Recent progress of small-molecule of RET inhibitors against Non-small cell lung cancer

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Abstract

Since the oncogenic rearranged during transfection (RET) gene fusion was discovered in non-small cell lung cancer (NSCLC) in 2012, multiple-targeted kinase inhibitors (MKIs) cabozantinib and vandetanib have been explored in the clinic for RET positive NSCLC patients. As the nonselective nature of these inhibitors, patients have off-target adverse effects. The discovery of highly potent selective RET inhibitors such as pralsetinib and selpercatinib improve the clinic efficiency and more favorable toxicity profile. However, acquired resistance mediated by secondary mutations in the solvent-front region of the kinase (e.g. G810C/S/R) become a new challenge for selective RET inhibitor therapies. In this review, we highlight typical RET inhibitors developed during these years and provide a reference for more potential RET inhibitors exploration in the future.

Keywords REarranged during Transfection (RET), Non-small cell lung cancer (NSCLC), Resistance, Inhibitors

Introduction

Lung cancer is responsible for 11.6% of global cancer morbidity and 18.4% of global cancer mortality. It was the most common oncological disease and it was classified for non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). NSCLC accounts for about 85% in lung cancer and is subdivided for squamous and non-squamous histological types (Bray et al. 2018). The oncogenic rearrange during transfection (RET) gene was first identified in 1985 (Takahashi et al. 1985) which encodes a receptor tyrosine kinase protein composed by1143 amino acid residues. It was identified in approximately 1–2% of NSCLCs in 2012 (Roberto et al. 2017). Up to now, 48 unique fusion partners in RET have been identified (Lipson et al. 2012) and these fusions lead to ligand-independent constitutive activation of the RET pathway which increased oncogenic signaling, resulting in RET gene overexpression.

Several multiple-targeted kinase inhibitors (MKIs) cabozantinib and vandetanib were approved for the treatment of RET positive NSCLC patients (Drilon et al. 2018; Subbiah et al. 2020). But the limited clinical benefits of these inhibitors prevent the application of these multiple-targeted drugs (Drilon et al. 2016; Lee et al. 2017; Hida et al. 2019; Gupta-Abramson et al. 2008; Wells et al. 2012; Elisei et al. 2013; Brose et al. 2014). In 2020, US Food and Drug Administration (FDA) approved two selective RET inhibitors, selpercatinib and pralsetinib. However, acquired resistance conferred by secondary mutations were identified. Several other highly promising selective RET inhibitors were also developed in different stages of clinical investigation. In this review, we focus on the present state of the RET inhibitors in the treatment of NSCLC, discuss the future perspectives for RET positive NSCLC patients and provide an updated panorama of this topic.

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The structure and functions of RET

In 1985, Takahashi et al. (1985) identified the pro-tooncogene RET is a transforming gene located in the long arm of human chromosome 10. It was derived by DNA rearrangement during transfection of mouse NIH3T3 cells with human lymphoma DNA. The RET gene encodes a receptor tyrosine kinase (RTK) protein composed of 1143 transmembrane amino acid residues and consist of three regions, a large extracellular domain, a transmembrane domain and an intracellular tyrosine kinase domain. The RET protein formed by in-frame fusion of the 5'-terminus of a chaperone gene with the 3'-terminus of RET containing its kinase structural domain (Takahashi et al. 1988; Masahide and Geoffrey 1987). The extracellular domain contains four cadherin-like domains(CLD1-4), a calcium binding site that between CLD2 and CLD3, a cysteine-richdomain and a conserved cysteinerich domain (Fig.1). As the intracellular region contains a tyrosine kinase domain and tyrosine phosphorylation sites located next to the C terminal region. The G1063 diverge C-terminal tail into three major forms, which are a short 9-amino acid one (RET9), a 43-amino acid one (RET43) and a long 51-amino acid one (RET51). These three isoforms share a largely common sequence and are coexpressed in many tissues.

