 14 nM; RET^{S891A} IC_{50} = 8.3 nM; RET^{M918T}, IC_{50} = 5.7 nM). In xenograft studies, alectinib displayed antitumor activity in tumors driven by RET fusion genes and blocked cell growth driven by fusion genes with a $RET^{V804L/M}$ gatekeeper mutation.[202]

To understand ligand-receptor binding interactions, alectinib was modeled in the RET kinase domain. It was found that the naphtha-[2,3-*b*]benzofuran-11(6*H*)-one moiety binds to the backbone NH of the ALA807 hinge residue, the *N*-piperidinyl morpholine orients towards the solvent front, and the benzonitrile enters the back pocket (Figure 5B). Structural modelling of V804L/M mutations demonstrate that these mutations do not cause steric clashes that would interfere with the binding of alectinib to RET. This indicates the potential for alectinib to inhibit RET gatekeeper mutations that are resistant to vandetanib and other first-generation RET inhibitors.

A phase I/II study of alectinib was completed to examine efficacy in NSCLC with RET gene fusions.[203] In the study, twenty-two patients had a KIF5B-RET fusion gene, eight patients had a CCDC6-RET fusion, and five were not distinguishable. Twenty-five RET inhibitor-naïve patients were treated with alectinib, of which one achieved an objective response and thirteen achieved disease control at 8 weeks.[203] The median progression-free survival was 3.4 months (95% CI 2.0–5.4), and the median overall survival was 19.0 months (5.4-NE). In patients treated with 450 mg alectinib twice daily, adverse effects included neutropenia, pneumonitis, diarrhea, hyponatremia, increased CPK, and blood bilirubin (4%). Despite exhibiting broad RET activity in pre-clinical studies, alectinib was found to have limited, clinical benefit in patients with RET-rearranged NSCLC.[204]

Nintedanib

Nintedanib (BIBF1120) is an angiokinase inhibitor and antifibrotic agent active against three major signaling pathways involved in angiogenesis and fibrosis mediated by VEGFR2, FGFR, and PDGFR. The biochemical enzyme inhibitory activities are listed in Table 3.[206] Nintedanib is approved by the FDA for the treatment of idiopathic pulmonary fibrosis by blocking fibroblast proliferation and reducing deposition of the extracellular matrix.[207–208] Nintedanib was found to be active against 34 kinases, but *in vitro* kinase activity did not necessarily translate to cellular activity. For example, CUTO-3.29 and KM-12 cell lines that harbor a TRK oncogene were resistant to nintedanib.[209]

Discovery of nintedanib was initiated by hit identification of VEGFR2 inhibitors.[207] VEGFR2 inhibition, along with selectivity screening to avoid CDK2 inhibition, led to the generation of lead compound **13** (Scheme 9). The perpendicular conformation of the central phenyl ring and the oxindole scaffold were thought to promote aqueous solubility. For this reason, the oxindole motif and the central phenyl ring were unchanged. SAR was explored around R_1 (C6 substitution) and R_2, and SAR around R_1 was found to be responsible

for kinase selectivity. Nitro and chloro substitutions produced lower selectivity whereas ester substitutions generated potent inhibitors albeit with risk of metabolic degradation. Optimization at R_2 was straightforward to fine-tune cellular properties and solubility. Substitutions with imidazole and morphinyl moieties did not provide improved solubility whereas 4-(NMe)$COCH_2$-(4-methylpiperazin-1-yl) had high exposure and displayed in vivo target inhibition after oral administration. Additional *in vivo* studies led to the clinical development of nintedanib.[207]

Nintedanib was identified as a potent RET inhibitor similar to other multikinase inhibitors.[209] Nintedanib inhibited KIF5B-RET-dependent BaF3/KR cells with an IC_{50} of 0.14 μM in comparison to parental BaF3 cells (IC_{50} = 1.67 μM), demonstrating the specificity of nintedanib for the KIF5B-RET gene fusion. Two nintedanib-resistant RET mutations were identified through long-term culture of KIF5B-RET-dependent cells in medium containing nintedanib.[193, 210] Sensitivities of these RET mutations were then cross profiled with known RET TKIs (cabozantinib, lenvatinib, vandetanib, and nintedanib).[205, 210] The L730I, V738A, V804L/M, Y806N, and G810S mutants were pan resistant to all four TKIs. The L730V/V804M double mutant had a higher degree of drug resistance to all four TKIs compared to the L730V or V804M single-site mutants. Apoptotic assays revealed that BaF3/KR (E732K) cells were resistant to cabozantinib-induced apoptosis, whereas they were sensitive to apoptosis induced by lenvatinib, vandetanib, and nintedanib. Unsurprisingly, the V804L/M gatekeeper mutants were resistant to all four TKIs. The G810S solvent front mutation resulted in resistance to all four TKIs, but the smaller alanine mutation at G810 was inhibited by cabozantinib, lenvatinib, and nintedanib. The RET^{M918T} mutation is prevalent in MTC, and nintedanib was not significantly affected by that mutation or by the V871I and F998V mutations in the C-lobe, which suggests nintedanib could be effective for treating RET^{M918T}-positive MTC.[191, 205]

The crystal structure of wild type RET-nintedanib shows nintedanib binds to the DFG-in confirmation of the kinase.[205] Nintedanib engages in four hydrogen bonds with RET, and also engages in a series of hydrophobic interactions. It is hypothesized that these interactions cause a shift in PHE776, along with other hydrophobic residues, which generates a novel, nintedanib-induced confirmation of RET (Figure 5C).

Comparing the structures of the RET-nintedanib complex with RET^{G810A}, it was predicted that ALA810 on RET would make hydrophobic contacts with the methyl group of nintedanib and unfavorable contacts with the phenyl ring (Figure 6A & 6B). [205] This may cause a shift of nintedanib in the binding pocket. Also, introduction of a bulkier residue at 810 could cause steric clashes with both the methyl group and the phenyl ring of nintedanib (Figure 6C). This structural insight suggests an explanation as to why the RET^{G810S} mutant is resistant to nintedanib. Nintedanib is active on RET^{L881V}, a novel vandetanib resistant germline mutation in FMTC. This can be explained by the nintedanib RET co-crystal structure where the phenyl ring and C6 of the indole form favorable interactions with LEU881 (Figure 6D).[205] When leucine is replaced with valine, the phenyl ring sits in a shallow notch between the side chain methyl group and the backbone of 810, which restores binding of the phenyl ring (Figure 6E).[205] Hence, nintedanib was found active on the

L881V mutation, whereas this mutation confers resistance to vandetanib due to weak shape complementarity.

Agerafenib (RXDX-105)

Agerafenib (CEP-32496, RXDX-105) is a quinazoline based inhibitor of BRAF (WT BRAF and $BRAF^{V600E}$) with activity against RET. Table 4 lists the biochemical and cellular inhibitory activities of agerafenib.[212]

Agerafenib was discovered by Ambit using an internal library that was screened against a kinase panel (Scheme 10).[211] This led to the identification of diaryl amide derivatives exhibiting high affinity for $BRAF^{V600E}$. Hit to lead optimization led to the discovery of 4-quinazolinyloxy-diaryl urea derivative **16**, which, when explored for SAR around the left-hand aryl moiety, led to the clinical candidate agerafenib.[211]

To understand RET binding, agerafenib was modelled in the RET kinase domain and found to bind the DFG-out conformation. The quinazoline binds the ALA807 hinge residue and the urea moiety forms two hydrogen bonds with the α-helix in the DFG-out fold of the kinase (Figure 5D).

Agerafenib demonstrated oral efficacy in several $BRAF^{V600E}$-driven human carcinoma xenograft mouse models (Colo-205 and A375).[211] Although agerafenib was initially identified as a BRAF inhibitor, the compound was also shown to inhibit wild-type RET, select mutant proteins (e.g., RET M918T), and chimeric oncoproteins generated by RET fusions (KIF5B–RET, CCDC6–RET, NCOA4–RET, and PRKAR1A–RET). Agerafenib is active in xenografts harboring the most common fusions in NSCLC (KIF5B–RET) and thyroid cancers (CCDC6–RET and NCOA4–RET) (Table 4 depicting the respective biochemical IC_{50}s).[213] Treatment with agerafenib in engineered RET-dependent cell lines inhibits phosphorylation of RET, AKT, and ERK. Consistent with pathway inhibition, a growth inhibitory effect was observed *via* an alamar blue cell viability assay in cells treated with agerafenib. *In vivo* efficacy of agerafenib was evaluated in four RET fusion xenograft models, including a HBEC3KT-RET cell line–derived xenograft model (driven by CCDC6-RET), a NSCLC patient-derived xenograft (PDX) model (CTG-0838/CTG-1048 both driven by KIF5B-RET), and two colorectal cancer models (CRC) PDX models (CR2518 and CR1520 both driven by CCDC6-RET). Agerafenib was tolerated in all dose groups.[213]

In a Phase I/Ib trial of agerafenib, a total of 152 patients were enrolled.[214] Fifty-five patients were treated in the Phase I dose-escalation portion of the study, while ninety-seven were treated in the Phase Ib dose-expansion portion of the study. The major tumor types were NSCLC (54%), followed by colorectal cancer (18%) and thyroid cancer (11%). Patients were treated in 9 dose level cohorts where agerafenib administration ranged from 20–350 mg.[214] The overall response to agerafenib included no complete responses, 2 (4%) partial responses, 20 (36%) stable disease, 22 (40%) progressive disease, and 11 (20%) unevaluable. The two confirmed partial responses were observed in an MTC patient with a RET^{M918T} mutation (50% tumor regression) and a NSCLC patient with a $KRAS^{G12C}$ mutation (40% tumor regression).

A Phase IIb study included 8 cohorts of patients treated with agerafenib. The drug was found to be most active in patients with RET inhibitor–naïve RET fusion–positive lung cancers. Importantly, showcasing an improvement from the toxicity profile of vandetanib, QT prolongation and VEGFR2/KDR inhibition related toxicities were not observed with agerafenib.[214]

6. Second generation selective RET mutant inhibitors

The majority of RET targeted therapeutics are non-selective kinase inhibitors. Although non-selective agents exhibit varying potencies against RET-driven thyroid cancers and RET fusion lung cancers, off-target activities on other kinases, such as EGFR, MET, KIT, BRAF, and VEGFR2, increase the risk for adverse events triggering discontinuation of treatment or dose reductions.[215–217] As a result, non-selective RET targeted agents have pharmacodynamic profiles not optimized for RET, which limits the ability to safely shut down RET signaling. Although TKI response rates in RET-associated tumors are high, the responses observed suggest limited control due to off target toxicities as well as the inability to maintain inhibition on clinically relevant RET mutations.[215, 218] Prolonged exposure to TKIs results in acquired resistance to treatment, often through selection of tumor clones that harbor site-specific mutations in the RET.[215–216] Inevitably, partial responses necessitate increased exposure of TKIs for patients with RET-associated tumors to maintain efficacy and this promotes acquired drug resistance and adverse events. To ameliorate this clinical issue, the development of second-generation RET and RET mutant selective inhibitors was initiated to improve the toxicity profile as well as maintain activity on multiple, clinically relevant RET mutations.[6] As treatment promotes evolution of the tumor, RET mutant inhibitors, with inhibitory profiles against numerous RET mutations, should maintain blockade of RET signaling to sustain disease remission.

Selpercatinib (LOXO-292)

Selpercatinib is an ATP-competitive small molecule RET inhibitor that was approved in May 2020 for the treatment of patients with lung cancer or thyroid cancer harboring RET alterations.[219] In contrast to MKIs, selpercatinib possesses selective, nanomolar potency against RET and a diverse set of RET mutations, including anticipated acquired resistance mutations. Selpercatinib also has favorable pharmacokinetic properties, including high bioavailability and exposure. Approximately 25% of patients with RET fusion–positive lung cancers have brain metastases, and selpercatinib was found to have significant central nervous system (CNS) penetration.[217] The inhibitory profile of selpercatinib against RET alterations and VEGFR2 is listed in Table 5.

Selpercatinib exhibits potent activity on RET and RET mutants and is selective against VEGFR2. In RET dependent cell lines, treatment with selpercatinib reduces cell viability, while in non-RET dependent cell lines selpercatinib has little effect.[205] This contrasts with cabozantinib and vandetanib as inhibitory profiles of these compounds overlap for cell lines with and without RET alterations, suggesting cabozantinib and vandetanib inhibit multiple targets necessary for cell viability. Selpercatinib was found to be 60–1300-fold

more effective than other MKIs against cell lines engineered with KIF5B-RET$^{V804L/M}$ gatekeeper mutations.[221]

In preclinical studies, anti-tumor activities of selpercatinib was compared to cabozantinib in patient-derived RET fusion-positive and RET-mutant mouse tumor models, including two RET fusion-positive models harboring a V804M acquired resistance gatekeeper mutation.[221] Results revealed that at the maximum tolerated dose, cabozantinib caused mild regression but was inactive against models containing RET^{V804M}, whereas selpercatinib caused regression in all models.[221]

To understand the binding of selpercatinib to RET, the crystal structure of the RET kinase-selpercatinib complex was determined at 2.06 Å (Figure 7A).[222] Selpercatinib exhibits a unique binding mode, where both front and back pockets of RET (unlike other TKIs) are occupied without passing through the back-pocket wall between V804 and K758. The back pocket is accessed by wrapping around the conserved lysine to avoided steric clashes with gatekeeper mutations at V804.

Molecular modeling indicates that substitutions of the glycine residue at position 810 in the RET kinase solvent front with bulky, charged, or polar residues sterically clashes with the alkoxy group of selpercatinib (Figure 8A and 8B).[223] These structural clashes are confirmed by loss of inhibitory activities (*in vitro* experiments using selpercatinib, pralsetinib, cabozantinib, and vandetanib) against RET$^{G810S/R/A/C}$. Though selpercatinib exhibits diminished activity against RET solvent front mutations, the inhibitor maintains activity against RET^{V804} and RET^{S904F} mutations. RETG810 solvent front substitutions have only a minor effect on ATP affinity, indicating that inhibition of drug binding is the likely culprit for loss of inhibition.[223]

LIBRETTO-001 was the first-in-human, phase 1 clinical trial of selpercatinib (NCT03157128). Patients were enrolled to study dose escalation and drug exposure. The ORR exceeded 70% for cancers with RET fusions in patients with NSCLC and in those with other tumors, specifically thyroid and pancreatic cancers. In patients with MTC and a RET mutation, the ORR and confirmed ORRs were 45% and 33%, respectively, including 2 complete responses.[221] Disease regression was observed in the majority of patients with RET fusions and in patients with MTCs and a RET mutation, irrespective of cancer type and pretreatment with other FDA approved agents. Treatment-emergent adverse events were observed in at least 10% of patients that included reversible grade 3 tumor lysis syndrome and elevated liver enzymes.[204]

Brain metastases are prevalent in lung cancer patients, so intracranial antitumor activity of selpercatinib was also investigated. CCDC6-RET fusion positive PDX cell suspensions were injected into mice intracranially and treated orally with selpercatinib and ponatinib.[217, 222] At reduced doses, selpercatinib significantly prolonged survival compared to ponatinib, which suggest a RET mutant selective inhibitor may present a clinical advantage over non-selective TKI therapy in RET-driven disease.[222]

Pralsetinib (BLU-667)

Pralsetinib is a potent and highly selective RET and RET mutant inhibitor that targets mutations found in NSCLC, thyroid cancer, and other solid tumors.[224] Pralsetinib received FDA approval in September 2020 for the treatment of adult metastatic RET fusion NSCLC.[225] During pre-clinical studies, pralsetinib was found to be equally active across various RET fusions and mutants, including CCDC6–RET, KIF5B–RET, and clinically relevant mutations found at the gatekeeper region including V804L, V804M, and V804E. First generation RET targeted therapies are multikinase inhibitors, which have significant dose-limiting toxicities that limit amount and duration of therapy. Pralsetinib, like selpercatinib, was designed to overcome such therapeutic limitations while improving treatment efficacy by targeting multiple clinically relevant RET mutations.[224]

Pralsetinib was identified by screening a library of ~10,000 compounds against RET with 60 unique chemical scaffolds. The goal was to identify compounds with activity against wild-type RET and RET mutations (M918T, V804L, and V804M), while maintaining selectivity against other kinases.[220] After identifying a hit candidate, iterative medicinal chemistry was completed to optimize for potency, selectivity, and drug properties, which lead to the generation of pralsetinib.

In biochemical assays, pralsetinib inhibited the kinase activity of wild type RET (IC_{50} 0.4 nM) 8- to 28-times more than cabozantinib, vandetanib, and agerafenib (IC_{50} 11, 4, and 3 nM), respectively.[220] Pralsetinib was 88-fold more selective for RET over VEGFR2, whereas other MKIs have notable VEGFR2 activity (**Table 9**). Although inhibiting VEGFR2 is antiangiogenic, excessive VEGFR2 inhibition is cardiotoxic, which limits the therapeutic benefit of non-selective MKIs for RET-driven disease.[218]

A co-crystal structure of the RET kinase–pralsetinib complex was obtained at a resolution of 1.9 Å (Figure 7B).[222] The crystal structure shows that pralsetinib binds to the RET kinase in a novel way similar to selpercatinib, occupying the front and back cleft by wrapping around the conserved lysine. This binding style avoids inhibitor disruptions from gatekeeper mutations while allowing high-affinity binding.[222] Nevertheless, this binding mode is still sensitive to resistance from mutations at several non-gatekeeper residues including RET^{S904F} and $RET^{G810R/C/S/V}$ solvent front mutations (Figure 8C & D).

In a pre-clinical study, pralsetinib and multikinase inhibitors were dosed to Ba/F3 cells engineered to express a KIF5B–RET fusion, and pralsetinib inhibited RET autophosphorylation (IC_{50} = 5 nM) 10 times more potently than other multikinase inhibitors. Phosphorylation of RET, SHC, and ERK1/2 was measured in a panel of RET-driven cell lines including LC-2/ad (CCDC6–RET; NSCLC), MZ-CRC-1 (RETM918T; MTC), and TT (RETC634W; MTC) and it was found that pralsetinib inhibited phosphorylation of RET, SHC, and ERK1/2 at concentrations at or below 10 nM.[220] Pralsetinib suppresses proliferation of KIF5B–RET Ba/F3 cells harboring wild type RET as well as V804L, V804M, and V804E variants, which is in contrast to multikinase inhibitors that have reduced activity on gatekeeper mutants.[220] In xenograft models, pralsetinib demonstrated dose-dependent activity against both KIF5B–RET Ba/F3 and KIF5B–RET^{V804L} Ba/F3

allograft tumors. Pralsetinib also demonstrated activity in a RET^{C634W} MTC xenograft and KIF5B–RET NSCLC and CCDC6–RET colorectal cancer PDX models.[220]

Impact of pralsetinib on RET driven malignancies was measured by a first in-human phase 1/2 trial (ARROW) in patients with NSCLC, thyroid cancer, or other solid tumors (NCT03037385).[226] The ORR was 47% among 49 response-evaluable MTCs. Further, 96% of responding patients continued treatment, with 15 exhibiting a response duration greater than 6 months. Rapid plasma clearance of RET variants and marked reduction in carcinoembryonic antigen and calcitonin were observed, indicating proliferation of the medullary thyroid cells were reduced with pralsetinib treatment. On December 1, 2020 the FDA granted accelerated approval of pralsetinib to treat patients with advanced or metastatic RET-mutant MTC that requires systemic therapy or RET fusion-positive radioactive iodine-refractory thyroid cancer.[227]

7. Third generation improved RET mutant inhibitors

Oncogenic activation of the receptor tyrosine kinase RET *via* point mutations or genomic rearrangements have been identified in multiple cancers. MKIs and RET mutant inhibitors have demonstrated efficacy against thyroid cancers and NSCLC with RET-fusions. However, despite achieving initial efficacy, drug resistant mutations are selected with treatment even with second generation RET mutant inhibitors. Solomon and colleagues report $RET^{G810R/S/C/V}$ solvent front mutations mediate acquired resistance to selpercatinib in RET fusion NSCLC and RET-mutant MTC (Figure 6).[223] Therefore, third generation RET inhibitors are currently being investigated to inhibit additional RET mutations that confer resistance to MKIs and RET mutant inhibitors.

TPX-0046

TPX-0046 is a dual RET/SRC inhibitor with a small, rigid macrocyclic structure that was rationally designed to inhibit RET. The rationale behind designing a macrocycle was to generate a compact Type I inhibitor that binds to the ATP-binding site while maintaining anti-tumor activity without acquired resistance.[228] By inhibiting SRC, as well as RET, TPX-0046 can block SRC driven resistance that is often observed with RET inhibitors. Moreover, TPX-0046 does not inhibit VEGFR kinases, which are often associated with cardiovascular toxicities such as hypertension. In enzymatic assays, TPX-0046 demonstrated nanomolar potency against RET and RET mutants, as well as SRC.[228] TPX-0046 potently inhibits RET phosphorylation and cell proliferation in Ba/F3 KIF5B-RET, TT, and LC-2/ad cells with IC_{50}s of approximately 1 nM.[228] TPX-0046 is an inhibitor of the solvent front mutation RET^{G810R} with a mean IC_{50} of 17 nM, whereas pralsetinib and selpercatinib have IC_{50}s >500 nM. In a Ba/F3 KIF5B-RET xenograft model, a single dose of 5 mg/kg TPX-0046 inhibited more than 80% of RET phosphorylation. With dosing of 5 mg/kg twice daily, tumor regression was observed in RET-dependent xenografts.[229] Tumor regression was also observed in models with RET solvent front mutations, including TT, CTG-0838 PDX (NSCLC, KIF5B-RET), CR 1520 PDX (CRC, NCOA4-RET), Ba/F3 KIF5B-RET, and Ba/F3 KIF5B-RET^{G810R}.[229] A Phase I/II trial is currently underway to determine the safety

and efficacy of TPX-0046 in patients with advanced or metastatic solid tumors harboring RET mutations or alterations (NCT04161391).

