U.PORTO

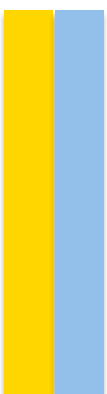
MESTRADO INTEGRADO
MEDICINA

Importance of the RET mutation in lung cancer

André Barroca Monteiro Aleixo Rocha

M

2023



Importance of the RET mutation in lung cancer

Dissertação de candidatura ao grau de Mestre em Medicina, submetida ao Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

André Barroca Monteiro Aleixo Rocha

Aluna do 6º ano profissionalizante do Mestrado Integrado em Medicina

Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

Rua de Jorge Viterbo Ferreira nº228, 4050-313 Porto

up201706279@up.pt

Orientador: Professor Doutor António Manuel Ferreira Araújo

Diretor do Serviço de Oncologia Médica, Centro Hospitalar Universitário de Santo António

Professor Catedrático Convidado, Instituto de Ciências Biomédicas Abel Salazar

antonio.araujo@chporto.min-saude.pt

Porto, maio de 2023

Assinado por: **André Barroca Monteiro Aleixo Rocha**
Num. de Identificação: 15001449
Data: 2023.05.24 11:04:08+01'00'

André Barroca Monteiro Aleixo Rocha

Assinado por: **ANTÓNIO MANUEL FERREIRA ARAÚJO**
Num. de Identificação: 05943839
Data: 2023.05.24 10:00:16+01'00'

Prof. Dr. António Manuel Ferreira Araújo

Dedicatórias

Para o meu avô, Raul Rocha

Acknowledgments (Agradecimentos)

A dissertação de mestrado é um marco duma vida. É o findar de um projeto de seis anos, anos de muito trabalho e esforço, só possíveis de concretizar com a ajuda de muitas pessoas, impossíveis todas de enumerar, mas a quem agradeço profundamente, mesmo que não estejam aqui explícitas.

Em primeiro lugar, aos meus pais, à minha irmã, e a toda a minha família, que foram aqueles que inicialmente me criaram e educaram, permitiram-me ser a pessoa que sou hoje, e também me forneceram todas as oportunidades, carinho e apoio que uma pessoa alguma vez poderia desejar.

De seguida, aos meus amigos, aos grupos académicos que pertenço, e em especial à Margarida, por durante todo o meu curso estarem ao meu lado, com boas palavras de aconselhamento, um ombro amigo, e um ouvido sempre pronto a tentar perceber e resolver qualquer problema, a qualquer hora. Foram sem dúvida uma das minhas pedras basilares.

Por último, mas não menos importante, ao Professor António Araújo, que desde o primeiro dia aceitou o desafio de ser meu orientador. Por se ter mostrado sempre disponível a ajudar-me, e a todo o apoio demonstrado desde o início, o meu muito obrigado.

Resumo

Contexto: O cancro do pulmão é a principal causa de morte por cancro em todo o mundo e também um dos cânceres mais diagnosticados, ficando atrás apenas dos dois principais cancros específicos por sexo: cancro de mama e cancro de próstata. O cancro do pulmão de não-pequenas células representa mais de 80% dos casos de cancro de pulmão, sendo a maioria diagnosticada em estadios avançados da doença, tornando-as inoperáveis. Portanto, há a necessidade de utilizar terapias sistémicas, com o objetivo atual do uso de medicamentos direcionados para mutações genéticas encontradas no tumor, que têm vindo a melhorar as taxas de sobrevida nestes pacientes. Uma dessas mutações envolve o gene *rearranged during transfection* (RET), presente em cerca de 1-2% dos casos de cancro do pulmão de não-pequenas células, identificado pela primeira vez em 2011. Esse gene codifica uma proteína que existe na membrana celular e é essencial para o desenvolvimento de numerosas células nervosas, bem como do rim e da espermatogénese. Ele pode se fundir com outro gene parceiro, tornando-se auto-sinalizador, o que leva à ativação contínua de suas vias de sinalização e à oncogénese. Esta revisão tem como objetivo identificar as terapias dirigidas à mutação RET já utilizadas e atuais para o cancro do pulmão de não-pequenas células.

Métodos: Esta revisão foi realizada utilizando as bibliotecas PubMed e *Cochrane Central Register of Controlled Trials* no período de janeiro de 2012 a janeiro de 2023. Os termos de busca utilizados foram: "*clinical trial*", "*lung cancer*" e "RET", com todos os sinónimos relevantes e variações de palavras, bem como os Descritores em Ciências da Saúde (*MeSH Terms*). Os registos de ensaios clínicos *Clinical Trials* e *International Clinical Trials Registry Platform* (ICTRP) foram pesquisados manualmente em busca de ensaios em curso relevantes sobre o tema. Além disso, revisões sobre o assunto foram consideradas para busca de citações.

Resultados: A pesquisa resultou num total de 97 resultados únicos, cujos títulos e resumos foram analisados. Um total de 19 estudos foram considerados para leitura do texto completo; a presente revisão englobou 11 estudos. Os cancros com alterações no gene RET foram inicialmente tratados com inibidores de quinases múltiplas. A sua eficácia foi modesta, com uma taxa de resposta geral (ORR) de 0-47%, com toxicidades proeminentes devido à sua atividade fora do alvo, o que frequentemente levava a reduções da dose ou interrupções de ciclos. Os novos inibidores altamente eficazes e específicos para o RET, selpercatinib e pralsetinib, mostraram grande promessa, com ORR de 58-91%, no entanto, mantendo alguns efeitos adversos.

Conclusão: Embora a eficácia destes novos fármacos, o desenvolvimento de resistências a estes inibidores e a ocorrência de alguns eventos adversos graves tenham levantado a novas preocupações, isto tem incentivado as empresas farmacêuticas a explorar este campo com fármacos que ultrapassem estas dificuldades.

Palavras-chave

Adenocarcinoma do pulmão, Ensaios Clínicos, c-RET

Abstract

Background: Lung cancer is the leading cause of death by cancer worldwide, and also one of the most diagnosed cancers, only behind the two main sex-specific cancers: breast and prostate cancer. Non-small cell lung cancer (NSCLC) accounts for more than 80% of the lung cancers, being most of them diagnosed in later stages of disease, inoperable. Therefore, there's a need to use systemic therapies, with the aim currently being in targeted drugs for genetic mutations found in the tumour, which have improved survival rates in these patients. One of these mutations is of the rearranged during transfection (RET) fusion gene, present in about 1-2% of the NSCLC, first identified in 2011. This gene codifies a protein that exists in the cell membrane and is essential to the development of numerous nerve cells, as well as the kidney and spermatogenesis. It can fuse with another partner gene, when it becomes self-signalling, which leads to continuous activation of its downstream activation and oncogenesis. This review aims to identify the previously used and ongoing targeted therapies for RET fusions in NSCLC.

Methods: This review was conducted using PubMed and Cochrane Central Register of Controlled Trials libraries in the time frame between January 2012 and January 2023. The search terms used were: "clinical trial", "lung cancer" and "RET", with all relevant synonyms and word variations, as well as Medical Subject Headings (MeSH Terms). The Clinical Trials and International Clinical Trials Registry Platform (ICTRP) were manually searched for relevant, on-going trials on the topic. In addition, reviews on the topic were considered for citation search.

Results: The search yielded a total of 97 unique results, all titles and abstract of which were analysed. A total of 19 studies were considered for full-text reading; the current review comprised 11 studies. RET-altered cancers were initially treated with multikinase inhibitors (MKIs). The efficacy of MKIs was modest, with an overall response rate (ORR) 0-47%, with prominent toxicities from their off-target activity, which frequently led to dose reductions or interruptions. New, highly effective and RET-specific inhibitors selpercatinib and pralsetinib have shown great promise, with ORR 58-91%, however mainting some adverse effects.

Conclusion: Although the efficacy of these new drugs, development of resistances to these inhibitors and the maintenance of some serious adverse events have brought new concerns, which is encouraging pharmaceuticals in exploring this field with drugs that can overcome these problems.

Keywords

Lung adenocarcinoma, Clinical Trials, c-RET

Acronyms

NSCLC - non-small cell lung cancer

SCLC - small cell lung cancer

EGFR - epidermal growth factor receptor

TK - tyrosine kinase

ALK - anaplastic lymphoma kinase

ROS1 - ROS proto-oncogene 1 kinase

BRAF - B-Raf proto-oncogene serine/threonine-protein kinase

NTRK - neurotrophic tyrosina kinase receptor

KRAS - Kirsten rat sarcoma viral oncogene

MET - mesenchymal epithelial transition

HER2 - human epidermal growth factor

RET - rearranged during trasnfection

OS - overall survival

PFS - progression-free survival

AEs - adverse events

DNA - deoxyribonucleic acid

mRNAs - messenger ribonucleic acids

GDNF - glial cell line-derived neurotrophic factor

GFLs - GDNF family ligands

GFRα - GDNF-family receptor-α

PI3K - phosphatidylinositol-4,5-biphosphonate 3-kinase

AKT - AKT serine/threonine kinase

RAS - Rat sarcoma virus

RAF - rapidly accelerated fibrosarcoma

ERK - extracellular signal-regulated kinases

STAT - signal transducer and activator of transcription proteins

JAK - Janus kinases

RNA - ribonucleic acid

KIF5B - kinesin family member 5B

CCDC6 - coiled-coil domain containing 6

NCOA4 - nuclear receptor coactivator 4

PD-L1 - programmed death-ligand 1

IHC - immunohistochemistry

FISH - fluorescent in situ hybridization

PCR - polymerase chain reaction

NGS - next-generation sequencing

MeSH Terms - Medical Subject Headings

ICTRP - International Clinical Trials Registry Platform

MKI - multikinase inhibitor

VEGF - vascular endothelial growth factor

ORR - overall response rate

DoR - duration of response

ALT - alanine aminotransferase

AST - aspartate aminotransferase

MYO5C - myosin VC

DCR - disease control rate

FGFR - fibroblast growth factor receptors

PDGFRα - platelet-derived growth factor receptor alpha

FDA - Federal Drug Administration

IC_{50} - half maximal inhibitory concentration

BCR-ABL - breakpoint cluster region protein-ABL protooncogene 1 nonreceptor tyrosine kinase

CNS - central nervous system

EMA - European Medical Agency

MAPK - mitogen-activated protein kinase

NRAS - neuroblastoma RAS viral oncogene homolog

Index

Table Index

Figure Index

Introduction

Lung cancer is the leading cause of death by cancer worldwide, with an estimated 1,796 million deaths per year (18,0 % of all cancers), and an yearly-incidence of 2,207 million (11,4%), being also one of the most diagnosed cancers, only behind the two main sex-specific cancers – breast and prostate cancers – , by estimations of the 2020 GLOBOCAN report. (1) In Portugal, the most up-to-date information on the epidemiology of cancers can be found in *Registo Oncológico Nacional*. (2) Lung cancer represents in Portugal the second most common cancer for men, with an incidence of 51/100 000 person-year, and the fourth most common for women, with an incidence of 17/100 000 person-year, showing an ever-increasing incidence since this registry began in 2010. (2)

However, it is important to look at its history and trend (Figure 1). (3) In the United States, lung cancer incidence has been steadily declining in the last three decades for men, at about 3% annually since 2009, but only since the mid-2000s there has been an inflexion point on the incidence curve for women, now declining at 1% annually, (4) which is in line with most of the developed world. (3, 5) Most of the differences between global and temporal trends on the incidence of lung cancer can be best defined by tobacco smoking, including its prevalence, habits, types, intensity, and age of initiation and cessation, which also explains the difference between sexes, as the smoking epidemic began later in women. (6, 7)

Traditionally, lung cancer is divided in two main histopathological categories – non-small -cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) – with clear differences in incidences: NSCLC accounts for 89% and SCLC for 11% of all lung cancers in the United States. (5, 8) However, due to the development of research in this area, there was a need for more precise and specific histologic typing. In the new 2021 WHO classification of Thoracic Tumours, lung carcinomas are generally divided in: adenocarcinomas, squamous cell carcinomas, adenosquamous carcinomas, sarcomatoid carcinomas, large cell carcinomas, neuroendocrine carcinomas (which include the SCLC), other epithelial tumours, and salivary gland-type tumours. In fact, each of these groups is further divided in subgroups, with special focus on the molecular and genetic classification, trying to provide greater diagnostic accuracy and improved outcomes with the new therapeutic strategies. (9)

In spite of the relative age of its work, Alberg *et al.* (10) made one of the best reviews of lung cancer risk factors and epidemiology. Tobacco smoking is the leading cause of lung cancer, accounting for 80 to 90% of the cases in the United States, with an up to 20-fold increased risk

compared with never-smokers. However, this risk is highly dependent on the tobacco consumption over the persons' lifetime (11). Among other known risk factors are the exposure to second-hand smoke (12), residential radon (13), silica (14), air pollution (15), asbestos (16, 17), occupation as painter (18), previous lung disease(s) (19), and history of lung cancer (20). Some commonly cited risk factors, such as socio-economic status and geography, can be explained by the historical patterns in cigarette smoking, with low-income households and/or lower education having a higher prevalence of tobacco consumption. (21)

In an early-stage tumour, surgery is the current mainstay of treatment, as it can be performed with a curative intent. (22) Nevertheless, close to half of the lung cancers are metastasized at the time of diagnosis, when surgery is no longer an option. (8) This has led to a need for research in this area, with the discovery of new therapeutic targets, new drugs, and better outcomes, which have been predominantly made by improvements in the NSCLC treatment. (23)