The structure of the protein always decide the function of it. A lot of studies have clarified the function of RET and much has been uncovered its role in cancer. To date, three general mechanisms of aberrant RET activation have been reported. One of them is in-frame RET gene fusions (Takahashi et al. 1985), the other is targeted mutation of the RET gene itself (Mulligan et al. 1993; Hofstra et al. 1994; Donis-Keller et al. 1993) and the third one is aberrant overexpression of the RET gene (Horibata et al. 2018; Mulligan 2018). The inappropriate activation of the tyrosine kinase is the common mechanism of these three. In consideration of the RET ligands, glial cell line derived neurotrophic factor (GDNF), neurturin, artemin and persephin, all belonging to the GDNF family (GFLs) (Arighi et al. 2005). These GFLs recruit RET for dimerization do not bind to RET directly but bind to GDNF family receptor-a (GFRa) coreceptors instead (Goodman et al. 2014; Worby et al. 1998; Amoresano et al. 2005; Wang 2013). GFL/GFRα/RET ternary complex active the phosphorylation of the intracellular tyrosine residues and multiple downstream signaling,which include RAS/MAPK, PI3K/AKT and JAK/STAT pathways to regulate cell migration, proliferation and differentiation in
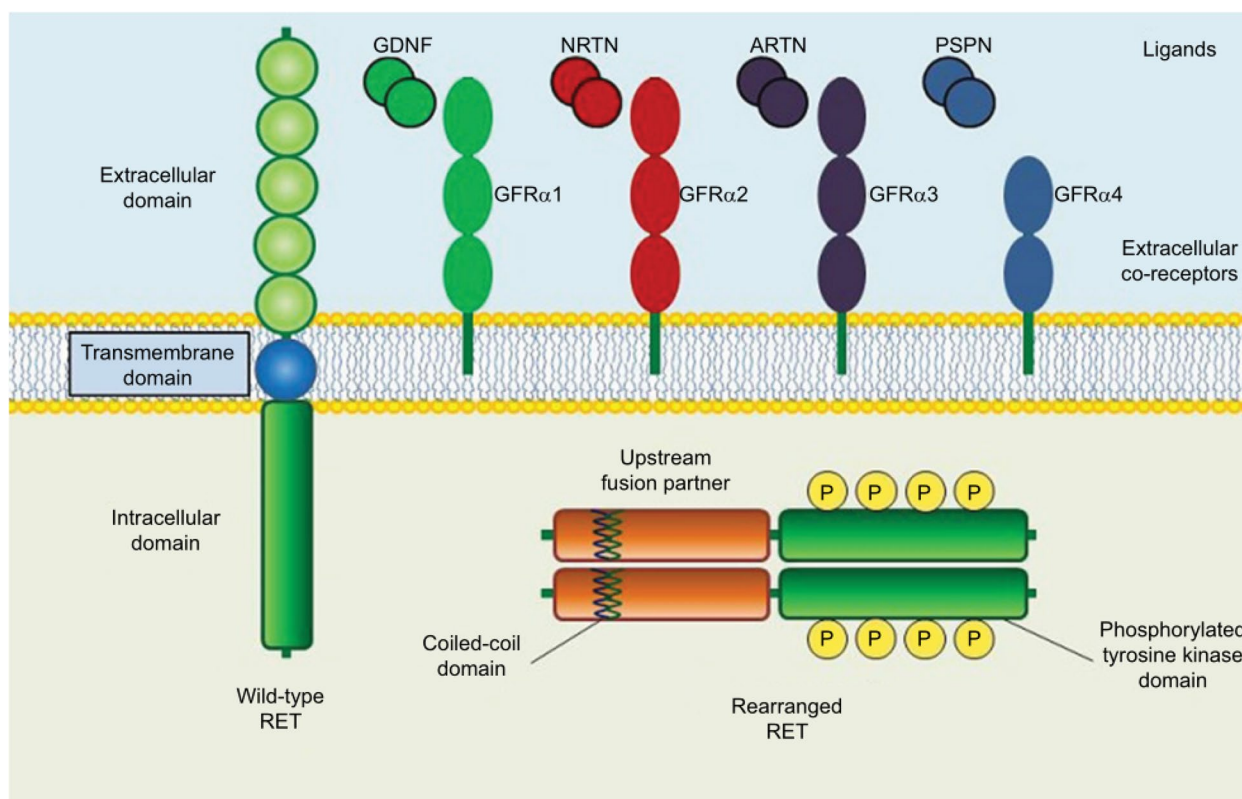
Fig.1 Structure of wild-type and rearranged RET proteins (Giuseppe et al. 2019)

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physiological conditions (Arighi et al. 2005; Mahato and Sidorova 2020) (Fig.2).

RET gain-of-function alterations have been identified in multiple solid tumours. More than 10,000 different metastatic tumours were sequenced, RET alterations have been found in 2.4% of all cases, thyroid cancers and NSCLC are primarily found. It is worth to notice that, abnormal activation of RET mediated by mutation, overexpression, or rearrangement with other oncogenic partners are identified as driver forces in a variety of human malignancies (Subbiah and Cote 2020; Wang et al. 2019a; Castinetti et al. 2018).
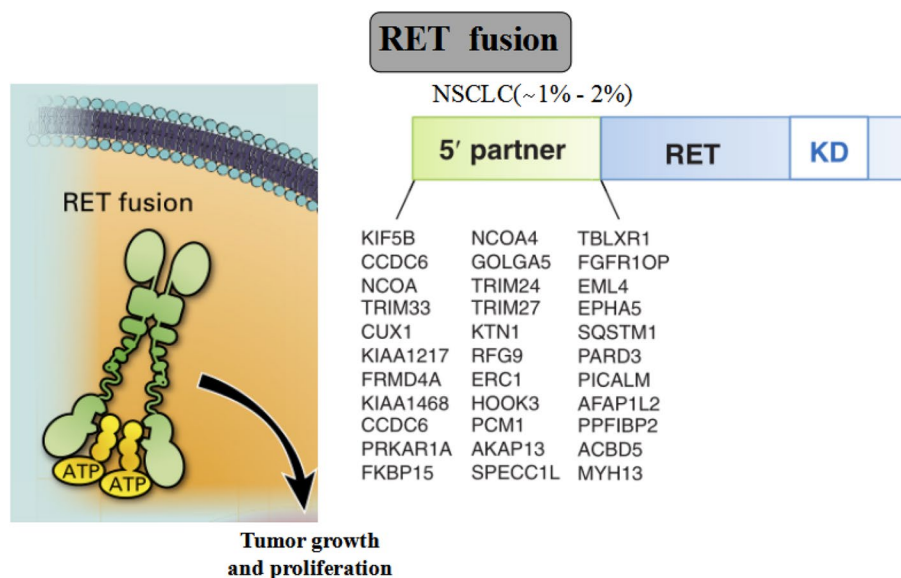
RET fusions

The first RET fusion rearrangement among NSCLC patients was identified by Kohno and Lipson, which was reported in 2012 (Roberto et al. 2017). KIF5B-RET is the most frequent and the best characterized RET fusion. It was derived from a 10.6 Mb pericentric inversion on chromosome 10. CCDC6, NCOA4 and TRIM33 are also partner 5 genes for RET fusion in NSCLCs (Romei et al. 2016). Until now, 48 unique fusion partners in RET have been identified and at least 12 fusion RET partner genes have been identified in NSCLCs (Lipson et al. 2012). RET fusions are thought to be oncogenic divers for two reasons. One of the reasons is fusion provides a mechanism to express RET aberrantly in a cell type where it is normally transcriptionally silent. The extracellular domain replaced with a protein dimerization domain is the other reason (Arighi et al. 2005). The outcome represent that

the production of an intracellular RET tyrosine kinase domain can be ligand-independent activation (Fig.3).