BOS-172738

BOS172738 (formerly DS-5010) is an orally available small-molecule RET inhibitor under clinical investigation. The inhibitor has been shown to have *in vitro* RET inhibitor activity and *in vivo* potency against transfected allograft and xenograft models.[230]

TAS0953 (HM06)

TAS0953/HM06 is an investigational oral treatment, which inhibits several RET abnormalities identified as oncogenic driver alterations in NSCLC, papillary, and MTCs.[231] Preclinical data showed several defining features in comparison to other targeted therapies acting on RET abnormalities. On April 1, 2020, the U.S. FDA reviewed Investigational New Drug (IND) application for TAS03/HM06 and released a “Study May Proceed” letter for the Phase I/II Study of TAS0953/HM06 in patients with advanced solid tumors with RET gene abnormalities (NCT04683250).[231]

SL-1001

SL-1001 is an oral RET inhibitor developed by the Cancer Research UK Manchester Institute at the University of Manchester, UK. The inhibitor exhibits potent, selective, preclinical anti-cancer activity in RET driven tumor models.[232–233] The same group recently developed a selective RET^{V804M} kinase inhibitor (RET^{V804M} IC_{50} = 19 nM) over *wt*-RET (16-fold) and VEGFR2 (410-fold). Development of mutant specific RET inhibitors may offer a clinical advantage over mixed wild-type/mutant inhibitors. Mutant selective inhibitors may provide an alternative therapeutic option to patients that develop significant tolerability issues and may serve an adjunct therapy alongside RET-selective agents.[234]

Conclusion and future perspectives

Over the last decade, there has been an explosion of approvals of kinase inhibitors for clinical use. Many of these inhibitors are utilized in an oncology setting because of the intimate relationship shared between rogue kinase signaling and cancer biology. The RET kinase was discovered in the 1980s and its oncogenic potential and action has since been realized and heavily researched. With the approval of imatinib in 2001, the pharmacological modulation of kinase activity became a clinical reality, and subsequently inhibitors for the RET kinase were pursued.

The first iteration of RET kinase inhibitors were discovered by repurposing multikinase inhibitors for the RET kinase. This taught a valuable clinical lesson that the broad activity profile of multikinase inhibitors does not determine, but instead restricts, clinical utility. With information obtained from the use of multikinase inhibitors, a new generation of RET inhibitors were developed with specificity for the target oncogene. These new inhibitors were also engineered to be dynamic by maintaining broad activity on variant forms of the RET oncogene, thereby blocking inherent tumor resistance mechanisms.

However, current research shows that even with RET inhibitors that possess broad activity on a variety of RET mutations resistance still occurs. This plays directly into the evolution paradigm that life, even rogue life, will find a way. Albeit, targeting the RET kinase has taught an important, clinical lesson—a kinase inhibitor active on a kinase and mutant forms of that kinase presents a significant, pharmacological advantage over a non-selective, multikinase inhibitor.

The current challenge for RET precision medicine is twofold. The first challenge is to develop scaffolds that can inhibit the next iteration of RET mutants, and the second challenge is to select patients with genetic criteria aligned with the pharmacology of the RET-targeted therapy. These two challenges are not mutually exclusive and must be developed in concert. In theory, next generation RET inhibitors will need to be continuously developed to block RET mutations that are selected for from prior therapy. In practice, however, these mutations are somewhat restricted as the novel mutants must be catalytically active—therefore, drug resistant mutations must bind ATP and phosphorylate the downstream substrate to act as an oncogene. Another important challenge is to address the off-target effects arising from the pleiotropic roles of RET. GNDF-RET signaling plays a significant role in maintenance of mature nerve lineages and kidney development. Prolonged inhibition of these signals may compromise nerve health and overall survival of the patient.

Constrained RET inhibitors, such as TPX-0046, occupy less space in the RET binding pocket and this will restrict areas of the kinase domain that can mutate to cause resistance to these molecules. This has already been clinically demonstrated with the TRK inhibitors LOXO-195 and repotrectinib (TPX-0005), which are constrained, cyclic versions of larotrectinib that are active against TRK solvent front mutations.[250–251] These next-generation inhibitors place a new selection pressure on the tumor where resistance mechanisms may not stem from a new TRK mutation.[252] Instead, resistance has been shown to occur through activation of the MAP kinase pathway via KRAS activating mutations.

It has also been demonstrated that resistance to RET inhibitors selpercatinib and pralsetinib is driven by RET-independent resistance mechanisms such as *MET* or *KRAS* amplification.[253] Therefore, RET-dependent and RET-independent resistance mechanisms can be considered to help identify biomarkers that can be utilized in clinical trials to improve outcomes in patients with RET-driven disease. This will involve selection of patients for a specific RET-inhibitor based on the genetic makeup of their tumor with the addition of another therapy to block RET-independent resistance mechanisms. To accomplish this, it is important to continue to assess and validate mechanisms of resistance to next generation RET inhibitors in large sample sizes in a variety of tumor types. This will help inform on tumor-specific or patient-specific therapeutic strategies to better combat RET-driven malignancies.

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Abbreviations Used

ARTN	artemin
ATC	anaplastic thyroid cancer
CML	chronic myelogenous leukemia
CNS	central nervous system
DC	discontinuation
CCDC6	coiled-coil domain containing 6
DFG	aspartic acid, phenylalanine, glycine
c-Kit	mast/stem factor receptor kit
DOK1/4/5/6	downstream of tyrosine kinase 1/4/5/6
DR	dose reduction
DTC	differentiated thyroid cancer
EGFR	epidermal growth factor receptor
ERK	RAS/extracellular signal-regulated kinase
FGFR1	fibroblast growth factor receptor 1
FMTC	familial medullary thyroid carcinoma
FRS2	fibroblast growth factor receptor substrate 2
GDNF	glial cell line-derived neurotrophic factor
GIST	gastrointestinal stromal tumors
HCC	hepatocellular carcinoma
IRS1/2	insulin receptor substrate ½
JNK	c-Jun N-terminal kinase
KIF5B	kinesin family 5B
LisH	Lis1 homology
MAPK	RAS/mitogen activated protein kinase
mCRC	metastatic colorectal cancer
MEN2	multiple endocrine neoplasia 2

MKI	multikinase inhibitor
MTC	medullary thyroid carcinoma
NCOA4	nuclear receptor co-activator 4
NSCLC	non-small-cell lung carcinoma
NRTN	neuturin
ORR	objective response rate
PDGFR	platelet-derived growth factor receptor
PDX	patient-derived xenograft
PI3K	phosphatidylinositol 3-kinase
PKC	protein kinase C
PLCγ	phospholipase Cγ
pNET	pancreatic neuroendocrine tumors
PSPN	persephin
PTC	papillary thyroid carcinoma
RCC	renal cell carcinoma
RECIST	response evaluation criteria in solid tumors
RET	rearranged during transfection
RR-DTC	radioiodine-refractory differentiated thyroid cancer
RTK	receptor tyrosine kinase
SAM	sterile α motif
SFM	solvent front mutation
SHC	src homology and collagen
TC	thyroid cancer
VEGFR	vascular endothelial growth factor receptor

References

1. Takahashi M; Buma Y; Iwamoto T; Inaguma Y; Ikeda H; Hiai H, Cloning and expression of the ret proto-oncogene encoding a tyrosine kinase with two potential transmembrane domains. Oncogene 1988, 3 (5), 571–578. [PubMed: 3078962]
2. Takahashi M; Ritz J; Cooper GM, Activation of a novel human transforming gene, RET, by DNA rearrangement. Cell 1985, 42 (2), 581–588, DOI:10.1016/0092-8674(85)90115-1. [PubMed: 2992805]

3. Schuchardt A; D'Agati V; Larsson-Blomberg L; Costantini F; Pachnis V, Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature 1994, 367 (6461), 380–383, DOI:10.1038/367380a0. [PubMed: 8114940]

4. Arighi E; Borrello MG; Sariola H, RET tyrosine kinase signaling in development and cancer. Cytokine & growth factor reviews 2005, 16 (4–5), 441–467, DOI:10.1016/j.cytogfr.2005.05.010. [PubMed: 15982921]

5. Jain S; Naughton CK; Yang M; Strickland A; Vij K; Encinas M; Golden J; Gupta A; Heuckeroth R; Johnson EM; Milbrandt J, Mice expressing a dominant-negative Ret mutation phenocopy human Hirschsprung disease and delineate a direct role of Ret in spermatogenesis. Development 2004, 131 (21), 5503–5513, DOI:10.1242/dev.01421. [PubMed: 15469971]

6. Mulligan LM, RET revisited: expanding the oncogenic portfolio. Nature reviews. Cancer 2014, 14 (3), 173–86, DOI:10.1038/nrc3680. [PubMed: 24561444]

7. Paratcha G; Ledda F, GDNF and GFRalpha: a versatile molecular complex for developing neurons. Trends in Neurosciences 2008, 31 (8), 384–391, DOI:10.1016/j.tins.2008.05.003. [PubMed: 18597864]

8. Lasrado R; Boesmans W; Kleinjung J; Pin C; Bell D; Bhaw L; McCallum S; Zong H; Luo L; Clevers H; Vanden Berghe P; Pachnis V, Lineage-dependent spatial and functional organization of the mammalian enteric nervous system. Science 2017, 356 (6339), 722–726, DOI:10.1126/science.aam7511. [PubMed: 28522527]

9. Meng X; Lindahl M; Hyvönen ME; Parvinen M; de Rooij DG; Hess MW; Raatikainen-Ahokas A; Sainio K; Rauvala H; Lakso M; Pichel JG; Westphal H; Saarma M; Sariola H, Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. Science 2000, 287 (5457), 1489–1493, DOI:10.1126/science.287.5457.1489. [PubMed: 10688798]

10. Fonseca-Pereira D; Arroz-Madeira S; Rodrigues-Campos M; Barbosa IAM; Domingues RG; Bento T; Almeida ARM; Ribeiro H; Potocnik AJ; Enomoto H; Veiga-Fernandes H, The neurotrophic factor receptor RET drives haematopoietic stem cell survival and function. Nature 2014, 514 (7520), 98–101, DOI:10.1038/nature13498. [PubMed: 25079320]

11. Shakya R; Watanabe T; Costantini F, The role of GDNF/Ret signaling in ureteric bud cell fate and branching morphogenesis. Developmental Cell 2005, 8 (1), 65–74, DOI:10.1016/j.devcel.2004.11.008. [PubMed: 15621530]

12. Pachnis V; Mankoo B; Costantini F, Expression of the c-ret proto-oncogene during mouse embryogenesis. Development 1993, 119 (4), 1005–1017. [PubMed: 8306871]

13. Gould TW; Yonemura S; Oppenheim RW; Ohmori S; Enomoto H, The neurotrophic effects of glial cell line-derived neurotrophic factor on spinal motoneurons are restricted to fusimotor subtypes. The Journal of Neuroscience 2008, 28 (9), 2131–2146, DOI:10.1523/JNEUROSCI.5185-07.2008. [PubMed: 18305247]

14. Kramer ER; Knott L; Su F; Dessaud E; Krull CE; Helmbacher F; Klein R, Cooperation between GDNF/Ret and ephrinA/EphA4 signals for motor-axon pathway selection in the limb. Neuron 2006, 50 (1), 35–47, DOI:10.1016/j.neuron.2006.02.020. [PubMed: 16600854]

15. Goodman KM; Kjær S; Beuron F; Knowles PP; Nawrotek A; Burns EM; Purkiss AG; George R; Santoro M; Morris EP; McDonald NQ, RET recognition of GDNF-GFRα1 ligand by a composite binding site promotes membrane-proximal self-association. Cell Reports 2014, 8 (6), 1894–1904, DOI:10.1016/j.celrep.2014.08.040. [PubMed: 25242331]

16. Ibáñez CF, Structure and physiology of the RET receptor tyrosine kinase. Cold Spring Harbor perspectives in Biology 2013, 5 (2), DOI:10.1101/cshperspect.a009134.

17. Wiesner T; He J; Yelensky R; Esteve-Puig R; Botton T; Yeh I; Lipson D; Otto G; Brennan K; Murali R; Garrido M; Miller VA; Ross JS; Berger MF; Sparatta A; Palmedo G; Cerroni L; Busam KJ; Kutzner H; Cronin MT; Stephens PJ; Bastian BC, Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. Nature Communications 2014, 5, 3116–3116, DOI:10.1038/ncomms4116.

18. Romei C; Ciampi R; Elisei R, A comprehensive overview of the role of the RET proto-oncogene in thyroid carcinoma. Nature Reviews Endocrinology 2016, 12 (4), 192–202, DOI:10.1038/nrendo.2016.11.

19. Paratala BS; Chung JH; Williams CB; Yilmazel B; Petrosky W; Williams K; Schrock AB; Gay LM; Lee E; Dolfi SC; Pham K; Lin S; Yao M; Kulkarni A; DiClemente F; Liu C; Rodriguez-Rodriguez L; Ganesan S; Ross JS; Ali SM; Leyland-Jones B; Hirshfield KM, RET rearrangements are actionable alterations in breast cancer. Nature Communications 2018, 9 (1), 4821–4821, DOI:10.1038/s41467-018-07341-4.
20. Pietrantonio F; Di Nicolantonio F; Schrock AB; Lee J; Morano F; Fucà G; Nikolinakos P; Drilon A; Hechtman JF; Christiansen J; Gowen K; Frampton GM; Gasparini P; Rossini D; Gigliotti C; Kim ST; Prisciandaro M; Hodgson J; Zaniboni A; Chiu VK; Milione M; Patel R; Miller V; Bardelli A; Novara L; Wang L; Pupa SM; Sozzi G; Ross J; Di Bartolomeo M; Bertotti A; Ali S; Trusolino L; Falcone A; de Braud F; Cremolini C, RET fusions in a small subset of advanced colorectal cancers at risk of being neglected. Annals of Oncology 2018, 29 (6), 1394–1401, DOI:10.1093/annonc/mdy090. [PubMed: 29538669]
21. Skálová A; Vanecek T; Uro-Coste E; Bishop JA; Weinreb I; Thompson LDR; de Sanctis S; Schiavo-Lena M; Laco J; Badoual C; Santana Conceiçao T; Ptáková N; Baněčkova M; Miesbauerová M; Michal M, Molecular profiling of salivary gland intraductal carcinoma revealed a subset of tumors harboring NCOA4-RET and novel TRIM27-RET fusions: A report of 17 cases. The American Journal of Surgical Pathology 2018, 42 (11), 1445–1455, DOI:10.1097/PAS.0000000000001133. [PubMed: 30045065]
22. Le Rolle A-F; Klempner SJ; Garrett CR; Seery T; Sanford EM; Balasubramanian S; Ross JS; Stephens PJ; Miller VA; Ali SM; Chiu VK, Identification and characterization of RET fusions in advanced colorectal cancer. Oncotarget 2015, 6 (30), 28929–28937, DOI:10.18632/oncotarget.4325. [PubMed: 26078337]
23. Stransky N; Cerami E; Schalm S; Kim JL; Lengauer C, The landscape of kinase fusions in cancer. Nature Communications 2014, 5, 4846–4846, DOI:10.1038/ncomms5846.
24. Drilon A; Hu ZI; Lai GGY; Tan DSW, Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes. Nature Reviews Clinical oncology 2018, 15 (3), 151–167, DOI:10.1038/nrclinonc.2017.175.
25. Liang J; Cai W; Feng D; Teng H; Mao F; Jiang Y; Hu S; Li X; Zhang Y; Liu B; Sun ZS, Genetic landscape of papillary thyroid carcinoma in the Chinese population. The Journal of Pathology 2018, 244 (2), 215–226, DOI:10.1002/path.5005. [PubMed: 29144541]
26. Mochizuki K; Kondo T; Nakazawa T; Iwashina M; Kawasaki T; Nakamura N; Yamane T; Murata S.-i.; Ito K; Kameyama K; Kobayashi M; Katoh R, RET rearrangements and BRAF mutation in undifferentiated thyroid carcinomas having papillary carcinoma components. Histopathology 2010, 57 (3), 444–450, DOI:10.1111/j.1365-2559.2010.03646.x. [PubMed: 20840674]
27. Grubbs EG; Ng PK-S; Bui J; Busaidy NL; Chen K; Lee JE; Lu X; Lu H; Meric-Bernstam F; Mills GB; Palmer G; Perrier ND; Scott KL; Shaw KR; Waguespack SG; Williams MD; Yelensky R; Cote GJ, RET fusion as a novel driver of medullary thyroid carcinoma. The Journal of Clinical Endocrinology and Metabolism 2015, 100 (3), 788–793, DOI:10.1210/jc.2014-4153. [PubMed: 25546157]
28. Zhang X; Li Y; Liu C; Wang W; Li M; Lv D; Sun G; Chen H; Dong X; Miao Z; Yao M; Wang K; Tian H, Identification of a novel KIF13A-RET fusion in lung adenocarcinoma by next-generation sequencing. Lung Cancer 2018, 118, 27–29, DOI:10.1016/j.lungcan.2017.08.019. [PubMed: 29571998]
29. Ballerini P; Struski S; Cresson C; Prade N; Toujani S; Deswarte C; Dobbelstein S; Petit A; Lapillonne H; Gautier EF; Demur C; Lippert E; Pages P; Mansat-De Mas V; Donadieu J; Huguet F; Dastugue N; Broccardo C; Perot C; Delabesse E, RET fusion genes are associated with chronic myelomonocytic leukemia and enhance monocytic differentiation. Leukemia 2012, 26 (11), 2384–2389, DOI:10.1038/leu.2012.109. [PubMed: 22513837]
30. Kato S; Subbiah V; Marchlik E; Elkin SK; Carter JL; Kurzrock R, RET aberrations in diverse cancers: next-generation sequencing of 4,871 patients. Clinical Cancer Research 2017, 23 (8), 1988–1997, DOI:10.1158/1078-0432.CCR-16-1679. [PubMed: 27683183]
31. Gozgit JM; Chen T-H; Song Y; Wardwell S; Wang F; Cai J; Li H; Edgren H; Rivera VM; Pritchard J, RET fusions observed in lung and colorectal cancers are sensitive to ponatinib. Oncotarget 2018, 9 (51), 29654–29664, DOI:10.18632/oncotarget.25664. [PubMed: 30038711]

32. Castinetti F; Moley J; Mulligan L; Waguespack SG, A comprehensive review on MEN2B. Endocrine-Related Cancer 2018, 25, T29–T39, DOI:10.1530/ERC-17-0209. [PubMed: 28698189]

33. Plaza-Menacho I, Structure and function of RET in multiple endocrine neoplasia type 2. Endocrine-Related Cancer 2018, 25, T79–T90, DOI:10.1530/ERC-17-0354. [PubMed: 29175871]

34. Kang J; Perry JK; Pandey V; Fielder GC; Mei B; Qian PX; Wu ZS; Zhu T; Liu DX; Lobie PE, Artemin is oncogenic for human mammary carcinoma cells. Oncogene 2009, 28 (19), 2034–2045, DOI:10.1038/onc.2009.66. [PubMed: 19363524]

35. Cavel O; Shomron O; Shabtay A; Vital J; Trejo-Leider L; Weizman N; Krelin Y; Fong Y; Wong RJ; Amit M; Gil Z, Endoneurial macrophages induce perineural invasion of pancreatic cancer cells by secretion of GDNF and activation of RET tyrosine kinase receptor. Cancer Research 2012, 72 (22), 5733–5743, DOI:10.1158/0008-5472.CAN-12-0764. [PubMed: 22971345]

36. He S; Chen CH; Chernichenko N; He S; Bakst RL; Barajas F; Deborde S; Allen PJ; Vakiani E; Yu Z; Wong RJ, GFRα1 released by nerves enhances cancer cell perineural invasion through GDNF-RET signaling. Proceedings of the National Academy of Sciences of the United States of America 2014, 111 (19), E2008–E2017, DOI:10.1073/pnas.1402944111. [PubMed: 24778213]

37. Amit M; Na'Ara S; Gil Z, Mechanisms of cancer dissemination along nerves. Nature Reviews Cancer 2016, 16, 399–408, DOI:10.1038/nrc.2016.38. [PubMed: 27150016]

38. Camós M; Esteve J; Jares P; Colomer D; Rozman M; Villamor N; Costa D; Carrió A; Nomdedéu J; Montserrat E; Campo E, Gene expression profiling of acute myeloid leukemia with translocation t(8;16)(p11;p13) and MYST3-CREBBP rearrangement reveals a distinctive signature with a specific pattern of HOX gene expression. Cancer Research 2006, 66 (14), 6947–6954, DOI:10.1158/0008-5472.CAN-05-4601. [PubMed: 16849538]

39. Rudat S; Pfaus A; Cheng YY; Holtmann J; Ellegast JM; Bühler C; Marcantonio DD; Martinez E; Göllner S; Wickenhauser C; Müller-Tidow C; Lutz C; Bullinger L; Milsom MD; Sykes SM; Fröhling S; Scholl C, RET-mediated autophagy suppression as targetable co-dependence in acute myeloid leukemia. Leukemia 2018, 32 (10), 2189–2202, DOI:10.1038/s41375-018-0102-4. [PubMed: 29654265]

40. Dawson DM; Lawrence EG; MacLennan GT; Amini SB; Kung HJ; Robinson D; Resnick MI; Kursh ED; Pretlow TP; Pretlow TG, Altered expression of RET proto-oncogene product in prostatic intraepithelial neoplasia and prostate cancer. Journal of the National Cancer Institute 1998, 90 (7), 519–523, DOI:10.1093/jnci/90.7.519. [PubMed: 9539247]

41. Ban K; Feng S; Shao L; Ittmann M, RET signaling in prostate cancer. Clinical Cancer Research 2017, 23 (16), 4885–4896, DOI:10.1158/1078-0432.CCR-17-0528. [PubMed: 28490466]

