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RET rearrangements: targeted therapy in lung cancer

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Resumo

Introdução: O cancro do pulmão, apesar de ser a neoplasia mais letal em todo o mundo, apresenta um mau prognóstico, uma vez que o diagnóstico é feito em estádios avançados. A identificação de oncogenes condutores, no entanto, abriu as portas a uma nova opção de tratamento – a terapia dirigida. Entre eles, estão as fusões do *RET*, responsáveis por até 2% dos casos de cancro do pulmão.

Caso clínico: Uma paciente do sexo feminino, 45 anos, foi diagnosticada com cancro do pulmão positivo para a fusão *KIF5B-RET*. Foi inicialmente tratada com cisplatina/pemetrexedo, com resposta parcial, tendo mantido terapêutica de manutenção com pemetrexedo. Quando a doença começou a progredir, iniciou tratamento com alectinib, sem resposta, e, posteriormente, nova quimioterapia, como docetaxel e nintedanibe.

Revisão da literatura: Os inibidores da tirosina cinase, como o cabozantinib, o vandetanib e o lenvatinib, foram os primeiros fármacos com potencial anti-*RET* testados em ensaios clínicos, mas os resultados obtidos em ensaios clínicos foram inferiores às verificados com outros oncogenes. Para superar estas limitações, inibidores seletivos do *RET* começaram a ser desenvolvidos. Selpercatinib e pralsetinib têm sido avaliados em ensaios clínicos, com muito bons resultados, o que levou à sua aprovação pela *Food and Drug Administration* no cancro do pulmão induzido por fusões do *RET*.

Discussão: A paciente foi tratada com alectinib, sem resposta ao tratamento. Em doentes com fusões do *RET*, o alectinib foi apenas avaliado num estudo de pequena dimensão, com quatro pacientes com alterações do *RET*, sendo que a sobrevida foi apenas de 20%. Por ter apresentado progressão da doença mesmo sob alectinib, um dos inibidores seletivos, particularmente o selpercatinib, que apresenta maior evidência, poderia ser uma boa opção terapêutica.

Conclusão: Os inibidores seletivos do *RET* representam um avançado num tratamento do cancro do pulmão associado a fusões do *RET*, e os estudos em curso certamente darão uma melhor perspetiva da sua relevância para o futuro.

Palavras-chave: Proto-oncogene RET; neoplasias do pulmão

Abstract

Introduction: Lung cancer, despite being the most lethal malignant neoplasm worldwide, still has a very poor prognosis, with most cases are identified only in later stages. The identification of oncogenic drivers, however, opened the door to a new treatment option – targeted therapy. Among them, are the *RET*-rearrangements, responsible for up to 2% of lung cancer cases.

Clinical case: A 45-year old woman was diagnosed with stage IV *KIF5B-RET*-positive lung cancer. She initially treated with cisplatin/pemetrexed, with partial response, and after remained on maintenance treatment with pemetrexed. When the disease started progressing, she started targeted therapy with alectinib, with no response, and then began a new chemotherapy treatment, with docetaxel and nintedanib.

Literature review: Multi-kinase inhibitors, such as cabozantinib, vandetanib and lenvatinib, were the first drugs with *RET*-targeting potential tested in clinical trials, but the overall response rate and progression free survival were much lower when compared to other oncogenic drivers. To surpass their limitations, highly specific *RET* inhibitors started being developed. Selpercatinib e pralsetinib were both assessed in clinical trials, with much better results than multi-kinase inhibitors, which lead to their approval for stage IV *RET*-rearranged lung cancer by the Food and Drug Administration.

Discussion: The patient was treated with alectinib, with no response to the drug. In *RET*-positive patients, alectinib was just evaluated in a small study, with five patients (four with *RET* rearrangements), with overall survival being only 20%. Being a stage IV patient with progression under a multi-kinase inhibitor, one of the newest selective inhibitors, mainly selpercatinib due to the higher level of evidence, could be a good treatment alternative.