The prevalence of RET fusion genes was 1.8% in the overall population. Also, Takeuchi et al. examined 1529 Japanese NSCLC patients, indicating that RET chromosomal rearrangement is 0.9% in those patients (Takeuchi et al. 2012). In total, the prevalence of the RET fusion gene in lung adenocarcinoma is 0.9–1.8%. It is important to found out that key regulatory mechanisms of RET inactivation, such as endocytosis and recruitment of membrane associated ubiquitin ligases, do not appear to have influence on the fusion proteins, but may enhance the oncogenicity (Kohno et al. 2012; Richardson et al. 2009). Also, the mechanism of activation of RET fusion proteins is similar with the oncogenic activation of rearranged ALK in NSCLC. In vitro,the Ba/F3 (pro-B lymphocyte) (Roberto et al. 2017) or NIH3T3 (fibroblast) cell lines (Hyndman et al. 2017; Suzuki et al. 2013), and CCDC6-RET-positive LC-2 lung adenocarcinoma cells demonstrated the tumorigenic potential of RET fusion proteins (Matsubara et al. 2012; Planchard et al. 2016). In vivo, the athymic mice through subcutaneous injection of KIF5B-RET transfected NIH3T3 cells (Kohno et al. 2012), and transgenic immunocompetent KIF5B-RET-rearranged mice were also evaluated (Horibata et al. 2018). To be noted, in vivo models, after tumour development, continuous KIF5B–RET fusion gene expression was required for lung tumour survival in the latter. Furthermore, RET-rearranged lung adenocarcinoma in transgenic mice presented a strong desmoplastic reaction and aggressive features (Chau and Haddad 2013).
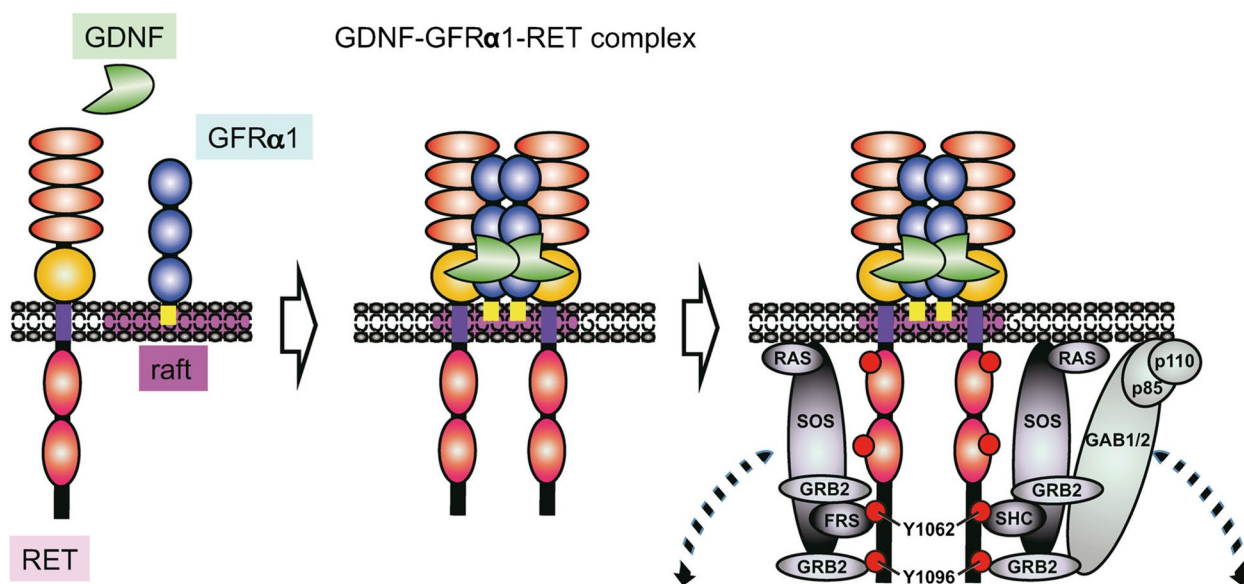
Fig. 2 GDNF signaling via the GDNF-GFRα1-RET complex (Kawai and Takahashi 2020)

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Fig. 3 RET fusion (Vivek et al. 2020; Wade and Christine 2018)

As RET was first identified more than several decades ago, which is activated in cancer mainly through chromosomal rearrangements that generate fusion genes containing the kinase domain of RET, implying that RET addicted malignancies are sensitive to targeted inhibition. With the study of RET in NSCLC, clinical treatments and inhibitors of it were gradually from bench to bedside, especially opening the door for small molecule inhibitors. We will discuss the MKIs, the selective inhibitors and some other reported inhibitors as followed.

RET inhibitors
multiple-targeted kinase inhibitors
#### Cabozantinib
The glimmer of hope for the patients with RET-rearranged NSCLC came with the discovery of MKIs (Fig. 4). The first MKIs of RET we discuss hear is cabozantinib (1), which was approved by the US FDA in 2016. Cabozantinib (XL-184), with the structure of N-(4-((6,7-dimethoxyquinolin-4-yl)oxy)phenyl)-N-(4-fluorophenyl) cyclopropane-1,1-dicarboxamide, was developed by Exelixis Inc. The drug has low nanomolar activity against RET (the IC_{50} for RET is 5.2 nM), and it also has activity against ROS1, MET, VEGFR2, AXL, TIE2, and vkit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue (KIT) (Chau and Haddad 2013). Twenty-six patients with advanced RET-rearranged NSCLC were evaluated the safety and activity of cabozantinib by Drilon et al., which in an open-label, single-arm and phase II trial (Hida et al. 2019). It was the first study of cabozantinib demonstrated the activity of a RET

inhibitor in a molecularly enriched cohort of patients with advanced-stage, RET-rearranged NSCLC (Drilon et al. 2013). In this trial, the primary objective overall response rate (ORR) was 28%, with 7 of 25 evaluable patients achieving a partial response, including patients with several other fusions. The median progression-free (mPFS) survival was 5.5 months and median overall survival was 9.9 months.

In this disease context, this trial was followed by two phase II trials of vandetanib, one conducted in Japan (LURET) (Planchard et al. 2016) and the other one in South Korea (Gupta-Abramson et al. 2008), which will be discussed next. The common adverse reactions caused by cabozantinib include abdominal complex signs of diarrhea, cavity inflammation, palmar-plantar erythrodysesthesia syndrome (PPES), body mass reduction, appetite lethargy, nausea, fatigue, oral pain, hair color changed, taste disturbance, high blood pressure, abdominal pain and constipation (Sara et al. 2016; Houvras and Wirth 2011).