Targeted therapy in lung cancer

Although all the improvements in early-diagnosis, lung cancer is most frequently diagnosed when it is already metastized, at what time treatment with curative intent is no longer an option. (8) Until the beginning of this century, these patients were treated with aggressive chemotherapy, often with poor results. (24) A new era came with the advent of targeted therapies in tumours possessing oncogenic mutations, beginning with gefitinib, an epidermal growth-factor receptor (EGFR) inhibitor demonstrating positive results in NSCLC with this mutation in 2001. (25)

These mutations are commonly referred as driver mutations. All cancers derive from mutations in the DNA of cancerous cells, but only a subset of these mutations is oncogenic, meaning a minority confers growth advantage and is positively selected in the microenvironment of the tissue in cause, leading downstream to the arise of a cancer. (26)

In this line of thought, other targets for tyrosine kinase (TK) inhibitors with clinical significance were found, mainly the anaplastic lymphoma kinase (ALK), ROS proto-oncogene kinase (ROS1), B-Raf proto-oncogene serine/threonine-protein kinase (BRAF) and neurotrophic tyrosine kinase receptor (NTRK). Other targets currently with approved drugs are the Kirsten rat sarcoma viral oncogene homolog (KRAS), mesenchymal epithelial transition (MET), human epidermal growth factor receptor 2 (HER2), and rearranged during transfection (RET). (27)

These new drugs have shown an improvement in longer overall survival (OS), progression--free survival (PFS), and better symptom and adverse events (AEs) control. (28-30)

Moreover, the cancer is a dynamic and ever-changing pathological tissue and, worryingly, can also adapt to its environment. Several mechanisms of resistance to targeted therapy have already been described, the main ones being on-target resistance, either by another mutation of the kinase or upregulation of the driver mutation, activation of a bypass pathway that mimics the original driver mutation, or transformation of the whole lineage, making it independent of the original driver mutation. This occurs because the targeted therapy cannot eliminate every tumour cell, enabling the surviving ones to proliferate and adapt to its new microenvironment, thus undermining its success in the long term. (26, 31)

RET structure and function

The RET gene, which resides in the pericentromeric region of chromosome 10 (region 10q11.2), was first detected in 1985 by Takashi *et al.* whom, by transfection of NIH 3T3 cells with deoxyribonucleic acid (DNA) from human T-cell lymphoma, induced transformation with high efficiency. (32, 33) Later, it was found that this gene coded a transmembrane receptor of the TK family, required for normal development and maintenance of multiple tissues and cell types. (34)

The TK receptor encoded by RET is composed by three different domains: a large extracellular domain, a hydrophobic transmembrane domain, and an intracellular kinase domain. (34-36) The extracellular domain includes a segment of four cadherin-like domains, with a calcium-binding motif in between each domain, and a cysteine-rich region close to the plasma membrane, all essential and necessary for correct ligand interaction. (37, 38)

Its expression is regulated in multiple ways, either by DNA-binding factors that attach to its upstream promoter sequences, or by messenger ribonucleic acids (mRNAs) that bind to the 3' region of the RET introns, which induce alternative splicing. (39-42) This alternative splicing in the C-terminal creates three isoforms of the receptor, of which two – RET9 and RET51 – are well conserved throughout the species, from *Drosophila melanogaster* to higher primates, with the highest rates of similarity between mammals, implying the essential function of this gene. (37, 43)

RET is the signalling receptor of a multimolecular complex that binds the growth factors of the glial cell line-derived neurotrophic factor (GDNF), neurturin, artemin and persephin, all belonging to the GDNF family ligands (GFLs) with neurotrophic effects. (44-49) However, RET does not link itself directly to GFLs, but to a complex of a GFL and one of the four GDNF-family

receptor-α (GFRα) proteins, which are co-receptors anchored to the cell surface, more specifically to a lipid-rich raft that floats on the plasma membrane. (50, 51) However, RET is located mostly outside the rafts, and after GFL and GFRα binding, RET is rapidly recruited to the raft to form a GFL-GFRα-RET complex, followed by homodimerization with another complex and autophosphorylation of the intracellular tyrosine residues. (52-54) The lipid raft act as a meeting point between the complex, where the intracellular tyrosine residues act as a docking site and meet with the downstream signalling cascades, such as the phosphatidylinositol-4,5-biphosphonate 3-kinase (PI3K)/AKT serine/threonine kinase (AKT), Rat sarcoma virus (RAS)/rapidly accelerated fibrosarcoma (RAF)/extracellular signal-regulated kinases (ERK), and the signal transducer and activator of transcription proteins (STAT)/Janus kinases (JAK) pathways, all of which induce and promote cell proliferation, differentiation, as well as tumorigenesis (Figure 2). (53, 55, 56)

RET-mediated signalling has been found in kidney and nervous system development, neuronal differentiation and survival, and maintenance of hematopoietic and spermatogonial stem cells. (57-61)

RET oncogenic mechanisms in lung cancer

The RET gene can induce oncogenic transformation by two mechanisms: chromosomal rearrangements, or gain-of-function mutations, although only the first has been described in lung cancer. (60, 62) RET rearrangements occur predominantly with the 3' of the sequence that encodes the TK domain, often breaking off at intron 11, with the 5' sequence of its fusion partner, who shares the dimerization domain, leading to a TK activation regardless of ligand status, continuous downstream activation and, consequently, oncogenesis. The intron 11 break implies that only the intracytoplasmic portion of the RET protein is maintained which, when fused with its partner, avoids endosomal transport and lysosomal processing that would down-regulate its expression. (53, 54, 62-64)

The RET fusion in lung cancer was first identified in 2012 by a Korean group (65), who reported, after massive parallel DNA and RNA sequencing, a patient with an intrachromosomal fusion of two remote genes – the kinesin family member 5B (KIF5B) and the RET gene. The KIF5B is a kinesin motor that contains a coiled-coil domain which induces homodimerization, a necessary step in RET activation. Importantly, it is also a gene that is universally expressed, allowing expression of RET in cells in which it is normally transcriptionally silent. (65, 66)

Furthermore, it has been shown that the KIF5B-RET fusion can induce a 2 to 30-fold higher RET transcription than in normal lung tissues, which supports its oncogenic addiction. (67)

In a recent database study, the most common fusion partners of RET in NSCLC were KIF5B (66%), coiled-coil domain containing 6 (CCDC6) (18,2%), and nuclear receptor coactivator 4 (NCOA4) (2,9%), all of whom are located in the chromosome 10. (68) This is just a short list, as the total number of RET fusion partners is always expanding, with 61 new mutations added to the list from this same study. (68)

Characteristics of RET-rearranged NSCLC

With the almost synchronous discovery of the RET fusion on NSCLC by multiple investigation teams (65, 67, 69, 70), prompt characterization of its clinicopathologic characteristics proceeded. (71) RET fusions are present in 1-2% of all NSCLCs, with adenocarcinoma as its main histology. (72, 73) A recent database study of 523 patients with RET-fusion positive NSCLC confirmed previous findings (72) – a prevalence of 1.14% of a RET-fusion, a younger age (median age = 64 vs 68) and a never-smoker/ low pack-year predominance (tobacco exposure 0,6% vs 13%). (68) Another recent multicentre study, the RET-MAP, with 218 patients reported similar findings. (73) Although, the first studies suggested that there was no sex predominance (71, 72), the more recent studies agree in a slightly higher incidence in females, with a majority of 56%. (68, 73)

Unfortunately, most patients are only diagnosed when disease is advanced, as 72-75% of RET-fusion positive NSCLC patients are diagnosed at stage IV. (72, 73) Adenocarcinomas of the lung have a particularly high incidence of brain metastases, being present in approximately 14% of patients at diagnosis, and occurring frequently on the progression of disease. (74) However, RET gene rearrangements have been independently associated as risk factor for brain metastasis, present in 21-25% of patients at diagnosis of stage IV disease, which further negatively impacts the prognosis of these patients. (73, 75, 76)

Furthermore, the majority of RET fusions are mutually exclusive with other known targetable mutations, as only 4% has other co-occurring targetable driver mutation, although both EGFR and KRAS represent 3% each of concurrent drivers. (68) Moreover, a mechanism of acquired EGFR TK inhibitor-resistance in EGFR-mutant NSCLC is acquired RET fusion, mainly with the fusion partner CCDC6. This resistance has been described even with third generation

metastasis inhibitors, such as osimertinib, which luckily could be overcome with the use of an additional RET-specific inhibitor. (77-81)

Another purpose of the RET-MAP was to ascertain the controversy of whether RET-rearranged NSCLC was associated with a low programmed death-ligand 1 (PD-L1) expression and low tumour mutational burden, which it confirmed. (73) However, RET-rearranged NSCLC can benefit from pemetrexed-based chemotherapy, as these tumours have shown to have a longer median PFS than in patients treated with other chemotherapy regimens, making it the currently first-line chemotherapy in this subset of patients. (30, 82, 83)

RET fusion detection in NSCLC

RET fusions can be detected by immunohistochemistry (IHC), fluorescent *in situ* hybridization (FISH), polymerase chain reaction (PCR) assays, or by next-generation sequencing (NGS). In the case of IHC, it was traditionally used as a diagnostic tool in thyroid pathology, but due to its variable sensitivity and specificity in the detection of RET fusions in NSCLC, it is currently not recommended as a screening tool. (84-86) On the contrary, FISH or reverse transcription PCR is the current standard for the detection of RET fusions. It has a good overall sensitivity and specificity, and it is comparatively less expensive than the alternatives. (85) However, since the approval of RET-specific inhibitors, RET-fusion detection is a must when screening for targetable biomarkers. As such, NGS has multiple advantages – possibility of probing multiple genes in the same assay, reducing the amount of required tissue, and close to 100% sensibility and specificity – being presently the recommended screening assay where feasible. (85, 86) If no solid tumour specimens can be obtained, liquid biopsy is recommended, as it is non-invasive. Nevertheless, a negative NGS on liquid biopsy does not rule-out RET fusion, as its positivity depends on the nanograms of cell-free DNA or RNA that can usually be isolated from the plasma. (85, 87)

Methods

Search strategy

As the first evidence of the presence of a RET mutation in NSCLC dates back to 2012, a search was performed in MEDLINE (PubMed) and Cochrane Central Register of Controlled Trials libraries in the time frame between January 2012 and January 2023. The search terms used were: “clinical trial”, “lung cancer” and “RET”, with all relevant synonyms and word variations, as well as

Medical Subject Headings (MeSH Terms); for more information consult Appendix A. Published data of trials assessing the efficacy and safety of RET inhibiting drug therapies on NSCLC were identified.

Only studies in peer-reviewed journals were considered. The Clinical Trials and International Clinical Trials Registry Platform (ICTRP) were manually searched for relevant, on-going trials on the topic. In addition, reviews on the topic were considered for citation search.

Inclusion criteria consisted of clinical trials including patients (i) with pathologically diagnosed RET-fusion positive NSCLC and (ii) receiving RET-inhibiting therapy. Of the selected trials, the most up to date information and reports were searched and selected.

The following types of papers were excluded: (i) papers reporting on duplicate results, (ii) study protocols, (iii) reviews, (iv) editorials, (v) case reports, (vi) papers in non-English languages, (vii) retrospective studies, (viii) meta-analyses, (ix) research papers, (x) poster or meeting abstracts, (xi) patients with other known targetable mutations, and (xii) studies with less than 5 patients enrolled.

Results and Discussion

The search yielded a total of 97 unique results, all titles and abstract of which were analysed. Applying the previously mentioned exclusion criteria, a total of 19 studies were considered for full-text reading; the current review comprised 11 studies (Figure 3). The results of the trials are summarized in Table I

Cabozantinib

Cabozantinib (XL184) is a small-molecule multikinase inhibitor (MKI) with activity against RET, in addition to potent activity toward MET and vascular endothelial growth factor (VEGF) receptor 2 , as well as a number of other TK receptors, showing firstly activity *in vitro* and in mouse models, with a decrease in tumor size and endothelial cell proliferation, being first approved in 2012 for patients with medullary thyroid cancer, regardless of RET status. (88, 89)

In July 2012, a phase II study involving cabozantinib was initiated (NCT01639508) in three patients, the first study targeting RET-positive NSCLC. In 2013 the first results were published, in which two of these patients showed marked improvement both clinically and radiographically, with progression-free at a minimum of 4 months, while another patient with a KIF5B-RET fusion

had a roughly 8-month period of stable illness. However, two of the patients needed a dose reduction due to adverse events of the MKI. (90)

In 2016, the results of this trial were updated. At this point 26 patients had been enrolled in the trial, receiving a cabozantinib dose of 60 mg once daily, although one was excluded of analysis because it failed to undergo repeat protocol imaging. Disease was assessed clinically and by CT-scan of the chest, abdomen, and pelvis at baseline, 4 weeks after cabozantinib initiation, and every 8 weeks thereafter. Notably, the main mutation was the KIF5B-RET fusion, present in 62% of patients. Although achieved a complete response, the overall response rate (ORR) was 28%, with a median PFS of 5,5 months and a median duration of response (DoR) of 7,0 months. (91)

The main grade three or higher treatment-related AEs was increased lipase (15%), increased alanine aminotransferase (ALT) or aspartate aminotransferase (AST) (8%/8%), thrombocytopenia (8%), and hypophosphatemia (8%), all of which resolved with dose modifications. (91)

Results are still awaited from the CRETA trial, a phase II Italian study with an expected enrolment of 25 patients started in 2019 (NCT04131543). (92)

Currently, no drug agencies have approved the use of cabozantinib in RET-positive NSCLC. However, it is featured in guidelines as a first-line or subsequent therapy option in RET-rearranged metastatic NSCLC. (93)

Vandetanib

Vandetanib is a MKI developed in 2002 that inhibits mainly RET, VEGF receptors and EGFR TKs. (94) Since then, it has been approved for the treatment of unresectable, locally advanced or metastatic medullary thyroid cancer, regardless of RET status. (89) In what regards lung cancer, it first showed promise after the demonstration of it being the most significant suppressant of RET's signalling pathways in a case of NSCLC with CCDC6-RET fusion from the available MKIs. (95) Afterwards, two trials were initiated to evaluate vandetanib efficacy in NSCLC with RET rearrangements. It is important to note that both studies were single-arm, with patients that had undergone platinum-based chemotherapy and progressed.