Conclusion: Highly specific *RET* inhibitors represent an incredible advance in the treatment of *RET*-positive lung cancer, and the ongoing studies will certainly give us an even better understanding of their role in the future.

Keywords: Proto-Oncogene Protein RET; Lung Neoplasms

Abbreviations

ABL	ABL proto-oncogene 1, non-receptor tyrosine kinase
AE	Adverse effects
AKT	AKT serine/threonine kinase
ALK	ALK receptor tyrosine kinase
ALT	Alanine aminotransferase
ASCO	American Society of Clinical Oncology
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AXL	AXL receptor tyrosine kinase
BCR	BCR activator of RhoGEF and GTPase
BRAF	B-Raf proto-oncogene, serine/threonine kinase
CCDC6	Coiled-coil domain containing 6
CHEK2	Checkpoint kinase 2
CHUP	*Centro Hospitalar e Universitário do Porto*
c-KIT	KIT proto-oncogene, receptor tyrosine kinase
CLIP1	CAP-Gly domain containing linker protein 1 (CLIP1)
c-RAF	RAF proto-oncogene serine/threonine-protein kinase
CT	Computed tomography
DNA	Deoxyribonucleic acid
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
EML4	EMAP like 4
ER	Emergency Room
ERK	Extracellular signal-regulated kinases
FDA	Food and Drug Administration

FGFR	Fibroblast growth receptor
FISH	Fluorescence in situ hybridization
FLT3	Fms related receptor tyrosine kinase 3
GDNF	Glial-derived neurotrophic factors
HDI	Human development index
HER2	Human epidermal growth factor receptor 2
hERG	Potassium voltage-gated channel subfamily H member 2
HPV	Human papillomavirus
IC_{50}	Half Maximal inhibitory concentration
JAK	Janus kinase
KIF5B	Kinesin family member 5B
KRAS	KRAS proto-oncogene, GTPase
LTK	Leukocyte receptor tyrosine kinase
MAPK	Mitogen-activated protein kinases
Mb	Megabases
MEN2	Multiple endocrine neoplasia type 2
MET	Hepatocyte growth factor receptor
mg	Miligrams
mm	Milimeters
MRI	Magnetic resonance imaging
MYO5C	Myosin VC
nM	Nanomolar
NGS	Next-generation sequencing
NRG1	Neuregulin 1
NSCLC	Non-small cell lung cancer
NTRK	Neurotrophic tyrosine receptor kinase
OH	Ontario Health

ORR	Overall response rate
OS	Overall survival
PDGFR	Platelet derived growth factor receptor
PET	Positron emission tomography
PI3K	Phosphatidylinositol-4,5-biphosphonate 3-kinase
PICALM	Phosphatidylinositol binding clathrin assembly protein
PFS	Progression free survival
RET	Rearranged during transfection
RON	*Registo Oncológico Nacional* (National Oncologic Registry)
ROS1	ROS proto-oncogene 1, receptor tyrosine kinase
RT-PCR	Reverse transcription polymerase chain reaction
SCLC	Small cell lung cancer
SH2	SRC homology 2
SRC	SRC proto-oncogene, non-receptor tyrosine kinase
STAT	Signal transducer and activator of transcription proteins
TKI	Tyrosine kinase inhibitor
TNM	Tumor, Node, Metastasis
US	United States of America
VEGFR	Endothelial growth factor receptor

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1. Introduction

1.1. Epidemiology

Worldwide, in 2020, lung cancer was the second most incident solid malignant neoplasm (11.4%), behind only female breast cancer, and the most lethal one, accounting for approximately 18% of all cancer deaths, with over 2 million people being diagnosed and around 1.8 million dying from it.[1-4]