Vandetanib
Vandetanib (ZD6474) is a 4-anilinoquinazoline-like drug molecule, with the structure of N-(2,4-difluorophenyl)-6-methoxy-7-((1-methylpiperidin-4-yl)methoxy) quinazoline-4 amine 2), which was developed by AstraZeneca (UK) and approved by the US FDA in 2011. Vandetanib is an orally active low-molecular multitargeted tyrosine kinase inhibitor with activity against EGFR, VEGFR-2 and RET(the IC_{50} for RET is 100 nM) (Song 2015). Preclinical studies demonstrated the antitumor activity of
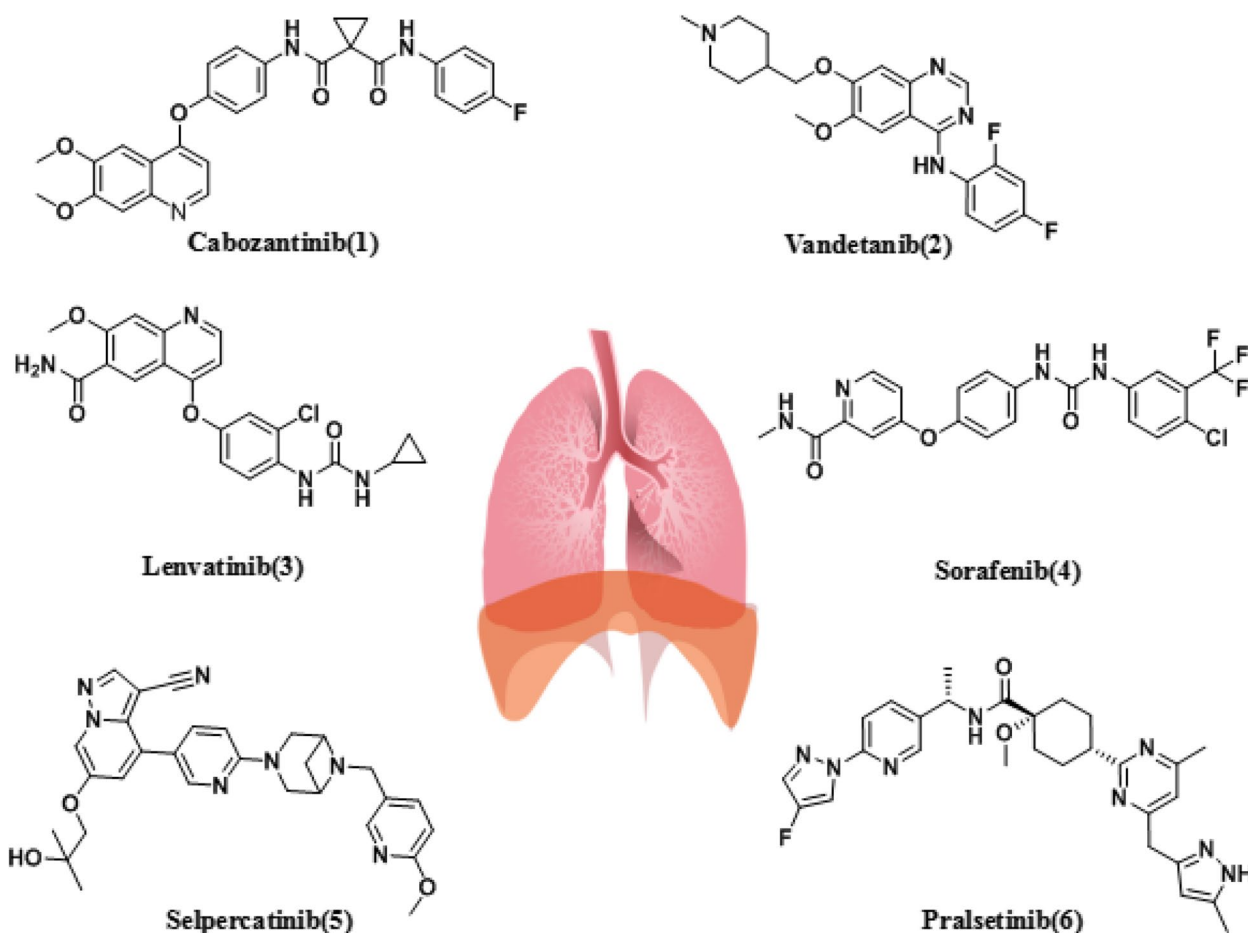
Fig. 4 MKIs and RET-selective inhibitors in patients with RET-positive lung cancer

vandetanib both in vitro and in vivo against LC-2/ad cells carrying the CCDC6-RET fusion (Wang et al. 2019b). In Japan, the LURET phase II study, 19 Japanese RET fusion patients received the vandetanib treatment, the mPFS was 4.7 months, the median OS was 11.1 months, and the OS at 12 months was 52.6%. Eleven patients (57.9%) had adverse events leading to a dose reduction. In Korean, patients received the vandetanib treatment in another phase II study explored the efficacy of it with metastatic or recurrent RET fusion NSCLC. This result displayed the mPFS was 4.5 months, the median OS was 11.6 months. The most common grade 3 adverse events (AEs) were hypertension (17%), a prolonged QTc interval (11%), and transaminitis (6%) (Wang et al. 2019b).

In the clinical trial of vandertanib for NSCLC described above, patients died most are caused by disease progression, but there are also side effects that cannot be tolerated discontinue the medication. Common side effects were diarrhea, rash, hypertension, and asymptomatic prolonged QT interval, nausea, vomiting, neutropenia, anemia, fatigue, etc. But most can be tolerated or can be relieved after symptomatic treatment. Also, the adverse reaction was higher of vandertanib combined application (Heymach et al. 2007; Heymach et al. 2008; Herbst et al. 2010; Socinski et al. 2003).