The first is Korean phase II trial (NCT01823068), who enrolled a total of 18 patients treated with a 300mg dose of vandetanib daily, with an intention-to-treat. Radiologic tumour assessment was performed at baseline, every 8 weeks thereafter and, if there wasn't disease progression, continued every 12 weeks for 1 year, and then every 24 weeks for another year. No

brain imaging was required if not for clinical guidance. Only eight patients were evaluated for the RET fusion partner, with five being with KIF5B, two with CCDC6, and one with myosin VC (MYO5C). Moreover, 72% of the patients had already performed two or more lines of chemotherapy before the initiation of vandetanib. (96, 97)

The ORR was 18%, with a disease control rate (DCR) of 65%, as eight patients achieved stable disease. The median PFS was 4,5 months, the median DoR was not calculated, but median OS was 11,6 months. (97)

The most common AEs were grade 1 or 2 hypertension (89%), rash (72%), and diarrhea (44%), with five patients experiencing AEs of grade 3 – three with hypertension, two with asymptomatic QTc prolongation and one with elevated serum level of AST/ALT, which led to dose reduction. No grade 4 or 5 AEs were stated. (97)

The second main trial that evaluated vandetanib's efficacy in RET-rearranged NSCLC is the LURET trial (UMIN000010095). It enrolled 19 patients receiving a 300mg dose of vandetanib daily. Radiologic tumour assessment was performed at baseline, every 4 weeks for 3 months, and every 8 weeks afterwards until disease progression. As in the last trial, no brain imaging was required. The main RET fusion partner was KIF5B, present in 52,6% of the cohort, followed by the CCDC6, present in 31,6%. As previously stated, all patients had previously undergone chemotherapy, 89,5% with a pemetrexed-based regimen. (98, 99)

The ORR was 47,4%, with nine patients being able to achieve a partial response, with a median DoR of 6,5 months. The median PFS was 6,5 months and the median OS was 13,5 months. Regarding safety, all patients exhibited AEs, with 84,2% having grade 3 or 4; the most common grade 3 or 4 were hypertension (68,4%) and rash (15,8%). It ultimately led to the discontinuation of treatment (21,1%), dose interruption (89,5%) or dose reduction (57,9%). (99)

Lenvatinib

Lenvatinib is a MKI with potent activity against VEGF receptors, fibroblast growth factor receptors (FGFR), platelet-derived growth factor receptor alpha (PDGFRα), RET, and proto-oncogene c-Kit. It is currently approved by the Federal Drug Administration (FDA) as a first-line treatment of patients with unresectable hepatocellular carcinoma, as well as endometrial cancer, when combined with pembrolizumab; also in advanced renal cell carcinoma, when in combination with pembrolizumab or everolimus, and in radioactive iodine-refractory differentiated thyroid cancer. (100, 101)

A phase II trial was conducted to assess lenvatinib's efficacy and safety in RET-rearranged NSCLC (NCT01877083). It enrolled 25 patients, 13 with the fusion partner KIF5B and 12 with CCDC6, receiving a dose of 24mg of lenvatinib daily. Radiologic tumour assessment was performed at baseline and followed by an assessment every 8 weeks. Only two patients were treatment-naïve, while five had already received RET-targeted therapy. (102, 103)

At the end of follow-up, the ORR was 16%, all of which partial responses, with 80% of patients experiencing a tumour size reduction. The median PFS was 7,3 months, with a significant difference when accounting for the partner gene, with 9,1 months for CCDC6 and 3,6 months for KIF5B, while the median OS was not reached. (102)

All patients reported at least one treatment-emergent AEs, and grade 3 or higher treatment-emergent AEs were reported in all but two patients (92%), the most common being hypertension (56%), hyponatremia (20%), proteinuria (16%), and pneumonia (16%). These required either treatment interruption (76%), dose reduction (64%), or discontinuation altogether (24%). Unfortunately, three patients died due to AEs, with one considered treatment related. (102)

RXDX-105

RXDX-105 was developed as a dual RET and BRAF MKI, but sparing VEGF receptor 1/2, and it showed potent inhibition in patient-derived xenografts with KIF5B, CCDC6 and NCOA4 RET-rearranged NSCLC. As such, it was hypothesized that it would allow an increase in drug dosage, with further RET inhibition, without the AEs frequently associated with angiogenesis inhibition. (104)

A phase I/Ib trial (NCT01877811) was initiated to evaluate its efficacy in RET-rearranged NSCLC, colorectal cancer, hepatocellular carcinoma, medullary thyroid cancer, among other solid tumours. A total of 152 patients, of which 40 with RET-rearranged NSCLC, were enrolled, receiving a RXDX-105 daily dose of 275 mg. At the beginning of phase Ib, tumour assessment was performed with thoraco-abdominal CT scan, and every 8 weeks thereafter, with brain imaging at screening for NSCLC patients. The most common fusion partners were KIF5B (65%) and CCDC6 (20%), and 31 patients (78%) had not received prior MKI-treatment. (105, 106)

The ORR was 19% in the MKI-naïve cohort, all of which partial responses, while the 9 MKI-pretreated patients achieved no response, with disease progression within the first two to four cycles. Additionally, a statistically significant difference in response was evident between KIF5B and non-KIF5B fusion partners, with no responses being observed in KIF5B–RET tumours, while

tumours with a non-KIF5B partner achieved an ORR of 67%. (105) To investigate this discrepancy, the authors produced Ba/F3 cell lines with KIF5B-RET, CCDC6-RET, and NCOA4-RET, and observed a near two-fold increase in the half maximal inhibitory concentration (IC_{50}) of KIF5B-RET cells when compared to others. Moreover, KIF5B-RET cells have a significantly higher mRNA expression, which the authors argue translates into a higher number of oncogenic proteins that the targeted therapy needs to silence. In summary, this would explain the inability of RXDX-105 to have efficacy in the most common RET gene fusion. (105)

The AEs were not differentiated for tumour type, being reported for the total number of patients of phase I. The most common grade 3 or higher treatment-related AEs were hypophosphatemia (9%), elevated ALT (8%), maculopapular rash (7%), elevated AST (5%), and diarrhea (5%), which required either dose reduction (28%) or dose discontinuation (16%). (105)

Alectinib

Alectinib is a second generation ALK inhibitor, currently approved for ALK-rearranged NSCLC. (83, 93) It first showed that it could inhibit ALK at a IC_{50} = 1,9nmol/L, while its RET inhibition was also significant (IC_{50} = 4,8nmol/L) *in vitro* and *in vivo*, with an overall lower inhibitory concentration needed when compared to cabozantinib and lenvatinib in the KIF5B-RET fusion. (107, 108) Thus, it was hypothesized that alectinib would have a clinical advantage when compared to MKIs, as it was well-tolerated in previous trials, and avoids common MKI AEs such as hypertension. (108)

Alectinib was tested in RET-rearranged NSCLC in a phase I/II Japanese trial (UMIN000020628). A total of 34 patients were enrolled in phases I and II. Phase I was conducted with 9 patients to determine the dose regimen of either 600mg or 450mg twice daily. Because of the development of grade 3 or higher AEs in 3 out of 6 patients in the 600mg cohort, it was agreed that the 450mg twice daily was the recommended dose. Radiologic tumour assessment was performed with full body CT scan at baseline, at 21 days after beginning of treatment, and every 6 weeks afterwards. Rearrangements were detected by NGS, with KIF5B-RET being the most common fusion gene, present in 61,8% of the patients, followed by the CCDC6-RET fusion, identified in 26,5%. (109, 110)

The efficacy was determined for the MKI-naïve patients – 22 in phase II and 3 in the 450mg twice daily cohort of phase I. The ORR was 4%, as only one patient had a partial response, but DCR was achieved in 52%. Moreover, PFS was calculated to be at 3,4 months and OS at 19 months. (110)

In what regards AEs, the most common in those treated with 450mg of alectinib twice daily were grade 1 or 2 constipation (42,9%), increase in creatine phosphokinase (46,4%), and creatinine increase (28,6%). Grade 3 AEs included diarrhea, pneumonitis, increase in creatine phosphokinase and bilirubin, hyponatremia, and neutropenia, all of which with only one patient developing them; no grade 4 AEs were observed. (110)

The ALERT-lung trial was a phase II trial started in 2018 (NCT03445000) also studying alectinib in RET-rearranged NSCLC. But it was prematurely terminated in 2021 due to its low recruitment rate (14 out of the expected 44 patients) and no results have yet been published. (111)

Another phase I/II study was conducted (NCT03131206) to assess alectinib's efficacy. Despite being a trial designed for RET-rearranged NSCLC and RET-mutated medullary thyroid cancer, 1 out of the 5 patients enrolled had an ALK rearrangement. In phase I it enrolled only NSCLC patients. Partial response was only seen in 1 patient (ORR = 20%), and no grade 3 or higher AEs were reported, while fatigue, hypokalemia and dyspnea were the most frequent AEs. The study ended due to slow recruitment and lack of efficacy. (112)

Ponatinib

Ponatinib is an oral MKI that potently inhibits breakpoint cluster region protein-ABL protooncogene 1 nonreceptor tyrosine kinase (BCR-ABL), as well as MKIs, including RET, with an IC_{50} of 25,8 nmol/L, when tested for the most frequent fusion partner, KIF5B, being the most potent inhibitor when compared to cabozantinib, vandetanib and lenvatinib. (113, 114)

Later, a phase II trial (NCT01813734) was planned to know if the prediction translated to real-world efficacy, evaluating ponatinib's efficacy and safety in treating RET-rearranged NSCLC. It enrolled a total of 9 patients that were given 30 mg daily of ponatinib, with tumour assessment at baseline and every month afterwards. The ORR was 0%, but 55,6% of patients achieved a DCR, with a median PFS of 3.8 months. All patients reported AEs, with constipation, diarrhea, non-cardiac chest pain and hypertension being the most frequent ones, while two patients reported grade 3 dry skin and maculo-papular rash. (115)

Selpercatinib

Selpercatinib, formerly known as LOXO-292, is a highly selective adenosine triphosphate-competitive inhibitor of RET kinase, which demonstrated potent pre-clinical activity in RET-positive NSCLC, higher than that provided by MKIs. (116, 117)

Currently, the main study assessing its safety and efficacy is the LIBRETTO-001, a phase I/II trial (NCT03157128). In phase I, patients were given selpercatinib in doses ranging from 20mg once daily to 240 mg twice a day. In phase II, all patients started by receiving the recommended phase II dose - 160 mg twice a day. (118, 119)

Radiologic tumor assessments were performed at baseline, every 8 weeks for 1 year, and then every 12 weeks, as well as brain imaging at baseline for all phase II patients. A total of 356 patients enrolled in the study, a more than doubling the initial 105 patients (116), of which 69 were treatment-naïve, and 247 had previously been treated with platinum-based chemotherapy, as well as other therapies, including immunotherapy and MKIs. The main mutations were fusions of the RET gene with the KIF5B or CCDC6 gene, present in 56,5% and 17,7% of the patients, respectively. (120)

The ORR was high for both cohorts, with 84% for treatment-naïve patients and 61% for pretreated patients, which highlights selpercatinib's efficacy. Moreover, selpercatinib also showed great impact in central nervous system (CNS) metastasis, with an ORR of 85%, and a median DoR intracrannialy of 9,4 months. As CNS metastasis is a poor prognostic factor and a significant source of clinical morbidity, selpercatinib's efficacy in reducing their burden indoubetly improves these patients' lifes. (120-122) Furthermore, with a median overall DoR and PFS of 20,2 and 22,0 months, respectively, in treatment-naïve patients, and 28,6 and 24,9 months in pretreated patients, respectively, this drug has a durable and sustained efficacy. (120)

Regarding AE, the most common grade 3 or 4 treatment-related AE include hypertension (13.2%), AST/ALT elevations (6.3%/9.0%) and QTc prolongation (3,4%). In addition with other AE, it led to dose reduction in 41% of patients. (120)

LIBRETTO-321 is a phase II study being conducted in 15 Chinese centers (NCT04280081), as LIBRETTO-001 did not include this population. It had the same procedures as LIBRETTO-001, with the same inclusion and exclusion criteria, dose of the drug, and radiologic tumour assessement. It enrolled a total of 47 patients with RET-fusion positive NSCLC, of which a subset of 26 were included for analysis, owing to a need for central laboratory confirmation of RET-fusion. 18 had previously received chemotherapy or immunotherapy, and 8 were treatment naïve. Results were similar to those of LIBRETTO-001 trial, with an ORR of 87.5% in treatment-naïve patients, and 61.1% in previously treated patients, although median PFS and OS were still not reached at data cut-off. In what regards intracranial response and AEs, the results were also overlapping. (123, 124)