42. Mulligan LM, GDNF and the RET receptor in cancer: new insights and therapeutic potential. Frontiers in Physiology 2018, 9, 1873–1873, DOI:10.3389/fphys.2018.01873. [PubMed: 30666215]

43. Gattei V; Degan M; Aldinucci D; De Iuliis A; Rossi FM; Tassan Mazzocco F; Rupolo M; Zagonel V; Pinto A, Differential expression of the RET gene in human acute myeloid leukemia. Annals of Hematology 1998, 77 (5), 207–210, DOI:10.1007/s002770050444. [PubMed: 9858145]

44. Kan Z; Jaiswal BS; Stinson J; Janakiraman V; Bhatt D; Stern HM; Yue P; Haverty PM; Bourgon R; Zheng J; Moorhead M; Chaudhuri S; Tomsho LP; Peters BA; Pujara K; Cordes S; Davis DP; Carlton VEH; Yuan W; Li L; Wang W; Eigenbrot C; Kaminker JS; Eberhard DA; Waring P; Schuster SC; Modrusan Z; Zhang Z; Stokoe D; De Sauvage FJ; Faham M; Seshagiri S, Diverse somatic mutation patterns and pathway alterations in human cancers. Nature 2010, 466 (7308), 869–873, DOI:10.1038/nature09208. [PubMed: 20668451]

45. Morandi A; Martin LA; Gao Q; Pancholi S; Mackay A; Robertson D; Zvelebil M; Dowsett M; Plaza-Menacho I; Isacke CM, GDNF-RET signaling in ER-positive breast cancers is a key determinant of response and resistance to aromatase inhibitors. Cancer Research 2013, 73 (12), 3783–3795, DOI:10.1158/0008-5472.CAN-12-4265. [PubMed: 23650283]

46. Nik-Zainal S; Davies H; Staaf J; Ramakrishna M; Glodzik D; Zou X; Martincorena I; Alexandrov LB; Martin S; Wedge DC; Van Loo P; Ju YS; Smid M; Brinkman AB; Morganella S; Aure MR; Lingjærde OC; Langerød A; Ringnér M; Ahn SM; Boyault S; Brock JE; Broeks A; Butler A; Desmedt C; Dirix L; Dronov S; Fatima A; Foekens JA; Gerstung M; Hooijer GKJ; Jang SJ; Jones DR; Kim HY; King TA; Krishnamurthy S; Lee HJ; Lee JY; Li Y; McLaren S; Menzies A; Mustonen V; O'Meara S; Pauporté I; Pivot X; Purdie CA; Raine K; Ramakrishnan K; Rodríguez-

González FG; Romieu G; Sieuwerts AM; Simpson PT; Shepherd R; Stebbings L; Stefansson OA; Teague J; Tommasi S; Treilleux I; Van Den Eynden GG; Vermeulen P; Vincent-Salomon A; Yates L; Caldas C; Veer L. V. t.; Tutt A; Knappskog S; Tan BKT; Jonkers J; Borg Å; Ueno NT; Sotiriou C; Viari A; Futreal PA; Campbell PJ; Span PN; Van Laere S; Lakhani SR; Eyfjord JE; Thompson AM; Birney E; Stunnenberg HG; Van De Vijver MJ; Martens JWM; Børresen-Dale AL; Richardson AL; Kong G; Thomas G; Stratton MR, Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 2016, 534 (7605), 47–54, DOI:10.1038/nature17676. [PubMed: 27135926]

47. Plaza-Menacho I; Morandi A; Robertson D; Pancholi S; Drury S; Dowsett M; Martin LA; Isacke CM, Targeting the receptor tyrosine kinase RET sensitizes breast cancer cells to tamoxifen treatment and reveals a role for RET in endocrine resistance. Oncogene 2010, 29 (33), 4648–4657, DOI:10.1038/onc.2010.209. [PubMed: 20531297]
48. Gattelli A; Nalvarte I; Boulay A; Roloff TC; Schreiber M; Carragher N; Macleod KK; Schlederer M; Lienhard S; Kenner L; Torres-Arzayus MI; Hynes NE, Ret inhibition decreases growth and metastatic potential of estrogen receptor positive breast cancer cells. EMBO Molecular Medicine 2013, 5 (9), 1335–1350, DOI:10.1002/emmm.201302625. [PubMed: 23868506]
49. Veit C; Genze F; Menke A; Hoeffert S; Gress TM; Gierschik P; Giehl K, Activation of phosphatidylinositol 3-kinase and extracellular signal-regulated kinase is required for glial cell line-derived neurotrophic factor-induced migration and invasion of pancreatic carcinoma cells. Cancer Research 2004, 64 (15), 5291–5300, DOI:10.1158/0008-5472.CAN-04-1112. [PubMed: 15289335]
50. Zeng Q; Cheng Y; Zhu Q; Yu Z; Wu X; Huang K; Zhou M; Han S; Zhang Q, The relationship between overexpression of glial cell-derived neurotrophic factor and its RET receptor with progression and prognosis of human pancreatic cancer. Journal of International Medical Research 2008, 36 (4), 656–664, DOI:10.1177/147323000803600406. [PubMed: 18652760]
51. Gil Z; Cavel O; Kelly K; Brader P; Rein A; Gao SP; Carlson DL; Shah JP; Fong Y; Wong RJ, Paracrine regulation of pancreatic cancer cell invasion by peripheral nerves. Journal of the National Cancer Institute 2010, 102 (2), 107–118, DOI:10.1093/jnci/djp456. [PubMed: 20068194]
52. Gattei V; Celetti A; Cerrato A; Degan M; De Iuliis A; Rossi FM; Chiappetta G; Consales C; Improta S; Zagonel V; Aldinucci D; Agosti V; Santoro M; Vecchio G; Pinto A; Grieco M, Expression of the RET receptor tyrosine kinase and GDNFR-α in normal and leukemic human hematopoietic cells and stromal cells of the bone marrow microenvironment. Blood 1997, 89 (8), 2925–2937, DOI:10.1182/blood.V89.8.2925. [PubMed: 9108413]
53. Saha D; Ryan KR; Lakkaniga NR; Smith EL; Frett B, Pyrazoloadenine inhibitors of the RET lung cancer oncoprotein discovered by a fragment optimization approach. ChemMedChem 2021, DOI:10.1002/cmdc.202100013.
54. Lakkaniga NR; Gunaganti N; Zhang L; Belachew B; Frett B; Leung Y-K; Li H. y., Pyrrolo[2,3-d]pyrimidine derivatives as inhibitors of RET: Design, synthesis and biological evaluation. European Journal of Medicinal Chemistry 2020, 206, 112691, DOI:10.1016/j.ejmech.2020.112691. [PubMed: 32823007]
55. Moccia M; Frett B; Zhang L; Lakkaniga NR; Briggs DC; Chauhan R; Brescia A; Federico G; Yan W; Santoro M; McDonald NQ; Li H.-y.; Carlomagno F, Bioisosteric discovery of NPA101.3, a second-generation RET/VEGFR2 inhibitor optimized for single-agent polypharmacology. Journal of Medicinal Chemistry 2020, 63 (9), 4506–4516, DOI:10.1021/acs.jmedchem.9b01336. [PubMed: 32298114]
56. Frett B; Carlomagno F; Moccia ML; Brescia A; Federico G; De Falco V; Admire B; Chen Z; Qi W; Santoro M; Li H.-y., Fragment-based discovery of a dual pan-RET/VEGFR2 kinase inhibitor optimized for single-agent polypharmacology. Angewandte Chemie International Edition 2015, 54 (30), 8717–8721, DOI:10.1002/anie.201501104. [PubMed: 26126987]
57. Wang X, Structural studies of GDNF family ligands with their receptors - Insights into ligand recognition and activation of receptor tyrosine kinase RET. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 2013, 1834, 2205–2212, DOI:10.1016/j.bbapap.2012.10.008. [PubMed: 23085183]

58. Subbiah V; Yang D; Velcheti V; Drilon A; Meric-Bernstam F, State-of-the-art strategies for targeting RET-dependent cancers. Journal of Clinical Oncology 2020, 38 (11), 1209–1221, DOI:10.1200/JCO.19.02551. [PubMed: 32083997]
59. Mulligan LM, 65 years of the double helix: Exploiting insights on the RET receptor for personalized cancer medicine. Endocrine-Related Cancer 2018, 25 (8), T189–T200, DOI:10.1530/ERC-18-0141. [PubMed: 29743166]
60. Lian EY; Maritan SM; Cockburn JG; Kasaian K; Crupi MJF; Hurlbut D; Jones SJM; Wiseman SM; Mulligan LM, Differential roles of RET isoforms in medullary and papillary thyroid carcinomas. Endocrine-Related Cancer 2017, 24 (1), 53–69, DOI:10.1530/ERC-16-0393. [PubMed: 27872141]
61. Tahira T; Ishizaka Y; Itoh F; Sugimura T; Nagao M, Characterization of ret proto-oncogene mRNAs encoding two isoforms of the protein product in a human neuroblastoma cell line. Oncogene 1990, 5 1, 97–102. [PubMed: 2181380]
62. Carter MT; Yome JL; Marcil MN; Martin CA; Vanhorne JB; Mulligan LM, Conservation of RET proto-oncogene splicing variants and implications for RET isoform function. Cytogenetics and Cell Genetics 2001, 95 (3–4), 169–76, DOI:10.1159/000059341. [PubMed: 12063395]
63. De Graaff E; Srinivas S; Kilkenny C; D'Agati V; Mankoo BS; Costantini F; Pachnis V, Differential activities of the RET tyrosine kinase receptor isoforms during mammalian embryogenesis. Genes and Development 2001, 15 (18), 2433–2444, DOI:10.1101/gad.205001. [PubMed: 11562352]
64. Tsui-Pierchala BA; Ahrens RC; Crowder RJ; Milbrandt J; Johnson EM, The long and short isoforms of Ret function as independent signaling complexes. The Journal of Biological Chemistry 2002, 277 (37), 34618–34625, DOI:10.1074/jbc.M203580200. [PubMed: 12091387]
65. Rossel M; Pasini A; Chappius S; Geneste O; Fournier L; Schuffenecker I; Takahishi M; van Grunsven LA; Urdiales JL; Rudkin BB; Lenoir GM; Billaud M, Distinct biological properties of two RET isoforms activated by MEN 2A and MEN 2B mutations. Oncogene 1997, 14 (3), 265–275, DOI:10.1038/sj.onc.1200831. [PubMed: 9018112]
66. Le Hir H; Charlet-Berguerand N; Gimenez-Roqueplo AP; Mannelli M; Plouin PF; De Franciscis V; Thermes C, Relative expression of the RET9 and RET51 isoforms in human pheochromocytomas. Oncology 2000, 58 (4), 311–318, DOI:10.1159/000012118. [PubMed: 10838497]
67. Ibáñez CF; Andressoo J-O, Biology of GDNF and its receptors — Relevance for disorders of the central nervous system. Neurobiology of Disease 2017, 97, 80–89, DOI:10.1016/j.nbd.2016.01.021. [PubMed: 26829643]
68. Worby CA; Vega QC; Chao HHJ; Seasholtz AF; Thompson RC; Dixon JE, Identification and characterization of GFRα−3, a novel co-receptor belonging to the glial cell line-derived neurotrophic receptor family. Journal of Biological Chemistry 1998, 273 (6), 3502–3508, DOI:10.1074/jbc.273.6.3502. [PubMed: 9452475]
69. Tsui CC; Gabreski NA; Hein SJ; Pierchala BA, Lipid rafts are physiologic membrane microdomains necessary for the morphogenic and developmental functions of glial cell line-derived neurotrophic factor in vivo. Journal of Neuroscience 2015, 35 (38), 13233–13243, DOI:10.1523/JNEUROSCI.2935-14.2015. [PubMed: 26400951]
70. Amoresano A; Incoronato M; Monti G; Pucci P; De Franciscis V; Cerchia L, Direct interactions among Ret, GDNF and GFRα1 molecules reveal new insights into the assembly of a functional three-protein complex. Cellular Signalling 2005, 17 (6), 717–727, DOI:10.1016/j.cellsig.2004.10.012. [PubMed: 15722196]
71. Tansey MG; Baloh RH; Milbrandt J; Johnson EM Jr., GFRα-mediated localization of RET to lipid rafts is required for effective downstream signaling, differentiation, and neuronal survival. Neuron 2000, 25 (3), 611–623, DOI:10.1016/S0896-6273(00)81064-8. [PubMed: 10774729]
72. Mahato AK; Sidorova YA, RET receptor tyrosine kinase: role in neurodegeneration, obesity, and cancer. International Journal of Molecular Sciences 2020, 21 (19), DOI:10.3390/ijms21197108.
73. Manié S; Santoro M; Fusco A; Billaud M, The RET receptor: function in development and dysfunction in congenital malformation. Trends in Genetics 2001, 17 (10), 580–9, DOI:10.1016/s0168-9525(01)02420-9. [PubMed: 11585664]
74. Perrinjaquet M; Vilar M; Ibáñez CF, Protein-tyrosine phosphatase SHP2 contributes to GDNF neurotrophic activity through direct binding to phospho-Tyr687 in the RET receptor

tyrosine kinase. Journal of Biological Chemistry 2010, 285 (41), 31867–31875, DOI:10.1074/jbc.M110.144923. [PubMed: 20682772]

75. Encinas M; Crowder RJ; Milbrandt J; Johnson EM, Tyrosine 981, a novel Ret autophosphorylation site, binds c-Src to mediate neuronal survival. Journal of Biological Chemistry 2004, 279 (18), 18262–18269, DOI:10.1074/jbc.M400505200. [PubMed: 14766744]
76. Schuringa JJ; Wojtachnio K; Hagens W; Vellenga E; Buys CHCM; Hofstra R; Kruijer W, MEN2A-RET-induced cellular transformation by activation of STAT3. Oncogene 2001, 20 (38), 5350–5358, DOI:10.1038/sj.onc.1204715. [PubMed: 11536047]
77. Borrello MG; Alberti L; Arighi E; Bongarzone I; Battistini C; Bardelli A; Pasini B; Piutti C; Rizzetti MG; Mondellini P; Radice MT; Pierotti MA, The full oncogenic activity of Ret/ptc2 depends on tyrosine 539, a docking site for phospholipase Cgamma. Molecular and Cellular Biology 1996, 16 (5), 2151–2163, DOI:10.1128/mcb.16.5.2151. [PubMed: 8628282]
78. Besset V; Scott RP; Ibáñez CF, Signaling complexes and protein-protein interactions involved in the activation of the Ras and phosphatidylinositol 3-kinase pathways by the c-Ret receptor tyrosine kinase. Journal of Biological Chemistry 2000, 275 (50), 39159–39166, DOI:10.1074/jbc.M006908200. [PubMed: 10995764]
79. Coulpier M; Anders J; Ibáñez CF, Coordinated activation of autophosphorylation sites in the RET receptor tyrosine kinase: Importance of tyrosine 1062 for GDNF mediated neuronal differentiation and survival. Journal of Biological Chemistry 2002, 277 (3), 1991–1999, DOI:10.1074/jbc.M107992200. [PubMed: 11713247]
80. Hayashi H; Ichihara M; Iwashita T; Murakami H; Shimono Y; Kawai K; Kurokawa K; Murakumo Y; Imai T; Funahashi H; Nakao A; Takahashi M, Characterization of intracellular signals via tyrosine 1062 in RET activated by glial cell line-derived neurotrophic factor. Oncogene 2000, 19 (39), 4469–4475, DOI:10.1038/sj.onc.1203799. [PubMed: 11002419]
81. Ichihara M; Murakumo Y; Takahashi M, RET and neuroendocrine tumors. Cancer Letters 2004, 204 (2), 197–211, DOI:10.1016/S0304-3835(03)00456-7. [PubMed: 15013219]
82. Segouffin-Cariou C; Billaud M, Transforming ability of MEN2A-RET requires activation of the phosphatidylinositol 3-kinase/AKT signaling pathway. Journal of Biological Chemistry 2000, 275 (5), 3568–3576, DOI:10.1074/jbc.275.5.3568. [PubMed: 10652352]
83. Liu X; Vega QC; Decker RA; Pandey A; Worby CA; Dixon JE, Oncogenic RET receptors display different autophosphorylation sites and substrate binding specificities. Journal of Biological Chemistry 1996, 271 (10), 5309–5312, DOI:10.1074/jbc.271.10.5309. [PubMed: 8621380]
84. De Vita G; Melillo RM; Carlomagno F; Visconti R; Castellone MD; Bellacosa A; Billaud M; Fusco A; Tsichlis PN; Santoro M, Tyrosine 1062 of RET-MEN2A mediates activation of Akt (protein kinase B) and mitogen-activated protein kinase pathways leading to PC12 cell survival. Cancer research 2000, 60 (14), 3727–31. [PubMed: 10919641]
85. Santoro M; Carlomagno F, Central role of RET in thyroid cancer. Cold Spring Harbor Perspectives in Biology 2013, 5 (12), DOI:10.1101/cshperspect.a009233.
86. Xi HQ; Wu XS; Wei B; Chen L, Eph receptors and ephrins as targets for cancer therapy. Journal of Cellular and Molecular Medicine 2012, 16 (12), 2894–2909, DOI:10.1111/j.1582-4934.2012.01612.x. [PubMed: 22862837]
87. Bossi D; Carlomagno F; Pallavicini I; Pruneri G; Trubia M; Raviele PR; Marinelli A; Anaganti S; Cox MC; Viale G; Santoro M; Di Fiore PP; Minucci S, Functional characterization of a novel FGFR1OP-RET rearrangement in hematopoietic malignancies. Molecular Oncology 2014, 8 (2), 221–31, DOI:10.1016/j.molonc.2013.11.004. [PubMed: 24315414]
88. Gautschi O; Milia J; Filleron T; Wolf J; Carbone DP; Owen D; Camidge R; Narayanan V; Doebele RC; Besse B; Remon-Masip J; Janne PA; Awad MM; Peled N; Byoung CC; Karp DD; Van Den Heuvel M; Wakelee HA; Neal JW; Mok TSK; Yang JCH; Ou SHI; Pall G; Froesch P; Zalcman G; Gandara DR; Riess JW; Velcheti V; Zeidler K; Diebold J; Früh M; Michels S; Monnet I; Popat S; Rosell R; Karachaliou N; Rothschild SI; Shih JY; Warth A; Muley T; Cabillic F; Mazières J; Drilon A, Targeting RET in patients with RET-rearranged lung cancers: Results from the global, multicenter RET registry. Journal of Clinical Oncology 2017, 35 (13), 1403–1410, DOI:10.1200/JCO.2016.70.9352. [PubMed: 28447912]

89. Ferrara R; Auger N; Auclin E; Besse B, Clinical and translational implications of RET rearrangements in non–small cell lung cancer. Journal of Thoracic Oncology 2018, 13, 27–45, DOI:10.1016/j.jtho.2017.10.021. [PubMed: 29128428]

90. Mizukami T; Shiraishi K; Shimada Y; Ogiwara H; Tsuta K; Ichikawa H; Sakamoto H; Kato M; Shibata T; Nakano T; Kohno T, Molecular mechanisms underlying oncogenic RET fusion in lung adenocarcinoma. Journal of Thoracic Oncology 2014, 9 (5), 622–630, DOI:10.1097/JTO.0000000000000135. [PubMed: 24722152]

91. Seki Y; Mizukami T; Kohno T, Molecular process producing oncogene fusion in lung cancer cells by illegitimate repair of DNA double-strand breaks. Biomolecules 2015, 5 (4), 2464–2476, DOI:10.3390/biom5042464. [PubMed: 26437441]

92. Salvatore D; Santoro M; Schlumberger M, The importance of the RET gene in thyroid cancer and therapeutic implications. Nature Reviews Endocrinology 2021, 17 (5), 296–306, DOI:10.1038/s41574-021-00470-9.

93. Gandhi M; Dillon LW; Pramanik S; Nikiforov YE; Wang YH, DNA breaks at fragile sites generate oncogenic RET/PTC rearrangements in human thyroid cells. Oncogene 2010, 29 (15), 2272–2280, DOI:10.1038/onc.2009.502. [PubMed: 20101222]

94. Ameziane-El-Hassani R; Boufraqech M; Lagente-Chevallier O; Weyemi U; Talbot M; Métivier D; Courtin F; Bidart JM; El Mzibri M; Schlumberger M; Dupuy C, Role of H2O2 in RET/PTC1 chromosomal rearrangement produced by ionizing radiation in human thyroid cells. Cancer Research 2010, 70 (10), 4123–4132, DOI:10.1158/0008-5472.CAN-09-4336. [PubMed: 20424115]

95. Ricarte-Filho JC; Li S; Garcia-Rendueles MER; Montero-Conde C; Voza F; Knauf JA; Heguy A; Viale A; Bogdanova T; Thomas GA; Mason CE; Fagin JA, Identification of kinase fusion oncogenes in post-Chernobyl radiation-induced thyroid cancers. Journal of Clinical Investigation 2013, 123 (11), 4935–4944, DOI:10.1172/JCI69766. [PubMed: 24135138]

96. Elisei R; Romei C; Vorontsova T; Cosci B; Veremeychik V; Kuchinskaya E; Basolo F; Demidchik EP; Miccoli P; Pinchera A; Pacini F, RET/PTC rearrangements in thyroid nodules: Studies in irradiated and not irradiated, malignant and benign thyroid lesions in children and adults. The Journal of Clinical Endocrinology & Metabolism 2001, 86 (7), 3211–3216, DOI:10.1210/jcem.86.7.7678. [PubMed: 11443191]

97. Rabes HM; Demidchik EP; Sidorow JD; Lengfelder E; Beimfohr C; Hoelzel D; Klugbauer S, Pattern of radiation-induced *RET* and *NTRK1* rearrangements in 191 post-Chernobyl papillary thyroid carcinomas: Biological, phenotypic, and clinical implications. Clinical Cancer Research 2000, 6 (3), 1093–1103. [PubMed: 10741739]

98. Hamatani K; Eguchi H; Ito R; Mukai M; Takahashi K; Taga M; Imai K; Cologne J; Soda M; Arihiro K; Fujihara M; Abe K; Hayashi T; Nakashima M; Sekine I; Yasui W; Hayashi Y; Nakachi K, RET/PTC rearrangements preferentially occurred in papillary thyroid cancer among atomic bomb survivors exposed to high radiation dose. Cancer Research 2008, 68 (17), 7176–7182, DOI:10.1158/0008-5472.CAN-08-0293. [PubMed: 18757433]

99. Qian YY; Chai S; Liang Z; Wang Y; Zhou Y; Xu X; Zhang C; Zhang M; Si J; Huang F; Huang Z; Hong W; Wang K, KIF5B-RET fusion kinase promotes cell growth by multilevel activation of STAT3 in lung cancer. Molecular Cancer 2014, 13 (1), DOI:10.1186/1476-4598-13-176.