Lenvatinib

Lenvatinib (E7080) with the structure of 4-(3-chloro-4-(3-cyclopropylureido)phenoxy)-7-methoxyquinoline-6-carboxamide (3) is a MKI of VEGFR1-3, fibroblast growth factor receptors (FGFR)1–4, platelet-derived growth factor receptor alpha (PDGFRα), KIT, and RET (the IC_{50} for RET is 1.5 nM) (Matsui et al. 2008a, b; Okamoto et al. 2013; Tohyama et al. 2014; Toyoaki et al. 2019; Horiike et al. 2016). It was developed by Eisai Inc. and was approved by the US FDA in 2015 (Okamoto et al. 2013). In a phase II trial, 25 patients with RET-rearranged NSCLC was tested 24 mg a day. Among them, 13 patients (52%) had a KIF5B-RET rearrangement and 12 patients (48%) had different known RET fusion genes. Interestingly, 7 patients (28%) of patients received lenvatinib after a previous line of anti-RET therapy. The ORR, disease

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control rates (DCR), and mPFS time were 16%, 76%, and 7.3 months, respectively. In seven patients (28%) who had received RET therapy before, ORR with lenvatinib was superimposable (14%) on the response seen in RET TKI-naive patients. The different known RET fusion genes has the equivalent (15%—17%) ORR in patients with the KIF5B-RET rearrangement, but the mPFS was lower in patients with the KIF5B-RET rearrangement (3.6 versus 9.1 months). Lenvatinib induced grade 3 to 4 AEs in 92% of the patients (hypertension in 58% and proteinuria in 16%); dose reduction and drug discontinuation occurred in 64% and 76% of patients, respectively. Lenvatinib has side effects like high blood pressure, diarrhea, and thrombocytopenia, also (Toyoaki et al. 2019).

Sorafenib

Sorafenib (BAY 43–9006) with the structure of 4-(4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido) phenoxy)-N-methylpicolinamide (4), targets VEGFR1-3, platelet derived growth factor receptor beta (PDGFRB), c-KIT, fms-like tyrosine kinase 3 (FLT3), and also RET (the IC_{50} for RET inhibition was 15–150 nM) (Heymach et al. 2007). It was developed by Bayer and was approved by the US FDA in 2005. In *vitro*, sorafenib suppressed the growth of KIF5B-RET-transfected Ba/F3 pro-B lymphocytes and the efficacy of sorafenib has been tested in a limited number of patients (*n*=3). One of these three patients displayed stable disease (SD) while two others showed progressive disease (PD) as best responses to treatment. However, the antitumor activity of sorafenib does not appear to be significant. The most common side effects were palmar metatarsal syndrome, hypertension, and diarrhea (Horiike et al. 2016).

In spite of MKIs are active in patients with RET-driven NSCLCs, response rates achieved in prospective series are lower than those observed in other driver-positive, advanced-stage tumours with matched targeted therapies. One possible explanation for the limited efficacy of RET-directed therapy with MKIs relates to the inhibition of non-RET kinases, as well as non-kinase targets.

It has been validated that selectively inhibiting the kinase is a promising therapeutic strategy for patients harboring RET aberrations. Subsequently, two highly potent and selective RET TKIs, selpercatinib and pralsetinib have been developed and their activity has been investigated, which will be discussed below.

Selective RET inhibitors

With the aim of overcoming treatment-related toxicities commonly seen with non-selective RET inhibitors, small and highly selective RET inhibitors have been developed.

As a result, FDA approved selpercatinib and pralsetinib for the treatment of NSCLC harbouring RET alterations.

Selpercatinib

Selpercatinib (LOXO-292) with the structure of 6-(2-hydroxy-2-methylpropoxy)-4-(6-(6-((6-methoxypyridin-3-yl)methyl)-3,6-diazabicyclo[3.1.1] heptan-3-yl)pyridin-3-yl)pyrazolo[1,5-a]pyridine-3-carbonitrile(5) has been developed as a selective, ATP-competitive RET-inhibitor (the IC_{50} for RET is 2.0 nM), which was developed by Lilly. Selpercatinib is a highly selective RET inhibitor because it could block the adenosine triphosphate binding site of RET receptor tyrosine kinase (Drilon et al. 2020). Data on its preclinical characterization and activity was published in 2018 (Subbiah et al. 2018b). On May 8th of 2020, the US FDA approved selpercatinib as the first targeted therapy for RET-rearranged NSCLC (Markham 2020).

Selpercatinib based on the ORR with prolonged duration of responses seen in a multicenter, open-label, multicohort clinical trial (LIBRETTO-001, NCT03157128)and the benefit-risk evaluation of the results of LIBRETTO-001 was accelerated approved by the US FDA (Bradford et al. 2021). 105 RET fusion-positive NSCLC patients previously treated with platinum chemotherapy and 39 treatment-naive were evaluated. ORRs for previously treated and treatment-naive patients were 64% and 85%, respectively. Also, patients with RET-mutant MTC were divided into two cohorts: one is previously treated with cabozantinib or vandetanib (N=55) and the other is cabozantinib and vandetanib naive (N=88). ORRs for the cohorts were 69% and 73%, respectively (Kim et al. 2021).

Selpercatinib was approved by the European Medicines Agency (EMA) and Swiss-Medic for second line or posterior line therapy in 2021. LIBRETTO-321 was evaluated the efficacy of selpercatinib for Chinese RET fusion NSCLC patients and the ORR was 61.1%. This trail displayed that selpercatinib was a promising therapeutic option for Chinese RET fusion NSCLC patients. Central nervous system (CNS) progression on selpercatinib were low in patients with RET fusion-positive lung cancers (Yonina et al. 2023).

Common side effects of selpercatinib included increased glutamic transaminase (AST) levels (51%), increased alanine aminotransferase (ALT) levels (45%), dry mouth (39%), diarrhea (37%), hypertension (35%) and rash (27%) (Subbiah et al. 2021a). The high cost of treatment is also an issue to consider, selpercatinib is priced at $20,600 per month, equivalent to 145,707 yuan per month, or about 1.748 million yuan per year. It is difficult for the average Chinese patients benefit from it.