Due to LIBRETTO-001 initial findings, selpercatinib was first approved by the FDA in 2020, and subsequently by European Medical Agency (EMA) in 2021; since then it has been included in international guidelines. (93, 125-127) Presently there are multiple ongoing trials with selpercatinib: (NCT04268550), (NCT05364645), (NCT03899792), (NCT03944772) and chiefly the phase III LIBRETTO-431 (NCT04194944), which seeks to assess the PFS of selpercatinib in RET-positive NSCLC, comparing it to a platinum and pemetrexed-based chemotherapy, with or without pembrolizumab; also, the phase III LIBRETTO-432 (NCT04819100), comparing selpercatinib as an adjuvant treatment in delaying cancer return in patients with early-stage RET positive NSCLC, who have already had surgery or radiation, to placebo. (111, 128-134)

Pralsetinib

Pralsetinib, formerly known as BLU-667, is a new, highly selective inhibitor of RET kinase, either the wild-type or the mutated one, with higher pre-clinical activity in cancer models than MKIs, including its main fusion partners (KIF5B and CCD6). (135)

The ARROW trial (NCT03037385) is a phase I/II study assessing its safety, efficacy, pharmacodynamics and cynetics, as well as tolerability. Patients were initially enrolled in phase I with pralsetinib dose escalation, from 30mg to 600 mg, in order to determine optimal phase II dose. It was determined that 400 mg once daily was the recommended phase II dose. (136, 137)

Radiologic tumor assessments were performed at baseline, every 8 weeks while the patient was on treatment, and then every 3 to 4 months after last dose for patients who discontinued without disease progression. Brain imaging at baseline was also performed for all patients. A total of 233 patients had enrolled at the time of data cut-off point, of which 75 were treatment-naïve, and 158 had previously been treated with platinum-based chemotherapy or other systemic therapy. The main mutations were fusions of the RET gene with the KIF5B or CCDC6 gene, present in 70,4% and 17,6% of the patients, respectively. (138)

The ORR was 64% for both cohorts, with 72% for treatment-naïve patients, 59% for patients previously treated with platinum-based chemotherapy and 73% for patients previously teated with other systemic therapies. Moreover, pralsetinib showed tumor size reduction in all treatment-naïve patients, and in 97% of those pretreated with platinum-based chemotherapy. Until the latest results, the median DoR was still not established in the treatment-naïve population, but it was 22,3 months in those previously teated with platinum-based chemotherapy. As with selpercatinib, pralsetinib also showed a response in CNS metastases, with an ORR of 70% and a median DoR intracrannialy of 10,5 months. Furthermore, the PFS was estimated to be 13,0 months in the treatment-naïve patients, as end-point was still not reached,

and determined as 16,5 months in the patients previously teated with platinum-based chemotherapy. (138)

In what regards safety, the most common grade 3 or 4 treatment-related AEs were neutropenia (20,3%), anaemia (12,5%) and hypertension (12,1%). The patients achieved a median dose intensity of 92%, which supports the tolerability and clinical maneagibility of pralsetinib, as ORR remained high. (138)

Pralsetinib has been approved by the FDA in 2020 and by the EMA in 2021, as well as by other agencies, and it has been included in international guidelines. (93, 126, 139-141) Currently there are some ongoing trials featuring pralsetinib: (NCT04302025), (NCT05170204); mainly the phase III AcceleRET (NCT04222972), which seeks to assess the PFS of pralsetinib in RET-positive NSCLC, comparing it to 1 of 6 platinum chemotherapy-based regimens chosen by investigators, all integrating current standard of care practices. (142-145)

Resistance to RET Inhibitors

In patients undergoing molecularly targeted therapy, cancer resistance is frequently seen. Globally, these mechanisms can be divided in primary resistance, when there is a lack of oncogene addiction or the presence of multiple targets in the same tumour due to its heterogeneity, and secondary or acquired resistance. In kinase inhibitors, one of the main mechanism of resistance is on-target mutations, often seen in the intracellular kinase domain. (146)

The first example of resistance to RET inhibitors was a RET S904F secondary mutation that induced lower affinity to vandetanib, the MKI being used, which ultimately led to progression due to decreased effectiveness. (147) Other case-reports of vandetanib resistance in RET-rearranged NSCLC surfaced, mainly by the gatekeeper RET V804M/L mutations, which increase adenosine triphosphate affinity in its phosphorilating domains, leading to increased kinase activity, similar to that seen in ALK and ROS1. (148, 149) The current selective RET inhibitors, selpercatinib and pralsetinib, were designed to have action against these mutations, first preclinically, and later, in 2018, showing evidence in patients. (150, 151)

More recently, Solomon *et al.* reported two patients whom, in the course of the LIBRETTO-001 trial, relapsed due to the surgence of RET G810 C-lobe solvent front mutations. The substitution of this glycine residue with residues that have large side chains causes an alteration

in the protein structure that disrupts the drug's binding site, directly inhibiting it. Moreover, in one patient the RET V804 gatekeeper mutation developed *de novo* with the RET G810 mutation, a phenomenon which was replicated in a xenograft. (152) The mutation of RET G810 has also been found in the cell-free DNA of two NSCLC patients treated with pralsetinib. (153) Similarly, mutations at the L730 N-lobe solvent front have shown to be resistant to pralsetinib in patients, but sensitive to selpercatinib in xenografts, due to pralsetinib's stronger steric conflict. (153, 154) Other mutations that caused the relapse of patients were at the RET Y806 hinge site, which borders the adenosine triphosphate pocket that selpercatinib and pralsetinib occupy. The tyrosine residue has a hydrophobic side that, when substituted by residues that contain non-hydrophobic, shorter side chains, interferes with the drug interactions and induces resistance. (155, 156) Moreover, the V738 β2 strand has also shown to be resistant to selpercatinib and pralsetinib in xenografts. (155)

The other main mechanism of resistance to kinase inhibitors is off-target, primarily by bypass and alternative activation of one of the oncogenic signaling pathways. (146) In what regards RET, this has been shown particularly with the kinases that activate the mitogen-activated protein kinase (MAPK) pathway, mainly EGFR, KRAS, neuroblastoma RAS viral oncogene homolog (NRAS) or BRAF mutations, or MET and fibroblast growth factor receptor 1 amplification. Rosen *et al.* analysed the mechanisms of resistance in patients enrolled in LIBRETTO-001 trial, in which they report two patients with primary resistance to treatment due to KRAS G12 mutations, detected in liquid biopsy cfDNA at a low allele frequency, but not in the tissue. During treatment, an inversion in prevalence was observed – RET rearrangements decreased, while KRAS mutations=allele frequency increased, at time of disease progression. This was also observed in another patient that developed a KRAS G12V mutation, suggesting the presence of multiple heterogeneous subclones in the tumour. (156)

Moreover, MET amplification has also shown to be one of the reasons for poor results to both selpercatinib and pralsetinib, with all patients, except one, achieving a PFS no longer than 6 months, which validates the previous reports of its agressiveness. (156-159) Combining selpercatinib treatment with crizotinib, a MET inhibitor, enabled the patients to overcome the resistance, even though for a short time, due to disease progression. (160)

There has also been a case report of a KH domain-containing, RNA-binding, signal transduction-associated protein 1- NTRK3 fusion occurring in a KIF5B-RET rearranged NSCLC patient after treatment with selpercatinib, which induced transformation in cell cultures but,

when exposed to both RET and NTRK3 inhibitors, the kinases were suppressed and apoptosis ensued. (161)

TPX-0046 is a drug purposedly built to overcome the the G810 solvent front mutation currently under a phase I/II trial (NCT04161391) that has shown to be a potent RET inhibitor, with an IC50 < 10 nM . However, it has also been shown that it is also susceptible to other mutations, mainly the gatekeeper mutations V804L/M. (162, 163)

Another RET inhibitor that has shown ability to overcome mutations is the SYHA1815. It has shown *in vitro* and *in vivo* that it can inhibit wild-type RET and overcome the V804L/M mutation. Moreover, it has also shown to have a nearly 20-fold selectivity for RET over VEGFR2, which is important to avoid common AEs associated with its inhibition, mainly hypertension. (164) It is also currently under a phase I/II trial to evaluate its safety, tolerability and preliminary efficacy (NCT05105464). (165)

Similarly, BOS172738 showed nanomolar potency for the wild-type RET and the V804L/M gatekeeper mutants, all with a 300-fold selectivity against VEGFR2. (166) BOS172738 is currently in an active phase I trial (NCT03780517). (150)

LOXO-260, previously known as LOX-18228, is a selective next-generation RET inhibitor. It has shown in a pre-clinical study to inhibit multiple RET mutations, including both V804M and G810S, and is currently undergoing a phase I trial to assess its safety, side effects and efficacy (NCT05241834). (167, 168)

Conclusion

The discovery of RET rearrangements in NSCLC implemented a race for the discovery of alternative therapies to the chemo and immunotherapy mainstay that could improve these patients' prognosis and, ultimately, lives. Firstly, available therapies with success in cancers with different drivers were tried, with somewhat positive results, mainly the MKIs vandetanib and cabozantinib, as RET was a prime target for them. However, the lack of specificity and, consequently, prominent adverse events, led ultimately to dose reductions and a lack of efficacy.

This led to the development of the new drugs and targeted therapies for RET specific inhibitors, e.g., selpercatinib and pralsetinib, which have proven to be safe and effective in both medullary thyroid carcinoma and NSCLC patients. As previously mentioned, both drugs are currently in phase III trials with the purpose of consolidating their status as first-line standard of

care in patients with advanced RET-rearranged NSCLC. Nevertheless, concerns have already been observed, mainly the development of resistances to these inhibitors and the maintenance of some serious adverse events. Presently, there is an array of drugs under clinical trial to overcome these resistances, mainly the solvent front G810 mutation, that have shown promising results.

Other RET-inhibitors, including TPX-0046, SY-5007, SYHA1815, BOS172738, LOXO-260, and TAS0953/HM06, are also underway to expand the current portfolio of available therapies.

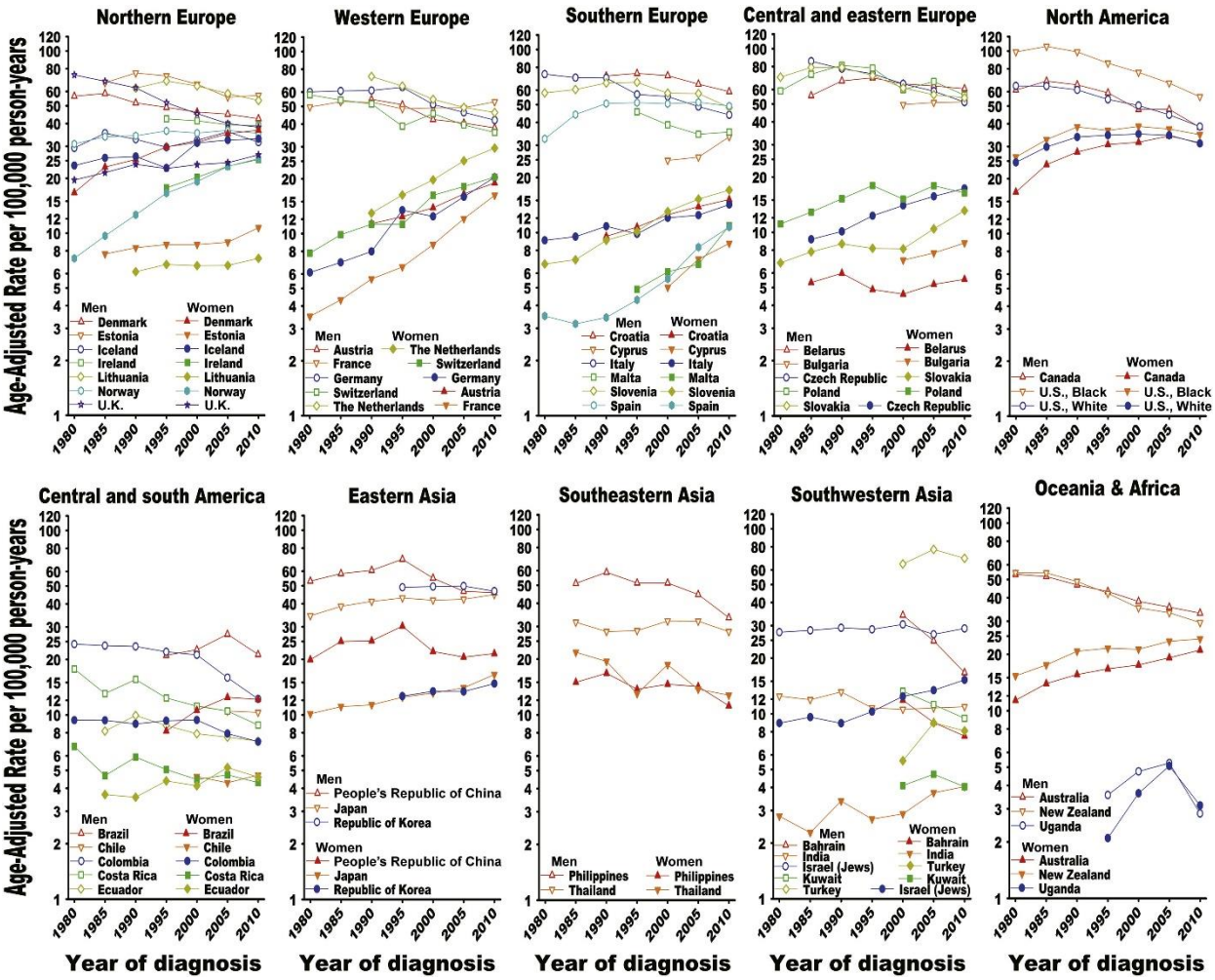


Figure 1 - Trends in lung cancer incidence rates by country for both sexes, from 1978 – 1982 to 2008 – 2012. Rates are per 100,000 person-years and age-adjusted to the world standard population.

