Title

Targeting the RET tyrosine kinase in neuroblastoma: A review and application of a novel selective drug design strategy

Authors

Steen, Erica A

Basilaia, Mariam

Kim, William

et al.

Publication Date

2023-10-01

DOI

10.1016/j.bcp.2023.115751

Peer reviewed

HHS Public Access

Author manuscript

Biochem Pharmacol. Author manuscript; available in PMC 2024 October 01.

Published in final edited form as:

Biochem Pharmacol. 2023 October ; 216: 115751. doi:10.1016/j.bcp.2023.115751.

Targeting the RET tyrosine kinase in neuroblastoma: A review and application of a novel selective drug design strategy

Erica A. Steen[a], **Mariam Basilaia**[b,e], **William Kim**[c], **Taelor Getz**[a], **Jeffrey L. Gustafson**[b], **Peter E. Zage**[a,d,*]

[a]Department of Pediatrics, Division of Hematology-Oncology, University of California San Diego, La Jolla, CA

[b]Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA

[c]Department of Medicine, University of California San Diego, La Jolla, CA

[d]Peckham Center for Cancer and Blood Disorders, Rady Children's Hospital, San Diego, CA

[e]Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA

Abstract

The *RET* (*RE*arranged during *T*ransfection) gene, which encodes for a transmembrane receptor tyrosine kinase, is an established oncogene associated with the etiology and progression of multiple types of cancer. Oncogenic *RET* mutations and rearrangements resulting in gene fusions have been identified in many adult cancers, including medullary and papillary thyroid cancers, lung adenocarcinomas, colon and breast cancers, and many others. While genetic *RET* aberrations are much less common in pediatric solid tumors, increased RET expression has been shown to be associated with poor prognosis in children with solid tumors such as neuroblastoma, prompting an interest in RET inhibition as a form of therapy for these children. A number of kinase inhibitors currently in use for patients with cancer have RET inhibitory activity, but these inhibitors also display activity against other kinases, resulting in unwanted side effects and limiting their safety and efficacy. Recent efforts have been focused on developing more specific RET inhibitors, but due to high levels of conservation between kinase binding pockets, specificity remains a drug design challenge. Here, we review the background of RET as a potential therapeutic target in neuroblastoma tumors and the results of recent preclinical studies and clinical trials evaluating the safety and efficacy of RET inhibition in adults and children. We also present a novel approach to drug discovery leveraging the chemical phenomenon of atropisomerism to develop specific RET inhibitors and present preliminary data demonstrating the efficacy of a novel RET inhibitor against neuroblastoma tumor cells.

Keywords

Neuroblastoma; RET; Atropisomerism; Getretinib

*Corresponding author. pzage@ucsd.edu (P.E. Zage).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

The *RET* (*RE*arranged during *T*ransfection) gene encodes a transmembrane-spanning receptor tyrosine kinase with restricted tissue expression that plays a critical role in numerous cellular processes. The *RET* proto-oncogene is located on the long arm of chromosome 10 (10q11.2; [1]), and the *RET* gene was initially identified and cloned in 1985 from transformed mouse NIH/3T3 fibroblast cells that underwent DNA rearrangement after transfection with human T-cell lymphoma DNA [2]. The *RET* gene was subsequently found to be homologous to other receptor tyrosine kinase genes [3]. The RET kinase was initially notable for its ability to regulate growth, survival and differentiation of neural-derived cell types, with well-characterized roles in the survival and differentiation of developing neurons in the central (CNS) and peripheral nervous systems (PNS) through binding to neurotrophin ligands, leading to downstream signaling in target cells. However, additional research has also defined roles for RET in other cell types, where it can contribute to cell growth, differentiation, migration, and tissue maturation and other physiological processes such as early embryogenesis, enteric nervous system development, kidney morphogenesis, spermatogenesis, and hematopoiesis.

Aberrant expression and activation of the RET kinase have been shown to be critical drivers of growth and proliferation of cancer cells from a variety of tumors, making RET expression and function potentially valuable therapeutic targets. Prior attempts to inhibit RET for cancer therapy have employed nonselective multi-kinase inhibitors with anti-RET activity, but these agents have multiple kinase targets and have shown limited clinical activity, with the lack of target specificity and consequently increased side effects leading to dose reduction, drug discontinuation, and reduced efficacy in patients. New, more selective RET inhibitors are showing promising efficacy, improved response rates, and more favorable toxicity profiles in early clinical trials. This review discusses the known functional roles of RET in different tumors, focusing on the role of RET expression and activity in the pediatric solid tumor neuroblastoma, and the results of prior clinical trials employing nonselective RET inhibitors in these patients. We also review early results using more selective RET inhibitors as well as describe a novel approach to develop stereoselective agents that are more specific for RET inhibition.

2. RET expression and biology in normal tissues

2.1. RET molecular biology

The unmodified RET receptor is a 120-kDa protein monomer with a 150 kDa immature glycosylated form [4–6], and three distinct isoforms of RET are produced as a result of alternative splicing: RET9 (1072 amino acids), RET43 (1106 amino acids, and RET51 (1114 amino acids). While these isoforms are all co-expressed, the RET9 and RET51 isoforms are by far the most predominant [7]. RET undergoes maturation via glycosylation during transit through the endoplasmic reticulum, resulting in two proteins differing in molecular weight. The 150 kDa protein, present in the cytoplasmic particulate fraction, represents the immature form of RET, whereas the 170 kDa protein, which is transported to the plasma membrane, represents the mature glycosylated form [8]. The RET receptor kinase domain

consists of two lobes connected via a hinge region, with the N-terminal lobe consisting of β-sheets, and the C-terminal lobe containing α-helices. The catalytic cleft containing the ATP binding site is located between these two lobes (Fig. 1). The RET receptor also contains four cadherin-like domains which have been speculated to be associated with cell adhesion, and these cadherin domains harbor 11 out of the 12 glycosylation sites in RET, indicating their significance for RET structure and folding.

The mature RET receptor protein is a subunit of a multi-protein complex that binds growth factors of the glial cell line-derived neurotropic factor (GDNF) family [9]. RET activation is secondary to the formation of this complex, which includes one of four soluble ligands, GDNF, neurturin (NRTN), artemin (ARTN), or persephin (PSPN), and one of four GPI-linked co-receptors (GFRα1–4) [10], leading to RET dimerization and transphosphorylation of tyrosine residues in the intracellular kinase domain (Tyr806, Tyr809, Tyr900, Tyr905, Tyr981, Tyr1015, Tyr1062, Tyr1090, and Tyr1096) (Fig. 1); [11,12]. Dimerization of the RET extracellular domain triggers the activation of its intracellular tyrosine kinase domains, which then transphosphorylate each other [13]. Further studies demonstrated that although GDNF does not bind to RET directly, GDNF family ligand binding is necessary for RET activation [14].

Phosphorylation as well as binding of adaptor proteins to phosphorylated tyrosine residues in the intracellular domain of the RET receptor leads to the activation of various signaling pathways, including the RAS/extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K)/AKT, p38 mitogen-activated protein kinase (p38 MAPK), and c-Jun N-terminal kinase (JNK) pathways [7,15]. Phosphorylation of specific tyrosine residues has been shown to activate specific downstream signaling pathways, and the signaling pathways that become activated following RET ligand binding are highly dependent on intracellular localization. For example, RET localized outside lipid rafts on the cell surface has the capacity to activate SHC (Src Homology 2 domain-Containing), while RET inside lipid rafts can activate fibroblast growth factor receptor substrate 2 (FRS2) [16,17]. RET kinase activity also stimulates the JAK/STAT pathway through the recruitment of adaptor proteins such as SOCS2 and SHP2, contributing to the development and survival of various cell types [18]. Additionally, RET can activate AKT from the cell surface, but the RAS/ERK pathway is activated only after RET has become internalized [11]. After this activation, RET associates with the ubiquitin ligase Cbl and undergoes ubiquitination, leading to RET degradation and downregulation of RET-mediated signaling [19].

2.2. RET cellular functions

RET-mediated activation of multiple downstream signaling pathways impacts many essential cellular functions, such as cell proliferation, differentiation, migration, metabolism, and survival. The RET receptor also provides positional information and plays key roles in cell adhesion and migration in both normal and cancer cells. RET can activate signaling pathways that trigger cell migration in some contexts and survival, proliferation, or differentiation in others [13].

Recently, RET inhibitors have shown efficacy in preventing migration of pancreatic cancer cells, suggesting key roles for RET in cell migration and ultimately tumor invasion

and metastasis [20]. RET depletion also reduced cell migration and induced a flattened epithelial-like morphology in thyroid cancer cells, and RET depletion further decreased the expression of mesenchymal markers and matrix metalloproteinases and reduced invasive potential [21]. Interestingly, RET expression promotes a more mesenchymal phenotype with reduced cell–cell adhesion and increased invasiveness in papillary thyroid cancer cells but is associated with tumor cell survival and proliferation in medullary thyroid cancer cells, suggesting cell-type specific roles for RET [21]. During the process of sympathetic neuron adhesion, cleavage of RET generates an N-terminal truncated fragment that functions as a cadherin accessory protein, modifying the cadherin environment and potentiating cadherin-mediated cell aggregation [22].

RET also induces cell adhesion and migration via the activation of the β1 and β3 integrin subunits *in vitro*, with β1 expression required for RET-induced cell adhesion and migration and with β3 expression correlated with RET-mediated invasion in a mouse tumor xenograft model [23], suggesting that coordinated signaling through these pathways is important for cell interactions with the microenvironment during tumor invasion and progression. In further support of a role for RET activity in cell migration, RET activation leads to cellular focal adhesion formation and to phosphorylation of critical molecules present in focal adhesions, including paxillin, focal adhesion kinase, and p130cas [24].

While these prior studies have identified roles for RET isoforms in cell migration and invasion, previous work demonstrated that RET9 and RET51 isoforms are associated with distinct functions in tumorigenesis, epithelial-mesenchymal transition (EMT) and metastasis, with RET51 expression more strongly correlated with malignant phenotypes and enhanced migration and invasion than RET9 [21]. While the molecular contributions of RET isoforms to tumorigenesis and metastasis are not fully defined, RET9 and RET51 recruit distinct combinations of proteins to promote different signaling events and cellular processes, such as cell proliferation or migration [18,25,26]. Although RET9 is generally more highly expressed, RET51 is significantly more effective at promoting cell proliferation and anchorage-independent growth *in vitro* [21,27–30].

Further studies demonstrated that the RET ligand GDNF promoted activation, interaction and colocalization of the RET51 isoform with Ezrin, an intracellular protein that serves to link cell membrane-associated proteins with the cytoskeleton. GDNF enhanced the formation of actin-rich filopodia containing both RET and Ezrin and promoted RhoA-GTPase activity and chemotaxis, while RET inhibition suppressed filopodia formation, reduced Ezrin colocalization with RET, and impaired cell migration [31]. Together, these results define a role for RET in regulating the mesenchymal gene expression profile and promoting a motile, invasive cell phenotype in thyroid, breast, and pancreatic cancers, where it has been linked to tumor invasion and metastasis [21,32,33].

RET is also known for its essential role in cell survival, but the mechanisms by which RET promotes survival and prevents cell death remain poorly defined. RET depletion increased thyroid cancer cell death, with RET expression specifically associated with tumor cell survival, proliferation and anoikis resistance in medullary thyroid cancer cells [21]. RET cleavage furthermore generates an intracellular domain that can trigger cell

death in apoptotic permissive settings [22]. RET depletion in thyroid cancer cells also increased chemotherapy-induced apoptosis via expression of and direct binding to ATF4, a transcription factor that activates proapoptotic genes NOXA and PUMA [34].

While RET has an important role for the normal development of both the PNS and CNS, RET also has functions outside the nervous system. RET signaling contributes to the regulation and function of hematopoietic cells and spermatogenesis [35,36], and RET has been shown to drive hematopoietic stem cell survival, expansion, and function, with RET ablation in hematopoietic stem cells leading to impaired survival and reduced cell numbers. During *in vitro* expansion, RET is active at the cell surface and mediates sustained cellular growth, resistance to stress, and improved hematopoietic stem cell survival [37]. Interestingly, hematopoietic stem cells deprived of RET retain normal differentiation potential, but display loss of cell-autonomous stress response and reconstitution potential [36].

2.3. RET function in cell and tissue development

The RET kinase has been shown to have critical roles in the normal development of many tissues, including the embryonic nervous system, the neural crest, and the enteric nervous system [4,38,39], along with roles in spermatogenesis [35], renal organogenesis [40], and intestine organogenesis during embryonic life [41]. Loss-of-function RET mutations in humans are associated with intestinal disorders, congenital malformations of the kidney and urinary tract, and congenital hypo-ventilation syndrome [42]. In mouse embryos, transcripts of *RET9* were detected in all cranial ganglia, in sensory and autonomic ganglia of the trunk, in a subset of neurons of the dorsal root ganglion, in motor neurons of the spinal cord, in the developing lungs and excretory systems, in the enteric neuroblasts of the enteric nervous system, and in the thyroid lobes. In contrast, *RET51* expression was weak and restricted to the motor column of the spinal cord, the dorsal root ganglion, the enteric neuroblasts, the lung bud, and the kidney [43]. Transgenic mice expressing a homozygous inactivating RET mutation die soon after birth with renal agenesis and absence of enteric neurons in the digestive tract [44], further demonstrating the significance of RET expression and function in development and organogenesis.

The role of *RET* in CNS and PNS development begins during early embryogenesis. *RET* transcripts were identified in mice beginning at day 8.5 of embryogenesis in PNS and CNS cell lineages as well as in the excretory system. Within the cranial region, *RET* mRNA was restricted to a small population of neural crest cells whereas at later stages, *RET* was observed in all cranial ganglia [45]. *RET* expression appears gradually in all cranial ganglia irrespective of origin of the contributing neural crests, suggesting that *RET* expression is associated with cranial ganglia development [45]. In early murine organogenesis, *RET* expression was observed in a small group of neural crest cells migrating from rhom-bomere 4 (r4) of the hindbrain. *RET* expression was also observed in a region of the epibranchial placodes. In later stage embryos, *RET* expression was shown to be downregulated by the time the r4 crest had completed migration to the second branchial arch.

During intestinal development, normal activity of the RET receptor is required for the migration of enteric nervous system progenitors throughout the gut. In vertebrates, the

enteric nervous system is derived from the vagal neural crest, and RET is required for the directional migration of enteric nervous system progenitors from the neural tube to the gut wall. In the enteric nervous system, *RET* was expressed in the presumptive enteric neuroblasts of the vagal crest (day 9.0–11.5) and in the myenteric ganglia of the gut (day 13.5–14.5) [45]. In zebrafish, enteric progenitor cells and the majority of enteric nervous system neurons express *RET* during development [46]. Loss of function mutations in murine *RET* lead to characteristic defects of neural crest cell migration within the developing gut [47], and loss-of-function *RET* mutations in humans are associated with Hirschsprung disease, a rare congenital anomaly of the enteric nervous system that is characterized by the absence of enteric ganglia in variable lengths of the distal intestinal tract [48,49]. Loss-of-function *RET* mutations also resulted in a failure to colonize the distal colon in transgenic mice, and mice with *RET* mutations displayed reduced proliferation and differentiation of enteric nervous system progenitors in the ganglionic proximal gut [39].

Embryonic kidney development begins with the outgrowth of the ureteric bud. Activation of RET in the ureteric bud epithelium signals through PI3K to control outgrowth and branching morphogenesis [50], and, similar to its function in enteric neuronal precursor cells, activation of RET results in chemotaxis as RET-expressing cells invade the surrounding GDNF-expressing tissue in the developing kidney [51]. RET signaling through transcription factors ETV4 and ETV5 also promotes competitive cell rearrangements in the nephric duct, in which the cells with the highest level of RET signaling preferentially migrate to form the first ureteric bud tip [52,53].