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Pralsetinib

Pralsetinib (BLU-667) with the structure of (1s,4R)-N-((S)-1-(6-(4-fluoro-1H-pyrazol-1-yl)pyridin-3-yl)ethyl)-1-methoxy-4-(4-methyl-6-((5-methyl-1H-pyrazol-3-yl)methyl)pyrimidin-2-yl)cyclohexane-1-carboxamide(**6**), is an oral tyrosine kinase inhibitors (TKI) with potent and specific activity against the RET kinase domain(the IC_{50} for RET inhibition was 0.4 nM), including multiple RET alterations such as fusions, activating point mutations and predicted acquired resistance mutations, which was developed by Blueprint Medicines Corporation.

In vitro, pralsetinib is 8 to 28-fold more potent against the wild-type RET kinase domain compared with cabozantinib and vandetanib. Besides, the clinical activity and safety of pralsetinib also display a strong activity against common oncogenic RET alterations, such as RET^{M918T}, KIF5B–RET and CCDC6–RET fusions (Subbiah et al. 2018a).

The ARROW study (Global multicentric single-arm phase I/II trial) investigated the clinical activity and safety of pralsetinib (Gainor et al. 2021). Based on this study, pralsetinib was approved as first-line or post-line treatment for RET fusion NSCLC by the US FDA in 2020 (FDA 2020; Wright 2020). The American Society of Clinical Oncology(ASCO) in 2021 showed that the ORR was 17.1 months, the CR was 6%, and the mPFS was 16.5 months ($n=136$) of pralsetinib. Nine patients with measurable brain metastases all showed an intracranial reduction to a certain extent (intracranial response rate (RR) 56%, intracranial complete remission (CR) 33%). As the significant efficacy and low off-target toxicity in RET cancer patients, pralsetinib was also approved by China's State Food and Drug Administration (SFDA) in 2021 for the first time (Sun and McCoach 2021; Horvath and Pircher 2021; Fu et al. 2021).

Considering the side effects, pralsetinib has been well tolerated with mainly low grade toxicities (28% had ≥ grade 3 events). The most commonly observed side effects were AST and ALT increase (22% and 17%, respectively), hypertension (18%), constipation (17%), neutropenia (15%) and fatigue (15%) (Gainor et al. 2019).

Other RET inhibitors in development

Except for selpercatinib and pralsetinib, BOS172738/Zeteletinib (Phase I, NCT03780517) (Schoffski et al. 2021; Schoffski et al. 2019), GSK3179106 (phase I, NCT02727283) (Eidam et al. 2018), SY-5007 (phase I, NCT05278364), KL590586 (phase I/II, NCT05265091) and HS-10365 (phase I, NCT05207787), were also developed in different stages of clinical investigation. For example, BOS172738(**7**) is a targeted inhibitor of aberrant mutations in RET. The phase I clinical trial of BOS172738 showed good safety in long-term administration. The ORR of BOS172738 was 33% ($n=18/54$) and the NSCLC cohort ORR was 33% ($n=10/30$) (Lin et al. 2020; Piotrowska et al. 2018). Currently, multiple clinical trials are being conducted, including LIBRETTO-431, LIBRETTO-531, NCT04211337, and NCT03780517 (Suda and Mitsudomi 2020).

Moreover, several N-phenyl-7,8-dihydro-6H-pyrimido[5,4-b][1,4]oxazin-4-amine derivatives have been reported as a new class of RET inhibitors. One of the representative compounds 17d (8) (Yang et al. 2018), with the structure of 1-(5-(tert-butyl)isoxazol-3-yl)-3-(4-((6,7,8,9-tetrahydropyrimido[5,4-b][1,4]oxazepin-4-yl)amino)phenyl)urea, inhibits RET(the IC_{50} for RET is 10 nM) and its mutants RET^{V804M} and RET^{V804L} potently. Lakkaniga et al. (2020) investigated a series of derivatives based on pyrrolo[2,3-d]pyrimidine skeleton and one of the represent compounds 59(9) is a type II inhibitor of RET, which potently inhibits RET(the IC_{50} for RET is 6.8 nM) and RET^{V804M} (the IC_{50} for RET is 13.5 nM). Moccia et al. (2020) identified the clinical drug candidates Pz-1(10) and NPA101.3(11), both with the IC_{50} for RET less than 1.0 nM. Also, Pz-1(10) and NPA101.3(11) lacking the structural liability for demethylation exhibited a selective inhibitory profile for both VEGFR2 and RET (WT and V804M).

During the last decade, Wang et al. (2019b) invested various nicotinamide analogs based on the scaffold of benzamide aminonaphthyridine. HSN356 was reported to inhibit RET kinase (Larocque et al. 2017). One of the nicotinamide analog of HSN356, HSN608(12) exerts strong RET inhibition and also inhibit $RET^{V804M/L}$ and RET^{S905F} mutants better than sorafenib, and vandetanib, which was almost as same as pralsetinib. (Fig. 5).

RET inhibitors can discovered by virtual screening of natural product (NP) libraries. There are four NP libraries encompassing Otava NP, NPASS (Natural Product Activity & Species Source) NP, IBS (InterBioScreen) NP, and LC (Life Chemicals). A 3D query as the model was employed to screen four NP libraries, including 102,829 NPs in total. As a result 198 compounds were procured by subsequent virtual screening, which were subjected to computer-aided drug designing (CADD). Among these natural product candidates, STOCK1N-98911 and STOCK1N-84953 exhibited favorable activites against RET and another kinases (Solomon et al. 2020). Even the compounds did not have the activity as much as other inhibitors, but it shows another way to discovery new structure RET inhibitors.