Retrieved from: *Zhang Y, Luo G, Etxeberria J, Hao Y. Global Patterns and Trends in Lung Cancer Incidence: A Population-Based Study. Journal of Thoracic Oncology. 2021;16(6):933-44*, with CCC® authorization

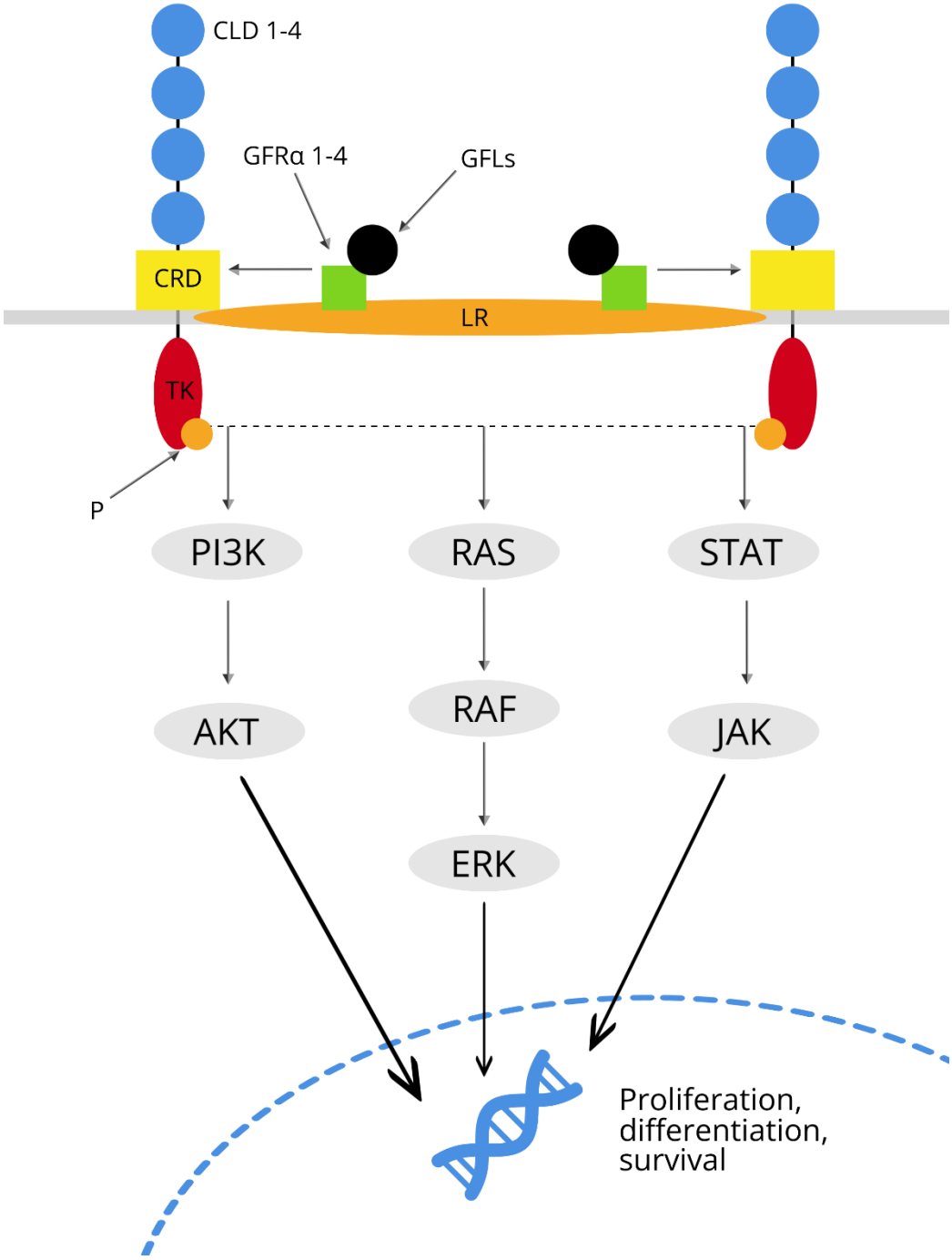


Figure 2 - Rearranged during transfection (RET) normal function and pathway

Notes: **CLD 1-4** – cadherin-like domain 1-4; **CRD** – cysteine rich domain; **TK** – tyrosine kinase; **P** – phosphorylation site; **GFLs** - glial cell line-derived neurotrophic factor (GDNF), neurturin, artemin and persephin; **GFRα 1-4** – GDNF-family receptor-α 1-4; **LR** – lipid raft; **PI3K** - phosphatidylinositol-4,5-biphosphonate 3-kinase; **AKT** - AKT serine/threonine kinase; **RAS** - Rat sarcoma virus; **RAF** - rapidly accelerated fibrosarcoma; **ERK** - extracellular signal-regulated kinases; **STAT** - signal transducer and activator of transcription proteins; **JAK** - Janus kinases

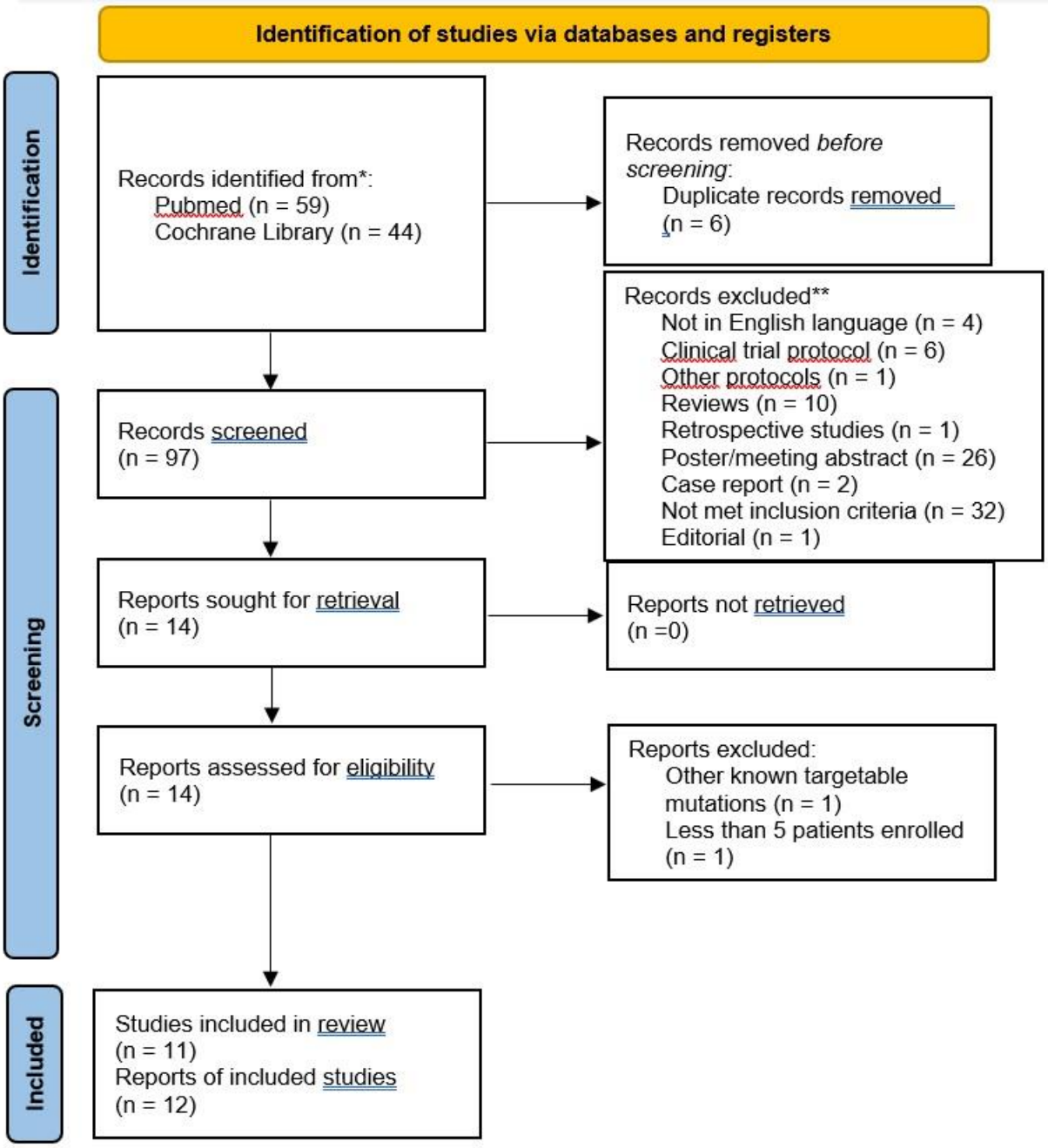


Figure 3 - PRISMA 2020 flowchart for study selection

Table I - Clinical data and results for RET inhibitors in patients with advanced RET-rearranged NSCLC by trial

Trial	Type of Trial	RET Inhibitor	Number of patients with RET-rearranged NSCLC	RET-fusion partner/ Number of patients	Pretreated patients (% of patients)	ORR (%)	Median PFS (months)	Median OS (months)	Grade 3 or 4 AEs (% of patients)
Drillon *et al.* (2016) (91)	Phase II single arm	Cabozantinib 60 mg/daily	26	KIF5B (16) CCDC6 (1) TRIM33 (1) CLIP1 (1) ERC1 (1) Unknown (6)	Chemotherapy 1 regimen – 13 (50%) Chemotherapy ≥ 2 regimens – 7 (27%)	28%	5,3 months (3,8-9,2)	9,9 months (8,6-NR)	Increased lipase (15%) Increased AST/ALT (8%/8%) Hypertension (4%)
Lee *et al.* (2017) LURET (97)	Phase II single arm	Vandetanib 300mg/daily	18	KIF5B (5) CCDC6 (2) MYO5C (1) Unknown (10)	Chemotherapy 1 regimen – 5 (28%) Chemotherapy 2 regimens – 2 (11%) Chemotherapy ≥3 regimens – 11 (61%)	18%	4,5 months	11,6 months	Hypertension (17%) Prolonged QT interval (11%) Increased transaminases (6%)

Yoh *et al.* (2021) (99)	Phase II single arm	Vandetanib 300mg/daily	19	KIF5B (10) CCDC6 (6) Unknown (3)	Surgery – 5 (26%) Radiotherapy – 4 (21%) Chemotherapy ≥3 regimens – 8 (42%)	47% (24,4-71,1)	6,5 months (3,9-9,3)	13,5 months (9,8-28,1)	Hypertension (68%) Acneiform rash (16%) Diarrhea (11%) Prolonged QT interval (11%)
Hida *et al.* (2019) (102)	Phase II single arm	Lenvatinib 24mg/daily	25	KIF5B (13) CCDC6 (12)	1 - anticancer therapy – 8 (32%) 2 anticancer therapies – 6 (24%) ≥3 anticancer therapies – 9 (36%)	16% (4,5-36,1)	7,3 months (3,6-10,2)	NR	Hypertension (56%) Hyponatremia (20%) Proteinuria (16%) Pneumonia (16%)
Drillon *et al.* (2019) (105)	Phase Ib single arm	RXDX-105 275mg/daily	40	KIF5B (20) CCDC6 (6) EML4 (2) PARD3 (1) Unknown (2)	≥1 MKI – 9 (23%)	19% (8-38) in MKI naïve 0% (0-34) in MKI pretreated	NA	NA	Maculopapular rash (10%) Increased ALT (8%) Hypophosphatemia (7%) Increased AST (5%)

Takeuchi *et al.* (2021) ALL-RET (110)	Phase II single arm	Alectinib 450mb/twice daily	34	KIF5B (21) CCDC6 (9) Unknown (4)	Chemotherapy 1 regimen – 7 (21%) Chemotherapy 2 regimens – 11 (32%) Chemotherapy ≥3 regimens – 16 (47%)	4% (0-20) in MKI naïve	3,4 months (2,0-5,4) in MKI naïve	19 months (5,4-NR) in MKI naïve	Increased bilirubin (1%) Hyponatremia (1%) Neutropenia (1%) Increased creatine phosphokinase (1%)
Awad *et al.* (2018) (112)	Phase I single arm	Alectinib in dose escalation/twice daily	5	NA	NA	20%	NA	NA	None reported
Shaw *et al.* (2019) (115)	Phase II single arm	Ponatinib 30mg/daily	9	NA	NA	0%	3,8 months (1,8-5,3)	NA	Rash maculopapular (11%) Dry skin (11%)

Drillon *et al.* (2023) LIBRETTO-001 (120)	Phase II single arm	Selpercatinib 160mg/twice daily	356	KIF5B (201) CCDC6 (63) NCOA4 (6) Others (48)	Chemotherapy – 247 (69%) Immunotherapy – 144 (40%) MKI – 85 (24%)	84% (73-92) in chemotherapy naïve 61% (55-67) in chemotherapy pretreated	22,0 months (13,8-NR) in chemotherapy naïve 24,9 months (13,9-NR) in chemotherapy pretreated	NA	Hypertension (20%) Increased ALT (11%) Increased AST (9%) Diarrhea (5%) Prolonged QT interval (5%)
Lu *et al.* (2022) LIBRETTO-321 (123)	Phase II single arm	Selpercatinib 160mg/twice daily	47	KIF5B/ CCDC6/ NCOA4 (42) Others (5)	Chemotherapy – 34 (72%) Immunotherapy – 11 (23%) MKI – 2 (4%)	91% (58,7-99,8) in treatment naïve 58% (40,8-74,5) in pretreated	NR	NR	Hypertension (20%) Increased AST/ALT (16%/16%) Thrombocytopenia (10%) Prolonged QT interval (8%)