While the role of RET in the development of embryonic tissues has been well established, the physiological role of RET in adult tissues remains unclear, and very little is known about RET function in adulthood. In normal adult human tissues, RET is mainly expressed in normal and malignant cells and organs derived from neural crest cells [54], with high levels of *RET* gene expression only found in a limited number of different human tissues, including the cerebellum and the substantia nigra, the adrenal and pituitary glands, and in C-cells in the thyroid (Fig. 2). No *RET* transcripts were found in a study examining other adult human tissues including the liver, lung, kidney, stomach, duodenum, colon, urinary bladder, spleen, thymus, placenta, uterus, atrium, ventricle, cerebral cortex, and medulla oblongata [54].

3. RET expression and biology in adult cancer

Oncogenic mutations that result in ligand-independent constitutive RET activation have been recognized for many years [55,56], and different oncogenic *RET* mutations are consistently associated with distinct tumor types. *RET* gene fusions and rearrangements, oncogenic *RET* gene mutations, and *RET* overexpression have each been associated with multiple cancers and diseases.

RET fusions from chromosomal rearrangements or inversions are genetic alterations that result in the fusion of the *RET* kinase domain with dimerization motifs from other genes [57], leading to spontaneous cytosolic dimerization and constitutive activation of the RET kinase that promotes sustained intracellular signaling and activation of cell growth and

survival pathways [58]. In human patients, the first oncogenic *RET* gene rearrangement leading to gene fusion of the RET tyrosine kinase domain with the 5′ terminal region of the *CCDC6* gene was identified in a papillary thyroid carcinoma (PTC) tumor [59]. Since then, *RET* gene rearrangements resulting in *RET* fusions have also been identified in up to 70% of PTCs and in 1–3% of non-small-cell lung carcinomas (NSCLC), and are less commonly found in colorectal and breast adenocarcinomas (0.1–0.3%) [57,60,61]. Recent approaches using more sensitive techniques have identified rare *RET* rearrangements in other cancer types including chronic myelogenous leukemia and pancreatic, ovarian, and head and neck tumors [61–66].

Over a dozen of *RET* fusion partner genes have been identified in PTCs to date, with dozens more identified in other tumor types, with the distribution of different gene fusion partners varying among tumor types. The most common *RET* fusions are *RET/PTC1* and *RET/PTC3* in PTC, which account for over 90% of all observed rearrangements and are the result of the fusion of the *RET* gene with either the *CCDC6* or *NCOA4* genes, respectively [67,68]. *RET* rearrangements and fusions have additionally been found much more frequently in childhood PTC patients and in those with prior significant radiation exposure. The most common gene rearrangement in NSCLC results from a fusion of the *KIF5B* gene with *RET* (*KIF5B-RET*) that is rare in other tumor types [69,70]. While RET fusions have not been extensively studied in solid tumors other than thyroid and lung cancer, the *CCDC6-RET*, *NCOA4-RET*, *KIF5B-RET*, and *RASGEF1A-RET* gene fusions have been identified in rare cases of colorectal and breast carcinomas [64,71].

Oncogenic *RET* gene mutations, unlike *RET* gene fusions, are rare outside of neuroendocrine tumors. Hereditary gain-of-function *RET* point mutations are responsible for multiple endocrine neoplasia type 2 (MEN2), a dominant inherited cancer syndrome that affects neuroendocrine organs and that is characterized by constitutive oncogenic RET activation, leading to medullary thyroid cancers (MTC) and adrenal pheochromocytomas [4,33,72]. These mutant RET receptors are able to stimulate unregulated signaling observed with wild-type RET activity from the cell membrane [73,74], and can also associate with RET ligand-GFRα complexes, leading to enhanced activity [75–77]. Genotype-phenotype relationships do exist between *RET* mutations and phenotype in MEN2, with mutations at specific positions being correlated with either MTC, pheochromocytoma, and/or hyperparathyroidism [78], and mutations with increased oncogenic RET activity are associated with more severe disease [78,79]. Mutations in the extracellular cysteine-rich domain of RET, such as C634R, C618Y, and C634Y, are associated with MEN type 2A (MEN2A), while type 2B (MEN2B) is associated with a specific activating RET mutation, M918T, located in the intracellular tyrosine kinase domain. Familial MTC is also associated with various RET mutations, including V804M, Y791F, C634W, E768D, and S891A [80–82].

Oncogenic *RET* point mutations are also the most common mutations identified in sporadic MTC, and these mutations occur in 40–65% of MTCs and are associated with more aggressive disease [33,72,83]. The most common and most aggressive somatic MTC mutation is M918T (exon 16), while a variety of other mutations affecting exons 10, 11

and 15 have been described. Single amino acid substitutions and small insertions/deletions are also associated with sporadic MTC as well as pheochromocytoma [84,85].

In addition to gene fusions and mutations, varying levels of *RET* expression have been identified in several different solid tumor types. *RET* expression occurs in up to 70% of invasive breast cancers and is more commonly found in ER + and HER2 + breast cancers, where it is associated with treatment resistance [86–89]. Increased *RET* expression that is associated with poor prognosis has been observed in 40–60% of breast tumors and in 40–65% pancreatic ductal adenocarcinomas (PDAC)[90–92]. Increased *RET* expression in PDAC has been linked to lymph node metastasis, and decreased *RET* expression reduces pancreatic cell invasion [93]. Similarly, 20–75% of prostate cancers display increased expression of *RET*, where increased *RET* expression is associated with poor tumor differentiation [94]. Melanoma, glioma, renal cell carcinoma, NSCLC, and endometrial and head and neck cancers also show increased *RET* levels [95,96] Interestingly, increased *RET* expression has been found in breast cancer brain metastases compared to the corresponding primary tumors, indicating a potential role for RET in metastasis [97].

4. RET expression and biology in neuroblastoma

4.1. RET associations with neuroblastoma pathogenesis and patient outcomes

In 1990, five years after the discovery of *RET* as an oncogene, a panel of human tumor cell lines was examined for expression of *RET* mRNA. *RET* expression was observed in all 11 neuroblastoma cell lines examined, whereas no detectable levels of *RET* mRNA was observed in 19 non-neuroblastoma tumor cell lines and a human diploid fibroblast line [98]. The specific expression in neuroblastoma caused speculation that RET may have cellular functions specific to neuroblastoma cells or for neuroblastoma oncogenesis. More recent investigations have demonstrated that neuroblastoma tumor cells express significantly higher levels of *RET* when compared across 1378 cancer cell lines from the Cancer Cell Line Encyclopedia (CCLE) (https://depmap.org) that includes gene expression data from a wide range of adult normal and cancer cells [99–101]; (Fig. 3A). However, while RET deregulation in other cancers has been a product of activating mutations or rearrangements, neither of these phenomena are observed in neuroblastoma [102,103]. Early studies also failed to find correlations between *RET* gene expression and neuroblastoma tumor clinical stage, *MYCN* amplification, or patient age [104], and two additional centers found that *RET* mRNA was equally distributed across different neuroblastoma tumor stages [105,106], raising questions about the role of RET in the tumorigenesis of neuroblastoma.

Subsequent studies have further demonstrated *RET* expression on neuroblastoma tumor cells and patient tumor samples [107–111], and have more firmly established the likely functional role of RET in neuroblastoma pathogenesis. Initial studies demonstrated that transgenic mice overexpressing *RET* develop neuroblastoma tumors [112]. *RET* expression was also shown to be associated with increased neuroblastoma metastases *in vivo* [109], and *RET* expression is higher in neuroblastoma tumors from patients with stage 4 and high-risk disease [113]. Neuroblastoma cell lines were also found to be the most sensitive of cancer cell lines in the CCLE to RET depletion with RNAi (Fig. 3B), further reaffirming the important role of RET in neuroblastoma cell viability.

In children with neuroblastoma, recent studies have further demonstrated significant associations between *RET* expression and both clinical and biological prognostic features and with patient outcomes. When expression levels of all receptor tyrosine kinase genes were compared across 10 independent patient cohorts, *RET* ranked as the top kinase whose expression robustly correlated to an unfavorable outcome [99]. Further analyses of gene expression profiles from neuroblastoma patient tumors also demonstrated significant associations between *RET* expression and patient outcomes (Fig. 4A), and elevated *RET* expression was also significantly associated with risk of relapse, *MYCN* amplification, and high-risk tumors (Fig. 4B–D), reaffirming the likely critical role of *RET* in neuroblastoma pathogenesis.

4.2. RET associations with neuroblastoma cell proliferation, metastasis, and differentiation

In addition to the demonstrated associations of *RET* expression with neuroblastoma patient outcomes and prognostic features, the RET kinase has also been shown to play important roles in neuroblastoma cell proliferation, survival, and metastasis. RET is an important mediator of survival, growth, differentiation, and migration of neural crest-derived cells [44,45], and RET signaling through the sonic hedgehog pathway stimulated by GDNF induces neuroblastoma cell proliferation and tumor growth [116]. GDNF was also shown to induce neuroblastoma cell proliferation by targeting and activating p70S6 kinase independent of the RAS/ERK signaling pathway [117] and by inducing GFRα1 clustering and RET activation [118]. RET signaling has also been shown to prevent neuroblastoma cell death induced by retinoic acid treatment [119]. RET expression may also contribute to neuroblastoma metastasis, as RET expression promotes non-adherent growth of the NB-39-nu neuroblastoma cell line [120] and neuroblastoma cells expressing the MEN2B oncogenic mutant *RET* were able to grow more readily in suspension and induced metastatic tumors at a significantly higher rate than control mice [109].

During normal development, RET kinase signaling also is essential for the differentiation of neural crest cells into mature neurons and other cell types, and *RET* expression in neural crest-derived cells is important for the maturation of sympathetic neurons [121]. In mouse tissues, elevated *RET* expression has been identified in enteric, sympathetic, motor, sensory, dopaminergic, and adrenergic neurons, further suggesting a functional role for the RET kinase in neuronal differentiation [122]. In neuroblastoma cell lines, retinoic acid treatment induced neurite outgrowth, increased neurofilament gene expression, and increased *RET* expression, demonstrating the association of *RET* expression with neuroblastoma tumor differentiation [123]. Neuroblastoma cell lines transfected with oncogenic *RET* also displayed a reduced growth rate and acquired a neurite-bearing phenotype, with enhanced expression of neuroblastoma differentiation markers [124]. Furthermore, induction of *RET* expression by retinoic acid occurred in advance of differentiation and in the absence of *de novo* protein synthesis, indicating that the positive transcriptional regulation of *RET* is closely associated with early neuronal differentiation [125].

More recent studies demonstrated that, in neuroblastoma tumor cells, retinoic acid-induced differentiation was mediated by a positive autocrine loop that sustained RET

downstream signaling and was dependent on GDNF expression and release, suggesting that RET activation is an upstream mechanism necessary to mediate retinoic acid-induced differentiation [126]. RET gene and protein expression were also primarily found in both neuroblastoma tumor and normal cells with gangliocytic differentiation and also identified *RET* as a consistently upregulated gene in neuroblastoma cells undergoing differentiation induced by retinoic acid treatment, with *RET* depletion resulting in inhibition of morphologic differentiation as well as reduced expression of differentiation marker genes [127].

4.3. RET signaling in neuroblastoma

The associations of RET with neuroblastoma cell growth and survival are likely mediated by a number of downstream signaling pathways that are activated by RET kinase ligand binding and autophosphorylation, including the PI3K/AKT, RAS/ERK, c-Jun NH2-terminal kinase (JNK), JAK/STAT, and p38 MAPK pathways [15,128]. Autophosphorylation sites within the intracellular tyrosine residues of RET serve as docking sites for downstream signaling effectors carrying Src homology 2 (SH2) or phosphotyrosine-binding (PTB) domains. Recruitment of PTB domain-containing adaptor protein SHC results in activation of the RAS/ERK and PI3K/AKT pathways, whereas recruitment of FRS2 activates the RAS/ERK and PI3K/AKT pathways, and studies have shown that competitive recruitment of SHC mediates cell survival signaling from RET whereas an engineered RET that recruits only FRS2 does not [129]. SHC recruitment by RET is both required and sufficient for cell survival partly via activation of PI3K/AKT but possibly also via other SHC-activated signaling pathways, such as NF-κB [6]. SHCD interaction with RET has also been shown to inhibit RAS/ERK and PI3K/AKT signaling and to reduce neuroblastoma cell viability and migration [130].

RET activity has also been shown to be mediated by interactions with other cell surface receptors in neuroblastoma cells, such as the anaplastic lymphoma kinase (ALK) and the TRK receptors TrkA and TrkB. *ALK* is altered by gain-of-function point mutations in over 10% of high-risk neuroblastoma tumors [131], and ALK activation induces RET upregulation in mouse sympathetic ganglia and in murine and human neuroblastoma tumors [132]. Neuroblastoma cells with mutant *ALK* also were found to have increased expression of *RET* and RET-driven sympathetic neuronal markers along with altered RAS/ERK pathway activity [133]. The ERK-ETV5-RET pathway has also been identified as a critical axis driving neuroblastoma oncogenesis downstream of activated ALK. ETV5 is a transcription factor regulated both at the protein and mRNA levels by ALK activity, and ETV5 has been shown to regulate *RET* expression in a MEK/ERK dependent manner [134]. RET and TrkA have also been shown to interact, with NGF-mediated TrkA activation inducing RET phosphorylation and ARTN-mediated RET activation leading to TrkA phosphorylation in neuroblastoma cells and with RET and TrkA co-expression in patient tumors [135].

5. RET inhibition for cancer therapy

5.1. RET inhibition in adult cancer patients

With the expanding knowledge of the critical roles that RET kinase expression and activity play in a variety of cancers, RET inhibition has become an increasingly important therapeutic strategy. Initial efforts to target RET activity utilized multikinase inhibitors originally developed to inhibit other kinases but that also inhibit RET activity [42,136]. The multikinase inhibitors vandetanib, a VEGFR2/EGFR/RET inhibitor, and cabozantinib, a VEGFR2/MET/RET inhibitor, have been approved by the FDA for treatment of patients with advanced thyroid cancers and have recently been evaluated in clinical trials in adults with lung and breast cancers, where some patients have experienced partial responses but with limited overall clinical benefit [68,69,137–142]. A number of other multikinase inhibitors with RET inhibitory activity, including agerafenib, alectinib, lenvatinib, ponatinib, and sorafenib, are currently in clinical trials or are undergoing additional preclinical testing for RET-associated cancers [42,143]. Unfortunately, use of these multikinase inhibitors has been associated with significant side effects, including hypertension, nausea, diarrhea, skin rash, fatigue, decreased appetite, and weight loss, likely due to the inhibition of alternative kinase targets and frequently leading to treatment dose reduction or discontinuation [42,136,144]. Furthermore, recent reports of acquired resistance due to the acquisition of additional gene mutations such as the Val804 gatekeeper mutation or other oncogenic events in patients treated with these agents [68,145] suggest that new and more selective RET inhibitors are more likely to improve patient outcomes.

Recently, a number of new kinase inhibitors with increased specificity for RET inhibition have been developed and are undergoing preclinical and clinical testing. Pralsetinib (BLU-667) and selpercatinib (LOXO-292) each have more than 100-fold greater selectivity for RET [145] and have recently been been given breakthrough designations by the FDA in 2020 for treatment of patients with thyroid cancers with oncogenic RET mutations [146,147], as they have demonstrated high rates of disease responses even in the presence of gatekeeper mutations combined with greater patient tolerability. Additional efforts have been made to design and synthesize selective RET inhibitors based off of the structure of pralsetinib, one of which displayed increased potency and decreased “off-target” effects compared to pralsetinib [148]. However, these RET-selective inhibitors still interact with other intracellular proteins, including off-target kinases such as DDR1, JAK1, and TRKC [149], and additional resistance mechanisms such as RET solvent front mutations have evolved in some patients [150], demonstrating the need for new strategies to inhibit RET and leading to ongoing efforts towards designing novel, highly selective RET inhibitors.