100. Richardson DS; Gujral TS; Peng S; Asa SL; Mulligan LM, Transcript level modulates the inherent oncogenicity of RET/PTC oncoproteins. Cancer Research 2009, 69 (11), 4861–4869, DOI:10.1158/0008-5472.CAN-08-4425. [PubMed: 19487296]

101. Xing M, Molecular pathogenesis and mechanisms of thyroid cancer. Nature Reviews Cancer 2013, 13, 184–199, DOI:10.1038/nrc3431. [PubMed: 23429735]

102. Kohno T; Ichikawa H; Totoki Y; Yasuda K; Hiramoto M; Nammo T; Sakamoto H; Tsuta K; Furuta K; Shimada Y; Iwakawa R; Ogiwara H; Oike T; Enari M; Schetter AJ; Okayama H; Haugen A; Skaug V; Chiku S; Yamanaka I; Arai Y; Watanabe SI; Sekine I; Ogawa S; Harris CC; Tsuda H; Yoshida T; Yokota J; Shibata T, KIF5B-RET fusions in lung adenocarcinoma. Nature Medicine 2012, 18 (3), 375–377, DOI:10.1038/nm.2644.

103. Lu C; Zhou Q, Diagnostics, therapeutics and RET inhibitor resistance for RET fusion–positive non-small cell lung cancers and future perspectives. Cancer Treatment Reviews 2021, 96, DOI:10.1016/j.ctrv.2021.102153.

104. Amit M; Na'Ara S; Leider-Trejo L; Binenbaum Y; Kulish N; Fridman E; Shabtai-Orbach A; Wong RJ; Gil Z, Upregulation of RET induces perineurial invasion of pancreatic adenocarcinoma. Oncogene 2017, 36 (23), 3232–3239, DOI:10.1038/onc.2016.483. [PubMed: 28092668]

105. Wiesenhofer B; Stockhammer G; Kostron H; Maier H; Hinterhuber H; Humpel C, Glial cell line-derived neurotrophic factor (GDNF) and its receptor (GFR-α1) are strongly expressed in human gliomas. Acta Neuropathologica 2000, 99 (2), 131–137, DOI:10.1007/PL00007416. [PubMed: 10672319]

106. Narita N; Tanemura A; Murali R; Scolyer RA; Huang S; Arigami T; Yanagita S; Chong KK; Thompson JF; Morton DL; Hoon DS, Functional RET G691S polymorphism in cutaneous malignant melanoma. Oncogene 2009, 28 (34), 3058–3068, DOI:10.1038/onc.2009.164. [PubMed: 19561646]

107. Pandey V; Qian PX; Kang J; Perry JK; Mitchell MD; Yin Z; Wu ZS; Liu DX; Zhu T; Lobie PE, Artemin stimulates oncogenicity and invasiveness of human endometrial carcinoma cells. Endocrinology 2010, 151 (3), 909–920, DOI:10.1210/en.2009-0979. [PubMed: 20118197]

108. Flavin R; Finn SP; Choueiri TK; Ingoldsby H; Ring M; Barrett C; Rogers M; Smyth P; O'Regan E; Gaffney E; O'Leary JJ; Loda M; Signoretti S; Sheils O, RET protein expression in papillary renal cell carcinoma. Urologic Oncology: Seminars and Original Investigations 2012, 30 (6), 900–905, DOI:10.1016/j.urolonc.2010.08.025. [PubMed: 21396847]

109. Chuang JY; Tsai CF; Chang SW; Chiang IP; Huang SM; Lin HY; Yeh WL; Lu DY, Glial cell line-derived neurotrophic factor induces cell migration in human oral squamous cell carcinoma. Oral Oncology 2013, 49 (12), 1103–1112, DOI:10.1016/j.oraloncology.2013.08.009. [PubMed: 24070603]

110. Kosari F; Ida CM; Aubry MC; Yang L; Kovtun IV; Klein JLS; Li Y; Erdogan S; Tomaszek SC; Murphy SJ; Bolette LC; Kolbert CP; Yang P; Wigle DA; Vasmatzis G, ASCL1 and RET expression defines a clinically relevant subgroup of lung adenocarcinoma characterized by neuroendocrine differentiation. Oncogene 2014, 33 (29), 3776–3783, DOI:10.1038/onc.2013.359. [PubMed: 24037524]

111. Lin C; Lu W; Ren Z; Tang Y; Zhang C; Yang R; Chen Y; Cao W; Wang L; Wang X; Ji T, Elevated RET expression enhances EGFR activation and mediates EGFR inhibitor resistance in head and neck squamous cell carcinoma. Cancer Letters 2016, 377 (1), 1–10, DOI:10.1016/j.canlet.2016.04.023. [PubMed: 27090738]

112. Takeuchi K; Soda M; Togashi Y; Suzuki R; Sakata S; Hatano S; Asaka R; Hamanaka W; Ninomiya H; Uehara H; Lim Choi Y; Satoh Y; Okumura S; Nakagawa K; Mano H; Ishikawa Y, RET, ROS1 and ALK fusions in lung cancer. Nature medicine 2012, 18 (3), 378–381, DOI:10.1038/nm.2658.

113. Nikiforov YE; Nikiforova MN, Molecular genetics and diagnosis of thyroid cancer. Nature Reviews Endocrinology 2011, 7 (10), 569–580, DOI:10.1038/nrendo.2011.142.

114. Amit M; Na'ara S; Fridman E; Vladovski E; Wasserman T; Milman N; Gil Z, RET, a targetable driver of pancreatic adenocarcinoma. International Journal of Cancer 2019, 144 (12), 3014–3022, DOI:10.1002/ijc.32040. [PubMed: 30515799]

115. Elisei R; Cosci B; Romei C; Bottici V; Renzini G; Molinaro E; Agate L; Vivaldi A; Faviana P; Basolo F; Miccoli P; Berti P; Pacini F; Pinchera A, Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: A 10-year follow-up study. Journal of Clinical Endocrinology and Metabolism 2008, 93 (3), 682–687, DOI:10.1210/jc.2007-1714. [PubMed: 18073307]

116. Mendes Oliveira D; Grillone K; Mignogna C; De Falco V; Laudanna C; Biamonte F; Locane R; Corcione F; Fabozzi M; Sacco R; Viglietto G; Malanga D; Rizzuto A, Next-generation sequencing analysis of receptor-type tyrosine kinase genes in surgically resected colon cancer: identification of gain-of-function mutations in the RET proto-oncogene. Journal of Experimental & Clinical Cancer Research 2018, 37 (1), 84, DOI:10.1186/s13046-018-0746-y. [PubMed: 29665843]

117. Wells SA; Asa SL; Dralle H; Elisei R; Evans DB; Gagel RF; Lee N; MacHens A; Moley JF; Pacini F; Raue F; Frank-Raue K; Robinson B; Rosenthal MS; Santoro M; Schlumberger M; Shah M; Waguespack SG, Revised American thyroid association guidelines for the management

of medullary thyroid carcinoma. Thyroid 2015, 25 (6), 567–610, DOI:10.1089/thy.2014.0335. [PubMed: 25810047]

118. Wells SA, Advances in the management of MEN2: From improved surgical and medical treatment to novel kinase inhibitors. Endocrine-Related Cancer 2018, 25 (2), T1–T13, DOI:10.1530/ERC-17-0325. [PubMed: 29142004]
119. Verga U; Fugazzola L; Cambiaghi S; Pritelli C; Alessi E; Cortelazzi D; Gangi E; Beck-Peccoz P, Frequent association between MEN 2A and cutaneous lichen amyloidosis. Clinical Endocrinology 2003, 59 (2), 156–161, DOI:10.1046/j.1365-2265.2003.01782.x. [PubMed: 12864791]
120. Sipple JH, Multiple endocrine neoplasia type 2 syndromes: Historical perspectives. Henry Ford Hospital Medical Journal 1984, 32 (4), 219–222. [PubMed: 6152453]
121. Romei C; Pardi E; Cetani F; Elisei R, Genetic and clinical features of multiple endocrine neoplasia types 1 and 2. Journal of Oncology 2012, 2012, DOI:10.1155/2012/705036.
122. Margraf RL; Crockett DK; Krautscheid PMF; Seamons R; Calderon FRO; Wittwer CT; Mao R, Multiple endocrine neoplasia type 2 RET protooncogene database: Repository of MEN2-associated RET sequence variation and reference for genotype/phenotype correlations. Human Mutation 2009, 30, 548–556, DOI:10.1002/humu.20928. [PubMed: 19177457]
123. Mulligan LM; Eng C; Healey CS; Clayton D; Kwok JBJ; Gardner E; Ponder MA; Frilling A; Jackson CE; Lehnert H; Neumann HPH; Thibodeau SN; Ponder BAJ, Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. Nature Genetics 1994, 6 (1), 70–74, DOI:10.1038/ng0194-70. [PubMed: 7907913]
124. Eng C; Clayton D; Schuffenecker I; Lenoir G; Cote G; Gagel RF; van Amstel HK; Lips CJ; Nishisho I; Takai SI; Marsh DJ; Robinson BG; Frank-Raue K; Raue F; Xue F; Noll WW; Romei C; Pacini F; Fink M; Niederle B; Zedenius J; Nordenskjöld M; Komminoth P; Hendy GN; Mulligan LM, The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA 1996, 276 (19), 1575–1579, DOI:10.1001/jama.1996.03540190047028. [PubMed: 8918855]
125. Santoro M; Carlomagno F; Romano A; Bottaro DP; Dathan NA; Grieco M; Fusco A; Vecchio G; Maťoškova B; Kraus MH; Di Fiore PP, Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science 1995, 267 (5196), 381–383, DOI:10.1126/science.7824936. [PubMed: 7824936]
126. Asai N; Iwashita T; Matsuyama M; Takahashi M, Mechanism of activation of the ret proto-oncogene by multiple endocrine neoplasia 2A mutations. Molecular and Cellular Biology 1995, 15 (3), 1613–1619, DOI:10.1128/mcb.15.3.1613. [PubMed: 7532281]
127. Machens A; Dralle H, Familial prevalence and age of RET germline mutations: Implications for screening. Clinical Endocrinology 2008, 69 (1), 81–87, DOI:10.1111/j.1365-2265.2007.03153.x. [PubMed: 18062802]
128. Gujral TS; Singh VK; Jia Z; Mulligan LM, Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2B. Cancer Research 2006, 66 (22), 10741–10749, DOI:10.1158/0008-5472.CAN-06-3329. [PubMed: 17108110]
129. Knowles PP; Murray-Rust J; Kjær S; Scott RP; Hanrahan S; Santoro M; Ibáñez CF; McDonald NQ, Structure and chemical inhibition of the RET tyrosine kinase domain. Journal of Biological Chemistry 2006, 281 (44), 33577–33587, DOI:10.1074/jbc.M605604200. [PubMed: 16928683]
130. Gimm O; Marsh DJ; Andrew SD; Frilling A; Dahia PLM; Mulligan LM; Zajac JD; Robinson BG; Eng C, Germline dinucleotide mutation in codon 883 of the RET proto-oncogene in multiple endocrine neoplasia type 2B without codon 918 mutation. Journal of Clinical Endocrinology and Metabolism 1997, 82 (11), 3902–3904, DOI:10.1210/jcem.82.11.4508. [PubMed: 9360560]
131. Jasim S; Ying AK; Waguespack SG; Rich TA; Grubbs EG; Jimenez C; Hu MI; Cote G; Habra MA, Multiple endocrine neoplasia type 2B with a RET proto-oncogene A883F mutation displays a more indolent form of medullary thyroid carcinoma compared with a RET M918T mutation. Thyroid 2011, 21 (2), 189–192, DOI:10.1089/thy.2010.0328. [PubMed: 21186952]
132. Mathiesen JS; Habra MA; Bassett JHD; Choudhury SM; Balasubramanian SP; Howlett TA; Robinson BG; Gimenez-Roqueplo AP; Castinetti F; Vestergaard P; Frank-Raue K, Risk profile of the RET A883F germline mutation: An international collaborative study. Journal of

Clinical Endocrinology and Metabolism 2017, 102 (6), 2069–2074, DOI:10.1210/jc.2016-3640. [PubMed: 28323957]

133. Nakao KT; Usui T; Ikeda M; Mori Y; Yamamoto T; Kawashima ST; Nanba K; Yuno A; Tamanaha T; Tagami T; Naruse M; Asato R; Shimatsu A, Novel tandem germline RET proto-oncogene mutations in a patient with multiple endocrine neoplasia type 2B: Report of a case and a literature review of tandem RET mutations with in silico analysis. Head and Neck 2013, 35 (12), DOI:10.1002/hed.23241.
134. Cranston AN; Carniti C; Oakhill K; Radzio-Andzelm E; Stone EA; McCallion AS; Hodgson S; Clarke S; Mondellini P; Leyland J; Pierotti MA; Whittaker J; Taylor SS; Bongarzone I; Ponder BAJ, RET is constitutively activated by novel tandem mutations that alter the active site resulting in multiple endocrine neoplasia type 2B. Cancer Research 2006, 66 (20), 10179–10187, DOI:10.1158/0008-5472.CAN-06-0884. [PubMed: 17047083]
135. Plaza-Menacho I; Mologni L; Sala E; Gambacorti-Passerini C; Magee AI; Links TP; Hofstra RMW; Barford D; Isacke CM, Sorafenib functions to potently suppress RET tyrosine kinase activity by direct enzymatic inhibition and promoting RET lysosomal degradation independent of proteasomal targeting. Journal of Biological Chemistry 2007, 282 (40), 29230–29240, DOI:10.1074/jbc.M703461200. [PubMed: 17664273]
136. Carlomagno F; Anaganti S; Guida T; Salvatore G; Troncone G; Wilhelm SM; Santoro M, BAY 43–9006 inhibition of oncogenic RET mutants. Journal of the National Cancer Institute 2006, 98 (5), 326–334, DOI:10.1093/jnci/djj069. [PubMed: 16507829]
137. Kloos RT; Ringel MD; Knopp MV; Hall NC; King M; Stevens R; Liang J; Wakely PE; Vasko VV; Saji M; Rittenberry J; Wei L; Arbogast D; Collamore M; Wright JJ; Grever M; Shah MH, Phase II trial of sorafenib in metastatic thyroid cancer. Journal of Clinical Oncology 2009, 27 (10), 1675–1684, DOI:10.1200/JCO.2008.18.2717. [PubMed: 19255327]
138. Wilhelm S; Carter C; Lynch M; Lowinger T; Dumas J; Smith RA; Schwartz B; Simantov R; Kelley S, Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. Nature Reviews Drug Discovery 2006, 5 (10), 835–844, DOI:10.1038/nrd2130. [PubMed: 17016424]
139. Smith RA; Barbosa J; Blum CL; Bobko MA; Caringal YV; Dally R; Johnson JS; Katz ME; Kennure N; Kingery-Wood J; Lee W; Lowinger TB; Lyons J; Marsh V; Rogers DH; Swartz S; Walling T; Wild H, Discovery of heterocyclic ureas as a new class of raf kinase inhibitors: identification of a second generation lead by a combinatorial chemistry approach. Bioorganic & Medicinal Chemistry Letters 2001, 11 (20), 2775–2778, DOI:10.1016/S0960-894X(01)00571-6. [PubMed: 11591521]
140. Timothy BL; Bernd R; Jacques D; Roger AS, Design and discovery of small molecules targeting Raf-1 kinase. Current Pharmaceutical Design 2002, 8 (25), 2269–2278, DOI:10.2174/1381612023393125. [PubMed: 12369855]
141. Wilhelm SM; Carter C; Tang L; Wilkie D; McNabola A; Rong H; Chen C; Zhang X; Vincent P; McHugh M; Cao Y; Shujath J; Gawlak S; Eveleigh D; Rowley B; Liu L; Adnane L; Lynch M; Auclair D; Taylor I; Gedrich R; Voznesensky A; Riedl B; Post LE; Bollag G; Trail PA, BAY 43–9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Research 2004, 64 (19), 7099 LP–7109, DOI:10.1158/0008-5472.CAN-04-1443. [PubMed: 15466206]
142. Gupta-Abramson V; Troxel AB; Nellore A; Puttaswamy K; Redlinger M; Ransone K; Mandel SJ; Flaherty KT; Loevner LA; O'Dwyer PJ; Brose MS, Phase II trial of sorafenib in advanced thyroid cancer. Journal of Clinical Oncology 2008, 26 (29), 4714–4719, DOI:10.1200/JCO.2008.16.3279. [PubMed: 18541894]
143. FDA approves Nexavar to treat metastatic differentiated thyroid cancer. https://www.drugs.com/newdrugs/fda-approves-nexavar-metastatic-differentiated-thyroid-cancer-3971.html.
144. Wilhelm SM; Adnane L; Newell P; Villanueva A; Llovet JM; Lynch M, Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Molecular Cancer Therapeutics 2008, 7 (10), 3129–3140, DOI:10.1158/1535-7163.MCT-08-0013. [PubMed: 18852116]
145. FDA approves nexavar (sorafenib) for advanced renal cell carcinoma. https://www.drugs.com/newdrugs/fda-approves-nexavar-sorafenib-advanced-renal-cell-carcinoma-67.html.

146. FDA approves nexavar (sorafenib) for unresectable hepatocellular carcinoma. https://www.drugs.com/newdrugs/fda-approves-nexavar-sorafenib-unresectable-hepatocellular-carcinoma-4946.html.

147. Brose MS; Nutting CM; Jarzab B; Elisei R; Siena S; Bastholt L; de la Fouchardiere C; Pacini F; Paschke R; Shong YK; Sherman SI; Smit JWA; Chung J; Kappeler C; Peña C; Molnár I; Schlumberger MJ, Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. The Lancet 2014, 384 (9940), 319–328, DOI:10.1016/S0140-6736(14)60421-9.

148. Hong D; Ye L; Gagel R; Chintala L; El Naggar AK; Wright J; Kurzrock R, Medullary thyroid cancer: targeting the RET kinase pathway with sorafenib/tipifarnib. Molecular Cancer Therapeutics 2008, 7 (5), 1001–1006, DOI:10.1158/1535-7163.MCT-07-2422. [PubMed: 18445656]

149. Wilhelm SM; Dumas J; Adnane L; Lynch M; Carter CA; Schütz G; Thierauch K-H; Zopf D, Regorafenib (BAY 73–4506): A new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. International Journal of Cancer 2011, 129 (1), 245–255, DOI:10.1002/ijc.25864. [PubMed: 21170960]

150. Goel G, Evolution of regorafenib from bench to bedside in colorectal cancer: Is it an attractive option or merely a “me too” drug? Cancer Manag Res 2018, 10, 425–437, DOI:10.2147/CMAR.S88825. [PubMed: 29563833]

151. Arai H; Battaglin F; Wang J; Lo JH; Soni S; Zhang W; Lenz H-J, Molecular insight of regorafenib treatment for colorectal cancer. Cancer Treatment Reviews 2019, 81, 101912–101912, DOI:10.1016/j.ctrv.2019.101912. [PubMed: 31715423]

152. Miura K; Satoh M; Kinouchi M; Yamamoto K; Hasegawa Y; Philchenkov A; Kakugawa Y; Fujiya T, The preclinical development of regorafenib for the treatment of colorectal cancer. Expert Opinion on Drug Discovery 2014, 9 (9), 1087–1101, DOI:10.1517/17460441.2014.924923. [PubMed: 24896071]

153. Stivarga FDA Approval History. https://www.drugs.com/history/stivarga.html.

154. Chen Z; Zhao Y; Yu Y; Pang JC; Woodfield SE; Tao L; Guan S; Zhang H; Bieerkehazhi S; Shi Y; Patel R; Vasudevan A, S.; Yi JS; Muscal JA; Xu G-T; Yang J, Small molecule inhibitor regorafenib inhibits RET signaling in neuroblastoma cells and effectively suppresses tumor growth in vivo. Oncotarget 2017, 8 (61), 104090–104103, DOI:10.18632/oncotarget.22011. [PubMed: 29262623]

155. Fong TAT; Shawver LK; Sun L; Tang C; App H; Powell TJ; Kim YH; Schreck R; Wang X; Risau W; Ullrich A; Hirth KP; McMahon G, SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. Cancer Research 1999, 59 (1), 99–106. [PubMed: 9892193]

156. Faivre S; Demetri G; Sargent W; Raymond E, Molecular basis for sunitinib efficacy and future clinical development. Nature Reviews Drug Discovery 2007, 6 (9), 734–745, DOI:10.1038/nrd2380. [PubMed: 17690708]

157. Sun L; Liang C; Shirazian S; Zhou Y; Miller T; Cui J; Fukuda JY; Chu J-Y; Nematalla A; Wang X; Chen H; Sistla A; Luu TC; Tang F; Wei J; Tang C, Discovery of 5-[5-Fluoro-2-oxo-1,2- dihydroindol-(3Z)-ylidenemethyl]-2,4- dimethyl-1H-pyrrole-3-carboxylic Acid (2-Diethylaminoethyl)amide, a novel tyrosine kinase inhibitor targeting vascular endothelial and platelet-derived growth factor receptor tyrosine kinase. Journal of Medicinal Chemistry 2003, 46 (7), 1116–1119, DOI:10.1021/jm0204183. [PubMed: 12646019]