Griesinger *et al.* (2022) ARROW (138)	Phase II single arm	Pralsetinib 400mg/daily	281	KIFB5B (164) CCDC6 (41) NCOA4 (1) Others (27)	Chemotherapy – 138 (59%) Immunotherapy – 69 (30%) MKI – 44 (19%)	72% (60-80) in treatment naïve 59% (50-67) in chemotherapy pretreated	13,0 months (9,1-NR) in treatment naïve 16,5 months (10,5-24,1) in chemotherapy pretreated	NR	Neutropenia (20%) Anaemia (12%) Hypertension (12%) Lymphopenia (9%) Leukopenia (8%)

Notes: **AEs** – adverse events; **ALT** – alanine aminotransferase; **AST** – aspartate aminotransferase; **CCDC6** – coiled-coil domain containing 6; **CLIP1** - CAP-Gly domain containing linker protein 1; **ERC1** - ELKS/RAB6-Interacting/CAST family member 1; **MKI** – multi-kinase inhibitor; **MYO5C** – myosin VC; **NA** – not available; **NCOA4** – nuclear receptor coactivator 4; **NR** – not reached; **NSCLC** – non-small cell lung cancer; **ORR** – overall response rate; **OS** – overall survival; **PARD3** – Par-3 family cell polarity regulator; **PFS** – progression-free survival; **RET** – rearranged during transfection; **TRIM33** – tripartite motif containing

Appendix A

Search string for PubMed: (((((randomized controlled trial[pt]) OR (controlled clinical trial[pt]) OR (clinical trial[pt]) OR (randomized.ab) OR (placebo.ab) OR ("Clinical Trials as Topic"[Mesh]))) NOT ((animals not (humans and animals)).sh)) AND (("Lung Neoplasms"[Mesh]) OR (nsclc[tw]) OR (nsclcs[tw]))) AND (("Proto-Oncogene Proteins c-ret"[Mesh]) OR (RET [tw]))

Search string for Cochrane Library: ("non small cell lung cancer"):ti,ab,kw AND ("RET"):ti,ab,kw AND ("clinical trial"):ti,ab,kw

References

1. Global Cancer Observatory: cancer today [Internet]. World Health Organization. 2020 [cited 07 October 2022]. Available from: https://gco.iarc.fr/today,.
2. EP IPdOdPFG-. RON. Registo Oncológico Nacional de Todos os Tumores na População Residente em Portugal. Porto; 2019 2022.
3. Zhang Y, Luo G, Etxeberria J, Hao Y. Global Patterns and Trends in Lung Cancer Incidence: A Population-Based Study. Journal of Thoracic Oncology. 2021;16(6):933-44.
4. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA: A Cancer Journal for Clinicians. 2022;72(1):7-33.
5. Barta JA, Powell CA, Wisnivesky JP. Global Epidemiology of Lung Cancer. Ann Glob Health. 2019;85(1).
6. Harris JE. Cigarette Smoking Among Successive Birth Cohorts of Men and Women in the United States During 1900–1980. JNCI: Journal of the National Cancer Institute. 1983;71(3):473-9.
7. Lubin JH, Caporaso N, Wichmann HE, Schaffrath-Rosario A, Alavanja MC. Cigarette smoking and lung cancer: modeling effect modification of total exposure and intensity. Epidemiology. 2007;18(5):639-48.
8. SEER*Stat Database: Incidence - SEER Research Data, 8 Registries, Nov 2021 Sub (1975-2019) [Internet]. National Cancer Institute. 2022 [cited 10 October 2022]. Available from: www.seer.cancer.gov.
9. Nicholson AG, Tsao MS, Beasley MB, Borczuk AC, Brambilla E, Cooper WA, et al. The 2021 WHO Classification of Lung Tumors: Impact of Advances Since 2015. Journal of Thoracic Oncology. 2022;17(3):362-87.
10. Alberg AJ, Brock MV, Ford JG, Samet JM, Spivack SD. Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2013;143(5 Suppl):e1S-e29S.
11. Gandini S, Botteri E, Iodice S, Boniol M, Lowenfels AB, Maisonneuve P, et al. Tobacco smoking and cancer: a meta-analysis. Int J Cancer. 2008;122(1):155-64.
12. Kim AS, Ko HJ, Kwon JH, Lee JM. Exposure to Secondhand Smoke and Risk of Cancer in Never Smokers: A Meta-Analysis of Epidemiologic Studies. Int J Environ Res Public Health. 2018;15(9).
13. Cheng ES, Egger S, Hughes S, Weber M, Steinberg J, Rahman B, et al. Systematic review and meta-analysis of residential radon and lung cancer in never-smokers. European Respiratory Review. 2021;30(159):200230.
14. Pelucchi C, Pira E, Piolatto G, Coggiola M, Carta P, La Vecchia C. Occupational silica exposure and lung cancer risk: a review of epidemiological studies 1996-2005. Ann Oncol. 2006;17(7):1039-50.
15. Ciabattini M, Rizzello E, Lucaroni F, Palombi L, Boffetta P. Systematic review and meta-analysis of recent high-quality studies on exposure to particulate matter and risk of lung cancer. Environ Res. 2021;196:110440.
16. Straif K, Benbrahim-Tallaa L, Baan R, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part C: metals, arsenic, dusts, and fibres. Lancet Oncol. 2009;10(5):453-4.
17. Kwak K, Kang D, Paek D. Environmental exposure to asbestos and the risk of lung cancer: a systematic review and meta-analysis. Occup Environ Med. 2022;79(3):207-14.
18. Guha N, Merletti F, Steenland NK, Altieri A, Cogliano V, Straif K. Lung cancer risk in painters: a meta-analysis. Environ Health Perspect. 2010;118(3):303-12.
19. Ang L, Ghosh P, Seow WJ. Association between previous lung diseases and lung cancer risk: a systematic review and meta-analysis. Carcinogenesis. 2021;42(12):1461-74.
20. Ang L, Chan CPY, Yau W-P, Seow WJ. Association between family history of lung cancer and lung cancer risk: a systematic review and meta-analysis. Lung Cancer. 2020;148:129-37.

21. Reid JL, Hammond D, Driezen P. Socio-economic status and smoking in Canada, 1999-2006: has there been any progress on disparities in tobacco use? Can J Public Health. 2010;101(1):73-8.
22. Doerr F, Stange S, Michel M, Schlachtenberger G, Menghesha H, Wahlers T, et al. Stage I and II Small-Cell Lung Cancer-New Challenge for Surgery. Lung. 2022;200(4):505-12.
23. Howlader N, Forjaz G, Mooradian MJ, Meza R, Kong CY, Cronin KA, et al. The Effect of Advances in Lung-Cancer Treatment on Population Mortality. N Engl J Med. 2020;383(7):640-9.
24. Naruke T, Tsuchiya R, Kondo H, Asamura H, Nakayama H. Implications of staging in lung cancer. Chest. 1997;112(4 Suppl):242s-8s.
25. Ciardiello F, Tortora G. A Novel Approach in the Treatment of Cancer: Targeting the Epidermal Growth Factor Receptor1. Clinical Cancer Research. 2001;7(10):2958-70.
26. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature. 2009;458(7239):719-24.
27. Sankar K, Gadgeel SM, Qin A. Molecular therapeutic targets in non-small cell lung cancer. Expert Rev Anticancer Ther. 2020;20(8):647-61.
28. Singal G, Miller PG, Agarwala V, Li G, Kaushik G, Backenroth D, et al. Association of Patient Characteristics and Tumor Genomics With Clinical Outcomes Among Patients With Non–Small Cell Lung Cancer Using a Clinicogenomic Database. JAMA. 2019;321(14):1391-9.
29. Naylor EC, Desani JK, Chung PK. Targeted Therapy and Immunotherapy for Lung Cancer. Surg Oncol Clin N Am. 2016;25(3):601-9.
30. Alexander M, Kim SY, Cheng H. Update 2020: Management of Non-Small Cell Lung Cancer. Lung. 2020;198(6):897-907.
31. Meador CB, Hata AN. Acquired resistance to targeted therapies in NSCLC: Updates and evolving insights. Pharmacology & Therapeutics. 2020;210:107522.
32. Takahashi M, Ritz J, Cooper GM. Activation of a novel human transforming gene, ret, by DNA rearrangement. Cell. 1985;42(2):581-8.
33. Ishizaka Y, Itoh F, Tahira T, Ikeda I, Sugimura T, Tucker J, et al. Human ret proto-oncogene mapped to chromosome 10q11.2. Oncogene. 1989;4(12):1519-21.
34. Takahashi M, Cooper GM. ret transforming gene encodes a fusion protein homologous to tyrosine kinases. Mol Cell Biol. 1987;7(4):1378-85.
35. Mulligan LM. RET revisited: expanding the oncogenic portfolio. Nat Rev Cancer. 2014;14(3):173-86.
36. Takahashi M, Buma Y, Iwamoto T, Inaguma Y, Ikeda H, Hiai H. Cloning and expression of the ret proto-oncogene encoding a tyrosine kinase with two potential transmembrane domains. Oncogene. 1988;3(5):571-8.
37. Anders J, Kjar 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. 2001;276(38):35808-17.
38. Amoresano A, Incoronato M, Monti G, Pucci P, de Franciscis V, Cerchia L. Direct interactions among Ret, GDNF and GFRalpha1 molecules reveal new insights into the assembly of a functional three-protein complex. Cell Signal. 2005;17(6):717-27.
39. Jeanpierre C, Macé G, Parisot M, Morinière V, Pawtowsky A, Benabou M, et al. RET and GDNF mutations are rare in fetuses with renal agenesis or other severe kidney development defects. J Med Genet. 2011;48(7):497-504.
40. Díaz-Beyá M, Navarro A, Ferrer G, Díaz T, Gel B, Camós M, et al. Acute myeloid leukemia with translocation (8;16)(p11;p13) and MYST3-CREBBP rearrangement harbors a distinctive microRNA signature targeting RET proto-oncogene. Leukemia. 2013;27(3):595-603.
41. Andrew SD, Capes-Davis A, Delhanty PJ, Marsh DJ, Mulligan LM, Robinson BG. Transcriptional repression of the RET proto-oncogene by a mitogen activated protein kinase-dependent signalling pathway. Gene. 2002;298(1):9-19.
42. Lang D, Epstein JA. Sox10 and Pax3 physically interact to mediate activation of a conserved c-RET enhancer. Hum Mol Genet. 2003;12(8):937-45.

43. Carter MT, Yome JL, Marcil MN, Martin CA, Vanhorne JB, Mulligan LM. Conservation of RET proto-oncogene splicing variants and implications for RET isoform function. Cytogenet Cell Genet. 2001;95(3-4):169-76.
44. Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K, Suvanto P, et al. GDNF signalling through the Ret receptor tyrosine kinase. Nature. 1996;381(6585):789-93.
45. Jing S, Wen D, Yu Y, Holst PL, Luo Y, Fang M, et al. GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-alpha, a novel receptor for GDNF. Cell. 1996;85(7):1113-24.
46. Kotzbauer PT, Lampe PA, Heuckeroth RO, Golden JP, Creedon DJ, Johnson EM, Jr., et al. Neurturin, a relative of glial-cell-line-derived neurotrophic factor. Nature. 1996;384(6608):467-70.
47. Milbrandt J, de Sauvage FJ, Fahrner TJ, Baloh RH, Leitner ML, Tansey MG, et al. Persephin, a novel neurotrophic factor related to GDNF and neurturin. Neuron. 1998;20(2):245-53.
48. Baloh RH, Tansey MG, Lampe PA, Fahrner TJ, Enomoto H, Simburger KS, et al. Artemin, a novel member of the GDNF ligand family, supports peripheral and central neurons and signals through the GFRalpha3-RET receptor complex. Neuron. 1998;21(6):1291-302.
49. Airaksinen MS, Saarma M. The GDNF family: signalling, biological functions and therapeutic value. Nat Rev Neurosci. 2002;3(5):383-94.
50. Ibáñez CF. Structure and physiology of the RET receptor tyrosine kinase. Cold Spring Harb Perspect Biol. 2013;5(2).
51. Morales D. Lipid Raft-Excluded GFRα1/Ret Fails to Transmit GDNF Signaling In Vivo. J Neurosci. 2016;36(5):1442-4.
52. Wang X. Structural studies of GDNF family ligands with their receptors-Insights into ligand recognition and activation of receptor tyrosine kinase RET. Biochim Biophys Acta. 2013;1834(10):2205-12.
53. Arighi E, Borrello MG, Sariola H. RET tyrosine kinase signaling in development and cancer. Cytokine Growth Factor Rev. 2005;16(4-5):441-67.
54. Kawai K, Takahashi M. Intracellular RET signaling pathways activated by GDNF. Cell Tissue Res. 2020;382(1):113-23.
55. Regua AT, Najjar M, Lo HW. RET signaling pathway and RET inhibitors in human cancer. Front Oncol. 2022;12:932353.
56. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
57. Coulpier M, Anders J, Ibáñez CF. Coordinated activation of autophosphorylation sites in the RET receptor tyrosine kinase: importance of tyrosine 1062 for GDNF mediated neuronal differentiation and survival. J Biol Chem. 2002;277(3):1991-9.
58. Jain S, Encinas M, Johnson EM, Jr., Milbrandt J. Critical and distinct roles for key RET tyrosine docking sites in renal development. Genes Dev. 2006;20(3):321-33.
59. Jijiwa M, Kawai K, Fukihara J, Nakamura A, Hasegawa M, Suzuki C, et al. GDNF-mediated signaling via RET tyrosine 1062 is essential for maintenance of spermatogonial stem cells. Genes Cells. 2008;13(4):365-74.
60. Takahashi M. RET receptor signaling: Function in development, metabolic disease, and cancer. Proc Jpn Acad Ser B Phys Biol Sci. 2022;98(3):112-25.
61. Fonseca-Pereira D, Arroz-Madeira S, Rodrigues-Campos M, Barbosa IA, Domingues RG, Bento T, et al. The neurotrophic factor receptor RET drives haematopoietic stem cell survival and function. Nature. 2014;514(7520):98-101.
62. Subbiah V, Cote GJ. Advances in Targeting RET-Dependent Cancers. Cancer Discovery. 2020;10(4):498-505.
63. Mizukami T, Shiraishi K, Shimada Y, Ogiwara H, Tsuta K, Ichikawa H, et al. Molecular mechanisms underlying oncogenic RET fusion in lung adenocarcinoma. J Thorac Oncol. 2014;9(5):622-30.
64. Richardson DS, Gujral TS, Peng S, Asa SL, Mulligan LM. Transcript level modulates the inherent oncogenicity of RET/PTC oncoproteins. Cancer Res. 2009;69(11):4861-9.