5.2. RET inhibition in children with neuroblastoma and other solid tumors

A number of preclinical studies have demonstrated the potential efficacy of RET inhibition against neuroblastoma cells and tumors, and the sensitivity of neuroblastoma cell lines in the CCLE to RET depletion (Fig. 3B) further emphasizes the potential efficacy of RET inhibition for neuroblastoma patients. An early study revealed that the multikinase inhibitor vandetanib, an inhibitor of VEGFR2, EGFR, and RET, was able to inhibit RET phosphorylation in neuroblastoma cells and to reduce tumor cell

viability *in vitro*. Additionally, in a human neuroblastoma xenograft model, vandetanib inhibited tumor growth by 85% [110]. A follow-up study reported that the combination of vandetanib and 13-*cis*-retinoic acid demonstrated synergistic reduction in viability and growth inhibition [111], leading to a single-institution phase I clinical trial testing the combination of vandetanib with 13-*cis*-retinoic acid in children with recurrent neuroblastoma (NCT00533169). 10 patients between the ages of 3–26 years were enrolled and received either 50 (patients 1–7) or 65 mg/m^2 (patients 8–10) vandetanib daily with 80 mg/m^2 13-*cis*-retinoic acid given twice daily for 14 consecutive days out of each cycle. Patients had received between 2 and 9 prior chemotherapy regimens prior to enrollment. Study treatment was generally well tolerated, with one patient experiencing dose-limiting toxicity (grade 3 hemorrhage). 2 patients had prolonged stable disease (12 and 16 weeks), suggesting the potential efficacy of this combination in the treatment of neuroblastoma [151].

Sunitinib is a multikinase inhibitor that has been shown to inhibit the activity of a wide range of kinases in addition to RET, including PDGFRα, PDGFRβ, Flt-3,VEGFR-1, VEGFR-2, and VEGFR-3. While initial preclinical testing showed that sunitinib demonstrated little tumor growth inhibition against a panel of neuroblastoma cell lines [152], follow-up studies demonstrated that sunitinib treatment inhibited neuroblastoma tumor growth, angiogenesis, and metastasis in tumor xenograft models [153,154], leading to a phase I clinical trial evaluating sunitinib in children with recurrent solid tumors through the Children's Oncology Group (NCT00387920). Two children with recurrent neuroblastoma out of 23 total patients were enrolled. No objective responses were observed in any enrolled patients and the study was unfortunately limited by the development of cardiac toxicity in patients previously exposed to cardiotoxic treatment [155].

Cabozantinib is another multikinase inhibitor that targets RET as well as MET, VEGFR2, FLT3, and c-KIT, and cabozantinib was also shown to be effective against neuroblastoma cell lines and xenograft tumors alone and in combination with 13-*cis*-retinoic acid, with reduced RET and ERK phosphorylation in cell lines most sensitive to cabozantinib [156,157]. Cabozantinib was subsequently evaluated in a phase I clinical trial for children with recurrent solid tumors through the Children's Oncology Group (NCT01709435), which enrolled 41 total patients, including three with neuroblastoma. Cabozantinib was well tolerated, with observed dose-limiting toxicities including hypertension, PRES, headache, elevated liver enzymes and proteinuria. 10 patients experienced partial responses or had prolonged stable disease, although none of the 10 patients had neuroblastoma [158].
A follow-up institutional case series reported results of four children with recurrent neuroblastoma treated at the recommended cabozantinib dose of 40 mg/m^2/day [159]. All four children experienced extended disease control, with two who experienced complete responses and two with prolonged stable disease. Two patients required dose reduction due to toxicity. These promising results have led to follow-up studies, including an ongoing phase II clinical trial evaluating cabozantinib for children with relapsed or refractory neuroblastoma that is positive for RET or MET mutations (NCT02867592) and a phase 1 study investigating the safety and tolerability of cabozantinib combined with 13-*cis*-retinoic acid (NCI03611595).

While the multikinase inhibitor sorafenib was initially developed as an inhibitor of the RAS-ERK signaling pathway, further studies established that sorafenib inhibited a number of additional kinases, including VEGFR1–3, PDGFRβ, c-Kit, and RET [160], and initial studies demonstrated the efficacy of sorafenib against neuroblastoma cells and tumors [161,162]. While initial phase 1 and 2 clinical trials for children with recurrent solid tumors did not include any patients diagnosed with neuroblastoma [163,164]; (NCT01445080, NCT01502410), a subsequent case series of four children with recurrent neuroblastoma treated with sorafenib showed transient antitumor activity, but with disease progression observed in all four patients within 4 weeks [165].

Other more recently developed multikinase inhibitors with RET inhibitory activity, including agerafenib, alectinib, lenvatinib, ponatinib, and regorafenib, have also proven to be effective against neuroblastoma cells and tumors *in vitro* and *in vivo*. While ponatinib was found to be effective against neuroblastoma cells and tumors [166] and to possess more significant efficacy in neuroblastoma models compared to other similar kinase inhibitors [167,168], clinical trials have primarily focused on patients with leukemias that are likely to respond to ponatinib's activity against both wild-type and treatment-resistant ABL kinase, and so the safety and efficacy of ponatinib in children with neuroblastoma tumors remain unknown. The newer multikinase inhibitor agerafenib was also found to be effective against neuroblastoma in preclinical models [169,170], but has not yet been evaluated in clinical trials for children with neuroblastoma. Regorafenib is a multikinase inhibitor that is FDA approved for the treatment of metastatic colorectal cancer and that targets angiogenic (VEGFR1–3, TIE2), stromal (PDGFR-β, FGFR), and oncogenic receptor tyrosine kinases (KIT, RET, and RAF) [171]. Regorafenib has been shown to be effective against neuroblastoma cells and tumors *in vitro* and *in vivo*, with regorafenib treatment leading to reduced activity of a number of intracellular signaling pathways, including the RAS/ERK, PI3K/AKT/mTOR and Fos/Jun pathways [111,172]. A phase 1 clinical trial for children with recurrent or refractory solid tumors (NCT02085148) found that regorafenib was well tolerated, with dose-limiting toxicities including thrombocytopenia, hypertension, and skin rash, but only one child with neuroblastoma was included out of 41 enrolled patients and did not receive any noted clinical benefit [173]. Alectinib represents a promising therapy for neuroblastoma treatment due to its unique combined activity against RET and ALK. Alectinib was also found to be effective against neuroblastoma cells and tumors [174,175], and in a recent case report, alectinib treatment was associated with a partial response in a child with recurrent metastatic neuroblastoma [176]. An ongoing phase 1 clinical trial for children with recurrent solid tumors (NCT04774718) will hopefully provide further support for the use of alectinib in children with neuroblastoma.

Recent preclinical studies have begun to explore the efficacy of the specific RET inhibitors pralsetinib and selpercatinib against neuroblastoma. Preliminary results have confirmed RET inhibition and efficacy against neuroblastoma cells by pralsetinib *in vitro* [177], but pralsetinib has not yet been evaluated for safety and efficacy in children with neuroblastoma. Conversely, selpercatinib has been approved by the FDA for use in any RET-fusion driven cancer and was found to be effective in children with MEN2 [178]. Early phase clinical trials evaluating the efficacy of selpercatinib in children with recurrent solid tumors are ongoing, but results for children with neuroblastoma have not yet been reported [179,180].

6. Development of novel RET inhibitors using atropisomerism

6.1. Novel drug design strategy for the stereoselective inhibition of RET

Prior studies detailed above have demonstrated the efficacy of multiple RET inhibitors against NB both *in vitro* and *in vivo* through decreased viability and induction of apoptosis. However, these inhibitors frequently also inhibit other kinases in critical signaling pathways which may contribute to their antitumor effects but which also lead to well-characterized adverse events in patients and do not prevent the development of resistance mechanisms. Therefore, efforts towards designing highly selective RET inhibitors are of increasing interest in both adult and pediatric cancers because of their anticipated improved efficacy and safety. However, developing highly selective kinase inhibitors remains a challenge in drug design due to the high degree of active site conformations among kinases.

Kinase inhibitor specificity is determined by three-dimensional drug structure, and unstable atropisomerism is innate in many common scaffolds in drug discovery, commonly existing as freely rotating aryl – aryl bonds. Such compounds can access the majority of dihedral conformations around the bond axis; however, most small molecules bind their target within a narrow range of these available conformations. The remaining accessible conformations can interact with other proteins leading to compound promiscuity and reduced specificity (Fig. 5).

Atropisomerism, first observed in 1922 by Christie and Kenner [181], is a stereochemical phenomenon that arises due to asymmetry around a chemical bond. Atropisomers are stereoisomers formed by a spontaneous hindered rotation, typically around an sp2-sp2 axis. Atropisomers were classified by LaPlante into three classes based on their rates of racemization at physiological conditions [182]. Class-1 atropisomers have barriers to rotation ($\Delta G^{\pm}_{rot}$) < 20 kcal/mol racemize on the second or less time scale and are treated as achiral molecules. Class-2 atropisomers ($\Delta G^{\pm}_{rot}$ = 20–29 kcal/mol) have half-lives to racemization varying from hours to days at room temperature. Class-3 atropisomers ($\Delta G^{\pm}_{rot}$ ≥ 29 kcal/mol) do not racemize at physiological conditions and are considered to exist as stable enantiomers or diastereomers [183]. Around 30% of small molecules that were FDA-approved between 2019 and early 2022 possess at least one atropisomeric axis [184]. The majority of these small molecule drugs exist as class-1 atropisomers, however, they bind their targets in single set of chiral conformations [185]. Hypothetically, therefore, the target selectivity of a promiscuous lead compound could be improved by locking the inhibitor into an atropisomeric conformation preferred by the targeted kinase, thereby precluding any off-target inhibition contributed by other conformations of the molecule.

Proof of concept for this hypothesis was obtained by turning promiscuous, rapidly interconverting pyrrolopyrimidine (PPY) kinase inhibitors, a common class of multi-kinase inhibitors (i.e. PP1) [186], into atropisomerically stable analogs by the strategic addition of two chlorine atoms at the ortho position. These synthesized class-3 analogs demonstrated superior target selectivity, and different atropisomers of the same compounds displayed varying kinase inhibition profiles.

This discovery prompted the idea to improve the selectivity and potency of a lead compound towards the RET kinase. A series of in silico docking models of the lead inhibitor into the crystallographic model of RET demonstrated that the electron-withdrawing chlorine at the C2 position reduced the hydrogen bonding interaction between the active site and N-5 on the ligand (Fig. 6). Replacing this chlorine atom with an electron-donating methyl group significantly increased hydrogen bonding strength, resulting in an increase in potency towards RET. The docking experiments also revealed that the hydrophobic pocket in the RET kinase active site could better accommodate polycyclic aromatic groups in (R)-conformation, leading to the replacement of the benzyl group with the naphthyl group. Further analysis suggested that the non-conserved serine (Ser891) in the kinase active site could potentially engage in hydrogen bonding with the ligand. This insight led to the replacement of the naphthyl group with a quinoline moiety to successfully facilitate the hydrogen bonding interaction between nitrogen on the ring and Ser891. The final synthesized product, (R_a)-getretinib, demonstrated enhanced efficacy and was 35 times more potent at RET inhibition compared to the (S_a)-**2** atropisomer, with R-getretinib exhibiting RET inhibitory activity at 8 nM, compared to 292 nM for *S*-getretinib (Fig. 6). Both atropisomers of each analog were synthesized and tested in cells demonstrating different kinase inhibition profiles [187].

6.2. R-getretinib reduces neuroblastoma cell confluence and RET phosphorylation

R-getretinib therefore is a novel inhibitor of RET kinase that leverages atropisomerism through the restriction of accessible low-energy dihedral conformations otherwise available to a more promiscuous compound to achieve a highly potent and ultra-selective kinase inhibitor. Getretinib possesses promising efficacy in models of RET-driven cancer [187], and R-getretinib possesses similar potency and improved selectivity compared to that of other next generation RET inhibitors. However, getretinib is half the molecular weight and possesses significantly improved ligand efficiencies towards RET. Getretinib is effective against RET-driven cancer cells, with calculated IC50 values comparable to that of vandetanib, without the non-RET mediated activities (Fig. 7).

To evaluate the efficacy of R- and S-getretinib against neuroblastoma cells, a panel of established human neuroblastoma cell lines representing a range of biological phenotypes (SK-N-AS, SK-N-BE(2), CHP134, CHP212, IMR-32, Kelly, NBL-S, NGP, and SK-N-SH) were obtained, validated, and maintained as published [169,172]. Cells were tested for sensitivity *in vitro* to both S- and R-getretinib and monitored by continuous live-cell imaging. R-getretinib reduced cell confluence in a dose-dependent manner in all tested cell lines, while S-getretinib had no significant effect on cell confluence over time at any tested doses (Fig. 8A,B). R-getretinib treatment also induced notable morphologic changes in all tested cell lines (Fig. 8C). To determine whether R- or S-getretinib could inhibit RET kinase activation, we evaluated neuroblastoma cells after R- and S-getretinib treatment for inhibition of RET phosphorylation. R-getretinib treatment of neuroblastoma cells resulted in reduced phosphorylation of RET in a dose-dependent manner, while treatment with S-getretinib resulted in paradoxical increase in RET phosphorylation (Fig. 8D), suggesting that specific inhibition of the RET kinase is likely responsible for the demonstrated efficacy

of R-getretinib against neuroblastoma and that R-getretinib represents a promising novel inhibitor with significant potential for safety and efficacy in children with neuroblastoma.

7. Concluding remarks and future directions

Neuroblastoma is the most common extracranial solid tumor in children and accounts for approximately 10% of new pediatric malignancies diagnosed each year. Aggressive, high-risk neuroblastoma tumors respond poorly to therapy, and refractory and recurrent neuroblastoma respond even more poorly to salvage therapy. Novel therapies directed against biologically relevant targets are clearly needed for these children. While neuroblastoma tumors generally are not associated with oncogenic *RET* gene mutations or fusions, increased *RET* expression is a feature of high-risk neuroblastoma tumors and is associated with poor patient outcomes. Therefore, RET inhibition represents a promising strategy for neuroblastoma therapy.

While multikinase inhibitors with activity against RET and more selective RET inhibitors both demonstrate efficacy against neuroblastoma in *in vitro* and *in vivo* models, responses in patients with neuroblastoma and other solid tumors remain poor, and further research into optimal treatment strategies, mechanisms of drug resistance, long-term consequences of potent RET inhibition, and development of more effective agents against emerging mutations are clearly needed. However, designing inhibitors with high selectivity remain a challenge.

Here, we review the role of RET in normal cells and tissues and in the development and progression of adult cancers. We further review the role of RET expression and activity in the pediatric solid tumor neuroblastoma and review recent preclinical and clinical studies evaluating currently available RET inhibitors. We have developed a novel strategy for generating specific RET kinase inhibitors using the chemical property of atropisomerism. We present R-getretinib as a highly potent and highly selective inhibitor of RET and have shown its selectivity and efficacy in models of adult cancers as well as in *in vitro* models of neuroblastoma. The high selectivity of R-getretinib towards RET has potential to minimize unwanted side effects caused by off-target kinase binding, thereby increasing the potential for clinical utility. While additional work is needed to further characterize the role of RET overexpression in neuroblastoma, our results provide preliminary evidence that highly selective inhibition of RET via atropisomerically stable R-getretinib holds great promise as a form of therapy in RET-driven cancers.

Despite the promise of targeted therapy for RET-driven cancers, the longer term effects of RET inhibition in normal tissues will need to be carefully monitored, particularly for children with cancer. RET signaling is essential for nervous system development and has critical roles in the maintenance and survival of mature nervous system tissue. RET signaling is also critical for hematopoietic stem cell maintenance, and prolonged inhibition of these signals may compromise either or both nervous system function or hematopoietic cell development and negatively impact patient long-term outcomes, particularly with inhibitors that are readily able to cross the blood–brain barrier.