Until now, selective RET kinases inhibitors received significant progress and promising clinical outcomes. However, acquired resistance conferred by secondary mutations, e.g. G810C/S/R in solvent-front region (SF), Y806 C/N (in hinge residue) or V738A (in β2 strand)

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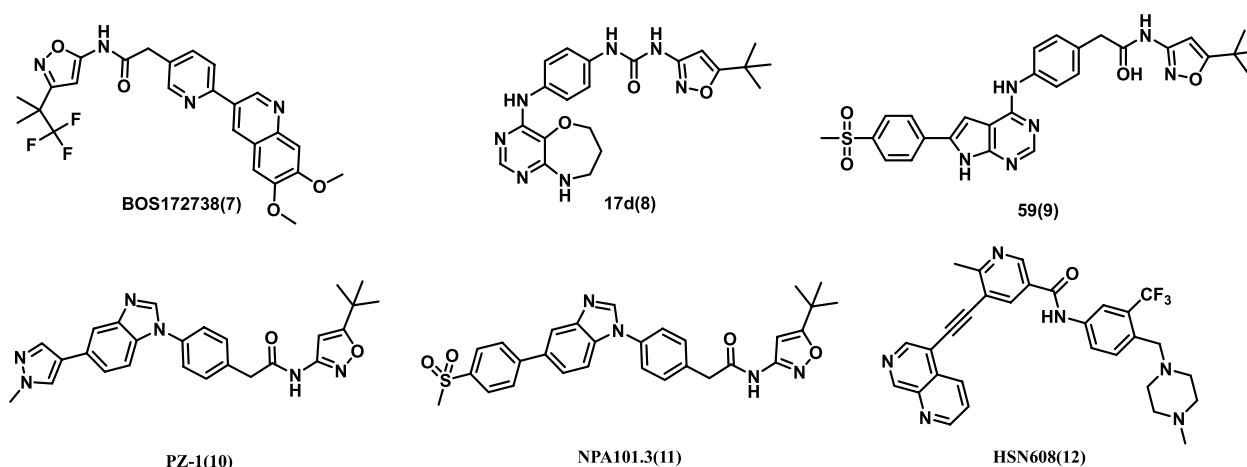
Fig. 5 Chemical structures of several other selective RET inhibitors

(Subbiah et al. 2021a; Solomon et al. 2020) were identified. As a result, substantial efforts have been devoted to discover new selective RET inhibitors for combating unsolved clinical needs (Zhang et al. 2022b; Moccia et al. 2021) and 4 candidates have been advanced into clinical trials at least. Such as TPX-0046 (Phase I/II, NCT04161391) (Drilon et al. 2019; Fancelli et al. 2021), LOXO-260 (Phase I, NCT05241834) (Kolakowski et al. 2021), TAS0953/HM06 (phase I/II, NCT04683250) (Miyazaki et al. 2017) and APS03118 with undisclosed structure which were based on the company's announcement (Subbiah et al. 2022) will be discussed next.

TPX-0046 is a small and rigid macrocyclic with the structure of (13E,14E,15aR,18aS,5S)-35-fluoro-5-methyl-15,15a,16,17,18,18a-hexahydro-4-oxa-7-aza-1(5,3)-cyclopenta[b]pyrazolo[1',5':1,2]pyrimido[4,5-e][1,4]oxazina-3(1,2)-benzenacyclooctaphan-8-one(13). It is a different structure from current RET inhibitors, which is developed by Turning Point Therapeutics and it is a potent and selective next-generation orally bioavailable RET/SRC kinase inhibitor. TPX-0046 demonstrated strong potency against many mutated RETs and WT, as well as SRC in enzymatic assays, which is except VEGFR2. In in-house engineered Ba/F3 KIF5B-RET, TT, and LC2/ad cells, TPX-0046 potently inhibited RET phosphorylation and cell proliferation with IC_{50} of nearly 1 nM.

TPX-0046 is also potent inhibit the Solvent Front Mutations (SFM) G810R in Ba/F3 cell proliferation assay with the IC_{50} value is 17 nM, whereas comparable molecules for pralsetinib and selpercatinib have $IC_{50} > 500$ nM. In vivo, TPX-0046 demonstrated marked anti-tumor efficacy in multiple RET-driven cancer cell-derived and patient-derived xenograft tumor models (Moccia et al. 2021). The clinical trial (ClinicalTrials.gov

Identifier: NCT04161391) employing TPX-0046 is on the way (Rebuzzi et al. 2021; Tan and Solomon 2020).

Ding's group (Zhang et al. 2022a), reported a serious of 1-methyl-3-((4-(quinolin-4-yloxy)phenyl)amino)-1H-pyrazole-4-carboxamide derivatives. The representative compounds 8q(14), strongly inhibit the wild-type RET kinase and the IC_{50} value is 13.7 nM. It is also potently suppressed the proliferation of BaF3 cells which stably expressing various oncogenic fusions of RET kinase with solvent-front mutations, such as CCDC6-RET[G810C], CCDC6-RET[G810R], KIF5BRET[G810C] and KIF5B-RET[G810R], the IC_{50} values are 15.4, 53.2, 54.2 and 120.0 nM, respectively. In Ba/F3-CCDC6-RET[G810C/R] cells, it dose-dependently inhibited the activation of RET and downstream signals and obviously triggered apoptosis. The compound 8q (14) also exhibited significant anti-tumor efficacy with a tumor growth inhibition (TGI) value of 66.9% at 30 mg/kg/day via i.p. in a Ba/F3-CCDC6-RET[G810C] xenograft mouse model.

HM06(vepafestinib, 15) (Isao et al. 2023) with the structure 4-amino-N-(4-(methoxymethyl)phenyl)-7-(1-methylcyclopropyl)-6-(3-morpholinoprop-1-yn-1-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide, was developed by Helsinn group and potently inhibited recombinant RET[WT], RET[V804] and solvent front (G810) kinase, the IC_{50} value of RET[WT] is 0.33 ± 0.01 nM. It is more selective than selpercatinib, pralsetinib and TPX-0046. Consistent with in vitro data, HM06 showed superior efficacy in tumor allografts derived from Ba/F3 cells expressing RET[WT] or RET[G810R] fusion proteins. It also increased the CNS availability and represented a possible effective strategy to overcome the emergence of acquired resistance to first-generation RET-selective inhibitors (Fig. 6).