When looking at lung cancer, there are some notable differences to consider regarding age, sex, race/ethnicity and socioeconomic status, which are generally related to tobacco and smoking epidemiology.[5] There are a few risk factors associated with lung cancer, with smoking, particularly cigarette smoking, being the most important one. In fact, more than 80% of lung cancer in the United States of America (US) and two-thirds of the deaths worldwide can be attributed to tobacco. The implementation of policies to prevent and cease smoking, however, lead to a decrease of the number of cases attributed to this cause.[4]

Lung cancer is typically a cancer of the elderly. Being closely associated with heavy smoking, the increased number of smoking years leads to an increased risk of lung cancer. Therefore, it is rarely diagnosed before 30 and its incidence peaks in older age groups, with a median age at diagnosis of 70 years in the US and more than half the cases occurring in patients aged between 55 and 74 years. Not surprisingly, mortality is also closely related to age, in a similar way to incidence, with higher mortality rates in older individuals. [5]

Lung cancer is more frequent in men, being the most incident cancer in this group (14.3%), although followed very closely by prostate cancer, and the most lethal one (21.5%), which is explained by the fact that, historically, smoking was a practice mostly associated with men. However, not very long after, with the empowerment of women, smoking among females became more and more common, and currently, despite not being as common as in males, lung cancer is the third most incident malignant neoplasm (8.4%) among women, after breast and colorectal cancer, and the second most lethal one (13.7%), behind only breast cancer.[1, 5]

The socioeconomic status differences that we can find are also related to smoking patterns. Individuals with lower levels of education (high school) have a higher

smoking prevalence compared with college graduates, which translates into a lower incidence of lung cancer in groups of higher socioeconomic status.[5]

The differences seen when looking at different races/ethnicities can also be explained by smoking patterns. According to US data, black men smoke at higher rates than men from other racial and ethnic groups, but there weren't many significant differences regarding women. Therefore, it is understandable that the highest incidence and mortality of lung cancer among males is in blacks, followed by whites, while the same isn't true for females, with the highest incidence being in whites, followed closely by American natives and blacks.[5]

Geographically, incidence and mortality rates are also higher in countries with high human development index (HDI), compared with countries with low HDI, with Africa having the lowest rates globally. This, however, is expected to change somewhat in future years, due to an increase of smokers in lower income countries.[1] Besides tobacco, air pollution is another important risk factor that plays an important role in geographic disparities, being most relevant in Eastern Asia, where the smoking patterns cannot fully explain the rates of lung cancer among women. The higher-than-expected values are thought to be due to air pollution and occupational exposures, with around 20% of the cases in China, for example, being attributed to this cause.[1, 5]

In Portugal, the last official epidemiologic data available is from the 2018's *Registo Oncológico Nacional* (RON) – the National Oncologic Registry – made available earlier in 2021. According to the data gathered, the incidence of lung cancer in Portugal, age-standardized for the world population, is 19.98/100000, being higher in males (31.38) and lower in females (10.64). It is the second most frequent malignant neoplasm in males and the fourth in females, with 4424 new cases being registered in a year, 3193 of them in the male population. The mortality rate, per 100000 malignant neoplasms, was the highest (16.63), increasing progressively with age. Lung cancer was more frequent between the ages of 50 and 79, with some notable regional differences – the Metropolitan Area of Lisbon had the highest incidence rate (25,85) and the central region the lowest (12.64), with the higher absolute number of cases being in the northern region of Portugal.[6]

Lung cancer has in general bad survival rates, with only 10 to 20% of the patients surviving past 5 years after the diagnosis. These rates depend greatly on the stage

of the disease at the time of the diagnosis, being as high as 54% for localized stage disease and as low as 4% for patients presenting with metastatic disease, with the majority of the patients presenting already in this stage at the time of diagnosis.[1, 4, 5] In the US, there are some survival differences according to race/ethnicity, with blacks having lower survival rates, due to delays in diagnosis and less likelihood of receiving standard care.[1, 5] To aid with early detection of lung cancer and consequent better prognosis, screening with low-dose computed tomography (CT) has been studied in high risk individuals (heavy smokers), with proved efficacy in clinical trials, but without any extrapolation to general population yet.[1]