Alternative strategies to target RET expression and activity and avoid the development of treatment resistance are actively being employed. A number of drug combinations are currently being evaluated for synergistic efficacy in clinical trials for adults and children. Antibody-drug conjugates targeted against RET or GFRα1 are also being tested in preclinical models, and additional studies have employed chimeric antigen receptor (CAR)-T cells to directly target RET via immune system activation. Further efforts to develop inhibitors specific for RET include the development of proteolysis-targeting chimeras (PROTACs) that could specifically and effectively target the RET kinase for proteasomal or lysosomal degradation, thereby eliminating or minimizing the oncogenic effects of *RET* overexpression.

In summary, the roles of RET expression and activity in a wide range of cancers has been clearly established over the past several decades. While the development of selective RET inhibitors represents an important clinical advance with significant benefits for patients, the need for new and more effective therapies has driven ongoing research into novel approaches toward targeting the RET kinase. The future treatment for many solid tumors is likely to incorporate many of these novel treatment strategies to inhibit RET activity, hopefully leading to improved patient outcomes in the future.

Acknowledgements

This project was supported by funding from a Hyundai Impact grant (to PEZ), a UCSD/SDSU Pilot grant (to PEZ, JLG), and NIGMS R35GM124637 (to JLG).

Data availability

Data will be made available on request.

Abbreviations:

ALK	anaplastic lymphoma kinase
ARTN	artemin
ATCC	American Type Culture Collection
CCLE	Cancer Cell Line Encyclopedia
CNS	Central nervous system
DMEM	Dulbecco’s Modified Eagle’s Medium
EMT	epithelial/mesenchymal transition
ERK	extracellular signal-regulated kinase
FBS	fetal bovine serum
FRS2	fibroblast growth factor receptor substrate 2
GDNF	glial cell line-derived neurotropic factor

GFL	growth factor ligand
JNK	c-Jun N-terminal kinase
p38 MAPK	p38 mitogen-activated protein kinase
MTC	medullary thyroid carcinoma
MTT	3-(4,5 dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide
NRTN	neurturin
NSCLC	non-small-cell lung carcinoma
PDAC	pancreatic ductal adenocarcinoma
PI3K	phosphatidylinositol 3-kinase
PNS	Peripheral nervous system
PSPN	persephin
PTB	phosphotyrosine-binding
PTC	papillary thyroid carcinoma
RET	*RE*arranged during *T*ransfection
SH2	Src Homology 2
SHC	Src Homology 2 domain-Containing

References

[1]. Ceccherini I, Bocciardi R, Luo Y, Pasini B, Hofstra R, Takahashi M, Romeo G, Exon structure and flanking intronic sequences of the human RET proto-oncogene, Biochem. Biophys. Res. Commun 196 (1993) 1288–1295, 10.1006/bbrc.1993.2392. [PubMed: 7902707]

[2]. Takahashi M, Ritz J, Cooper GM, Activation of a novel human transforming gene, ret, by DNA rearrangement, Cell 42 (1985) 581–588, 10.1016/0092-8674(85)90115-1. [PubMed: 2992805]

[3]. Takahashi M, Cooper GM, ret transforming gene encodes a fusion protein homologous to tyrosine kinases, Mol. Cell Biol 7 (1987) 1378–1385, 10.1128/mcb.7.4.1378-1385.1987. [PubMed: 3037315]

[4]. Arighi E, Borrello MG, Sariola H, RET tyrosine kinase signaling in development and cancer, Cytok. Growth Factor Rev 16 (2005) 441–467, 10.1016/j.cytogfr.2005.05.010.

[5]. Anders J, Kjær S, Ibáñez CF, Molecular modeling of the extracellular domain of the RET receptor tyrosine kinase reveals multiple cadherin-like domains and a calcium-binding site, J. Biol. Chem 276 (2001) 35808–35817, 10.1074/jbc.M104968200. [PubMed: 11445581]

[6]. Ibanez CF, Structure and physiology of the RET receptor tyrosine kinase, Cold Spring Harb. Perspect. Biol 5 (2) (2013) a009134. [PubMed: 23378586]

[7]. Takahashi M, Kawai K, Asai N, Roles of the RET Proto-oncogene in cancer and development, JMA J 3 (2020) 175–181, 10.31662/jmaj.2020-0021. [PubMed: 33150251]

[8]. Miyazaki K, Asai N, Iwashita T, Taniguchi M, Isomura T, Funahashi H, Takagi H, Matsuyama M, Takahashi M, Tyrosine kinase activity of the ret proto-oncogene products in vitro, Biochem. Biophys. Res. Commun 193 (2) (1993) 565–570. [PubMed: 7685595]

[9]. Santoro M, Melillo RM, Carlomagno F, Vecchio G, Fusco A, Minireview: RET: normal and abnormal functions, Endocrinology 145 (2004) 5448–5451, 10.1210/en.2004-0922. [PubMed: 15331579]

[10]. Airaksinen MS, Titievsky A, Saarma M, GDNF family neurotrophic factor signaling: four masters, one servant? Mol. Cell. Neurosci 13 (1999) 313–325, 10.1006/mcne.1999.0754. [PubMed: 10356294]

[11]. Richardson DS, Lai AZ, Mulligan LM, RET ligand-induced internalization and its consequences for downstream signaling, Oncogene 25 (2006) 3206–3211, 10.1038/sj.onc.1209349. [PubMed: 16418724]

[12]. Kawamoto Y, Takeda K, Okuno Y, Yamakawa Y, Ito Y, Taguchi R, Kato M, Suzuki H, Takahashi M, Nakashima I, Identification of RET autophosphorylation sites by mass spectrometry, J. Biol. Chem 279 (14) (2004) 14213–14224. [PubMed: 14711813]

[13]. Runeberg-Roos P, Saarma M, Neurotrophic factor receptor RET: structure, cell biology, and inherited diseases, Ann. Med 39 (2007) 572–580, 10.1080/07853890701646256. [PubMed: 17934909]

[14]. Takahashi M, RET receptor signaling: Function in development, metabolic disease, and cancer, Proc. Jpn. Acad. Ser. B Phys. Biol. Sci 98 (2022) 112–125, 10.2183/pjab.98.008.

[15]. Ichihara M, Murakumo Y, Takahashi M, RET and neuroendocrine tumors, Cancer Lett 204 (2004) 197–211, 10.1016/S0304-3835(03)00456-7. [PubMed: 15013219]

[16]. Paratcha G, Ledda F, Baars L, et al. , Released GFRalpha1 potentiates downstream signaling, neuronal survival, and differentiation via a novel mechanism of recruitment of c-Ret to lipid rafts, Neuron 29 (2001) 171–184, 10.1016/s0896-6273(01)00188-x. [PubMed: 11182089]

[17]. Kurokawa K, Iwashita T, Murakami H, Hayashi H, Kawai K, Takahashi M, Identification of SNT/FRS2 docking site on RET receptor tyrosine kinase and its role for signal transduction, Oncogene 20 (16) (2001) 1929–1938. [PubMed: 11360177]

[18]. 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, J. Biol. Chem 285 (2010) 31867–31875, 10.1074/jbc.M110.144923. [PubMed: 20682772]

[19]. Hyndman BD, Crupi MJF, Peng S, Bone LN, Rekab AN, Lian EY, Wagner SM, Antonescu CN, Mulligan LM, Differential recruitment of E3 ubiquitin ligase complexes regulates RET isoform internalization, J. Cell Sci 130 (2017) 3282–3296, 10.1242/jcs.203885. [PubMed: 28794017]

[20]. Funahashi H, Okada Y, Sawai H, Takahashi H, Matsuo Y, Takeyama H, Manabe T, The role of glial cell line-derived neurotrophic factor (GDNF) and integrins for invasion and metastasis in human pancreatic cancer cells, J. Surg. Oncol 91 (2005) 77–83, 10.1002/jso.20277. [PubMed: 15999351]

[21]. Lian EY, Maritan SM, Cockburn JG, Kasaian K, Crupi MJ, Hurlbut D, Jones SJ, Wiseman SM, Mulligan LM, Differential roles of RET isoforms in medullary and papillary thyroid carcinomas, Endocr. Relat. Cancer 24 (2017) 53–69, 10.1530/ERC-16-0393. [PubMed: 27872141]

[22]. Cabrera JR, Bouzas-Rodriguez J, Tauszig-Delamasure S, Mehlen P, RET modulates cell adhesion via its cleavage by caspase in sympathetic neurons, J. Biol. Chem 286 (2011) 14628–14638, 10.1074/jbc.M110.195461. [PubMed: 21357690]

[23]. Cockburn JG, Richardson DS, Gujral TS, Mulligan LM, RET-mediated cell adhesion and migration require multiple integrin subunits, J. Clin. Endocrinol. Metab 95 (2010) E342–E346, 10.1210/jc.2010-0771. [PubMed: 20702524]

[24]. Murakami H, Iwashita T, Asai N, Iwata Y, Narumiya S, Takahashi M, Rho-dependent and -independent tyrosine phosphorylation of focal adhesion kinase, paxillin and p130Cas mediated by Ret kinase, Oncogene 18 (1999) 1975–1982, 10.1038/sj.onc.1202514. [PubMed: 10208419]

[25]. Tsui-Pierchala BA, Ahrens RC, Crowder RJ, Milbrandt J, Johnson EM Jr., The long and short isoforms of Ret function as independent signaling complexes, J. Biol. Chem 277 (2002) 34618–34625, 10.1074/jbc.M203580200. [PubMed: 12091387]

[26]. Crupi MJF, Maritan SM, Reyes-Alvarez E, Lian EY, Hyndman BD, Rekab AN, Moodley S, Antonescu CN, Mulligan LM, GGA3-mediated recycling of the RET receptor tyrosine

kinase contributes to cell migration and invasion, Oncogene 39 (2020) 1361–1377, 10.1038/s41388-019-1068-z. [PubMed: 31645646]

[27]. Pasini A, Geneste O, Legrand P, Schlumberger M, Rossel M, Fournier L, Rudkin BB, Schuffenecker I, Lenoir GM, Billaud M, Oncogenic activation of RET by two distinct FMTC mutations affecting the tyrosine kinase domain, Oncogene 15 (1997) 393–402, 10.1038/sj.onc.1201199. [PubMed: 9242375]

[28]. Rossel M, Pasini A, Chappuis S, et al. , Distinct biological properties of two RET isoforms activated by MEN 2A and MEN 2B mutations, Oncogene 14 (1997) 265–275, 10.1038/sj.onc.1200831. [PubMed: 9018112]

[29]. Iwashita T, Kato M, Murakami H, Asai N, Ishiguro Y, Ito S, Iwata Y, Kawai K, Asai M, Kurokawa K, Kajita H, Takahashi M, Biological and biochemical properties of Ret with kinase domain mutations identified in multiple endocrine neoplasia type 2B and familial medullary thyroid carcinoma, Oncogene 18 (1999) 3919–3922, 10.1038/sj.onc.1202742. [PubMed: 10445857]

[30]. Le Hir H, Charlet-Berguerand N, Gimenez-Roqueplo A, Mannelli M, Plouin P, de Franciscis V, Thermes C, Relative expression of the RET9 and RET51 isoforms in human pheochromocytomas, Oncology 58 (2000) 311–318, 10.1159/000012118. [PubMed: 10838497]

[31]. Moodley S, Lian EY, Crupi MJF, Hyndman BD, Mulligan LM, RET isoform-specific interaction with scaffold protein Ezrin promotes cell migration and chemotaxis in lung adenocarcinoma, Lung Cancer 142 (2020) 123–131, 10.1016/j.lungcan.2020.02.004. [PubMed: 32146264]

[32]. 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, J. Natl Cancer Inst 102 (2010) 107–118, 10.1093/jnci/djp456. [PubMed: 20068194]

[33]. Mulligan LM, RET revisited: expanding the oncogenic portfolio, Nat. Rev. Cancer 14 (2014) 173–186, 10.1038/nrc3680. [PubMed: 24561444]

[34]. Bagheri-Yarmand R, Sinha KM, Gururaj AE, Ahmed Z, Rizvi YQ, Huang SC, Ladbury JE, Bogler O, Williams MD, Cote GJ, Gagel RF, A novel dual kinase function of the RET proto-oncogene negatively regulates activating transcription factor 4-mediated apoptosis, J. Biol. Chem 290 (2015) 11749–11761, 10.1074/jbc.M114.619833. [PubMed: 25795775]

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

[36]. 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 514 (7520) (2014) 98–101. [PubMed: 25079320]

[37]. Grey W, Chauhan R, Piganeau M, Huerga Encabo H, Garcia-Albornoz M, McDonald NQ, Bonnet D, Activation of the receptor tyrosine kinase RET improves long-term hematopoietic stem cell outgrowth and potency, Blood 136 (22) (2020) 2535–2547. [PubMed: 32589703]

[38]. Marcos C, Pachnis V, The effect of the ret-mutation on the normal development of the central and parasympathetic nervous systems, Int. J. Dev. Biol Suppl 1 (1996) 137S–138S. [PubMed: 9087731]

[39]. Natarajan D, McCann C, Dattani J, Pachnis V, Thapar N, Multiple Roles of Ret Signalling During Enteric Neurogenesis, Front. Mol. Neurosci 15 (2022), 832317, 10.3389/fnmol.2022.832317. [PubMed: 35694443]

[40]. Ivanchuk SM, Eng C, Cavenee WK, Mulligan LM, The expression of RET and its multiple splice forms in developing human kidney, Oncogene 14 (1997) 1811–1818, 10.1038/sj.onc.1201016. [PubMed: 9150387]

[41]. 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 Dev. 15 (18) (2001) 2433–2444. [PubMed: 11562352]

[42]. Drilon A, Hu ZI, Lai GGY, Tan DSW, Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes, Nat. Rev. Clin. Oncol 15 (2018) 151–167, 10.1038/nrclinonc.2017.188. [PubMed: 29134959]

[43]. Lee KY, Samy ET, Sham MH, Tam PKH, Lui VCH, 3' Splicing variants of ret receptor tyrosine kinase are differentially expressed in mouse embryos and in adult mice, Biochim. Biophys. Acta 1627 (2003) 26–38, 10.1016/s0167-4781(03)00068-x. [PubMed: 12759189]

[44]. 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 367 (6461) (1994) 380–383. [PubMed: 8114940]

[45]. Pachnis V, Mankoo B, Costantini F, Expression of the c-ret proto-oncogene during mouse embryogenesis, Dev. Camb. Engl 119 (4) (1993) 1005–1017, 10.1242/dev.119.4.1005.

[46]. Bandla A, Melancon E, Taylor CR, Davidson AE, Eisen JS, Ganz J, A new transgenic tool to study the ret signaling pathway in the enteric nervous system, Int. J. Mol. Sci 23 (2022) 15667, 10.3390/ijms232415667. [PubMed: 36555308]

[47]. Natarajan D, Marcos-Gutierrez C, Pachnis V, de Graaff E, Requirement of signalling by receptor tyrosine kinase RET for the directed migration of enteric nervous system progenitor cells during mammalian embryogenesis, Dev Camb Engl 129 (2002) 5151–5160, 10.1242/dev.129.22.5151.