158. Mohammadi M; McMahon G; Sun L; Tang C; Hirth P; Yeh BK; Hubbard SR; Schlessinger J, Structures of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. Science 1997, 276 (5314), 955, DOI:10.1126/science.276.5314.955. [PubMed: 9139660]

159. Laird AD; Vajkoczy P; Shawver LK; Thurnher A; Liang C; Mohammadi M; Schlessinger J; Ullrich A; Hubbard SR; Blake RA; Fong TAT; Strawn LM; Sun L; Tang C; Hawtin R; Tang F; Shenoy N; Hirth KP; McMahon G; Cherrington JM, SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. Cancer Research 2000, 60 (15), 4152. [PubMed: 10945623]

160. Yakes FM; Chen J; Tan J; Yamaguchi K; Shi Y; Yu P; Qian F; Chu F; Bentzien F; Cancilla B; Orf J; You A; Laird AD; Engst S; Lee L; Lesch J; Chou Y-C; Joly AH, Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. Molecular Cancer Therapeutics 2011, 10 (12), 2298–2308, DOI:10.1158/1535-7163.MCT-11-0264. [PubMed: 21926191]

161. Kinase Inhibitor Drugs. John Wiley & Sons, Inc 2009.

162. Potapova O; Laird AD; Nannini MA; Barone A; Li G; Moss KG; Cherrington JM; Mendel DB, Contribution of individual targets to the antitumor efficacy of the multitargeted receptor tyrosine kinase inhibitor SU11248. Molecular Cancer Therapeutics 2006, 5 (5), 1280–1289, DOI:10.1158/1535-7163.MCT-03-0156. [PubMed: 16731761]

163. Wu H; Shih J-Y; Yang JC-H, Rapid response to sunitinib in a patient with lung adenocarcinoma harboring KIF5B-RET fusion gene. Journal of Thoracic Oncology 2015, 10 (9), e95–e96, DOI:10.1097/JTO.0000000000000611. [PubMed: 26291023]

164. Mendel DB; Laird AD; Xin X; Louie SG; Christensen JG; Li G; Schreck RE; Abrams TJ; Ngai TJ; Lee LB; Murray LJ; Carver J; Chan E; Moss KG; Haznedar JÖ; Sukbuntherng J; Blake RA; Sun L; Tang C; Miller T; Shirazian S; McMahon G; Cherrington JM, *In Vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clinical Cancer Research 2003, 9 (1), 327–337. [PubMed: 12538485]

165. Ryan AJ; Wedge SR, ZD6474 – a novel inhibitor of VEGFR and EGFR tyrosine kinase activity. British Journal of Cancer 2005, 92 (1), S6–S13, DOI:10.1038/sj.bjc.6602603. [PubMed: 15928657]

166. Matsui J; Funahashi Y; Uenaka T; Watanabe T; Tsuruoka A; Asada M, Multi-Kinase inhibitor E7080 suppresses lymph node and lung metastases of human mammary breast tumor MDA-MB-231 via inhibition of vascular endothelial growth factor-receptor (VEGF-R) 2 and VEGF-R3 kinase. Clinical Cancer Research 2008, 14 (17), 5459–5465, DOI:10.1158/1078-0432.CCR-07-5270. [PubMed: 18765537]

167. Wedge SR; Ogilvie DJ; Dukes M; Kendrew J; Chester R; Jackson JA; Boffey SJ; Valentine PJ; Curwen JO; Musgrove HL; Graham GA; Hughes GD; Thomas AP; Stokes ESE; Curry B; Richmond GHP; Wadsworth PF; Bigley AL; Hennequin LF, ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. Cancer Research 2002, 62 (16), 4645–4655. [PubMed: 12183421]

168. Okamoto K; Ikemori-Kawada M; Jestel A; von König K; Funahashi Y; Matsushima T; Tsuruoka A; Inoue A; Matsui J, Distinct binding mode of multikinase inhibitor lenvatinib revealed by biochemical characterization. ACS Medicinal Chemistry Letters 2015, 6 (1), 89–94, DOI:10.1021/ml500394m. [PubMed: 25589937]

169. Pelizzo MR; Boschin IM; Bernante P; Toniato A; Piotto A; Pagetta C; Nibale O; Rampin L; Muzzio PC; Rubello D, Natural history, diagnosis, treatment and outcome of medullary thyroid cancer: 37 years experience on 157 patients. European Journal of Surgical Oncology 2007, 33 (4), 493–497, DOI:10.1016/j.ejso.2006.10.021. [PubMed: 17125960]

170. Paszko Z; Sromek M; Czetwertynska M; Skasko E; Czapczak D; Wisniewska A; Prokurat A; Chrupek M; Jagielska A; Kozlowicz-Gudzinska I, The occurrence and the type of germline mutations in the RET gene in patients with medullary thyroid carcinoma and their unaffected kindred's from Central Poland. Cancer Investigation 2007, 25 (8), 742–749, DOI:10.1080/07357900701518735. [PubMed: 18058472]

171. Carlomagno F; Guida T; Anaganti S; Vecchio G; Fusco A; Ryan AJ; Billaud M; Santoro M, Disease associated mutations at valine 804 in the RET receptor tyrosine kinase confer resistance to selective kinase inhibitors. Oncogene 2004, 23 (36), 6056–6063, DOI:10.1038/sj.onc.1207810. [PubMed: 15184865]

172. Carlomagno F; Santoro M, Identification of RET kinase inhibitors as potential new treatment for sporadic and inherited thyroid cancer. Journal of Chemotherapy 2004, 16 (sup4), 49–51, DOI:10.1179/joc.2004.16.Supplement-1.49.

173. Sim MW; Cohen MS, The discovery and development of vandetanib for the treatment of thyroid cancer. Expert Opinion on Drug Discovery 2014, 9 (1), 105–114, DOI:10.1517/17460441.2014.866942. [PubMed: 24299515]

174. Thornton K; Kim G; Maher VE; Chattopadhyay S; Tang S; Moon YJ; Song P; Marathe A; Balakrishnan S; Zhu H; Garnett C; Liu Q; Booth B; Gehrke B; Dorsam R; Verbois L; Ghosh D; Wilson W; Duan J; Sarker H; Miksinski SP; Skarupa L; Ibrahim A; Justice R; Murgo A; Pazdur R, Vandetanib for the treatment of symptomatic or progressive medullary thyroid cancer in patients with unresectable locally advanced or metastatic disease: U.S. food and drug administration drug approval summary. Clinical Cancer Research 2012, 18 (14), 3722, DOI:10.1158/1078-0432.CCR-12-0411. [PubMed: 22665903]

175. Chau NG; Haddad RI, Vandetanib for the treatment of medullary thyroid cancer. Clinical Cancer Research 2013, 19 (3), 524–529, DOI:10.1158/1078-0432.CCR-12-2353. [PubMed: 23231950]

176. Matsui J; Yamamoto Y; Funahashi Y; Tsuruoka A; Watanabe T; Wakabayashi T; Uenaka T; Asada M, E7080, a novel inhibitor that targets multiple kinases, has potent antitumor activities against stem cell factor producing human small cell lung cancer H146, based on angiogenesis inhibition. International Journal of Cancer 2008, 122 (3), 664–671, DOI:10.1002/ijc.23131. [PubMed: 17943726]

177. Okamoto K; Kodama K; Takase K; Sugi NH; Yamamoto Y; Iwata M; Tsuruoka A, Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion-driven tumor models. Cancer Letters 2013, 340 (1), 97–103, DOI:10.1016/j.canlet.2013.07.007. [PubMed: 23856031]

178. Yamamoto Y; Matsui J; Matsushima T; Obaishi H; Miyazaki K; Nakamura K; Tohyama O; Semba T; Yamaguchi A; Hoshi SS; Mimura F; Haneda T; Fukuda Y; Kamata J.-i.; Takahashi K; Matsukura M; Wakabayashi T; Asada M; Nomoto K.-i.; Watanabe T; Dezso Z; Yoshimatsu K; Funahashi Y; Tsuruoka A, Lenvatinib, an angiogenesis inhibitor targeting VEGFR/FGFR, shows broad antitumor activity in human tumor xenograft models associated with microvessel density and pericyte coverage. Vascular Cell 2014, 6 (1), 18, DOI:10.1186/2045-824X-6-18. [PubMed: 25197551]

179. Roskoski R, Classification of small molecule protein kinase inhibitors based upon the structures of their drug-enzyme complexes. Pharmacological Research 2016, 103, 26–48, DOI:10.1016/j.phrs.2015.10.021. [PubMed: 26529477]

180. Gild ML; Bullock M; Robinson BG; Clifton-Bligh R, Multikinase inhibitors: a new option for the treatment of thyroid cancer. Nature Reviews Endocrinology 2011, 7 (10), 617–624, DOI:10.1038/nrendo.2011.141.

181. Seghezzi G; Patel S; Ren CJ; Gualandris A; Pintucci G; Robbins ES; Shapiro RL; Galloway AC; Rifkin DB; Mignatti P, Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: An autocrine mechanism contributing to angiogenesis. Journal of Cell Biology 1998, 141 (7), 1659–1673, DOI:10.1083/jcb.141.7.1659. [PubMed: 9647657]

182. Tohyama O; Matsui J; Kodama K; Hata-Sugi N; Kimura T; Okamoto K; Minoshima Y; Iwata M; Funahashi Y, Antitumor activity of lenvatinib (E7080): An angiogenesis inhibitor that targets multiple receptor tyrosine kinases in preclinical human thyroid cancer models. Journal of Thyroid Research 2014, 2014, 638747–638747, DOI:10.1155/2014/638747. [PubMed: 25295214]

183. Matsuki M; Adachi Y; Ozawa Y; Kimura T; Hoshi T; Okamoto K; Tohyama O; Mitsuhashi K; Yamaguchi A; Matsui J; Funahashi Y, Targeting of tumor growth and angiogenesis underlies the enhanced antitumor activity of lenvatinib in combination with everolimus. Cancer Science 2017, 108 (4), 763–771, DOI:10.1111/cas.13169. [PubMed: 28107584]

184. Yamada K; Yamamoto N; Yamada Y; Nokihara H; Fujiwara Y; Hirata T; Koizumi F; Nishio K; Koyama N; Tamura T, Phase I dose-escalation study and biomarker analysis of E7080 in patients with advanced solid tumors. Clinical Cancer Research 2011, 17 (8), 2528 LP–2537, DOI:10.1158/1078-0432.CCR-10-2638. [PubMed: 21372218]

185. Boss DS; Glen H; Beijnen JH; Keesen M; Morrison R; Tait B; Copalu W; Mazur A; Wanders J; O'Brien JP; Schellens JHM; Evans TRJ, A phase I study of E7080, a multitargeted tyrosine kinase inhibitor, in patients with advanced solid tumours. British Journal of Cancer 2012, 106 (10), 1598–1604, DOI:10.1038/bjc.2012.154. [PubMed: 22516948]

186. Cabanillas ME; Schlumberger M; Jarzab B; Martins RG; Pacini F; Robinson B; McCaffrey JC; Shah MH; Bodenner DL; Topliss D; Andresen C; O'Brien JP; Ren M; Funahashi Y; Allison R;

Elisei R; Newbold K; Licitra LF; Sherman SI; Ball DW, A phase 2 trial of lenvatinib (E7080) in advanced, progressive, radioiodine-refractory, differentiated thyroid cancer: A clinical outcomes and biomarker assessment. Cancer 2015, 121 (16), 2749–2756, DOI:10.1002/cncr.29395. [PubMed: 25913680]

187. Schlumberger M; Tahara M; Wirth LJ; Robinson B; Brose MS; Elisei R; Habra MA; Newbold K; Shah MH; Hoff AO; Gianoukakis AG; Kiyota N; Taylor MH; Kim S-B; Krzyzanowska MK; Dutcus CE; de las Heras B; Zhu J; Sherman SI, Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. New England Journal of Medicine 2015, 372 (7), 621–630, DOI:10.1056/NEJMoa1406470. [PubMed: 25671254]
188. Brose MS; Worden FP; Newbold KL; Guo M; Hurria A, Effect of age on the efficacy and safety of lenvatinib in radioiodine-refractory differentiated thyroid cancer in the Phase III SELECT trial. Journal of Clinical Oncology 2017, 35 (23), 2692–2699, DOI:10.1200/JCO.2016.71.6472. [PubMed: 28613956]
189. Schlumberger M; Jarzab B; Cabanillas ME; Robinson B; Pacini F; Ball DW; McCaffrey J; Newbold K; Allison R; Martins RG; Licitra LF; Shah MH; Bodenner D; Elisei R; Burmeister L; Funahashi Y; Ren M; Brien JP; Sherman SI, A Phase II trial of the multitargeted tyrosine kinase inhibitor lenvatinib (E7080) in advanced medullary thyroid cancer. Clinical Cancer Research 2016, 22 (1), 44–53, DOI:10.1158/1078-0432.CCR-15-1127. [PubMed: 26311725]
190. Schoffski P; Elisei R; Müller S; Brose MS; Shah MH; Licitra LF; Jarzab B; Medvedev V; Kreissl M; Niederle B; Cohen EEW; Wirth LJ; Ali HY; Hessel C; Yaron Y; Ball DW; Nelkin B; Sherman SI; Schlumberger M, An international, double-blind, randomized, placebo-controlled phase III trial (EXAM) of cabozantinib (XL184) in medullary thyroid carcinoma (MTC) patients (pts) with documented RECIST progression at baseline. Journal of Clinical Oncology 2012, 30 (15_suppl), 5508–5508, DOI:10.1200/jco.2012.30.15_suppl.5508.
191. Drilon A; Wang L; Hasanovic A; Suehara Y; Lipson D; Stephens P; Ross J; Miller V; Ginsberg M; Zakowski MF; Kris MG; Ladanyi M; Rizvi N, Response to cabozantinib in patients with *RET* fusion-positive lung adenocarcinomas. Cancer Discovery 2013, 3 (6), 630–635, DOI:10.1158/2159-8290.CD-13-0035. [PubMed: 23533264]
192. Drilon A; Rekhtman N; Arcila M; Wang L; Ni A; Albano M; Van Voorthuysen M; Somwar R; Smith RS; Montecalvo J; Plodkowski A; Ginsberg MS; Riely GJ; Rudin CM; Ladanyi M; Kris MG, Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: an open-label, single-centre, phase 2, single-arm trial. The Lancet Oncology 2016, 17 (12), 1653–1660, DOI:10.1016/S1470-2045(16)30562-9. [PubMed: 27825636]
193. Liu X; Shen T; Mooers BHM; Hilberg F; Wu J, Drug resistance profiles of mutations in the RET kinase domain. British Journal of Pharmacology 2018, 175 (17), 3504–3515, DOI:10.1111/bph.14395. [PubMed: 29908090]
194. O'Hare T; Shakespeare WC; Zhu X; Eide CA; Rivera VM; Wang F; Adrian LT; Zhou T; Huang W-S; Xu Q; Metcalf CA III; Tyner JW; Loriaux MM; Corbin AS; Wardwell S; Ning Y; Keats JA; Wang Y; Sundaramoorthi R; Thomas M; Zhou D; Snodgrass J; Commodore L; Sawyer TK; Dalgarno DC; Deininger MWN; Druker BJ; Clackson T, AP24534, a Pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell 2009, 16 (5), 401–412, DOI:10.1016/j.ccr.2009.09.028. [PubMed: 19878872]
195. FH T; TL P; SS S; RB L, Ponatinib: a novel multi-tyrosine kinase inhibitor against human malignancies. OncoTargets and Therapy 2019, 12, 635–645, DOI:10.2147/OTT.S189391. [PubMed: 30705592]
196. Cortes JE; Kim DW; Pinilla-Ibarz J; le Coutre P; Paquette R; Chuah C; Nicolini FE; Apperley JF; Khoury HJ; Talpaz M; DiPersio J; DeAngelo DJ; Abruzzese E; Rea D; Baccarani M; Müller MC; Gambacorti-Passerini C; Wong S; Lustgarten S; Rivera VM; Clackson T; Turner CD; Haluska FG; Guilhot F; Deininger MW; Hochhaus A; Hughes T; Goldman JM; Shah NP; Kantarjian H, A Phase 2 trial of ponatinib in philadelphia chromosome–positive leukemias. New England Journal of Medicine 2013, 369 (19), 1783–1796, DOI:10.1056/NEJMoa1306494. [PubMed: 24180494]
197. Zhou T; Commodore L; Huang W-S; Wang Y; Thomas M; Keats J; Xu Q; Rivera VM; Shakespeare WC; Clackson T; Dalgarno DC; Zhu X, Structural mechanism of the Pan-BCR-ABL inhibitor ponatinib (AP24534): Lessons for overcoming kinase inhibitor resistance. Chemical

Biology & Drug Design 2011, 77 (1), 1–11, DOI:10.1111/j.1747-0285.2010.01054.x. [PubMed: 21118377]

198. De Falco V; Buonocore P; Muthu M; Torregrossa L; Basolo F; Billaud M; Gozgit JM; Carlomagno F; Santoro M, Ponatinib (AP24534) is a novel potent inhibitor of oncogenic RET mutants associated with thyroid cancer. The Journal of Clinical Endocrinology & Metabolism 2013, 98 (5), E811–E819, DOI:10.1210/jc.2012-2672. [PubMed: 23526464]
199. Song Z; Wang M; Zhang A, Alectinib: a novel second generation anaplastic lymphoma kinase (ALK) inhibitor for overcoming clinically-acquired resistance. Acta Pharmaceutica Sinica B 2015, 5 (1), 34–37, DOI:10.1016/j.apsb.2014.12.007. [PubMed: 26579422]
200. Latif M; Saeed A; Kim SH, Journey of the ALK-inhibitor CH5424802 to phase II clinical trial. Archives of Pharmacal Research 2013, 36 (9), 1051–1054, DOI:10.1007/s12272-013-0157-8. [PubMed: 23700294]
201. Kinoshita K; Kobayashi T; Asoh K; Furuichi N; Ito T; Kawada H; Hara S; Ohwada J; Hattori K; Miyagi T; Hong W-S; Park M-J; Takanashi K; Tsukaguchi T; Sakamoto H; Tsukuda T; Oikawa N, 9-Substituted 6,6-Dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazoles as highly selective and potent anaplastic lymphoma kinase inhibitors. Journal of Medicinal Chemistry 2011, 54 (18), 6286–6294, DOI:10.1021/jm200652u. [PubMed: 21823617]
202. Kodama T; Tsukaguchi T; Satoh Y; Yoshida M; Watanabe Y; Kondoh O; Sakamoto H, Alectinib shows potent antitumor activity against *RET*-rearranged non–small cell lung cancer. Molecular Cancer Therapeutics 2014, 13 (12), 2910–2918, DOI:10.1158/1535-7163.MCT-14-0274. [PubMed: 25349307]
203. Sakamoto H; Tsukaguchi T; Hiroshima S; Kodama T; Kobayashi T; Fukami Takaaki A.; Oikawa N; Tsukuda T; Ishii N; Aoki Y, CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. Cancer Cell 2011, 19 (5), 679–690, DOI:10.1016/j.ccr.2011.04.004. [PubMed: 21575866]
204. Takeuchi S; Murayama T; Yoshimura K; Kawakami T; Takahara S; Imai Y; Kuribayashi Y; Nagase K; Goto K; Nishio M; Hasegawa Y; Satouchi M; Kiura K; Seto T; Yano S, Phase I/II study of alectinib in lung cancer with RET fusion gene: study protocol. The Journal of Medical Investigation 2017, 64 (3.4), 317–320, DOI:10.2152/jmi.64.317. [PubMed: 28955006]
205. Terzyan SS; Shen T; Liu X; Huang Q; Teng P; Zhou M; Hilberg F; Cai J; Mooers BHM; Wu J, Structural basis of resistance of mutant RET protein-tyrosine kinase to its inhibitors nintedanib and vandetanib. Journal of Biological Chemistry 2019, 294 (27), 10428–10437, DOI:10.1074/jbc.RA119.007682. [PubMed: 31118272]
206. Hilberg F; Roth GJ; Krssak M; Kautschitsch S; Sommergruber W; Tontsch-Grunt U; Garin-Chesa P; Bader G; Zoephel A; Quant J; Heckel A; Rettig WJ, BIBF 1120: Triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. Cancer Research 2008, 68 (12), 4774–4782, DOI:10.1158/0008-5472.CAN-07-6307. [PubMed: 18559524]
207. Roth GJ; Heckel A; Colbatzky F; Handschuh S; Kley J; Lehmann-Lintz T; Lotz R; Tontsch-Grunt U; Walter R; Hilberg F, Design, synthesis, and evaluation of indolinones as triple angiokinase inhibitors and the discovery of a highly specific 6-methoxycarbonyl-substituted indolinone (BIBF 1120). Journal of Medicinal Chemistry 2009, 52 (14), 4466–4480, DOI:10.1021/jm900431g. [PubMed: 19522465]
208. Roth GJ; Binder R; Colbatzky F; Dallinger C; Schlenker-Herceg R; Hilberg F; Wollin S-L; Kaiser R, Nintedanib: From discovery to the clinic. Journal of Medicinal Chemistry 2015, 58 (3), 1053–1063, DOI:10.1021/jm501562a. [PubMed: 25474320]
209. Hilberg F; Tontsch-Grunt U; Baum A; Le AT; Doebele RC; Lieb S; Gianni D; Voss T; Garin-Chesa P; Haslinger C; Kraut N, Triple angiokinase inhibitor nintedanib directly inhibits tumor cell growth and induces tumor shrinkage via blocking oncogenic receptor tyrosine kinases. Journal of Pharmacology and Experimental Therapeutics 2018, 364 (3), 494–503, DOI:10.1124/jpet.117.244129. [PubMed: 29263244]
210. Huang Q; Schneeberger VE; Luetteke N; Jin C; Afzal R; Budzevich MM; Makanji RJ; Martinez GV; Shen T; Zhao L; Fung K-M; Haura EB; Coppola D; Wu J, Preclinical modeling of KIF5B–RET fusion lung adenocarcinoma. Molecular Cancer Therapeutics 2016, 15 (10), 2521–2529, DOI:10.1158/1535-7163.MCT-16-0258. [PubMed: 27496134]