65. Ju YS, Lee WC, Shin JY, Lee S, Bleazard T, Won JK, et al. A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res. 2012;22(3):436-45.
66. Saito M, Ishigame T, Tsuta K, Kumamoto K, Imai T, Kohno T. A mouse model of KIF5B-RET fusion-dependent lung tumorigenesis. Carcinogenesis. 2014;35(11):2452-6.
67. Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, et al. KIF5B-RET fusions in lung adenocarcinoma. Nat Med. 2012;18(3):375-7.
68. Parimi V, Tolba K, Danziger N, Kuang Z, Sun D, Lin DI, et al. Genomic landscape of 891 RET fusions detected across diverse solid tumor types. NPJ Precis Oncol. 2023;7(1):10.
69. Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med. 2012;18(3):382-4.
70. Li F, Feng Y, Fang R, Fang Z, Xia J, Han X, et al. Identification of RET gene fusion by exon array analyses in "pan-negative" lung cancer from never smokers. Cell Res. 2012;22(5):928-31.
71. Wang R, Hu H, Pan Y, Li Y, Ye T, Li C, et al. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. J Clin Oncol. 2012;30(35):4352-9.
72. Gautschi O, Milia J, Filleron T, Wolf J, Carbone DP, Owen D, et al. Targeting RET in Patients With RET-Rearranged Lung Cancers: Results From the Global, Multicenter RET Registry. J Clin Oncol. 2017;35(13):1403-10.
73. Aldea M, Marinello A, Duruisseaux M, Zrafi W, Conci N, Massa G, et al. RET-MAP: An international multi-center study on clinicobiologic features and treatment response in patients with lung cancer harboring a RET fusion. J Thorac Oncol. 2023.
74. Lamba N, Wen PY, Aizer AA. Epidemiology of brain metastases and leptomeningeal disease. Neuro Oncol. 2021;23(9):1447-56.
75. Wang H, Wang Z, Zhang G, Zhang M, Zhang X, Li H, et al. Driver genes as predictive indicators of brain metastasis in patients with advanced NSCLC: EGFR, ALK, and RET gene mutations. Cancer Med. 2020;9(2):487-95.
76. Drilon A, Lin JJ, Filleron T, Ni A, Milia J, Bergagnini I, et al. Frequency of Brain Metastases and Multikinase Inhibitor Outcomes in Patients With RET-Rearranged Lung Cancers. J Thorac Oncol. 2018;13(10):1595-601.
77. Piotrowska Z, Isozaki H, Lennerz JK, Gainor JF, Lennes IT, Zhu VW, et al. Landscape of Acquired Resistance to Osimertinib in EGFR-Mutant NSCLC and Clinical Validation of Combined EGFR and RET Inhibition with Osimertinib and BLU-667 for Acquired RET Fusion. Cancer Discov. 2018;8(12):1529-39.
78. Kim M, Na JM, Lee GW, Lee SJ, Kim JD, Yang JW. EGFR-mutated pulmonary adenocarcinoma with concurrent PIK3CA mutation, and with acquired RET fusion and EGFR T790M mutation after afatinib therapy. J Pathol Transl Med. 2021;55(1):79-82.
79. Zhu VW, Klempner SJ, Ou SI. Receptor Tyrosine Kinase Fusions as an Actionable Resistance Mechanism to EGFR TKIs in EGFR-Mutant Non-Small-Cell Lung Cancer. Trends Cancer. 2019;5(11):677-92.
80. Kim L, Chae YK, Jung CM, Lee AD, Yu E. Addition of selpercatinib to overcome osimertinib resistance in non-small cell lung cancer (NSCLC) with acquired RET fusion detected in ctDNA at very low allele frequency. Journal of Clinical Oncology. 2021;39(15_suppl):3046-.
81. Rotow J, Patel J, Hanley M, Yu H, Goldman J, Nechustan H, et al. FP14.07 Combination Osimertinib plus Selpercatinib for EGFR-mutant Non-Small Cell Lung Cancer (NSCLC) with Acquired RET fusions. Journal of Thoracic Oncology. 2021;16(3):S230.
82. Shih JY, Inoue A, Cheng R, Varea R, Kim SW. Does Pemetrexed Work in Targetable, Nonsquamous Non-Small-Cell Lung Cancer? A Narrative Review. Cancers (Basel). 2020;12(9).
83. Planchard D, Popat S, Kerr K, Novello S, Smit EF, Faivre-Finn C, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018;29(Suppl 4):iv192-iv237.
84. Hung YP, Sholl LM. Diagnostic and Predictive Immunohistochemistry for Non-Small Cell Lung Carcinomas. Adv Anat Pathol. 2018;25(6):374-86.

85. Belli C, Penault-Llorca F, Ladanyi M, Normanno N, Scoazec JY, Lacroix L, et al. ESMO recommendations on the standard methods to detect RET fusions and mutations in daily practice and clinical research. Ann Oncol. 2021;32(3):337-50.
86. Yang SR, Aypar U, Rosen EY, Mata DA, Benayed R, Mullaney K, et al. A Performance Comparison of Commonly Used Assays to Detect RET Fusions. Clin Cancer Res. 2021;27(5):1316-28.
87. Hasegawa N, Kohsaka S, Kurokawa K, Shinno Y, Takeda Nakamura I, Ueno T, et al. Highly sensitive fusion detection using plasma cell-free RNA in non-small-cell lung cancers. Cancer Sci. 2021;112(10):4393-403.
88. Yakes FM, Chen J, Tan J, Yamaguchi K, Shi Y, Yu P, et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. Mol Cancer Ther. 2011;10(12):2298-308.
89. Kim M, Kim BH. Current Guidelines for Management of Medullary Thyroid Carcinoma. Endocrinol Metab (Seoul). 2021;36(3):514-24.
90. Drilon A, Wang L, Hasanovic A, Suehara Y, Lipson D, Stephens P, et al. Response to Cabozantinib in patients with RET fusion-positive lung adenocarcinomas. Cancer Discov. 2013;3(6):630-5.
91. Drilon A, Rekhtman N, Arcila M, Wang L, Ni A, Albano M, et al. Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: an open-label, single-centre, phase 2, single-arm trial. Lancet Oncol. 2016;17(12):1653-60.
92. Bologna Uo, Molecular ASOM-UoO, Pathology T, Srl BA, Ipsen, SpA M. Phase II Study With Cabozantinib in Patients With RET Positive NSCLC. https://ClinicalTrials.gov/show/NCT04131543; 2019.
93. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A, et al. Non-Small Cell Lung Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2022;20(5):497-530.
94. Carlomagno F, Vitagliano D, Guida T, Ciardiello F, Tortora G, Vecchio G, et al. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. Cancer Res. 2002;62(24):7284-90.
95. Suzuki M, Makinoshima H, Matsumoto S, Suzuki A, Mimaki S, Matsushima K, et al. Identification of a lung adenocarcinoma cell line with CCDC6-RET fusion gene and the effect of RET inhibitors in vitro and in vivo. Cancer Science. 2013;104(7):896-903.
96. Center SM, AstraZeneca. Vandetanib in Advanced NSCLC With RET Rearrangement. https://ClinicalTrials.gov/show/NCT01823068; 2014.
97. Lee SH, Lee JK, Ahn MJ, Kim DW, Sun JM, Keam B, et al. Vandetanib in pretreated patients with advanced non-small cell lung cancer-harboring RET rearrangement: a phase II clinical trial. Ann Oncol. 2017;28(2):292-7.
98. JPRN-UMIN000010095. A Phase II, Open-label Single-Arm Study to Evaluate the efficacy and Safety of Vandetanib in Patients with RET Fusion-positive Unresectable Locally Advanced or Metastatic Non-Small Cell Lung Cancer. 2013.
99. Yoh K, Seto T, Satouchi M, Nishio M, Yamamoto N, Murakami H, et al. Final survival results for the LURET phase II study of vandetanib in previously treated patients with RET-rearranged advanced non-small cell lung cancer. Lung Cancer. 2021;155:40-5.
100. LENVIMA (lenvatinib) - Prescribing Information. In: Agency FD, editor. 2021.
101. Okamoto K, Kodama K, Takase K, Sugi NH, Yamamoto Y, Iwata M, et al. Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion-driven tumor models. Cancer Letters. 2013;340(1):97-103.
102. Hida T, Velcheti V, Reckamp KL, Nokihara H, Sachdev P, Kubota T, et al. A phase 2 study of lenvatinib in patients with RET fusion-positive lung adenocarcinoma. Lung Cancer. 2019;138:124-30.
103. Inc. E. Study of the Safety and Activity of Lenvatinib (E7080) in Subjects With KIF5B-RET-Positive Adenocarcinoma of the Lung. https://ClinicalTrials.gov/show/NCT01877083; 2013.

104. Li GG, Somwar R, Joseph J, Smith RS, Hayashi T, Martin L, et al. Antitumor Activity of RXDX-105 in Multiple Cancer Types with RET Rearrangements or Mutations. Clin Cancer Res. 2017;23(12):2981-90.
105. Drilon A, Fu S, Patel MR, Fakih M, Wang D, Olszanski AJ, et al. A Phase I/Ib Trial of the VEGFR-Sparing Multikinase RET Inhibitor RXDX-105. Cancer Discov. 2019;9(3):384-95.
106. Roche H-L. Study of RXDX-105, Potent RET Inhibitor in Patients With Advanced Lung Cancer and Other Solid Tumors. https://ClinicalTrials.gov/show/NCT01877811; 2013.
107. Sakamoto H, Tsukaguchi T, Hiroshima S, Kodama T, Kobayashi T, Fukami Takaaki A, et al. CH5424802, a Selective ALK Inhibitor Capable of Blocking the Resistant Gatekeeper Mutant. Cancer Cell. 2011;19(5):679-90.
108. Kodama T, Tsukaguchi T, Satoh Y, Yoshida M, Watanabe Y, Kondoh O, et al. Alectinib Shows Potent Antitumor Activity against RET-Rearranged Non–Small Cell Lung Cancer. Molecular Cancer Therapeutics. 2014;13(12):2910-8.
109. JPRN-UMIN000020628. A phase I/II, open-label, single-arm study of CH5424802 for patients with advanced non-small-cell lung cancer harboring a RET fusion gene. 2016.
110. Takeuchi S, Yanagitani N, Seto T, Hattori Y, Ohashi K, Morise M, et al. Phase 1/2 study of alectinib in RET-rearranged previously-treated non-small cell lung cancer (ALL-RET). Transl Lung Cancer Res. 2021;10(1):314-25.
111. Foundation EIP, Roche H-L. ALEctinib for the Treatment of Pretreated RET-rearranged Advanced Non-small Cell Lung Cancer. https://ClinicalTrials.gov/show/NCT03445000; 2018.
112. Institute D-FC, Genentech I. A Study of Alectinib in RET-rearranged Non-small Cell Lung Cancer or RET-mutated Thyroid Cancer. https://ClinicalTrials.gov/show/NCT03131206; 2017.
113. De Falco V, Buonocore P, Muthu M, Torregrossa L, Basolo F, Billaud M, et al. Ponatinib (AP24534) is a novel potent inhibitor of oncogenic RET mutants associated with thyroid cancer. J Clin Endocrinol Metab. 2013;98(5):E811-9.
114. Huang Q, Schneeberger VE, Luetteke N, Jin C, Afzal R, Budzevich MM, et al. Preclinical Modeling of KIF5B-RET Fusion Lung Adenocarcinoma. Mol Cancer Ther. 2016;15(10):2521-9.
115. Hospital MG. Ponatinib in Advanced NSCLC w/ RET Translocations. https://ClinicalTrials.gov/show/NCT01813734; 2013.
116. Drilon A, Oxnard GR, Tan DSW, Loong HHF, Johnson M, Gainor J, et al. Efficacy of Selpercatinib in RET Fusion-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2020;383(9):813-24.
117. Subbiah V, Velcheti V, Tuch BB, Ebata K, Busaidy NL, Cabanillas ME, et al. Selective RET kinase inhibition for patients with RET-altered cancers. Annals of Oncology. 2018;29(8):1869-76.
118. Goto K, Oxnard GR, Tan DS-W, Loong HHF, Bauer TM, Gainor JF, et al. Selpercatinib (LOXO-292) in patients with RET-fusion+ non-small cell lung cancer. Journal of Clinical Oncology. 2020;38(15_suppl):3584-.
119. Loxo Oncology I, Lilly E, Company. A Study of Selpercatinib (LOXO-292) in Participants With Advanced Solid Tumors, RET Fusion-Positive Solid Tumors, and Medullary Thyroid Cancer (LIBRETTO-001). https://ClinicalTrials.gov/show/NCT03157128; 2017.
120. Drilon A, Subbiah V, Gautschi O, Tomasini P, de Braud F, Solomon BJ, et al. Selpercatinib in Patients With RET Fusion-Positive Non-Small-Cell Lung Cancer: Updated Safety and Efficacy From the Registrational LIBRETTO-001 Phase I/II Trial. J Clin Oncol. 2023;41(2):385-94.
121. Cagney DN, Martin AM, Catalano PJ, Redig AJ, Lin NU, Lee EQ, et al. Incidence and prognosis of patients with brain metastases at diagnosis of systemic malignancy: a population-based study. Neuro Oncol. 2017;19(11):1511-21.
122. Kang Y, Jin Y, Li Q, Yuan X. Advances in Lung Cancer Driver Genes Associated With Brain Metastasis. Front Oncol. 2021;10.
123. Lu S, Cheng Y, Huang D, Sun Y, Wu L, Zhou C, et al. Efficacy and safety of selpercatinib in Chinese patients with advanced RET fusion-positive non-small-cell lung cancer: a phase II clinical trial (LIBRETTO-321). Ther Adv Med Oncol. 2022;14:17588359221105020.