[48]. Badner JA, Sieber WK, Garver KL, Chakravarti A, A genetic study of Hirschsprung disease, Am. J. Hum. Genet 46 (1990) 568–580. [PubMed: 2309705]

[49]. Parisi MA, Kapur RP, Genetics of Hirschsprung disease, Curr. Opin. Pediatr 12 (2000) 610–617, 10.1097/00008480-200012000-00017. [PubMed: 11106284]

[50]. Tang MJ, Cai Y, Tsai SJ, Wang YK, Dressler GR, Ureteric bud outgrowth in response to RET activation is mediated by phosphatidylinositol 3-kinase, Dev. Biol 243 (2002) 128–136, 10.1006/dbio.2001.0557. [PubMed: 11846482]

[51]. Kim D, Dressler GR, PTEN modulates GDNF/RET mediated chemotaxis and branching morphogenesis in the developing kidney, Dev. Biol 307 (2007) 290–299, 10.1016/j.ydbio.2007.04.051. [PubMed: 17540362]

[52]. Costantini F, GDNF/Ret signaling and renal branching morphogenesis: From mesenchymal signals to epithelial cell behaviors, Organogenesis 6 (2010) 252–262, 10.4161/org.6.4.12680. [PubMed: 21220964]

[53]. Lu BC, Cebrian C, Chi X, Kuure S, Kuo R, Bates CM, Arber S, Hassell J, MacNeil L, Hoshi M, Jain S, Asai N, Takahashi M, Schmidt-Ott KM, Barasch J, D'Agati V, Costantini F, Etv4 and Etv5 are required downstream of GDNF and Ret for kidney branching morphogenesis, Nat. Genet 41 (2009) 1295–1302, 10.1038/ng.476. [PubMed: 19898483]

[54]. Takaya K, Yoshimasa T, Arai H, Tamura N, Miyamoto Y, Itoh H, Nakao K, Expression of the RET proto-oncogene in normal human tissues, pheochromocytomas, and other tumors of neural crest origin, J. Mol. Med. (Berl) 74 (10) (1996) 617–621. [PubMed: 8912182]

[55]. Fusco A, Grieco M, Santoro M, Berlingieri MT, Pilotti S, Pierotti MA, Porta GD, Vecchio G, A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases, Nature 328 (6126) (1987) 170–172. [PubMed: 3600795]

[56]. Mulligan LM, Kwok JBJ, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, Ponder MA, Telenius H, Tunnacliffe A, Ponder BAJ, Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A, Nature 363 (6428) (1993) 458–460. [PubMed: 8099202]

[57]. Romei C, Ciampi R, Elisei R, A comprehensive overview of the role of the RET proto-oncogene in thyroid carcinoma, Nat. Rev. Endocrinol 12 (2016) 192–202, 10.1038/nrendo.2016.11. [PubMed: 26868437]

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

[59]. Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della Ports G, Fusco A, Vecchiot G, PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas, Cell 60 (4) (1990) 557–563. [PubMed: 2406025]

[60]. Stransky N, Cerami E, Schalm S, Kim JL, Lengauer C, The landscape of kinase fusions in cancer, Nat. Commun 5 (2014) 4846, 10.1038/ncomms5846. [PubMed: 25204415]

[61]. Kato S, Subbiah V, Marchlik E, Elkin SK, Carter JL, Kurzrock R, RET aberrations in diverse cancers: next-generation sequencing of 4,871 patients, Clin. Cancer Res 23 (2017) 1988–1997, 10.1158/1078-0432.CCR-16-1679. [PubMed: 27683183]

[62]. Ballerini P, Struski S, Cresson C, Prade N, Toujani S, Deswarte C, Dobbelstein S, Petit A, Lapillonne H, Gautier E-F, 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 26 (11) (2012) 2384–2389. [PubMed: 22513837]

[63]. 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 9 (51) (2018) 29654–29664. [PubMed: 30038711]

[64]. 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, Nat. Commun 9 (1) (2018), 10.1038/s41467-018-07341-4.

[65]. 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, Ann. Oncol 29 (6) (2018) 1394–1401. [PubMed: 29538669]

[66]. 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, Baňěckova 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, Am. J. Surg. Pathol 42 (11) (2018) 1445–1455. [PubMed: 30045065]

[67]. Li AY, McCusker MG, Russo A, Scilla KA, Gittens A, Arensmeyer K, Mehra R, Adamo V, Rolfo C, RET fusions in solid tumors, Cancer Treat. Rev 81 (2019) 101911. [PubMed: 31715421]

[68]. Vodopivec DM, Hu MI, RET kinase inhibitors for RET-altered thyroid cancers, Ther Adv Med Oncol 14 (2022), 10.1177/17588359221101691.

[69]. Ferrara R, Auger N, Auclin E, Besse B, Clinical and translational implications of RET rearrangements in non-small cell lung cancer, J. Thorac. Oncol 13 (2018) 27–45, 10.1016/j.jtho.2017.10.021. [PubMed: 29128428]

[70]. 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 C-C, Karp DD, Van Den Heuvel M, Wakelee HA, Neal JW, Mok TSK, Yang JCH, Ou S-H, 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 J-Y, 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, J. Clin. Oncol 35 (13) (2017) 1403–1410. [PubMed: 28447912]

[71]. 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 6 (30) (2015) 28929–28937. [PubMed: 26078337]

[72]. Wells SA, Advances in the management of MEN 2, Endocr. Relat. Cancer 25 (2018) T1–T13, 10.1530/ERC-17-0325. [PubMed: 29142004]

[73]. Asai N, Iwashita T, Matsuyama M, Takahashi M, Mechanism of activation of the ret proto-oncogene by multiple endocrine neoplasia 2A mutations, Mol. Cell Biol 15 (1995) 1613–1619, 10.1128/MCB.15.3.1613. [PubMed: 7532281]

[74]. Santoro M, Carlomagno F, Romano A, Bottaro DP, Dathan NA, Grieco M, Fusco A, Vecchio G, Matoskova B, Kraus MH, Di Fiore PP, Activation of RET as a dominant transforming gene

by germline mutations of MEN2A and MEN2B, Science 267 (5196) (1995) 381–383. [PubMed: 7824936]

[75]. Bongarzone I, Vigano' E, Alberti L, Borrello MG, Pasini B, Greco A, Mondellini P, Smith DP, Ponder BAJ, Romeo G, Pierotti MA, Full activation of MEN2B mutant RET by an additional MEN2A mutation or by ligand GDNF stimulation, Oncogene 16 (18) (1998) 2295–2301. [PubMed: 9620546]

[76]. Cranston AN, Carniti C, Oakhill K, Radzio-Andzelm E, Stone EA, McCallion AS, et al. , RET is constitutively activated by novel tandem mutations that alter the active site resulting in multiple endocrine neoplasia type 2B, Cancer Res 66 (2006) 10179–10187, 10.1158/0008-5472.CAN-06-0884. [PubMed: 17047083]

[77]. Gujral TS, Singh VK, Jia Z, Mulligan LM, Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2B, Cancer Res 66 (2006) 10741–10749, 10.1158/0008-5472.CAN-06-3329. [PubMed: 17108110]

[78]. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, et al. , The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2: international RET Mutation Consortium, J. Am. Med. Assoc 276 (1996) 1575–1579.

[79]. 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 25 (6) (2015) 567–610. [PubMed: 25810047]

[80]. Krampitz GW, Norton JA, RET gene mutations (genotype and phenotype) of multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma, Cancer 120 (13) (2014) 1920–1931, 10.1002/cncr.28661. [PubMed: 24699901]

[81]. Dabir T, Hunter SJ, Russell CF, McCall D, Morrison PJ, The RET mutation E768D confers a late-onset familial medullary thyroid carcinoma – only phenotype with incomplete penetrance: implications for screening and management of carrier status, Fam. Cancer 5 (2006) 201–204, 10.1007/s10689-006-6990-x. [PubMed: 16736292]

[82]. Qi X-P, Zhao J-Q, Chen Z-G, Cao J-L, Du J, Liu N-F, Li F, Sheng M, Fu E.r., Guo J, Jia H, Zhang Y-M, Ma J-M, RET mutation p. S891A in a Chinese family with familial medullary thyroid carcinoma and associated cutaneous amyloidosis binding OSMR variant p.G513D, Oncotarget 6 (32) (2015) 33993–34003. [PubMed: 26356818]

[83]. Vuong HG, Odate T, Ngo HTT, Pham TQ, Tran TTK, Mochizuki K, et al. , Clinical significance of RET and RAS mutations in sporadic medullary thyroid carcinoma: a meta-analysis, Endocr. Relat. Cancer 25 (2018) 633–641, 10.1530/ERC-18-0056. [PubMed: 29615431]

[84]. Salvatore D, Santoro M, Schlumberger M, The importance of the RET gene in thyroid cancer and therapeutic implications, Nat. Rev. Endocrinol 17 (2021) 296–306, 10.1038/s41574-021-00470-9. [PubMed: 33603219]

[85]. Komminoth P, Roth J, Muletta-Feurer S, Saremaslani P, Seelentag WK, Heitz PU, RET proto-oncogene point mutations in sporadic neuroendocrine tumors, J. Clin. Endocrinol. Metab 81 (1996) 2041–2046, 10.1210/jcem.81.6.8964826. [PubMed: 8964826]

[86]. Morandi A, Martin L-A, Gao Q, Pancholi S, Mackay A, Robertson D, et al. , GDNF-RET signaling in ER-positive breast cancers is a key determinant of response and resistance to aromatase inhibitors, Cancer Res 73 (2013) 3783–3795, 10.1158/0008-5472.CAN-12-4265. [PubMed: 23650283]

[87]. Esseghir S, Todd SK, Hunt T, Poulsom R, Plaza-Menacho I, Reis-Filho JS, et al. , A role for glial cell derived neurotrophic factor induced expression by inflammatory cytokines and RET/GFR alpha 1 receptor up-regulation in breast cancer, Cancer Res 67 (2007) 11732–11741, 10.1158/0008-5472.CAN-07-2343. [PubMed: 18089803]

[88]. Plaza-Menacho I, Morandi A, Robertson D, Pancholi S, Drury S, Dowsett M, Martin L-A, 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 29 (33) (2010) 4648–4657. [PubMed: 20531297]

[89]. 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 Mol. Med 5 (9) (2013) 1335–1350. [PubMed: 23868506]

[90]. Gattelli A, Hynes NE, Schor IE, Vallone SA, Ret receptor has distinct alterations and functions in breast cancer, J. Mammary Gland Biol. Neoplasia 25 (2020) 13–26, 10.1007/s10911-020-09445-4. [PubMed: 32080788]

[91]. 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, J. Int. Med. Res 36 (4) (2008) 656–664. [PubMed: 18652760]

[92]. Ito Y, Okada Y, Sato M, Sawai H, Funahashi H, Murase T, Hayakawa T, Manabe T, Expression of glial cell line-derived neurotrophic factor family members and their receptors in pancreatic cancers, Surgery 138 (4) (2005) 788–794. [PubMed: 16269310]

[93]. 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 36 (23) (2017) 3232–3239. [PubMed: 28092668]

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

[95]. Mulligan LM, GDNF and the RET receptor in cancer: new insights and therapeutic potential, Front. Physiol 9 (2018) 1873, 10.3389/fphys.2018.01873. [PubMed: 30666215]

[96]. Tan L, Hu Y, Tao Y, Wang B, Xiao J, Tang Z, Lu T, Tang H, Expression and copy number gains of the RET gene in 631 early and mid stage non-small cell lung cancer cases, Thorac Cancer 9 (4) (2018) 445–451. [PubMed: 29473341]

[97]. Varěslija D, Priedigkeit N, Fagan A, et al. , Transcriptome characterization of matched primary breast and brain metastatic tumors to detect novel actionable targets, J. Natl Cancer Inst 111 (2019) 388–398, 10.1093/jnci/djy110. [PubMed: 29961873]

[98]. Ikeda I, Ishizaka Y, Tahira T, Suzuki T, Onda M, Sugimura T, Nagao M, Specific expression of the ret proto-oncogene in human neuroblastoma cell lines, Oncogene 5 (1990) 1291–1296. [PubMed: 2216455]

[99]. Rozen EJ, Shohet JM, Systematic review of the receptor tyrosine kinase superfamily in neuroblastoma pathophysiology, Cancer Metastasis Rev 41 (2022) 33–52, 10.1007/s10555-021-10001-7. [PubMed: 34716856]

[100]. McFarland JM, Ho ZV, Kugener G, Dempster JM, Montgomery PG, Bryan JG, Krill-Burger JM, Green TM, Vazquez F, Boehm JS, Golub TR, Hahn WC, Root DE, Tsherniak A, Improved estimation of cancer dependencies from large-scale RNAi screens using model-based normalization and data integration, Nat. Commun 9 (2018) 4610, 10.1038/s41467-018-06916-5. [PubMed: 30389920]

[101]. Ghandi M, Huang FW, Jańe-Valbuena J, Kryukov GV, Lo CC, McDonald ER 3rd, Barretina J, Gelfand ET, Bielski CM, Li H, Hu K, Andreev-Drakhlin AY, Kim J, Hess JM, Haas BJ, Aguet F, Weir BA, Rothberg MV, Paolella BR, Lawrence MS, Akbani R, Lu Y, Tiv HL, Gokhale PC, de Weck A, Mansour AA, Oh C, Shih J, Hadi K, Rosen Y, Bistline J, Venkatesan K, Reddy A, Sonkin D, Liu M, Lehar J, Korn JM, Porter DA, Jones MD, Golji J, Caponigro G, Taylor JE, Dunning CM, Creech AL, Warren AC, McFarland JM, Zamanighomi M, Kauffmann A, Stransky N, Imielinski M, Maruvka YE, Cherniack AD, Tsherniak A, Vazquez F, Jaffe JD, Lane AA, Weinstock DM, Johannessen CM, Morrissey MP, Stegmeier F, Schlegel R, Hahn WC, Getz G, Mills GB, Boehm JS, Golub TR, Garraway LA, Sellers WR, Next-generation characterization of the cancer cell line encyclopedia, Nature 569 (2019) 503–508, 10.1038/s41586-019-1186-3. [PubMed: 31068700]

[102]. Peaston AE, Camacho ML, Norris MD, Haber M, Marsh DJ, Robinson BG, Hyland VJ, Marshall GM, Absence of MEN2A- or 2B-type RET mutations in primary neuroblastoma tumour tissue, Mol. Cell. Probes 12 (4) (1998) 239–242. [PubMed: 9727201]

[103]. Hofstra RMW, Stulp RP, Stelwagen T, Buys CHCM, Ching Cheng N, Caron H, Westerveld A, Versteeg R, Hansen C, Tommerup N, Clausen N, No mutations found by RET mutation scanning in sporadic and hereditary neuroblastoma, Hum. Genet 97 (3) (1996) 362–364. [PubMed: 8786083]

[104]. Nagao M, Ishizaka Y, Nakagawara A, Kohno K, Kuwano M, Tahira T, Itoh F, Ikeda I, Sugimura T, Expression of ret proto-oncogene in human neuroblastomas, Jpn. J. Cancer Res 81 (4) (1990) 309–312. [PubMed: 1694838]

[105]. Borrello MG, Bongarzone I, Plerotti MA, Luksch R, Gasparini M, Collini P, Pllotti S, Rlzzetti MG, Mondellini P, De Bernardi B, Di Martino D, Garaventa A, Brisigotti M, Tonini GP, trk and ret proto-oncogene expression in human neuroblastoma specimens: high frequency of trk expression in non-advanced stages, Int. J. Cancer 54 (4) (1993) 540–545. [PubMed: 8514446]

[106]. Shimada A, Hirato J, Kuroiwa M, Kikuchi A, Hanada R, Wakai K, Hayashi Y, Expression of KIT and PDGFR is associated with a good prognosis in neuroblastoma, Pediatr. Blood Cancer 50 (2) (2008) 213–217. [PubMed: 17941064]

[107]. Hishiki T, Nimura Y, Isogai E, Kondo K, Ichimiya S, Nakamura Y, Ozaki T, Sakiyama S, Hirose M, Seki N, Takahashi H, Ohnuma N, Tanabe M, Nakagawara A, Glial cell line-derived neurotrophic factor/neurturin-induced differentiation and its enhancement by retinoic acid in primary human neuroblastomas expressing c-Ret, GFRα−1, and GFRα−2, Cancer Res 58 (1998) 2158–2165. [PubMed: 9605760]

[108]. Nakamura T, Ishizaka Y, Nagao M, Hara M, Ishikawa T, Expression of the RET Proto-oncogene product in human normal and neoplastic tissues of neural crest origin, J. Pathol 172 (1994) 255–260, 10.1002/path.1711720305. [PubMed: 8195928]