211. Rowbottom MW; Faraoni R; Chao Q; Campbell BT; Lai AG; Setti E; Ezawa M; Sprankle KG; Abraham S; Tran L; Struss B; Gibney M; Armstrong RC; Gunawardane RN; Nepomuceno RR; Valenta I; Hua H; Gardner MF; Cramer MD; Gitnick D; Insko DE; Apuy JL; Jones-Bolin S; Ghose AK; Herbertz T; Ator MA; Dorsey BD; Ruggeri B; Williams M; Bhagwat S; James J; Holladay MW, Identification of 1-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)urea hydrochloride (CEP-32496), a highly potent and orally efficacious inhibitor of V-RAF murine sarcoma viral oncogene homologue B1 (BRAF) V600E. Journal of Medicinal Chemistry 2012, 55 (3), 1082–1105, DOI:10.1021/jm2009925. [PubMed: 22168626]

212. James J; Ruggeri B; Armstrong RC; Rowbottom MW; Jones-Bolin S; Gunawardane RN; Dobrzanski P; Gardner MF; Zhao H; Cramer MD; Hunter K; Nepomuceno RR; Cheng M; Gitnick D; Yazdanian M; Insko DE; Ator MA; Apuy JL; Faraoni R; Dorsey BD; Williams M; Bhagwat SS; Holladay MW, CEP-32496: A novel orally active $BRAF^{V600E}$ inhibitor with selective cellular and *in vivo* antitumor activity. Molecular Cancer Therapeutics 2012, 11 (4), 930–941, DOI:10.1158/1535-7163.MCT-11-0645. [PubMed: 22319199]

213. Li GG; Somwar R; Joseph J; Smith RS; Hayashi T; Martin L; Franovic A; Schairer A; Martin E; Riely GJ; Harris J; Yan S; Wei G; Oliver JW; Patel R; Multani P; Ladanyi M; Drilon A, Antitumor activity of RXDX-105 in multiple cancer types with *RET* rearrangements or mutations. Clinical Cancer Research 2017, 23 (12), 2981–2990, DOI:10.1158/1078-0432.CCR-16-1887. [PubMed: 28011461]

214. Drilon A; Fu S; Patel MR; Fakih M; Wang D; Olszanski AJ; Morgensztern D; Liu SV; Cho BC; Bazhenova L; Rodriguez CP; Doebele RC; Wozniak A; Reckamp KL; Seery T; Nikolinakos P; Hu Z; Oliver JW; Trone D; McArthur K; Patel R; Multani PS; Ahn M-J, A Phase I/Ib trial of the VEGFR-sparing multikinase RET inhibitor RXDX-105. Cancer Discovery 2019, 9 (3), 384–395, DOI:10.1158/2159-8290.CD-18-0839. [PubMed: 30487236]

215. Fox E; Widemann BC; Chuk MK; Marcus L; Aikin A; Whitcomb PO; Merino MJ; Lodish M; Dombi E; Steinberg SM; Wells SA; Balis FM, Vandetanib in children and adolescents with multiple endocrine neoplasia type 2B associated medullary thyroid carcinoma. Clinical Cancer Research 2013, 19 (15), 4239–4248, DOI:10.1158/1078-0432.CCR-13-0071. [PubMed: 23766359]

216. Kobayashi S; Boggon TJ; Dayaram T; Jänne PA; Kocher O; Meyerson M; Johnson BE; Eck MJ; Tenen DG; Halmos B, EGFR mutation and resistance of non–small-cell lung cancer to gefitinib. New England Journal of Medicine 2005, 352 (8), 786–792, DOI:10.1056/NEJMoa044238. [PubMed: 15728811]

217. Drilon AE; Filleron T; Bergagnini I; Milia J; Hatzoglou V; Velcheti V; Besse B; Mok T; Awad MM; Wolf J; Carbone DP; Camidge DR; Riely GJ; Peled N; Mazieres J; Kris MG; Gautschi O, Baseline frequency of brain metastases and outcomes with multikinase inhibitor therapy in patients with RET-rearranged lung cancers. Journal of Clinical Oncology 2017, 35 (15_suppl), 9069–9069, DOI:10.1200/JCO.2017.35.15_suppl.9069.

218. Touyz RM; Herrmann J, Cardiotoxicity with vascular endothelial growth factor inhibitor therapy. NPJ Precision Oncology 2018, 2 (1), 13, DOI:10.1038/s41698-018-0056-z. [PubMed: 30202791]

219. Lilly Receives U.S. FDA Approval for Retevmo™ (selpercatinib), the First Therapy Specifically for Patients with Advanced RET-Driven Lung and Thyroid Cancers. 2020.

220. Subbiah V; Gainor JF; Rahal R; Brubaker JD; Kim JL; Maynard M; Hu W; Cao Q; Sheets MP; Wilson D; Wilson KJ; DiPietro L; Fleming P; Palmer M; Hu MI; Wirth L; Brose MS; Ou S-HI; Taylor M; Garralda E; Miller S; Wolf B; Lengauer C; Guzi T; Evans EK, Precision targeted therapy with BLU-667 for RET-driven cancers. Cancer Discovery 2018, 8 (7), 836–849, DOI:10.1158/2159-8290.CD-18-0338. [PubMed: 29657135]

221. Subbiah V; Velcheti V; Tuch BB; Ebata K; Busaidy NL; Cabanillas ME; Wirth LJ; Stock S; Smith S; Lauriault V; Corsi-Travali S; Henry D; Burkard M; Hamor R; Bouhana K; Winski S; Wallace RD; Hartley D; Rhodes S; Reddy M; Brandhuber BJ; Andrews S; Rothenberg SM; Drilon A, Selective RET kinase inhibition for patients with RET-altered cancers. Annals of Oncology 2018, 29 (8), 1869–1876, DOI:10.1093/annonc/mdy137. [PubMed: 29912274]

222. Subbiah V; Shen T; Terzyan SS; Liu X; Hu X; Patel KP; Hu M; Cabanillas M; Behrang A; Meric-Bernstam F; Vo PTT; Mooers BHM; Wu J, Structural basis of acquired resistance to

selpercatinib and pralsetinib mediated by non-gatekeeper RET mutations. Annals of Oncology 2021, 32 (2), 261–268, DOI:10.1016/j.annonc.2020.10.599. [PubMed: 33161056]

223. Solomon BJ; Tan L; Lin JJ; Wong SQ; Hollizeck S; Ebata K; Tuch BB; Yoda S; Gainor JF; Sequist LV; Oxnard GR; Gautschi O; Drilon A; Subbiah V; Khoo C; Zhu EY; Nguyen M; Henry D; Condroski KR; Kolakowski GR; Gomez E; Ballard J; Metcalf AT; Blake JF; Dawson S-J; Blosser W; Stancato LF; Brandhuber BJ; Andrews S; Robinson BG; Rothenberg SM, RET solvent front mutations mediate acquired resistance to selective RET inhibition in RET-driven malignancies. Journal of Thoracic Oncology 2020, 15 (4), 541–549, DOI:10.1016/j.jtho.2020.01.006. [PubMed: 31988000]
224. Taylor MH; Gainor JF; Hu MIN; Zhu VW; Lopes G; Leboulleux S; Brose MS; Schuler MH; Bowles DW; Kim D-W; Baik CS; Garralda E; Lin C-C; Adkins D; Sarker D; Curigliano G; Zhang H; Clifford C; Turner CD; Subbiah V, Activity and tolerability of BLU-667, a highly potent and selective RET inhibitor, in patients with advanced RET-altered thyroid cancers. Journal of Clinical Oncology 2019, 37 (15_suppl), 6018–6018, DOI:10.1200/JCO.2019.37.15_suppl.6018.
225. Wright K FDA Approves Pralsetinib for Treatment of Adults with Metastatic RET Fusion-Positive NSCLC. https://www.cancernetwork.com/view/pralsetinib_fda.
226. Genentech Announces FDA Approval of Gavreto (pralsetinib) for People With Advanced or Metastatic RET-Mutant and RET Fusion-Positive Thyroid Cancers. 2020.
227. FDA approves pralsetinib for RET-altered thyroid cancers. https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pralsetinib-ret-altered-thyroid-cancers#:~:text=On%20December%201%2C%202020%2C%20the,fusion%2Dpositive%20thyroid%20cancer%20who.
228. Drilon A; Rogers E; Zhai D; Deng W; Zhang X; Lee D; Ung J; Whitten J; Zhang H; Liu J; Hu T; Zhuang H; Lu Y; Huang Z; Graber A; Zimmerman Z; Xin R; Cui JJ; Subbiah V, TPX-0046 is a novel and potent RET/SRC inhibitor for RET-driven cancers. Annals of Oncology 2019, 30, v190–v191, DOI:10.1093/annonc/mdz244.068.
229. Turning Point Therapeutics, Inc. Common Stock. 2019.
230. Schoffski P; Aftimos PG; Massard C; Italiano A; Jungels C; Andreas K; Keegan M; Ho PTC, A phase I study of BOS172738 in patients with advanced solid tumors with RET gene alterations including non-small cell lung cancer and medullary thyroid cancer. Journal of Clinical Oncology 2019, 37 (15_suppl), TPS3162–TPS3162, DOI:10.1200/JCO.2019.37.15_suppl.TPS3162.
231. Helsinn announces FDA acceptance of IND application for TAS0953/HM06 in Patients with Advanced Solid Tumors with RET Gene Abnormalities. https://www.helsinn.com/news-and-events/helsinn-announces-fda-acceptance-of-ind-application-for-tas0953hm06-in-patients-with-advanced-solid-tumors-with-ret-gene-abnormalities/.
232. Stemline In-Licenses Worldwide Rights to Novel Selective RET Inhibitor (SL-1001); Expands Oncology Pipeline. GLOBE NEWSWIRE.
233. 001-35619; Stemline Therapeutics Inc.: 12/31/2019.
234. Newton R; Waszkowycz B; Seewooruthun C; Burschowsky D; Richards M; Hitchin S; Begum H; Watson A; French E; Hamilton N; Jones S; Lin L-Y; Waddell I; Echalier A; Bayliss R; Jordan AM; Ogilvie D, Discovery and Optimization of wt-RET/KDR-Selective Inhibitors of RETV804M Kinase. ACS Medicinal Chemistry Letters 2020, 11 (4), 497–505, DOI:10.1021/acsmedchemlett.9b00615. [PubMed: 32292556]
235. Horiike A; Takeuchi K; Uenami T; Kawano Y; Tanimoto A; Kaburaki K; Tambo Y; Kudo K; Yanagitani N; Ohyanagi F; Motoi N; Ishikawa Y; Horai T; Nishio M, Sorafenib treatment for patients with *RET* fusion-positive non-small cell lung cancer. Lung Cancer 2016, 93, 43–46, DOI:10.1016/j.lungcan.2015.12.011. [PubMed: 26898613]
236. Ravaud A; de la Fouchardière C; Caron P; Doussau A; Do Cao C; Asselineau J; Rodien P; Pouessel D; Nicolli-Sire P; Klein M; Bournaud-Salinas C; Wemeau J-L; Gimbert A; Picat M-Q; Pedenon D; Digue L; Daste A; Catargi B; Delord J-P, A multicenter phase II study of sunitinib in patients with locally advanced or metastatic differentiated, anaplastic or medullary thyroid carcinomas: mature data from the THYSU study. European Journal of Cancer 2017, 76, 110–117, DOI:10.1016/j.ejca.2017.01.029. [PubMed: 28301826]

237. Bikas A; Kundra P; Desale S; Mete M; O'Keefe K; Clark BG; Wray L; Gandhi R; Barett C; Jelinek JS; Wexler JA; Wartofsky L; Burman KD, Phase 2 clinical trial of sunitinib as adjunctive treatment in patients with advanced differentiated thyroid cancer. European Journal of Endocrinology 2016, 174 (3), 373–380, DOI:10.1530/EJE-15-0930. [PubMed: 26671977]

238. Lam ET; Ringel MD; Kloos RT; Prior TW; Knopp MV; Liang J; Sammet S; Hall NC; Wakely PE; Vasko VV; Saji M; Snyder PJ; Wei L; Arbogast D; Collamore M; Wright JJ; Moley JF; Villalona-Calero MA; Shah MH, Phase II clinical trial of sorafenib in metastatic medullary thyroid cancer. Journal of Clinical Oncology 2010, 28 (14), 2323–2330, DOI:10.1200/JCO.2009.25.0068. [PubMed: 20368568]

239. Lee SH; Lee JK; Ahn MJ; Kim DW; Sun JM; Keam B; Kim TM; Heo DS; Ahn JS; Choi YL; Min HS; Jeon YK; Park K, Vandetanib in pretreated patients with advanced non-small cell lung cancer-harboring *RET* rearrangement: a phase II clinical trial. Annals of Oncology 2017, 28 (2), 292–297, DOI:10.1093/annonc/mdw559. [PubMed: 27803005]

240. Wells SA; Robinson BG; Gagel RF; Dralle H; Fagin JA; Santoro M; Baudin E; Elisei R; Jarzab B; Vasselli JR; Read J; Langmuir P; Ryan AJ; Schlumberger MJ, Vandetanib in Patients With Locally Advanced or Metastatic Medullary Thyroid Cancer: A Randomized, Double-Blind Phase III Trial. Journal of Clinical Oncology 2011, 30 (2), 134–141, DOI:10.1200/JCO.2011.35.5040. [PubMed: 22025146]

241. Leboulleux S; Bastholt L; Krause T; de la Fouchardiere C; Tennvall J; Awada A; Gómez JM; Bonichon F; Leenhardt L; Soufflet C; Licour M; Schlumberger MJ, Vandetanib in locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 2 trial. The Lancet Oncology 2012, 13 (9), 897–905, DOI:10.1016/S1470-2045(12)70335-2. [PubMed: 22898678]

242. Koyama S; Miyake N; Fujiwara K; Morisaki T; Fukuhara T; Kitano H; Takeuchi H, Lenvatinib for Anaplastic Thyroid Cancer and Lenvatinib-Induced Thyroid Dysfunction. European Thyroid Journal 2018, 7 (3), 139–144, DOI:10.1159/000485972. [PubMed: 30023346]

243. Elisei R; Schlumberger MJ; Müller SP; Schöffski P; Brose MS; Shah MH; Licitra L; Jarzab B; Medvedev V; Kreissl MC; Niederle B; Cohen EEW; Wirth LJ; Ali H; Hessel C; Yaron Y; Ball D; Nelkin B; Sherman SI, Cabozantinib in progressive medullary thyroid cancer. Journal of Clinical Oncology 2013, 31 (29), 3639–3646, DOI:10.1200/JCO.2012.48.4659. [PubMed: 24002501]

244. Cabanillas ME; de Souza JA; Geyer S; Wirth LJ; Menefee ME; Liu SV; Shah K; Wright J; Shah MH, Cabozantinib as salvage therapy for patients with tyrosine kinase inhibitor–refractory differentiated thyroid cancer: Results of a multicenter phase II international thyroid oncology group trial. Journal of Clinical Oncology 2017, 35 (29), 3315–3321, DOI:10.1200/JCO.2017.73.0226. [PubMed: 28817373]

245. Lin JJ; Kennedy E; Sequist LV; Brastianos PK; Goodwin KE; Stevens S; Wanat AC; Stober LL; Digumarthy SR; Engelman JA; Shaw AT; Gainor JF, Clinical activity of alectinib in advanced *RET*-rearranged non-small cell lung cancer. Journal of Thoracic Oncology 2016, 11 (11), 2027–2032, DOI:10.1016/j.jtho.2016.08.126. [PubMed: 27544060]

246. Lemmens L, Nintedanib in advanced NSCLC: management of adverse events. Lung Cancer Management 2015, 5 (1), 29–41, DOI:10.2217/lmt.15.33. [PubMed: 30643547]

247. Drilon AE; Liu S; Doebele R; Rodriguez C; Fakih M; Reckamp KL; Bazhenova L; Cho BC; Kowack E; Oliver J; Multani P; Ahn MJ, A phase 1b study of RXDX-105, a VEGFR-sparing potent RET inhibitor, in RETi-naïve patients with RET fusion-positive NSCLC. Annals of Oncology 2017, 28, v612, DOI:10.1093/annonc/mdx440.012.

248. Bradford D; Larkins E; Mushti SL; Rodriguez L; Skinner AM; Helms WS; Price LSL; Zirkelbach JF; Li Y; Liu J; Charlab R; Turcu FR; Liang D; Ghosh S; Roscoe D; Philip R; Zack-Taylor A; Tang S; Kluetz PG; Beaver JA; Pazdur R; Theoret MR; Singh H, FDA approval summary: Selpercatinib for the treatment of lung and thyroid cancers with *RET* gene mutations or fusions. Clinical Cancer Research 2020, DOI:10.1158/1078-0432.CCR-20-3558.

249. Gainor JF; Curigliano G; Kim D-W; Lee DH; Besse B; Baik CS; Doebele RC; Cassier PA; Lopes G; Tan DS-W; Garralda E; Paz-Ares LG; Cho BC; Gadgeel SM; Thomas M; Liu SV; Clifford C; Zhang H; Turner CD; Subbiah V, Registrational dataset from the phase I/II ARROW trial of pralsetinib (BLU-667) in patients (pts) with advanced RET fusion+ non-small cell lung

cancer (NSCLC). Journal of Clinical Oncology 2020, 38 (15_suppl), 9515–9515, DOI:10.1200/JCO.2020.38.15_suppl.9515.

250. Drilon A; Nagasubramanian R; Blake JF; Ku N; Tuch BB; Ebata K; Smith S; Lauriault V; Kolakowski GR; Brandhuber BJ; Larsen PD; Bouhana KS; Winski SL; Hamor R; Wu W-I; Parker A; Morales TH; Sullivan FX; DeWolf WE; Wollenberg LA; Gordon PR; Douglas-Lindsay DN; Scaltriti M; Benayed R; Raj S; Hanusch B; Schram AM; Jonsson P; Berger MF; Hechtman JF; Taylor BS; Andrews S; Rothenberg SM; Hyman DM, A next-generation TRK kinase inhibitor overcomes acquired resistance to prior TRK kinase inhibition in patients with TRK fusion–positive solid tumors. Cancer Discovery 2017, 7 (9), 963, DOI:10.1158/2159-8290.CD-17-0507. [PubMed: 28578312]

251. Drilon A; Ou S-HI; Cho BC; Kim D-W; Lee J; Lin JJ; Zhu VW; Ahn M-J; Camidge DR; Nguyen J; Zhai D; Deng W; Huang Z; Rogers E; Liu J; Whitten J; Lim JK; Stopatschinskaja S; Hyman DM; Doebele RC; Cui JJ; Shaw AT, Repotrectinib (TPX-0005) is a next-generation ROS1/TRK/ALK inhibitor that potently inhibits ROS1/TRK/ALK solvent-front mutations. Cancer Discovery 2018, 8 (10), 1227, DOI:10.1158/2159-8290.CD-18-0484. [PubMed: 30093503]

252. Cocco E; Schram AM; Kulick A; Misale S; Won HH; Yaeger R; Razavi P; Ptashkin R; Hechtman JF; Toska E; Cownie J; Somwar R; Shifman S; Mattar M; Selçuklu SD; Samoila A; Guzman S; Tuch BB; Ebata K; de Stanchina E; Nagy RJ; Lanman RB; Houck-Loomis B; Patel JA; Berger MF; Ladanyi M; Hyman DM; Drilon A; Scaltriti M, Resistance to TRK inhibition mediated by convergent MAPK pathway activation. Nature Medicine 2019, 25 (9), 1422–1427, DOI:10.1038/s41591-019-0542-z.

253. Lin JJ; Liu SV; McCoach CE; Zhu VW; Tan AC; Yoda S; Peterson J; Do A; Prutisto-Chang K; Dagogo-Jack I; Sequist LV; Wirth LJ; Lennerz JK; Hata AN; Mino-Kenudson M; Nardi V; Ou SHI; Tan DSW; Gainor JF, Mechanisms of resistance to selective RET tyrosine kinase inhibitors in RET fusion-positive non-small-cell lung cancer. Annals of Oncology 2020, 31 (12), 1725–1733, DOI:10.1016/j.annonc.2020.09.015. [PubMed: 33007380]

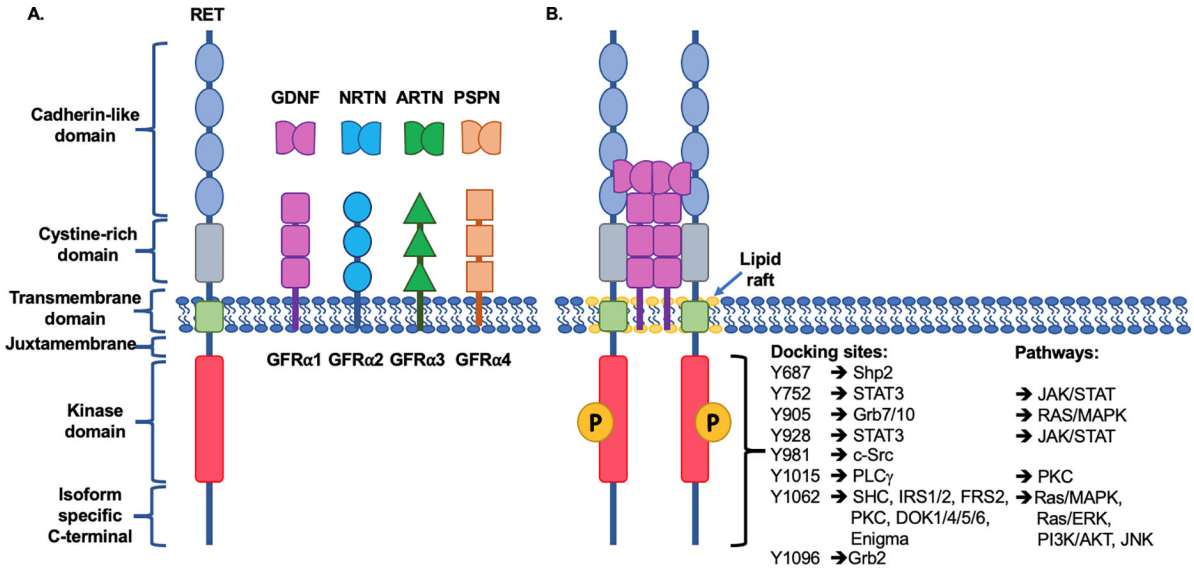


Figure 1.