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However, other compounds like LOXO-260 (Phase I, NCT05241834) (Subbiah et al. 2022) and APS03118 which is filed by Investigation New Drug (IND) haven't shown any clinical data now. The only thing about APS03118 is the IC_{50} for mutant RET inhibition was less than 0.4 nM which is based on the company's announcement, but the structure of it was undisclosed (Tan and Solomon 2020).

However, no drug is approved for overcoming acquired resistance against the selective RET inhibitor therapies until now. As mentioned, some mutations occured in RET-positivite NSCLCs, we will discuss next.

RET mutations
RET mutation was an in-traget kinase-acquired resistance. It dynamically evolves under kinase inhibitor selection pressure, making the kinase continuously activated under medication conditions. Gatekeeper mutations and solvent-front mutations were included. It has been reported that resistance mechanisms in MKIs include RET^{V804M} gatekeeper mutations and RET^{S904F} (Larocque et al. 2017). The primary V804M/L/E and S904F mutations of RET positive NSCLS patients formed steric clashes with the drugs because the mutations are in the kinase gatekeeper and activation loop, respectively (Román-Gil et al. 2022; Liu et al. 2018; Nakaoku et al. 2018).

Recent studies have demonstrated that $RET^{V804M/L}$ provides a gatekeeper function in oncogenic RET fusions, limiting efficacy of other MKIs such as cabozantinib (Liu et al. 2018) at the same time. For this reason, the selective RET inhibitors have been screened and designed for activity against gatekeeper mutations.

The selective RET inhibitor selpercatinib and pralsetinib induced a mutation (G810A/S) (Yoda et al. 2018), which demonstrated it's increased kinase activity and conferred resistance through allosteric effects. As a result, selective RET inhibitors have been designed to overcome gatekeeper mutations. The concurrent RET^{V804M} gatekeeper mutation was associated with a G810 resolute mutation in one NSCLC patient.

In addition, RET mutations located at the floor of the solvent-front (G810C/S/R), the hinge (Y806C/N), and the β2 strand (V738A) of the RET ATP-binding site (Yang et al. 2018) targeted-by pass mechanisms (Subbiah et al. 2021b; Rosen et al. 2021). The G810C/S/R mutations displayed the strongest resistance (Drilon et al. 2020) and were observed more often in patients whose tumors developed resistance to selpercatinib. The identification of selpercatinib-resistant RET mutations were cross-resistant to pralsetinib (Kim et al. 2021). Interestingly, Wu's group identified that the $RET^{L730V/I}$ mutations at the roof of the solvent-front site of the RET kinase were strongly resistant to pralsetinib but not to selpercatinib (Shen et al. 2021).

Conclusions and perspectives
In the last two decades, RET mutations and rearrangements represent a well-established mechanism that drive tumor growth across several types of neoplasms, including NSCLCs. Treatment with non-specific MKIs in RET fusion-positive NSCLC achieved modest clinical outcomes and limited response. Then, as pralsetinib and selpercatinib, the two highly selective RET inhibitors, were specifically developed to target RET kinase selectivity and to combat resistances to MKIs. But the emergence of off-target RET-independent mechanisms of resistance to pralsetinib and selpercatinib have highlighted the necessary to exploit further next-generation compounds and to explore new therapeutic strategies, including concurrent inhibition of RET and parallel signaling pathways of
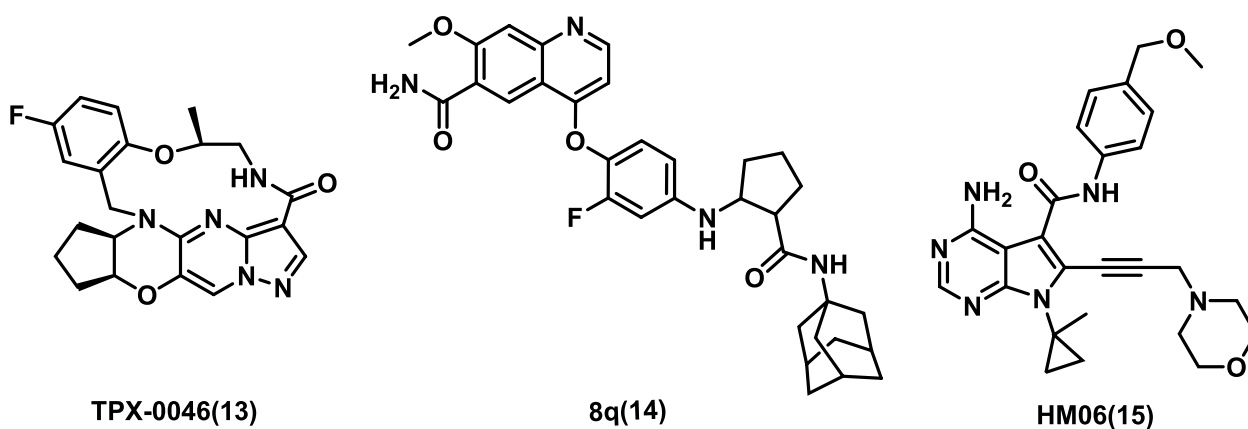
Fig. 6 Several RET inhibitors suppressing resistant mutants in solvent-front regions

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resistance. What's more, the treatment of two selective RET inhibitors costs are high. Within the next decade, the RET inhibition in NSCLC is on the verge of a break-through that will give physicians and patients promising new therapeutic options. Identifying potent, selective, and less toxic RET target agents, looking for compounds with RET activity from natural products, exploring the potential impact of different fusion variants, character-izing concomitant molecular alterations and mechanisms of resistance to RET inhibition to identify optimal thera-peutic combinations represent the challenges for future research in this field of NSCLC treatment.

Authors' contributions
Not applicable.

Declarations

Availability of data and materials
No dataset was generated or analyzed during this study.

Competing interests
All authors declare that they have no conflict of interest.

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