[109]. Marshall GM, Peaston AE, Hocker JE, Smith SA, Hansford LM, Tobias V, et al. , Expression of Multiple Endocrine Neoplasia 2B RET in Neuroblastoma Cells Alters Cell Adhesion in Vitro, Enhances Metastatic Behavior in Vivo, and Activates Jun Kinase, Cancer Res 57 (1997) 5399–5405. [PubMed: 9393766]

[110]. Beaudry P, Nilsson MB, Rioth M, et al. , Potent antitumor effects of ZD6474 on neuroblastoma via dual targeting of tumor cells and tumor endothelium, Mol. Cancer Ther 7 (2008) 418–424, 10.1158/1535-7163.MCT-07-0568. [PubMed: 18245671]

[111]. Zage PE, Zeng L, Palla S, Fang W, Nilsson MB, Heymach JV, Zweidler-McKay PA, A novel therapeutic combination for neuroblastoma: The VEGFR/EGFR/RET inhibitor vandetanib with 13-cis-retinoic acid, Cancer 116 (2010) 2465–2475, 10.1002/cncr.25017. [PubMed: 20225331]

[112]. Iwamoto T, Taniguchi M, Wajjwalku W, Nakashima I, Takahashi M, Neuroblastoma in a transgenic mouse carrying a metallothionein/ret fusion gene, Br. J. Cancer 67 (1993) 504–507, 10.1038/bjc.1993.94. [PubMed: 8439501]

[113]. Chen Z, Zhao Y, Yu Y, Pang JC, Woodfield SE, Tao L, Guan S, Zhang H, Bieerkehazhi S, Shi Y, Patel R, Vasudevan SA, 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 8 (61) (2017) 104090–104103. [PubMed: 29262623]

[114]. Dempster JM, Boyle I, Vazquez F, Root DE, Boehm JS, Hahn WC, Tsherniak A, McFarland JM, Chronos: a cell population dynamics model of CRISPR experiments that improves inference of gene fitness effects, Genome Biol 22 (2021) 343. [PubMed: 34930405]

[115]. Woodfield SE, Guo RJ, Liu Y, Major AM, Hollingsworth EF, Indiviglio S, Whittle SB, Mo Q, Bean AJ, Ittmann M, Lopez-Terrada D, Zage PE, Neuroblastoma patient outcomes, tumor differentiation, and ERK activation are correlated with expression levels of the ubiquitin ligase UBE4B, Genes Cancer 7 (1–2) (2016) 13–26. [PubMed: 27014418]

[116]. Ruan H, Luo H, Wang J, Ji X, Zhang Z, Wu J, Zhang X, Wu X, Smoothened-independent activation of hedgehog signaling by rearranged during transfection promotes neuroblastoma cell proliferation and tumor growth, Biochim. Biophys. Acta 1860 (9) (2016) 1961–1972. [PubMed: 27316313]

[117]. Hirata Y, Kiuchi K, Mitogenic effect of glial cell line-derived neurotrophic factor is dependent on the activation of p70S6 kinase, but independent of the activation of ERK and up-regulation of Ret in SH-SY5Y cells, Brain Res 983 (2003) 1–12, 10.1016/s0006-8993(03)02837-3. [PubMed: 12914961]

[118]. Kang MY, Kim KY, Yoon Y, Kang Y, Kim HB, Youn CK, Kim DH, Kim MH, Ape1/Ref-1 Stimulates GDNF/GFRalpha1-mediated Downstream Signaling and Neuroblastoma Proliferation, Korean J. Physiol. Pharmacol 13 (2009) 349–356, 10.4196/kjpp.2009.13.5.349. [PubMed: 19915696]

[119]. Takada N, Isogai E, Kawamoto T, Nakanishi H, Todo S, Nakagawara A, Retinoic acid-induced apoptosis of the CHP134 neuroblastoma cell line is associated with nuclear accumulation of p53 and is rescued by the GDNF/Ret signal, Med. Pediatr. Oncol 36 (2001) 122–126, 10.1002/1096-911X(20010101)36:1<122::AID-MPO1029>3.0.CO;2-R. [PubMed: 11464863]

[120]. Futami H, Sakai R, RET protein promotes non-adherent growth of NB-39-nu neuroblastoma cell line, Cancer Sci 100 (2009) 1034–1039, 10.1111/j.1349-7006.2009.01143.x. [PubMed: 19320641]

[121]. Enomoto H, Crawford PA, Gorodinsky A, Heuckeroth RO, Johnson EM Jr, Milbrandt J, RET signaling is essential for migration, axonal growth and axon guidance of developing sympathetic neurons, Development 128 (2001) 3963–3974, 10.1242/dev.128.20.3963. [PubMed: 11641220]

[122]. Zhou Y, Kato H, Asanoma K, et al. , Identification of FOXC1 as a TGF-beta1 responsive gene and its involvement in negative regulation of cell growth, Genomics 80 (2002) 465–472. [PubMed: 12408963]

[123]. Ikuno N, Shimokawa I, Nakamura T, Ishizaka Y, Ikeda T, Ret-oncogene expression correlates with neuronal differentiation of neuroblastic tumors, Pathol. Res. Pract 191 (1995) 92–99, 10.1016/S0344-0338(11)80558-3. [PubMed: 7567689]

[124]. D'Alessio A, De Vita G, Calì G, et al. , Expression of the RET oncogene induces differentiation of SK-N-BE neuroblastoma cells, Cell Growth Differ 6 (1995) 1387–1394. [PubMed: 8562477]

[125]. Bunone G, Borrello MG, Picetti R, Bongarzone I, Peverali FA, de Franciscis V, Valle GD, Pierotti MA, Induction of RET proto-oncogene expression in neuroblastoma cells precedes neuronal differentiation and is not mediated by protein synthesis, Exp. Cell Res 217 (1) (1995) 92–99. [PubMed: 7867726]

[126]. Cerchia L, D'Alessio A, Amabile G, et al. An autocrine loop involving ret and glial cell-derived neurotrophic factor mediates retinoic Acid-induced neuroblastoma cell differentiation. Mol Cancer Res 2006; 4: 481–8. 10.1158/1541-7786.MCR-06-0050. [PubMed: 16849523]

[127]. Oppenheimer O, Cheung NK, Gerald WL. The RET oncogene is a critical component of transcriptional programs associated with retinoic acid-induced differentiation in neuroblastoma. Mol Cancer Ther 2007;6(4):1300–1309. 10.1158/1535-7163.MCT-06-0587. [PubMed: 17431108]

[128]. Hansford JR, Mulligan LM, Multiple Endocrine Neoplasia type 2 and RET: from neoplasia to neurogenesis, J. Med. Genet 37 (2000) 817–827, 10.1136/jmg.37.11.817. [PubMed: 11073534]

[129]. Lundgren TK, Scott RP, Smith M, Pawson T, Ernfors P, Engineering the recruitment of phosphotyrosine binding domain-containing adaptor proteins reveals distinct roles for RET receptor-mediated cell survival, J. Biol. Chem 281 (2006) 29886–29896, 10.1074/jbc.M600473200. [PubMed: 16847065]

[130]. Mabruk ZA, Ahmed SBM, Thomas AC, Prigent SA, The role of the ShcD and RET interaction in neuroblastoma survival and migration, Biochem. Biophys. Rep 13 (2018) 99–108, 10.1016/j.bbrep.2018.01.007. [PubMed: 29556564]

[131]. Trigg RM, Turner SD, ALK in Neuroblastoma: Biological and Therapeutic Implications, Cancers 10 (2018) 113, 10.3390/cancers10040113. [PubMed: 29642598]

[132]. Cazes A, Lopez-Delisle L, Tsarovina K, Pierre-Eugène C, De Preter K, Peuchmaur M, Nicolas A, Provost C, Louis-Brennetot C, Daveau R, Kumps C, Cascone I, Schleiermacher G, Prignon A, Speleman F, Rohrer H, Delattre O, Janoueix-Lerosey I, Activated Alk triggers prolonged neurogenesis and Ret upregulation providing a therapeutic target in ALK-mutated neuroblastoma, Oncotarget 5 (9) (2014) 2688–2702. [PubMed: 24811913]

[133]. Lambertz I, Kumps C, Claeys S, et al. Upregulation of MAPK Negative Feedback Regulators and RET in Mutant ALK Neuroblastoma: Implications for Targeted Treatment. Clin Cancer Res 2015; 21: 3327–3339. 10.1158/1078-0432.CCR-14-2024. [PubMed: 25805801]

[134]. Lopez-Delisle L, Pierre-Eugène C, Louis-Brennetot C, Surdez D, Raynal V, Baulande S, Boeva V, Grossetête-Lalami S, Combaret V, Peuchmaur M, Delattre O, Janoueix-Lerosey I, Activated ALK signals through the ERK-ETV5-RET pathway to drive neuroblastoma oncogenesis, Oncogene 37 (11) (2018) 1417–1429. [PubMed: 29321660]

[135]. Tetri LH, Kolla V, Golden RL, Iyer R, Croucher JL, Choi JH, Macfarland SP, Naraparaju K, Guan P, Nguyen F, Gaonkar KS, Raman P, Brodeur GM, RET receptor expression and interaction

with TRK receptors in neuroblastomas, Oncol. Rep 44 (2020) 263–272, 10.3892/or.2020.7583. [PubMed: 32319659]

[136]. Belli C, Anand S, Gainor JF, Penault-Llorca F, Subbiah V, Drilon A, Andrè F, Curigliano G. Progresses Toward Precision Medicine in RET-altered Solid Tumors. Clin Cancer Res 2020; 26: 6102–6111. 10.1158/1078-0432.CCR-20-1587. [PubMed: 32665298]

[137]. 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, J. Clin. Oncol 30 (2) (2012) 134–141. [PubMed: 22025146]

[138]. Drilon A, Rekhtman N, Arcila M, Wang L.u., 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, Lancet Oncol. 17 (12) (2016) 1653–1660. [PubMed: 27825636]

[139]. Schlumberger M, Elisei R, Müller S, Schöffski P, Brose M, Shah M, Licitra L, Krajewska J, Kreissl MC, Niederle B, Cohen EEW, Wirth L, Ali H, Clary DO, Yaron Y, Mangeshkar M, Ball D, Nelkin B, Sherman S, Overall survival analysis of EXAM, a phase III trial of cabozantinib in patients with radiographically progressive medullary thyroid carcinoma, Ann. Oncol 28 (11) (2017) 2813–2819. [PubMed: 29045520]

[140]. Yoh K, Seto T, Satouchi M, Nishio M, Yamamoto N, Murakami H, Nogami N, Matsumoto S, Kohno T, Tsuta K, Tsuchihara K, Ishii G, Nomura S, Sato A, Ohtsu A, Ohe Y, Goto K, Vandetanib in patients with previously treated RET-rearranged advanced non-small-cell lung cancer (LURET): an open-label, multicentre phase 2 trial, Lancet Respir. Med 5 (1) (2017) 42–50. [PubMed: 27825616]

[141]. Miller KD, Trigo JM, Wheeler C, Barge A, Rowbottom J, Sledge G, Baselga J. A multicenter phase II trial of ZD6474, a vascular endothelial growth factor receptor-2 and epidermal growth factor receptor tyrosine kinase inhibitor, in patients with previously treated metastatic breast cancer. Clin Cancer Res 2005; 11: 3369–76. 10.1158/1078-0432.CCR-04-1923. [PubMed: 15867237]

[142]. 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 SI, 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, Mazìeres J, Drilon A, Targeting RET in Patients With RET-Rearranged Lung Cancers: Results From the Global, Multicenter RET Registry. J Clin Oncol 35 (2017) 1403–1410, 10.1200/JCO.2016.70.9352. [PubMed: 28447912]

[143]. Redaelli S, Plaza-Menacho I, Mologni L, Novel targeted therapeutics for MEN2, Endocr. Relat. Cancer 25 (2018) T53–T68, 10.1530/ERC-17-0297. [PubMed: 29348306]

[144]. Thein KZ, Velcheti V, Mooers BHM, Wu J, Subbiah V, Precision therapy for RET-altered cancers with RET inhibitors, Trends Cancer 7 (2021) 1074–1088, 10.1016/j.trecan.2021.07.003. [PubMed: 34391699]

[145]. 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, Ann. Oncol 29 (2018) 1869–1876, 10.1093/annonc/mdy137. [PubMed: 29912274]

[146]. 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. Clin Cancer Res 2021; 27: 2130–2135. 10.1158/1078-0432.CCR-20-3558. [PubMed: 33239432]

[147]. Kim J, Bradford D, Larkins E, Pai-Scherf LH, Chatterjee S, Mishra-Kalyani PS, Wearne E, Helms WS, Ayyoub A, Bi Y, Sun J, Charlab R, Liu J, Zhao H, Liang D, Ghosh S, Philip

R, Pazdur R, Theoret MR, Beaver JA, Singh H. FDA Approval Summary: Pralsetinib for the Treatment of Lung and Thyroid Cancers With RET Gene Mutations or Fusions. Clin Cancer Res 2021; 27: 5452–5456. 10.1158/1078-0432.CCR-21-0967. [PubMed: 34045295]

[148]. Luo Z, Wang L, Fu Z, Shuai B, Luo M, Hu G, Chen J, Sun J, Wang J, Li J, Chen S, Zhang Y, Discovery and optimization of selective RET inhibitors via scaffold hopping, Bioorg. Med. Chem. Lett 47 (2021) 128149. [PubMed: 34058344]

[149]. 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 SI, 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 Discov 2018; 8: 836–849. 10.1158/2159-8290.CD-18-0338. [PubMed: 29657135]

[150]. 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 SJ, 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, J. Thorac. Oncol 15 (2020) 541–549, 10.1016/j.jtho.2020.01.006. [PubMed: 31988000]

[151]. Zage PE, Zeng L, Palla S, Fang W, Nilsson MB, Heymach JV, Zweidler-McKay PA, The Multi-Kinase Inhibitor Vandetanib with 13-cis-Retinoic Acid: A Novel Therapeutic Combination for Neuroblastoma, Pediatr. Blood Cancer 52 (2009) 691.