Schematic of RET receptor tyrosine kinase, coreceptors, and ligands.

A. Domain structure of RET, glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs): GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN), GDNF family receptor-α (GFRα1–4). **B.** RET-coreceptor-ligand complex incorporated into a lipid raft, dimerization and autophosphorylation enabling adaptor and signaling proteins to bind to docking sites activating downstream signaling pathways.

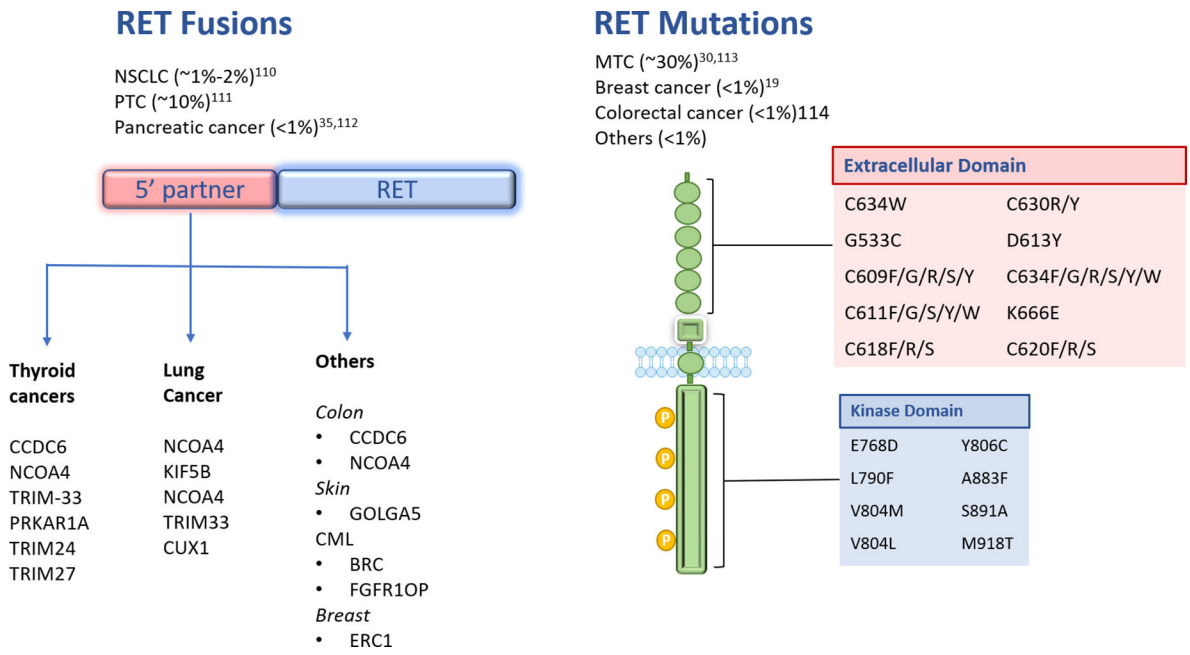


Figure 2.

RET activation in cancers through RET fusions or mutations[19, 30, 112–116]

CML: Chronic myeloid leukemia; NSCLC: Non-small cell lung cancer; PTC: Papillary thyroid cancer, MTC: Medullary thyroid cancer.

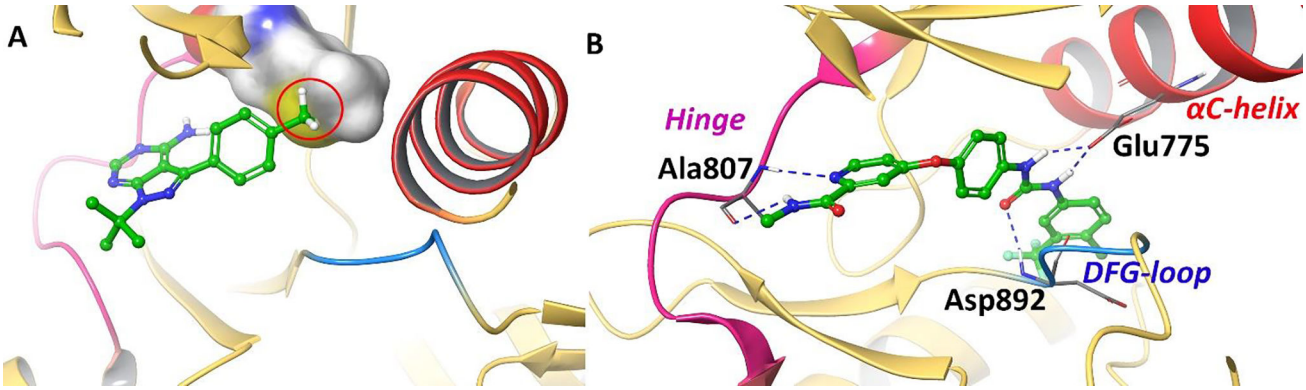


Figure 3:

PP1 and sorafenib in the RET kinase. (A) PP1 bound to RET (PDB ID: 2IVV) with a V804M mutant. The V804M mutation extends into the ATP pocket clashing with the isopropyl substitution on PP1. (B) Docking of sorafenib in a DFG-out RET kinase homology model. The backbone of ALA807 and sidechains of conserved residues GLU775 and ASP892 engage in hydrogen bonds with sorafenib. The hinge region, αC-helix, and DFG loop are illustrated in pink, red, and blue, respectively.[56, 129]

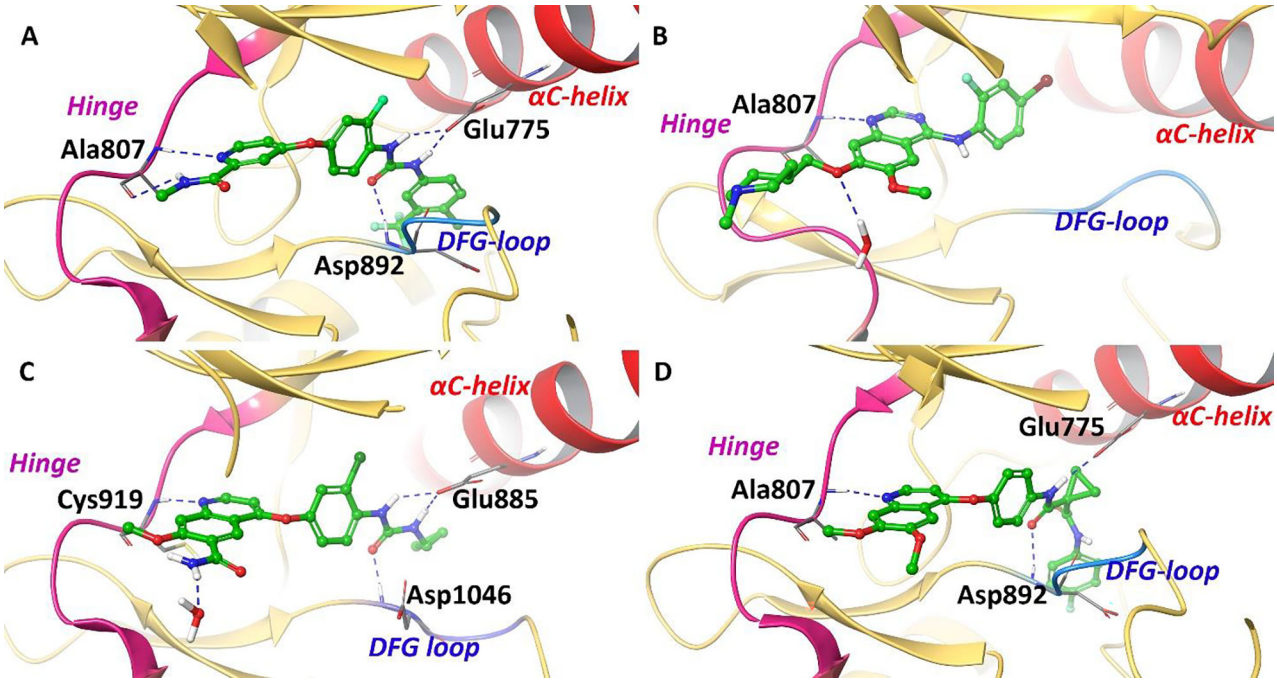


Figure 4:

(A) Regorafenib docked in a DFG-out RET kinase homology model. (B) Vandetanib and RET co-crystal structure (PDB ID: 2IVU). (C) Lenvatinib and VEGFR2 co-crystal structure (PDB ID: 3WZD). (D) Cabozantinib docked in a DFG-out RET kinase homology model. The hinge region, αC-helix, and DFG loop are illustrated in pink, red, and blue, respectively.[128, 160, 168]

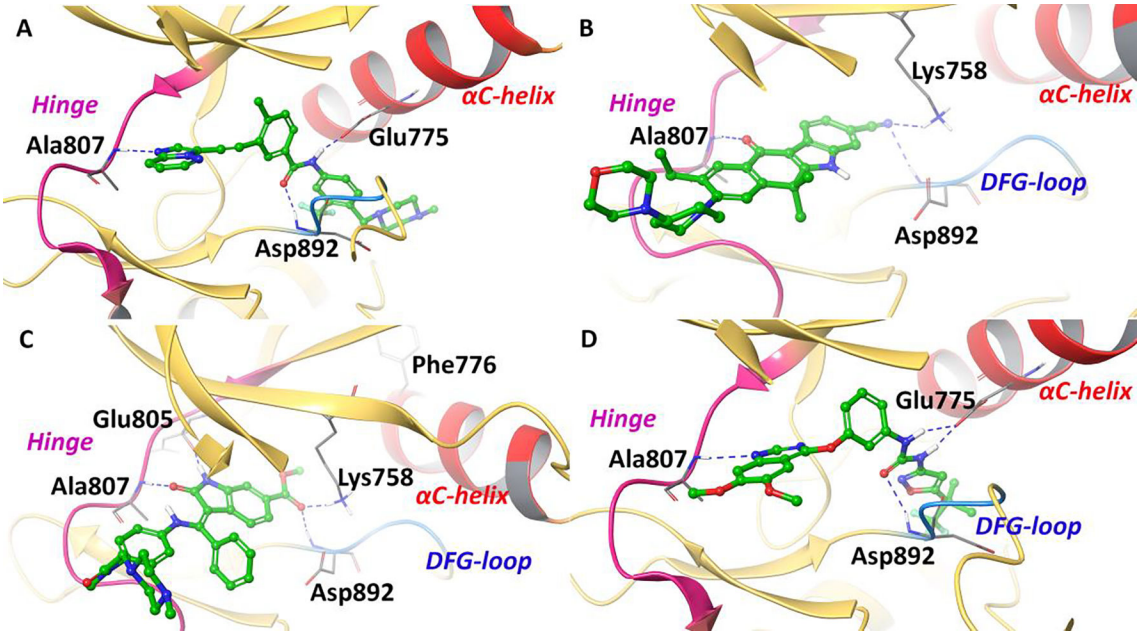


Figure 5:

(A) Ponatinib docked in a DFG-out RET kinase homology model.[197] (B) Alectinib docked in the RET kinase. (C) Nintedanib and RET co-crystal structure (PDB ID: NEC). (D) Agerafenib docked in a DFG-out RET kinase homology model.[189, 205] The hinge region, αC-helix, and DFG loop are illustrated in pink, red, and blue, respectively.

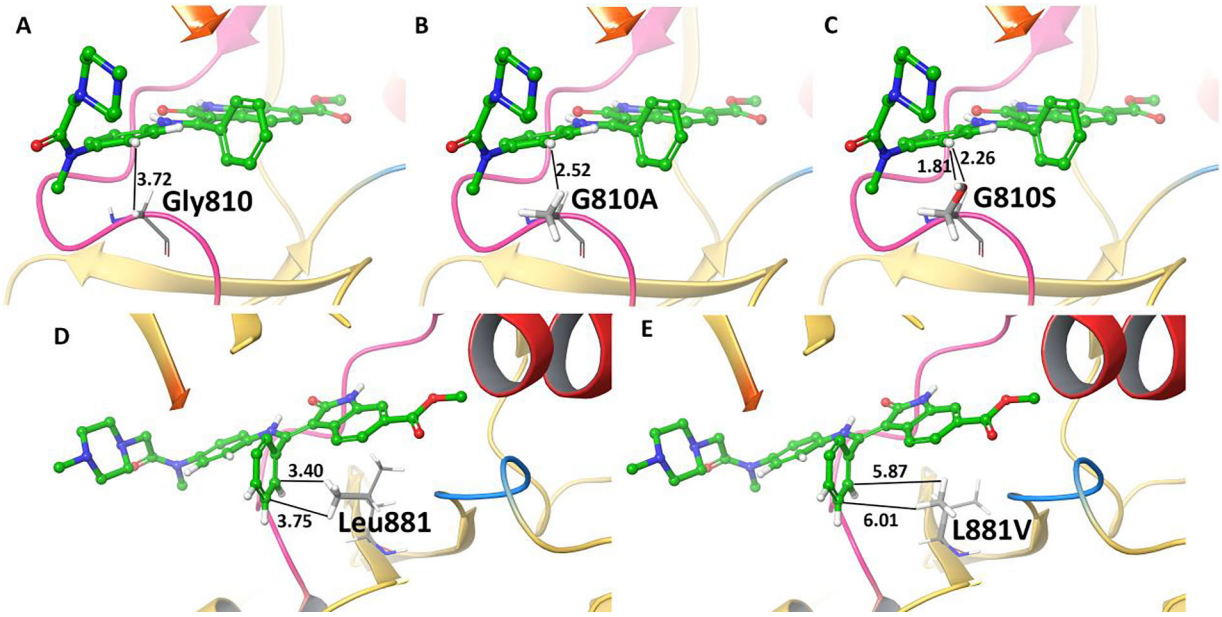


Figure 6:

Binding pose of nintedanib in the RET kinase. (A) Residue G810 is situated at around 3.9 Å from the phenyl group of p-phenylenediamine of nintedanib establishing hydrophobic contacts. (B) The G810A mutation reduces binding distance to nearly 2.5 Å, which increases steric strain with nintedanib. (C) The G810S mutation further reduces distance to ~2 Å, which significantly increases steric strain. (D) In wt-RET L881, the leucine side chain engages nintedanib in several hydrophobic contacts. (E) A L881V mutation increases interaction distance, resulting in a loss of hydrophobic contacts with nintedanib. The hinge region, αC-helix, and DFG loop are illustrated in pink, red, and blue, respectively.[205, 211]

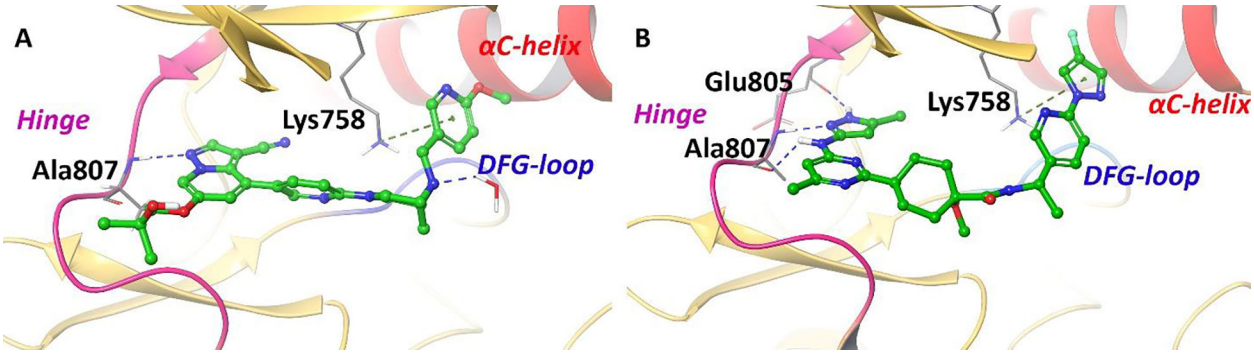


Figure 7.

Binding pose and interactions of third generation inhibitors in the RET kinase. (A) Selpercatinib (PDB ID: 7JU6) and (B) pralsetinib (PDB ID: 7JU5) RET co-crystal structures. Both compounds bind to the DFG-in conformation of the RET kinase. The hinge region, αC-helix, and DFG loop are illustrated in pink, red, and blue, respectively.[222]

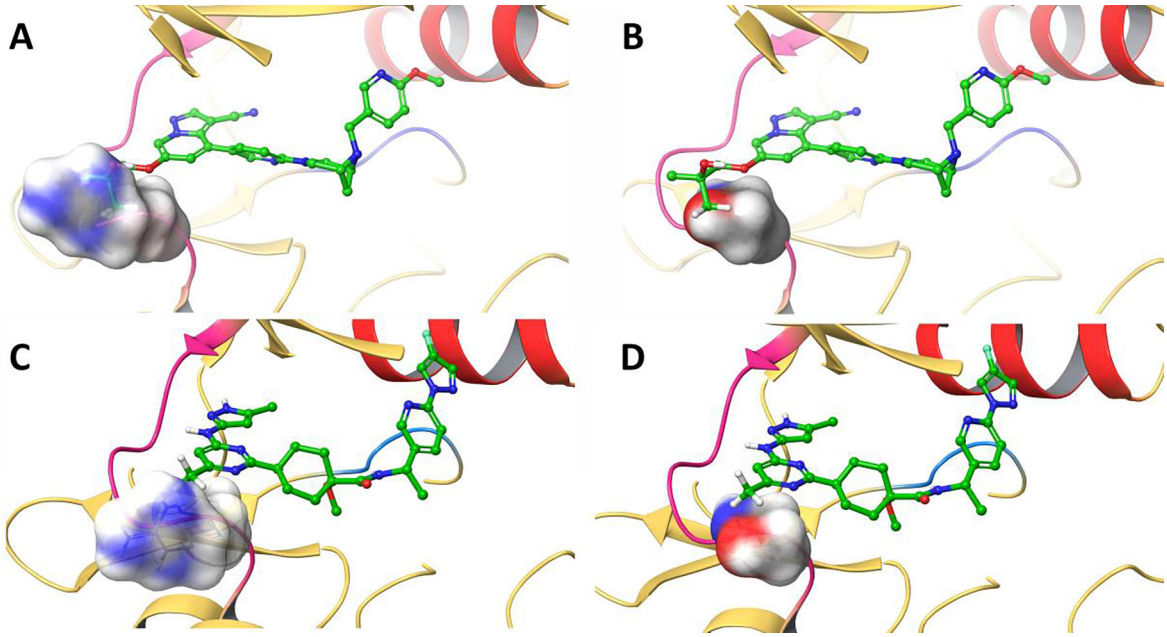
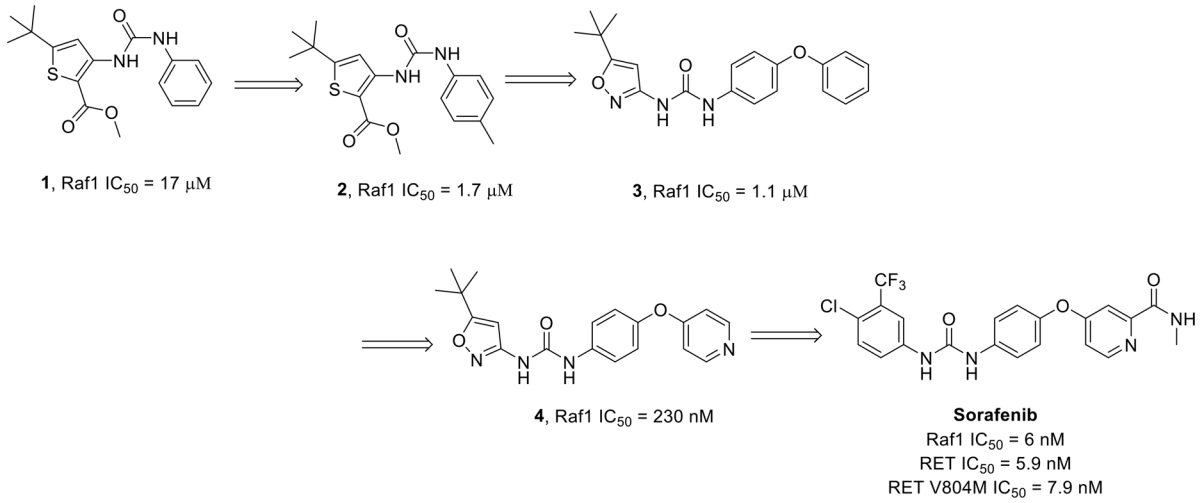
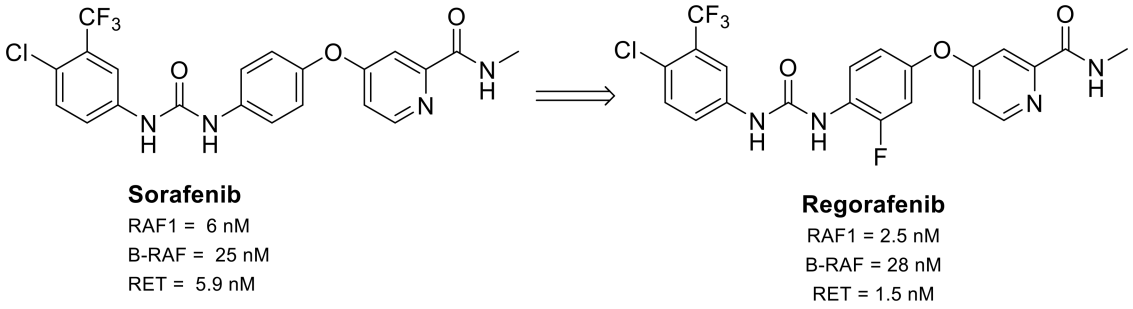