124. A Study of Selpercatinib (LY3527723) in Participants With Advanced Solid Tumors Including RET Fusion-positive Solid Tumors, Medullary Thyroid Cancer and Other Tumors With RET Activation. https://ClinicalTrials.gov/show/NCT04280081.
125. Markham A. Selpercatinib: First Approval. Drugs. 2020;80(11):1119-24.
126. Network NCC. Non-small Cell Lung Cancer. https://www.nccn.org/patientresources/patient-resources2022.
127. RETSEVMO (selpercatinib) - Product Information. In: Agency EM, editor. 2021.
128. Goto K, Wolf J, Elamin Y, Santini F, Soldatenkova V, Sashegyi A, et al. FP14.05 LIBRETTO-431: Selpercatinib in Treatment-Naïve Patients with RET Fusion-Positive Non-Small Cell Lung Cancer (NSCLC). Journal of Thoracic Oncology. 2021;16(3, Supplement):S228-S9.
129. Goldman J, Besse B, Wu Y, Yang JC, Paz-Ares L, Drilon A, et al. P01.01 LIBRETTO-432: A Placebo-Controlled Phase 3 Study of Adjuvant Selpercatinib in Stage IB-IIIA RET Fusion-Positive NSCLC. Journal of Thoracic Oncology. 2021;16(10, Supplement):S975-S6.
130. AstraZeneca. Phase 2 Platform Study in Patients With Advanced Non-Small Lung Cancer Who Progressed on First-Line Osimertinib Therapy (ORCHARD). https://ClinicalTrials.gov/show/NCT03944772; 2019.
131. Loxo Oncology I, Lilly E, Company. A Study of Oral LOXO-292 (Selpercatinib) in Pediatric Participants With Advanced Solid or Primary Central Nervous System (CNS) Tumors. https://ClinicalTrials.gov/show/NCT03899792; 2019.
132. Loxo Oncology I, Lilly E, Company. A Study of Selpercatinib (LY3527723) in Participants With Advanced or Metastatic RET Fusion-Positive Non-Small Cell Lung Cancer. https://ClinicalTrials.gov/show/NCT04194944; 2020.
133. Loxo Oncology I, Lilly E, Company. A Study of Selpercatinib After Surgery or Radiation in Participants With Non-Small Cell Lung Cancer (NSCLC). https://ClinicalTrials.gov/show/NCT04819100; 2021.
134. Group SO, Institute NC. Testing the Use of Targeted Treatment for RET Positive Advanced Non-small Cell Lung Cancer. https://ClinicalTrials.gov/show/NCT05364645; 2022.
135. Subbiah V, Gainor JF, Rahal R, Brubaker JD, Kim JL, Maynard M, et al. Precision Targeted Therapy with BLU-667 for RET-Driven Cancers. Cancer Discovery. 2018;8(7):836-49.
136. Gainor JF, Lee DH, Curigliano G, Doebele RC, Kim D-W, Baik CS, et al. Clinical activity and tolerability of BLU-667, a highly potent and selective RET inhibitor, in patients (pts) with advanced RET-fusion+ non-small cell lung cancer (NSCLC). Journal of Clinical Oncology. 2019;37(15_suppl):9008-.
137. Phase 1/2 Study of the Highly-selective RET Inhibitor, Pralsetinib (BLU-667), in Participants With Thyroid Cancer, Non-Small Cell Lung Cancer, and Other Advanced Solid Tumors. https://ClinicalTrials.gov/show/NCT03037385.
138. Griesinger F, Curigliano G, Thomas M, Subbiah V, Baik CS, Tan DSW, et al. Safety and efficacy of pralsetinib in RET fusion-positive non-small-cell lung cancer including as first-line therapy: update from the ARROW trial. Ann Oncol. 2022;33(11):1168-78.
139. GAVRETO (pralsetinib) - Prescribing Information. In: Agency FD, editor. 2020.
140. GAVRETO (pralsetinib) - Product Information. In: Agency EM, editor. 2021.
141. Markham A. Pralsetinib: First Approval. Drugs. 2020;80(17):1865-70.
142. Popat S, Felip E, Kim ES, de Marinis F, Cho BC, Wermke M, et al. AcceleRET Lung: A phase 3 study of first-line pralsetinib in patients with RET fusion–positive advanced/metastatic NSCLC. Journal of Clinical Oncology. 2022;40(16_suppl):TPS9159-TPS.
143. Genentech I, Corporation BM, Co. CP, Roche H-L. A Study of Multiple Therapies in Biomarker-Selected Patients With Resectable Stages IB-III Non-Small Cell Lung Cancer. https://ClinicalTrials.gov/show/NCT04302025; 2020.
144. Roche H-L. A Study of Pralsetinib Versus Standard of Care for First-Line Treatment of Advanced Non-Small Cell Lung Cancer (NSCLC). https://ClinicalTrials.gov/show/NCT04222972; 2020.

145. Roche H-L. A Study Evaluating the Efficacy and Safety of Multiple Therapies in Cohorts of Participants With Locally Advanced, Unresectable, Stage III Non-Small Cell Lung Cancer (NSCLC). https://ClinicalTrials.gov/show/NCT05170204; 2022.
146. Aldea M, Andre F, Marabelle A, Dogan S, Barlesi F, Soria J-C. Overcoming Resistance to Tumor-Targeted and Immune-Targeted Therapies. Cancer Discovery. 2021;11(4):874-99.
147. Nakaoku T, Kohno T, Araki M, Niho S, Chauhan R, Knowles PP, et al. A secondary RET mutation in the activation loop conferring resistance to vandetanib. Nat Commun. 2018;9(1):625.
148. Dagogo-Jack I, Stevens SE, Lin JJ, Nagy R, Ferris L, Shaw AT, et al. Emergence of a RET V804M Gatekeeper Mutation During Treatment With Vandetanib in RET-Rearranged NSCLC. J Thorac Oncol. 2018;13(11):e226-e7.
149. Wirth LJ, Kohno T, Udagawa H, Matsumoto S, Ishii G, Ebata K, et al. Emergence and Targeting of Acquired and Hereditary Resistance to Multikinase RET Inhibition in Patients With RET-Altered Cancer. JCO Precision Oncology. 2019(3):1-7.
150. Subbiah V, Velcheti V, Tuch BB, Ebata K, Busaidy NL, Cabanillas ME, et al. Selective RET kinase inhibition for patients with RET-altered cancers. Annals of Oncology. 2018;29(8):1869-76.
151. Subbiah V, Gainor JF, Rahal R, Brubaker JD, Kim JL, Maynard M, et al. Precision Targeted Therapy with BLU-667 for RET-Driven Cancers. Cancer Discov. 2018;8(7):836-49.
152. Solomon BJ, Tan L, Lin JJ, Wong SQ, Hollizeck S, Ebata K, et al. RET Solvent Front Mutations Mediate Acquired Resistance to Selective RET Inhibition in RET-Driven Malignancies. J Thorac Oncol. 2020;15(4):541-9.
153. Gainor J, Curigliano G, Doebele RC, Lin JJ, Ou SH, Miller S, et al. OA05.02 Analysis of Resistance Mmechanisms to Pralsetinib in Patients with RET Fusion-Positive Non-Small Cell Lung Cancer (NSCLC) from the ARROW Study. Journal of Thoracic Oncology. 2021;16(1):S5.
154. Shen T, Hu X, Liu X, Subbiah V, Mooers BHM, Wu J. The L730V/I RET roof mutations display different activities toward pralsetinib and selpercatinib. NPJ Precis Oncol. 2021;5(1):48.
155. Subbiah V, Shen T, Terzyan SS, Liu X, Hu X, Patel KP, et al. Structural basis of acquired resistance to selpercatinib and pralsetinib mediated by non-gatekeeper RET mutations. Ann Oncol. 2021;32(2):261-8.
156. Rosen EY, Won HH, Zheng Y, Cocco E, Selcuklu D, Gong Y, et al. The evolution of RET inhibitor resistance in RET-driven lung and thyroid cancers. Nat Commun. 2022;13(1):1450.
157. Zhu VW, Madison R, Schrock AB, Ou S-HI. Emergence of high level of MET amplification as off-target resistance to selpercatinib treatment in KIF5B-RET NSCLC. Journal of Thoracic Oncology. 2020;15(7):e124-e7.
158. Lin JJ, Liu SV, McCoach CE, Zhu VW, Tan AC, Yoda S, et al. Mechanisms of resistance to selective RET tyrosine kinase inhibitors in RET fusion-positive non-small-cell lung cancer. Annals of Oncology. 2020;31(12):1725-33.
159. Tong JH, Yeung SF, Chan AW, Chung LY, Chau SL, Lung RW, et al. MET Amplification and Exon 14 Splice Site Mutation Define Unique Molecular Subgroups of Non-Small Cell Lung Carcinoma with Poor Prognosis. Clin Cancer Res. 2016;22(12):3048-56.
160. Rosen EY, Johnson ML, Clifford SE, Somwar R, Kherani JF, Son J, et al. Overcoming MET-Dependent Resistance to Selective RET Inhibition in Patients with RET Fusion-Positive Lung Cancer by Combining Selpercatinib with Crizotinib. Clin Cancer Res. 2021;27(1):34-42.
161. Subbiah V, Shen T, Tetzlaff M, Weissferdt A, Byers LA, Cascone T, et al. Patient-driven discovery and post-clinical validation of NTRK3 fusion as an acquired resistance mechanism to selpercatinib in RET fusion-positive lung cancer. Annals of Oncology. 2021;32(6):817-9.
162. Repetto M, Crimini E, Ascione L, Boscolo Bielo L, Belli C, Curigliano G. The return of RET GateKeeper mutations? an in-silico exploratory analysis of potential resistance mechanisms to novel RET macrocyclic inhibitor TPX-0046. Invest New Drugs. 2022;40(5):1133-6.
163. Turning Point Therapeutics I. Study of TPX-0046, A RET/SRC Inhibitor in Adult Subjects With Advanced Solid Tumors Harboring RET Fusions or Mutations. https://ClinicalTrials.gov/show/NCT04161391; 2019.

164. Jiang Y, Peng X, Ji Y, Dai Y, Fang Y, Xiong B, et al. The Novel RET Inhibitor SYHA1815 Inhibits RET-Driven Cancers and Overcomes Gatekeeper Mutations by Inducing G(1) Cell-Cycle Arrest through c-Myc Downregulation. Mol Cancer Ther. 2021;20(11):2198-206.
165. Shanghai Runshi Pharmaceutical Technology Co. L, Hospital JPT. A Study to Investigate the Safety of SYHA1815 in Subjects With Unresectable Locally Advanced or Metastatic Solid Tumors. https://ClinicalTrials.gov/show/NCT05105464; 2021.
166. Schoffski P, Aftimos PG, Massard C, Italiano A, Jungels C, Andreas K, et al. A phase I study of BOS172738 in patients with advanced solid tumors with RET gene alterations including non-small cell lung cancer and medullary thyroid cancer. Journal of Clinical Oncology. 2019;37(15_suppl):TPS3162-TPS.
167. Kolakowski GR, Anderson ED, Ballard JA, Brandhuber BJ, Condroski KR, Gomez EB, et al. Abstract 1464: Pre-clinical characterization of potent and selective next-generation RET inhibitors. Cancer Research. 2021;81(13_Supplement):1464-.
168. Lilly E, Company, Loxo Oncology I. A Study of LOXO-260 in Cancer Patients With a Change in a Particular Gene (RET) That Has Not Responded to Treatment. https://ClinicalTrials.gov/show/NCT05241834; 2022.

INSTITUTO DE **CIÊNCIAS BIOMÉDICAS ABEL SALAZAR**