[152]. Maris JM, Courtright J, Houghton PJ, Morton CL, Kolb EA, Lock R, Tajbakhsh M, Reynolds CP, Keir ST, Wu J, Smith MA, Initial testing (stage 1) of sunitinib by the pediatric preclinical testing program, Pediatr. Blood Cancer 51 (1) (2008) 42–48. [PubMed: 18293383]

[153]. Zhang L, Smith KM, Chong AL, Stempak D, Yeger H, Marrano P, Thorner PS, Irwin MS, Kaplan DR, Baruchel S, In vivo antitumor and antimetastatic activity of sunitinib in preclinical neuroblastoma mouse model, Neoplasia 11 (2009) 426–435, 10.1593/neo.09166. [PubMed: 19412427]

[154]. Calero R, Morchon E, Johnsen JI, Serrano R, Castresana JS, Sunitinib suppress neuroblastoma growth through degradation of MYCN and inhibition of angiogenesis, PLoS One 9 (4) (2014) e95628. [PubMed: 24759734]

[155]. Dubois SG, Shusterman S, Ingle AM, Ahern CH, Reid JM, Wu B, Baruchel S, Glade-Bender J, Ivy P, Grier HE, Adamson PC, Blaney SM. Phase I and pharmacokinetic study of sunitinib in pediatric patients with refractory solid tumors: a children's oncology group study. Clin Cancer Res 2011; 17: 5113–22. 10.1158/1078-0432.CCR-11-0237. [PubMed: 21690570]

[156]. Daudigeos-Dubus E, Le Dret L, Bawa O, Opolon P, Vievard A, Villa I, Bosq J, Vassal G, Geoerger B, Dual inhibition using cabozantinib overcomes HGF/MET signaling mediated resistance to pan-VEGFR inhibition in orthotopic and metastatic neuroblastoma tumors, Int. J. Oncol 50 (1) (2017) 203–211. [PubMed: 27922668]

[157]. Zhang L, Scorsone K, Woodfield SE, Zage PE, Sensitivity of neuroblastoma to the novel kinase inhibitor cabozantinib is mediated by ERK inhibition, Cancer Chemother. Pharmacol 76 (2015) 977–987, 10.1007/s00280-015-2871-z. [PubMed: 26407819]

[158]. Chuk MK, Widemann BC, Minard CG, Liu X, AeRang Kim, Bernhardt MB, Kudgus RA, Reid JM, Voss SD, Blaney S, Fox E, Weigel BJ, A phase 1 study of cabozantinib in children and adolescents with recurrent or refractory solid tumors, including CNS tumors: Trial ADVL1211, a report from the Children's Oncology Group, Pediatr. Blood Cancer 65 (8) (2018) e27077. [PubMed: 29693796]

[159]. Perisa MP, Storey M, Streby KA, Ranalli MA, Skeens M, Shah N, Cabozantinib for relapsed neuroblastoma: Single institution case series, Pediatr. Blood Cancer 67 (2020) e28317. [PubMed: 32343886]

[160]. Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, et al. , Discovery and development: a multikinase inhibitor for treating cancer, Nat. Rev. Drug Discov 5 (2006) 835–844, 10.1038/nrd2130. [PubMed: 17016424]

[161]. Kakodkar NC, Peddinti RR, Tian Y, Guerrero LJ, Chlenski A, Yang Q, Salwen HR, Maitland ML, Cohn SL, Sorafenib inhibits neuroblastoma cell proliferation and signaling, blocks

angiogenesis, and impairs tumor growth, Pediatr. Blood Cancer 59 (2012) 642–647, 10.1002/pbc.24004. [PubMed: 22147414]

[162]. Yang F, Jove V, Buettner R, Xin H, Wu J, Wang Y, Nam S, Xu Y, Ara T, DeClerck YA, Seeger R, Yu H, Jove R, Sorafenib inhibits endogenous and IL-6/S1P induced JAK2-STAT3 signaling in human neuroblastoma, associated with growth suppression and apoptosis, Cancer Biol. Ther 13 (2012) 534–541, 10.4161/cbt.21781. [PubMed: 22406995]

[163]. Widemann BC, Kim A, Fox E, Baruchel S, Adamson PC, Ingle AM, Glade Bender J, Burke M, Weigel B, Stempak D, Balis FM, Blaney SM. A phase I trial and pharmacokinetic study of sorafenib in children with refractory solid tumors or leukemias: a Children's Oncology Group Phase I Consortium report. Clin Cancer Res 2012; 18: 6011–22. 10.1158/1078-0432.CCR-11-3284. [PubMed: 22962440]

[164]. Kim A, Widemann BC, Krailo M, Jayaprakash N, Fox E, Weigel B, Blaney SM, Phase 2 trial of sorafenib in children and young adults with refractory solid tumors: A report from the Children's Oncology Group, Pediatr. Blood Cancer 62 (2015) 1562–1566, 10.1002/pbc.25548. [PubMed: 26207356]

[165]. Okada K, Nakano Y, Yamasaki K, Nitani C, Fujisaki H, Hara J, Sorafenib treatment in children with relapsed and refractory neuroblastoma: an experience of four cases, Cancer Med 5 (2016) 1947–1949, 10.1002/cam4.784. [PubMed: 27264843]

[166]. Whittle SB, Patel K, Zhang L, Woodfield SE, Du M, Smith V, Zage PE, The novel kinase inhibitor ponatinib is an effective anti-angiogenic agent against neuroblastoma, Invest. New Drugs 34 (2016) 685–692, 10.1007/s10637-016-0387-y. [PubMed: 27586230]

[167]. Singh A, Meier-Stephenson V, Jayanthan A, Narendran A, In Vitro Sensitivity Profiling of Neuroblastoma Cells Against A Comprehensive Small Molecule Kinase Inhibitor Library to Identify Agents for Future Therapeutic Studies, Curr. Cancer Drug Targets 17 (2017) 569–584, 10.2174/1568009617666161122145219. [PubMed: 27875952]

[168]. Sidarovich V, De Mariano M, Aveic S, Pancher M, Adami V, Gatto P, Pizzini S, Pasini L, Croce M, Parodi F, Cimmino F, Avitabile M, Emionite L, Cilli M, Ferrini S, Pagano A, Capasso M, Quattrone A, Tonini GP, Longo L. A High-Content Screening of Anticancer Compounds Suggests the Multiple Tyrosine Kinase Inhibitor Ponatinib for Repurposing in Neuroblastoma Therapy. Mol Cancer Ther 2018; 17: 1405–1415. 10.1158/1535-7163.MCT-17-0841. [PubMed: 29695637]

[169]. Flynn SM, Lesperance J, Macias A, Phanhthilath N, Paul MR, Kim JW, Tamayo P, Zage PE, The multikinase inhibitor RXDX-105 is effective against neuroblastoma in vitro and in vivo, Oncotarget 10 (59) (2019) 6323–6333. [PubMed: 31695841]

[170]. Li H, Yu Y, Zhao Y, Wu D, Yu X, Lu J, Chen Z, Zhang H, Hu Y, Zhai Y, Su J, Aheman A, De Las CA, Jin J, Xu X, Shi Z, Woodfield SE, Vasudevan SA, Agarwal S, Yan Y, Yang J, Foster JH, Small molecule inhibitor agerafenib effectively suppresses neuroblastoma tumor growth in mouse models via inhibiting ERK MAPK signaling, Cancer Lett 457 (2019) 129–141, 10.1016/j.canlet.2019.05.011. [PubMed: 31100410]

[171]. Ettrich TJ, Seufferlein T, Regorafenib, Recent Results Cancer Res 211 (2018) 45–56, 10.1007/978-3-319-91442-8_3. [PubMed: 30069758]

[172]. Subramonian D, Phanhthilath N, Rinehardt H, Flynn S, Huo Y, Zhang J, Messer K, Mo Q, Huang S, Lesperance J, Zage PE, Regorafenib is effective against neuroblastoma in vitro and in vivo and inhibits the RAS/MAPK, PI3K/Akt/mTOR and Fos/Jun pathways, Br. J. Cancer 123 (4) (2020) 568–579. [PubMed: 32457362]

[173]. Geoerger B, Morland B, Jimenez I, Frappaz D, Pearson ADJ, Vassal G, Maeda P, Kincaide J, Mueller U, Schlief S, Teufel M, Ploeger BA, Cleton A, Agostinho AC, Marshall LV, Phase 1 dose-escalation and pharmacokinetic study of regorafenib in paediatric patients with recurrent or refractory solid malignancies, Eur. J. Cancer 153 (2021) 142–152, 10.1016/j.ejca.2021.05.023. [PubMed: 34157616]

[174]. Lu J, Guan S, Zhao Y, Yu Y, Woodfield SE, Zhang H, Yang KL, Bieerkehazhi S, Qi L, Li X, Gu J, Xu X, Jin J, Muscal JA, Yang T, Xu GT, Yang J, The second-generation ALK inhibitor alectinib effectively induces apoptosis in human neuroblastoma cells and inhibits tumor growth in a TH-MYCN transgenic neuroblastoma mouse model, Cancer Lett 400 (2017) 61–68, 10.1016/j.canlet.2017.04.022. [PubMed: 28455243]

[175]. Alam MW, Borenäs M, Lind DE, Cervantes-Madrid D, Umapathy G, Palmer RH, Hallberg B, Alectinib, an Anaplastic Lymphoma Kinase Inhibitor, Abolishes ALK Activity and Growth in ALK-Positive Neuroblastoma Cells, Front. Oncol 9 (2019) 579, 10.3389/fonc.2019.00579. [PubMed: 31334113]

[176]. Heath JA, Campbell MA, Thomas A, Solomon B, Good clinical response to alectinib, a second generation ALK inhibitor, in refractory neuroblastoma, Pediatr. Blood Cancer 65 (7) (2018) e27055. [PubMed: 29603581]

[177]. Gutierrez J, Flynn S, Huo Y, Lesperance J, Zage PE, Inhibition of RET via BLU-667 in an in vitro model of neuroblastoma, Cancer Res 80 (16 Suppl) (2020) 5402, 10.1158/1538-7445.AM2020-5402.

[178]. Shankar A, Kurzawinski T, Ross E, Stoneham S, Beale T, Proctor I, Hulse T, Simpson K, Gaze MN, Cattaneo E, Gevers E, Marshall L, Hubbard JG, Brain C, Treatment outcome with a selective RET tyrosine kinase inhibitor selpercatinib in children with multiple endocrine neoplasia type 2 and advanced medullary thyroid carcinoma, Eur. J. Cancer 158 (2021) 38–46, 10.1016/j.ejca.2021.09.012. [PubMed: 34649088]

[179]. Ortiz MV, Gerdemann U, Raju SG, Henry D, Smith S, Rothenberg SM, Cox MC, Proust S, Bender JG, Frazier AL, Anderson P, Pappo AS, Activity of the highly specific RET inhibitor selpercatinib (LOXO-292) in pediatric patients with tumors harboring RET gene alterations, JCO Precis. Oncol (4) (2020) 341–347.

[180]. Morgenstern D, Mascarenhas L, Campbell M, et al. , Oral selpercatinib in pediatric patients with advanced RET-altered solid or primary CNS tumors: preliminary results from the phase 1/2 LIBRETTO-121 trial, J. Clin. Oncol 39 (suppl 15) (2021) 10009, 10.1200/JCO.2021.39.15_suppl.10009.

[181]. Christie GH, Kenner J, LXXI.—The molecular configurations of polynuclear aromatic compounds. Part I. The resolution of γ−6 : 6′-dinitro- and 4 : 6 : 4′ : 6′-tetranitro-diphenic acids into optically active components, J. Chem. Soc. Trans 121 (0) (1922) 614–620.

[182]. LaPlante SR, Fader LD, Fandrick KR, Fandrick DR, Hucke O, Kemper R, Miller SPF, Edwards PJ, Assessing atropisomer axial chirality in drug discovery and development, J. Med. Chem 54 (2011) 7005–7022, 10.1021/jm200584g. [PubMed: 21848318]

[183]. Cardenas MM, Toenjes ST, Nalbandian CJ, Gustafson JL, Enantioselective synthesis of pyrrolopyrimidine scaffolds through cation-directed nucleophilic aromatic substitution, Org. Lett 20 (2018) 2037–2041, 10.1021/acs.orglett.8b00579. [PubMed: 29561161]

[184]. Basilaia M, Chen MH, Secka J, Gustafson JL, Atropisomerism in the Pharmaceutically Relevant Realm, Acc. Chem. Res 55 (2022) 2904–2919, 10.1021/acs.accounts.2c00500. [PubMed: 36153960]

[185]. Toenjes ST, Gustafson JL, Atropisomerism in medicinal chemistry: challenges and opportunities, Future Med. Chem 10 (2018) 409–422, 10.4155/fmc-2017-0152. [PubMed: 29380622]

[186]. Liu Y, Bishop A, Witucki L, Kraybill B, Shimizu E, Tsien J, Ubersax J, Blethrow J, Morgan DO, Shokat KM, Structural Basis for Selective Inhibition of Src Family Kinases by PP1, Chem. Biol 6 (1999) 671–678, 10.1016/s1074-5521(99)80118-5. [PubMed: 10467133]

[187]. Toenjes ST, Garcia V, Maddox SM, Dawson GA, Ortiz MA, Piedrafita FJ, Gustafson JL, Leveraging atropisomerism to obtain a selective inhibitor of RET kinase with secondary activities toward EGFR mutants, ACS Chem. Biol 14 (2019) 1930–1939, 10.1021/acschembio.9b00407. [PubMed: 31424197]

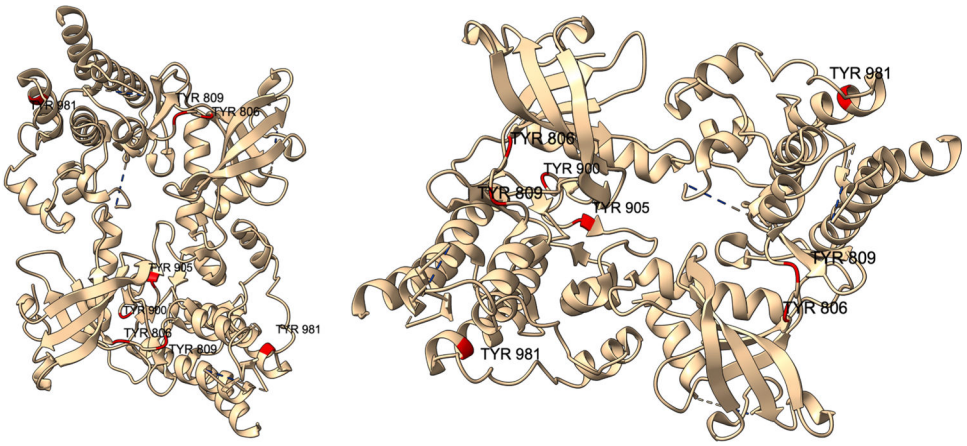

Fig. 1.

Autophosphorylation sites in the kinase domain of RET. The structure of the RET kinase domain (705–1013) solved via x-ray crystallography was modeled using UCSF ChimeraX (https://www.rbvi.ucsf.edu/chimerax/). Tyrosine residues known to become autophosphorylated upon RET activation (Tyr806, Tyr809, Tyr900, Tyr905, Tyr981) are shown in red.

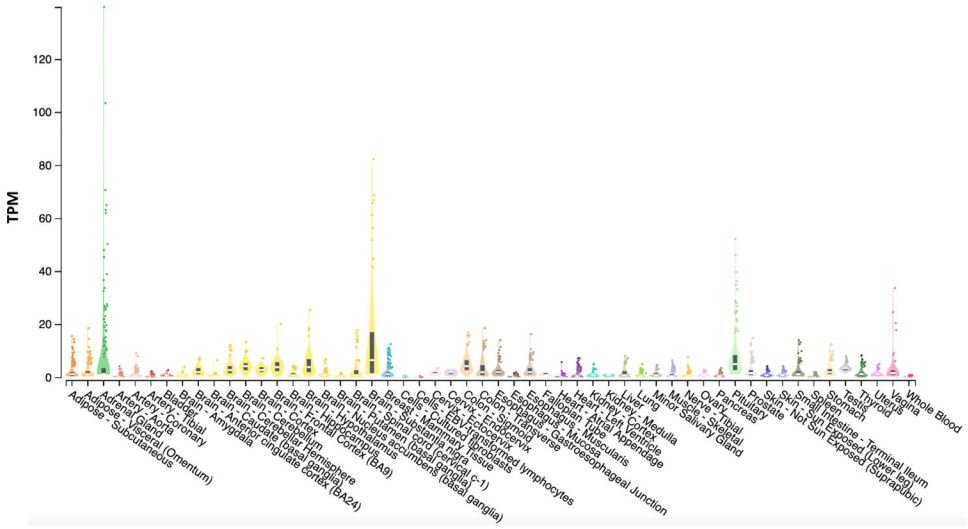

Fig. 2.

Bulk tissue *RET* gene expression patterns. Graph of RET tissue expression using gene expression data from adult tissues was generated from https://www.gtexportal.org/home/gene/RET.