Figure 8:

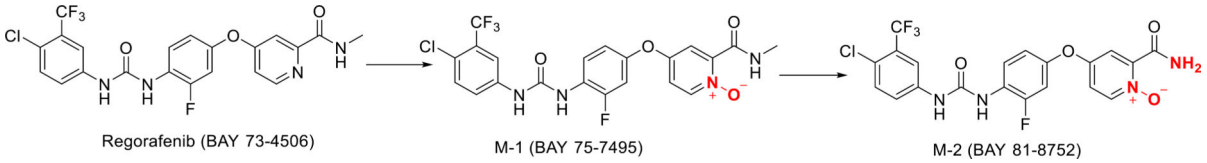
Residue G810 of RET when mutated to arginine (A) or serine (B) creates steric clashes with the solvent-exposed alkoxy group rendering both the mutated kinases resistant to selpercatinib and pralsetinib (C&D). These mutations are termed solvent front mutations (SFMs). The hinge region, αC-helix, and DFG loop are illustrated in pink, red, and blue respectively.[223]



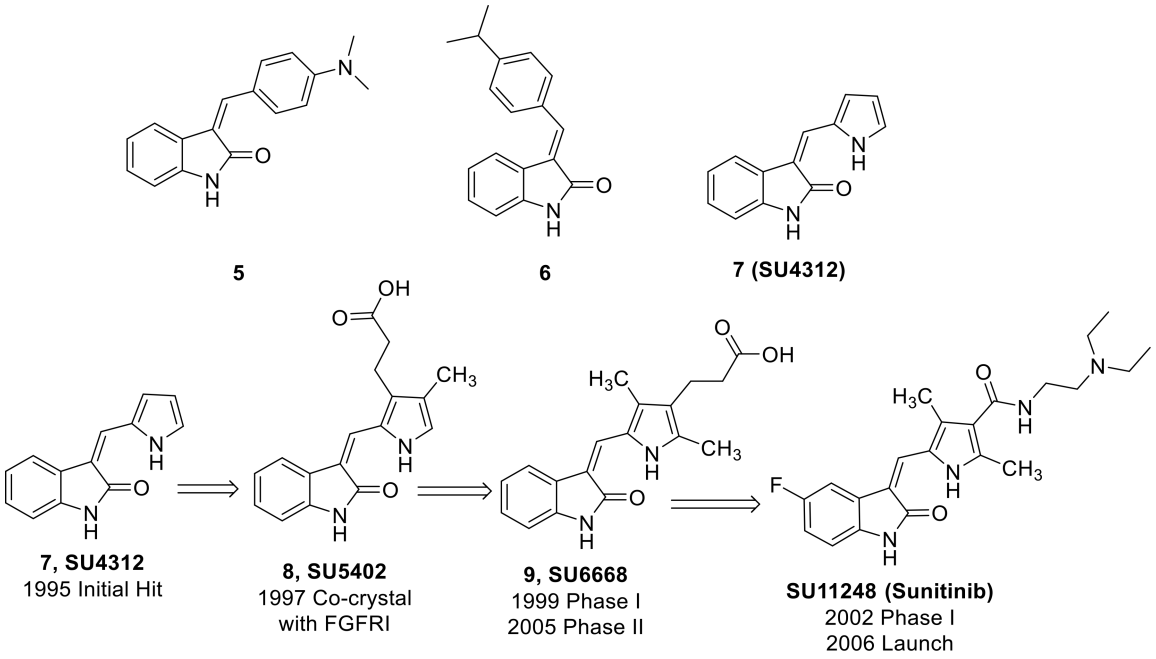
Scheme 1.
Discovery of sorafenib



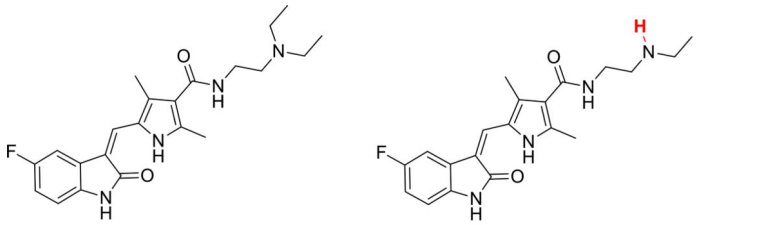
Scheme 2.
Discovery of regorafenib



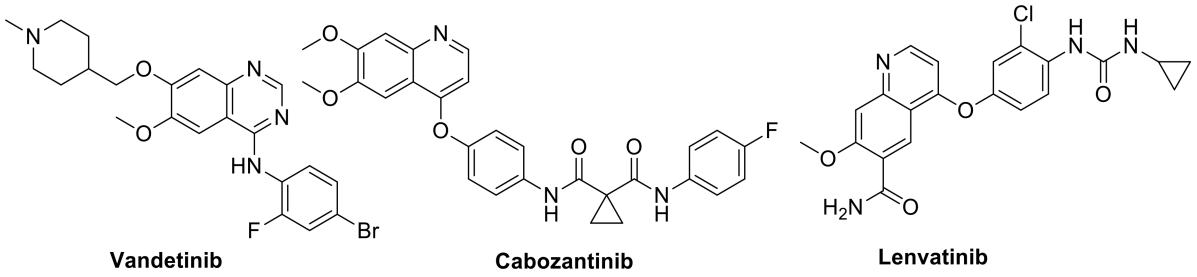
Scheme 3.
Regorafenib and metabolites **M-1** and **M-2**.



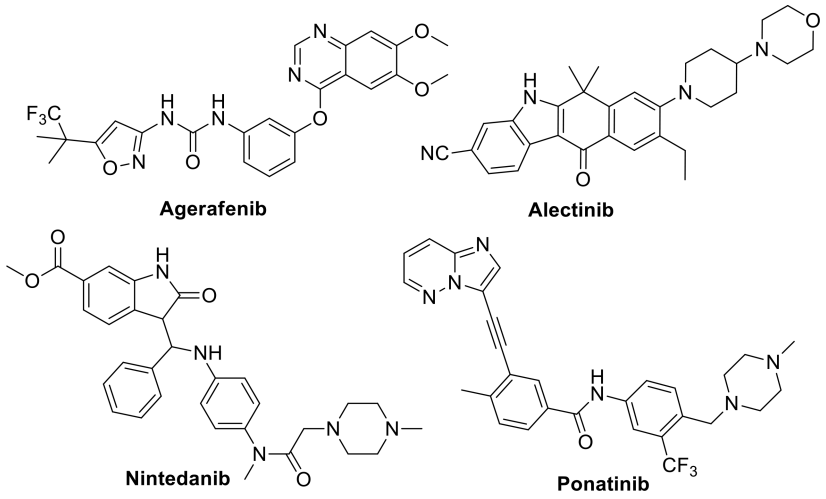
Scheme 4.
Discovery of sunitinib



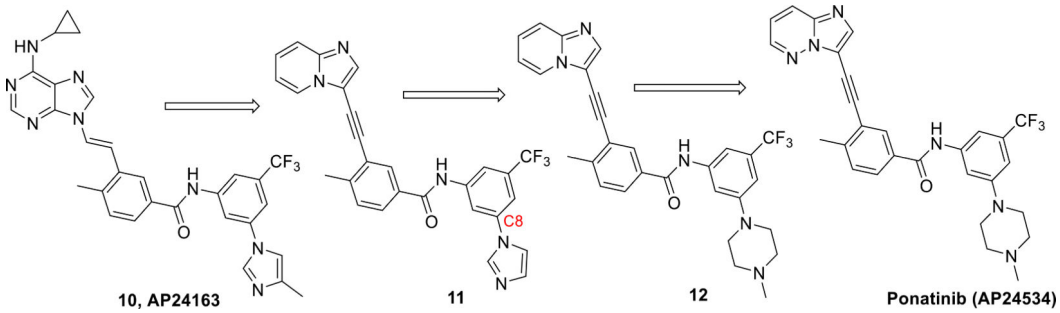
Scheme 5.
Structure of sunitinib and metabolite SU11248



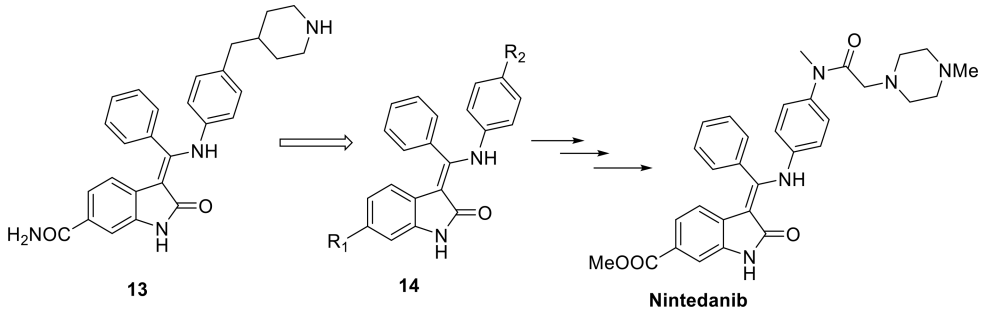
Scheme 6.
First generation multikinase inhibitors with RET activity



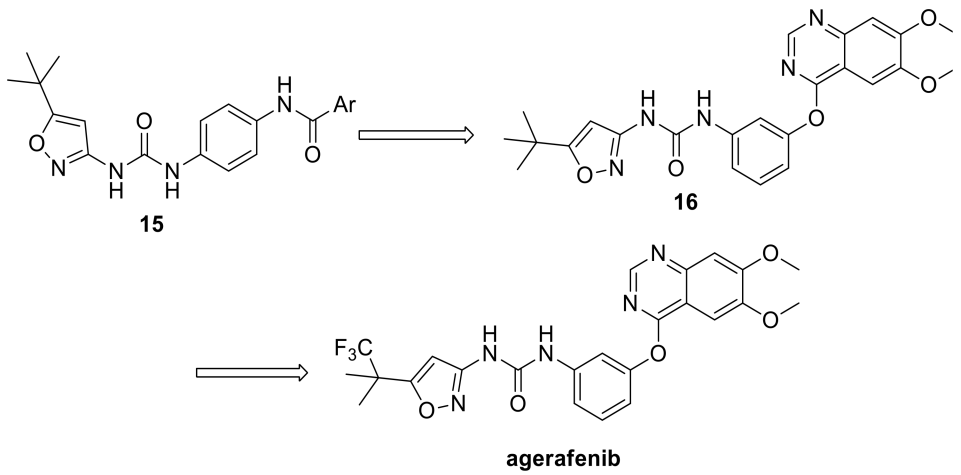
Scheme 7.
First generation MKIs with RET mutant activity



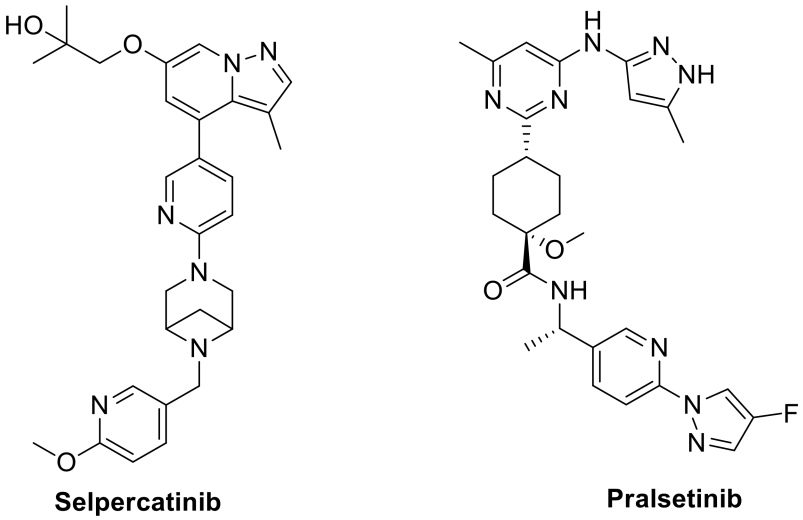
Scheme 8.
Discovery of ponatinib



Scheme 9.
Discovery of nintedanib



Scheme 10.
Discovery of agerafenib



Scheme 11.
Second generation selective RET mutant inhibitors

Table 1

Cellular inhibitory profile of sorafenib[135, 138]

Cellular kinase assay	**GI_{50} (nM)**
RET^{V804M} human thyroid carcinoma cells	147
RET^{V804L} human thyroid carcinoma cells	110
RET phosphorylation in human NIH3T3 fibroblasts	47
$BRAF^{V600E}$ in human thyroid carcinoma cells	1000

Table 2.

Comparative kinase inhibitory profile of first generation MKIs

Kinase	Sorafenib IC_{50} (nM)[135, 138]	Regorafenib IC_{50} (nM)[149–150]	Sunitinib IC_{50} (nM)[156]	Vandetinib IC_{50} (nM)[165]	Lenvatinib IC_{50} (nM)[166]	Cabozantinib IC_{50} (nM)[128, 160]
RET	5.9	1.5	5	0.13	1.5	5.2
RETV804M	7.9	--	--	--	--	--
Raf-1	6	2.5	--	--	--	--
BRAF	25	28.0	--	--	--	--
BRAFV600E	38	19.0	--	--	--	--
VEGFR1	26	13	ND	>1	22	--
VEGFR2	90	4.2	0.4	0.04	4.0	0.035
VEGFR3	20	46	ND	0.11	5.2	--
EGFR	--	--	--	0.5	6500	--
PDGFRα	--	--	6.9	--	51	--
PDGFRβ	57	--	3.9	>1	39	--
FGFR1	580	202	--	>1	46	--
FLT-3	33	--	2.5	--	--	11.3
FLT3-ITD	--	--	5	--	--	--
p38	38	--	--	--	--	--
c-Kit	68	7	--	>20	100	4.6
c-MET	--	--	--	--	--	1.3–14.6
AXL	--	--	--	--	--	7.0
Tie-2	--	311	--	--	--	14.3

Table 3.

Kinase inhibition profile of nintedanib[191]

Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)
RET	2±1	CSF1R	5±2
VEGFR1	99	TRKA	30±8
VEGFR2	3	TRKC	48±25
VEGFR3	4	ABL1	12±5
PDGFRα	18	KIT	6±3
PDGFRβ	28	FGFR4	421
FGFR1	41	FGFR3	96
FGFR2	47		

Table 4:

Inhibitory profile of agerafenib[211–212]

Target	Kd (nM)	IC_{50} (nM)	GI_{50} (nM)
RET	1.5	7	21 (TT-1)
BRAF	36	na	2,736 (Hs578T), 6,631 (LNCaP
BRAFV600E	14	na	60 (Colo-205), 84 (A375)
CRAF	39	146	≥3000 (HeLa)
ABL, BCR-ABL	2.8	6	39 (K562; ABL), 70 (K562; BCR
VEGFR2	7.9	43	≥700 (HUVEC)
FLT-1	14	1	≥1,000 (HUVEC)
CKIT	2.4	na	1,000 (A431)

Kinase	IC_{50} (nM) [196]
CCDC6-RET	0.33
NCOA4-RET	0.41
PRKA1A-RET	0.81
RETM918T	4.34
RETV804M	266
RETV804L	319

Table 5:

Biochemical activity of RET inhibitors and MKIs against RET mutants and VEGFR2[220]

Compound	Biochemical IC_{50} (nM)					
	WT RET	RET^{V804L}	RET^{V804M}	RET^{M918T}	VEGFR2	CCDC6-RET
Selpercatinib	0.4	0.42	0.8	0.7	100	--
Pralsetinib	0.4	0.3	0.4	0.4	35	0.4
Agerafenib	31	168	102	4	17	7
Cabozantinib	5.2	45	162	8	0.035	34
Vandetanib	130	3597	726	7	4	20

Table 6.

Kinase inhibitor drugs * with associated RET profile, classification, and FDA indication[24]

Name	RET Profile (nM)	Non-RET Profile (nM)	RET Indication	Dose Limiting Toxicity (Grade 3/4 AEs)	Dose Reduction (% of patients)	Discontinuation Rate (% of patients)	References
1st Generation							
Sorafenib	RET: 5.9 RET^{V804M}: 7.9	*Other targets*: Raf-1, BRAF, VEGFR1/3, Flt3, p38	No RET indication	Phase II: Hand and foot syndrome (25%)	NA	NA	235
Regorafenib	RET: 1.5	*Other targets*: Raf-1, BRAF VEGFR1/2/3, c-KIT, $BRAF^{V600E}$, PDGFRβ	No RET indication	Retrospective study: NA	NA	NA	88
Sunitinib	RET: 5	*Other targets*: VEGFR1/2/3, KIT, FLT3, CSF1R, PDGFR*β*	No RET indication	Phase II: Fatigue (4%), Diarrhea (4%), leukopenia (13%), hypertension (13%), hand–foot syndrome (9%), and Anorexia (9%)	26% (DTC)	13% (DTC)	88, 236–238
Vandetanib	RET: 130 RET^{M918T}: 7	*Other targets*: VEGFR1/2/3, EGFR	MTC	Phase II: Hypertension (58%), Rash (16%), Diarrhea (11%), Prolonged QT interval (11%)	50% (NSCLC)	21% (NSCLC)	239–241
Lenvatinib	RET: 1.5	*Other targets*: VEGFR 1/2/3, FGFR 1–4, PDGFRβ	DTC	Phase II: Hypertension (58%), Proteinuria (16%)	64% (NSCLC)	76% (NSCLC)	187, 242
Cabozantinib	RET: 5.2 RET^{M918T}: 7	*Other targets*: *c*-MET, *c*-KIT, FLT3, AXL	MTC	Phase II: Increased Lipase (15%), increased liver transaminase level (8%), Hypertension (4%)	73% (NSCLC)	8% (NSCLC)	243–244
Ponatinib	RET: 26 RET^{V804M}: 34	*Other targets*: BCR-Abl, Src, FLT3, KIT, FGFR, PDGFR, VEGFR	No RET indication	Retrospective study: NA	NA	NA	88
Alectinib	RET: 4.8 RET^{V804M}: 32 RET^{V804M}: 53 RET^{G691S}: 9.5 RET^{Y719F}: 14 RET^{S891A}: 8.3 RET^{M918T}: 5.7	*Other targets*: ALK, ALK^{L1196M}	No RET indication	Retrospective study: NA	NA	NA	88, 245
Nintedanib	RET: 2	*Other targets*: VEGFR1/2/3, FGFR1–4, CSF1R, Trk A/C, ABL1, PDGFR α/β	No RET indication	Fatigue (14%) and Diarrhea (13%)	1% (NSCLC)	No complete discontinuation of treatment	88, 246
Agerafenib	RET: 31 RET^{M918T}: 4	*Other targets*: BRAF, $BRAF^{V600E}$, Abl, BCR-Abl, VEGFR1/2, FLT-1, c-KIT	No RET indication	Rash (10%), increased aspartate aminotransferase (8%), increased aspartate aminotransferase level (5%)	NA	NA	247
2nd Generation							

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Name	RET Profile (nM)	Non-RET Profile (nM)	RET Indication	Dose Limiting Toxicity (Grade 3/4 AEs)	Dose Reduction (% of patients)	Discontinuation Rate (% of patients)	References
Selpercatinib	RET: 0.4 RET^{V804M}: 0.8 RET^{M918T}: 0.7 RET^{V804L}: 0.4	Selective for RET	NSCLC, MTC, TC	Phase II: Hypertension (14%), increased aspartate aminotransferase level (12%), Prolonged QT interval (5%)	NA	NA	[248]
Pralsetinib	RET: 0.4 RET^{V804M}: 0.7 RET^{M918T}: 0.7 RET^{V804L}: 0.3	Selective for RET	NSCLC	No grade 3/4 adverse effects	NA	NA	[249]
3rd Generation							
TPX-0046	RET	SRC	Advanced or metastatic solid tumors with RET mutations and alterations	NA	NA	NA	
BOS172738	RET	NA		NA	NA	NA	
TAS0953	RET	NA		NA	NA	NA	
SL-1001	RET	NA		NA	NA	NA	

AE: Adverse effects; HCC: hepatocellular carcinoma; RCC: renal cell carcinoma; DTC: differentiated thyroid carcinoma; CRC: colorectal cancer; GIST: gastrointestinal stromal tumors; pNET: pancreatic neuroendocrine tumors; MTC: medullary thyroid cancer; ALL: acute lymphoblastic leukemia; CML: chronic myeloid leukemia; NSCLC: non-small cell lung cancer; TC: thyroid cancer; DC: discontinuation; DR: dose reduction; ORR, objective response rate.

* Multikinase inhibitors (MKIs) sorafenib, regorafenib, and sunitinib exhibit RET activity but are not FDA approved for a RET indication. Grade 3/4 adverse effects associated with these MKIs include diarrhea, hypertension, and hand/foot syndrome. Vandetanib, lenvatinib, and cabozantinib are FDA approved for RET driven thyroid cancers but exhibit cardiovascular toxicities with >50% of patients developing Grade 3/4 hypertension at the dose limiting toxicity of vandetanib and lenvatinib. More than 50% of patients taking vandetanib, lenvatinib, and cabozantinib require a dose reduction. Selpercatinib and pralsetinib, second generation RET inhibitors with selectivity for RET and RET mutants, exhibit minor to no grade 3/4 adverse events at therapeutic doses. This supports that the development of selective RET therapies has improved toxicity profiles that demonstrate a significant drop in dose reduction and discontinuation rates at therapeutic doses. This can be attributed to selectivity of the kinase inhibitor for the RET oncogene and RET oncogene mutations.