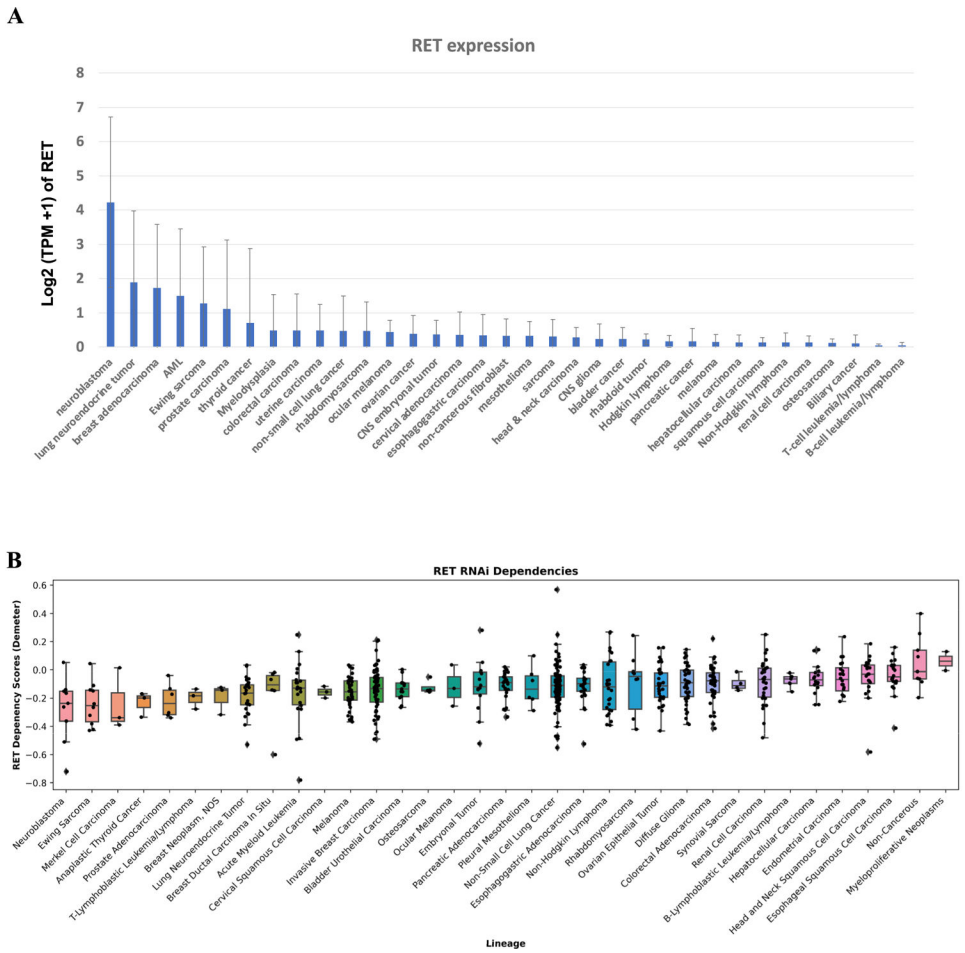

Fig. 3.

RET tumor cell line expression and outcomes of RET depletion. A. Graph of RET gene expression levels in cancer cell lines from the Cancer Cell Line Encyclopedia (CCLE) was generated from https://depmap.org. B. Graph of Chronos dependency scores [114] from RNAi datasets Achilles + DRIVE + Marcotte and DEMETER2 from the CCLE was generated from https://depmap.org. A lower Chronos score indicates a higher likelihood that the tested gene is essential for a given cell line, with a score of −1 indicating a gene comparable to the median of all pan-essential genes.

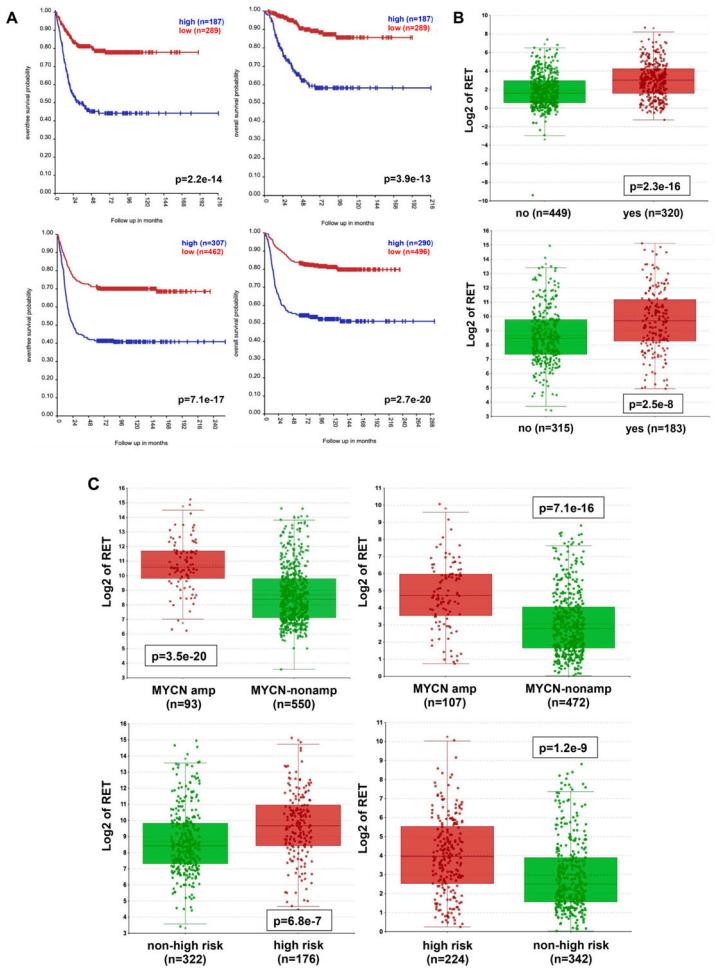

Fig. 4.

Neuroblastoma Patient Outcomes Based on RET Expression. A. Using the neuroblastoma Kocak (top) and Cangelosi (bottom) patient datasets in the R2 Genomics Analysis and Visualization Platform (http://r2.amc.nl), patients were divided into high (blue) and low (red) RET gene expression groups by median-centered Log2 ratios and survival curves were generated as previously published [115]. Event-free survival (left) and overall survival (right) curves are shown with patient numbers in parentheses. B. Relative RET gene expression in patients with and without experiencing an event, such as disease recurrence or death (top, Cangelosi dataset), or disease relapse (bottom, SEQC dataset) from in the R2 Genomics Analysis and Visualization Platform are shown. Graphs were generated as previously published [115]. C. Relative RET gene expression in patients with neuroblastoma tumors with and without MYCN amplification from the Kocak (top left) and Westermann (top right) datasets and in patients with high risk and non-high risk neuroblastoma tumors from the SEQC (bottom left) and Westermann (bottom right) datasets from in the R2 Genomics Analysis and Visualization Platform are shown. Graphs were generated as previously published [115].

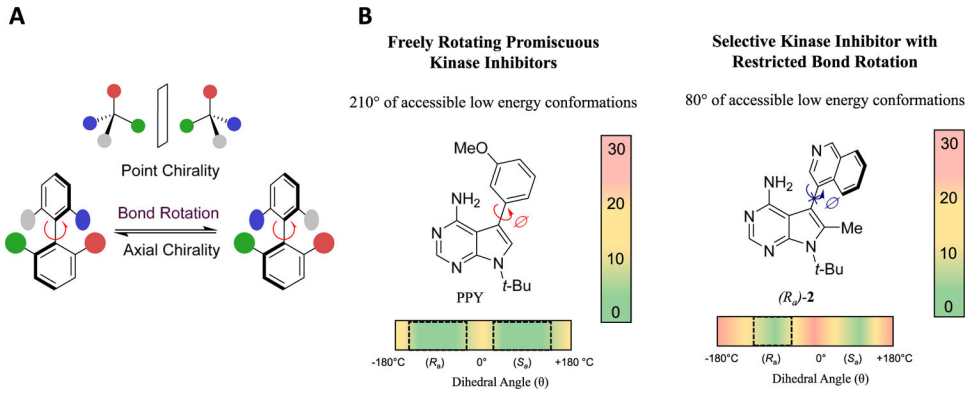

Fig. 5.

Point chirality versus axial chirality. A. Images representing chemical point chirality (top) and axial chirality via chemical bond rotation around an axis (bottom) B. Atropisomerism as a strategy to restrict free rotation around a chiral center to enhance drug selectivity. Unrestricted axis rotation allows for up to 210° of accessible low energy confirmations (left), while restricted rotation due to bulky side chains allows for only 80° of accessible low energy confirmations (right). Adapted from Ref. [179].

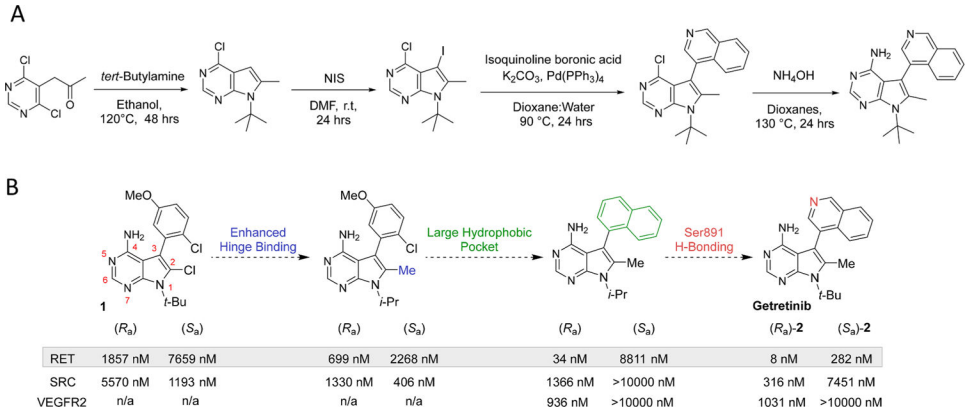

Fig. 6.

Atropisomeric Design and Synthesis of Getretinib. A. The PPY core of getretinib was synthesized via SNAr with *tert*-Butylamine followed by cyclization. The scaffold was iodinated using N-iodosuccinimide in dimethylformamide followed by Suzuki-Miyaura coupling with isoquinoline-4-boronic acid. To yield the final compound, scaffold was aminated vis SNAr with ammonium hydroxide in a pressure vial. The resulting racemic mixture was separated on a semi-preparative chiral HPLC column to yield each atropisomer. Racemization kinetics studies on HPLC showed the barrier of rotation to be 30.04 kcal/mol, classifying getretinib as a class-3 atropisomer with a half-life of 4.48 years at 37 °C. B. Chemical modification of the lead compound [1] began with replacing the C2 chlorine with a methyl group, replacement of the benzyl group with a naphthyl group, and replacement of the naphthyl group with a quinoline. In vitro IC_{50} values for inhibitors at each step of synthesis using ADP-Glo kinase inhibition assays are shown (bottom).

Cellular activity against RET driven cell lines (GI, nM)	(R_a)-**2**	(S_a)-**2**	Vandetanib
RET Driven cellular models			
LC-2/AD (NSCLC)	2810	>10000	1470
TT (Thyroid Cancer)	1450	>10000	1010
ED-MCF7 (Breast Cancer)	1150	>10000	250
non RET Driven cellular models			
BT474 (+ER, PR, HER2)	>10000	>10000	>5950
H292 (WT EGFR)	>10000	>10000	>1140

Fig. 7.

Cellular Activity Against RET-driven Cell Lines. R- and S-getretinib enantiomers were tested in cellular models of RET-driven and non-RET-driven control cell lines. (R_a)-getretinib displayed promising antiproliferative activities in RET-driven models of breast, thyroid, and non-small cell lung cancers. The *in vitro* selectivity also carried over as we observed reduced activity towards non-RET driven models. Activity in cell lines was measured in triplicate.

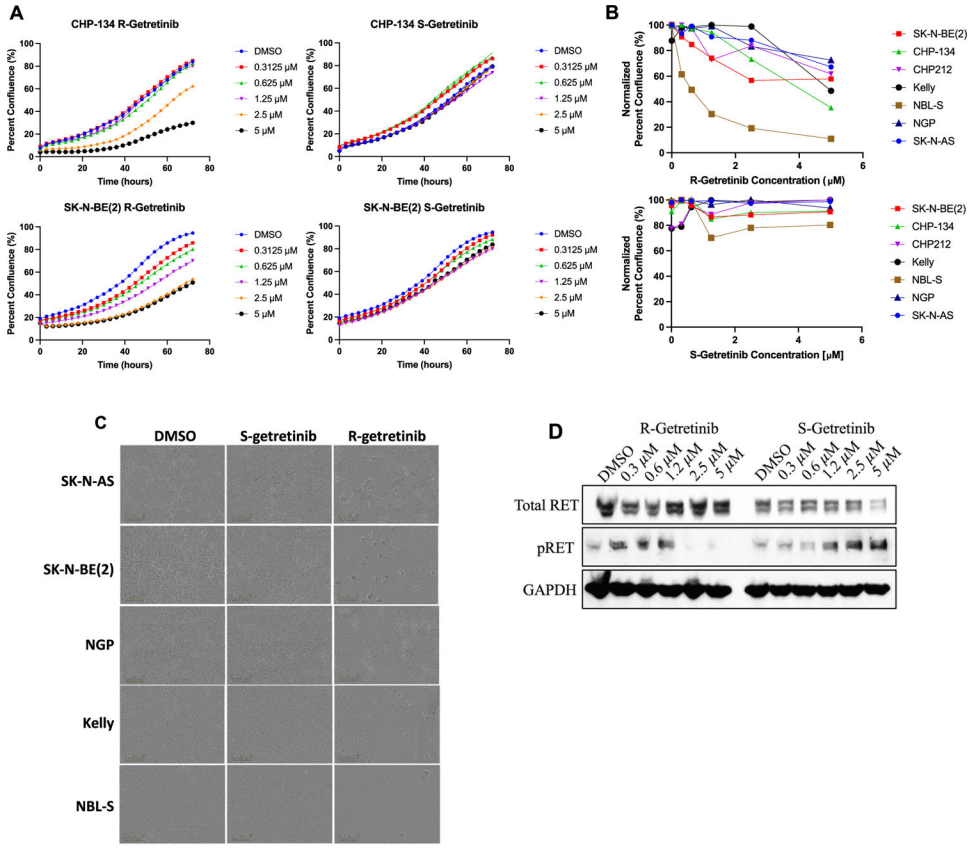

Fig. 8.

R-Getretinib reduces neuroblastoma cell confluence and inhibits RET phosphorylation. A. Neuroblastoma cell lines CHP-134 (top) and SK-N-BE(2) (bottom) were grown using standard conditions [164,167] and exposed to increasing doses of R- and S-getretinib. Cell confluence was assessed by continuous live cell imaging using the Incucyte ZOOM™ after 72 h of incubation as previously published [164,167]. Time-response curves for R-getretinib (left) and S-getretinib (right) are shown. B. Neuroblastoma cell lines SK-N-BE(2), CHP-134, CHP-212, Kelly, NBL-S, NGP, and SK-N-AS were obtained from the ATCC, validated by DNA sequence, and grown using standard conditions (164,167). Cell lines were exposed to increasing doses of R- and S-getretinib and cell confluence was assessed by continuous live cell imaging using the Incucyte ZOOM™ after 72 h of incubation as above. Dose-response curves for R-getretinib (top) and S-getretinib (bottom) are shown. C. Neuroblastoma cell lines grown and treated as above [164,167] with R- and S-getretinib were photographed at regular intervals, and 10X images taken from the Incucyte ZOOM™ after 72 h of treatment with 5μM of either S- or R-getretinib were compared to control cells. D. SK-N-BE(2) neuroblastoma cells were grown as above [164,167] and treated with 5 μM 13-*cis*-retinoic acid for 48 h, followed by treatment with increasing concentrations of either R- or S-getretinib for 72 h. Cells were lysed with RIPA buffer, and lysates were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by Western blot for total (3220, Cell Signaling Technology) and phosphorylated RET (3221S, Cell Signaling Technology) and GAPDH (5174S, Cell Signaling Technology), using anti-

rabbit or anti-mouse HRP-conjugated secondary antibodies (1:5000, Sigma-Aldrich). Signal was visualized using Amersham ECL (GE Healthcare Bio-Sciences).