Incidence and mortality trends in lung cancer reflect the changes in smoking patterns – in the countries with high HDI, where smoking started earlier, there has been a decline in these indicators, showing a cessation effort. However, in countries with lower HDI, smoking started much later and, as such, incidence and mortality have been increasing and are expected to continue doing so in the future. Trends also differ among women and men – since in most countries the smoking uptake started much later in women, while the rates among men have slowly started decreasing, among females there are yet a big number of countries with increasing rates.[1, 5]

1.2. Histological classification

Lung cancer is usually classified according to its histological characteristics in two broad groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC).[7]

NSCLCs account for about 80% of all lung cancers, and can be further divided according to histological subtypes into adenocarcinoma, (40%), squamous cell carcinoma (20%), large cell carcinoma (3%) and other rarer subtypes.[2, 8]

SCLCs can be grouped with other neuroendocrine lung cancers, representing in total the remaining 20%.[8] SCLC is the most frequent subtype, accounting for 15% of all lung cancers, with the remaining 5% encompassing more rarer subtypes: large cell neuroendocrine carcinomas (3%), typical carcinoids (1.8%) and atypical carcinoids (0.2%).[9]

According to 2010's RON, adenocarcinoma is the most frequent histological subtype of lung cancer in Portugal, corresponding to around 39% of the cases, followed by squamous cell carcinoma (22%).[10]

This classification is and has been of the utmost importance in decisions regarding the best way to treat these patients.[11] However, with the advent of targeted therapy, better classification of these tumors according to the presence of specific oncogene alterations has become increasingly more important.[11-13]

1.3. Staging

Lung cancer staging is done through the tumor, node, metastasis (TNM) system, with the primary objective of creating groups with different survival outcomes. Therefore, the three parameters evaluated are the extent of the primary tumor (T), the extent of nodal involvement in the thorax and supraclavicular regions (N) and the extent of metastatic involvement (M).[14]

The T component ranges between T0 and T4 and is defined by a set of factors, including size of primary site, presence (or absence) of satellite sites within lung parenchyma, and extension of the tumor into adjacent thoracic structures. The interpretation of the T component categories is as follows:

- T0: no primary tumor was identified;
- Tis: carcinoma *in situ;*
- T1: the primary tumor's size is smaller than 3 cm, inclusive. T1 can be further split into T1a, which includes tumors up to 1 cm, inclusive, and has two different identifiers ((mi) for minimally invasive adenocarcinoma) and (ss) for superficial spreading tumor in central airways, confined to tracheal or bronchial wall), T1b, which includes tumors between 1 and 2 cm, inclusive, and T1c, which includes tumors between 2 and 3 cm, inclusive;
- T2: the primary tumor's between 3 and 5 cm, inclusive, or there is tumor involving the visceral pleura (T2 Visc Pl) or main bronchus (excluding carina), with atelectasis to the hilum (T2 Centr). T2 can be divided into

T2a, if the tumor is between 3 and 4 cm, inclusive, and T2b, if the tumor is between 4 and 5 cm, inclusive;

- T3: the primary tumor's between 5 and 7 cm, inclusive, or there is invasion of the chest wall, pericardium or phrenic nerve (T3 Inv) or there are separate tumor nodules in the same lobe (T3 Satell);
- T4: the primary tumor's larger than 7 cm or it involves the mediastinum, diaphragm, heart, great vessels, recurrent laryngeal nerve, carina, trachea, esophagus or spine (T4 Inv) or there are tumor nodules in a different ipsilateral lobe (T4 Ipsi Nod);
- TX: T status was not assessed.[14]

The N component ranges between N0 and N3, with N0 meaning absence of regional lymph node involvement, N1 the involvement of ipsilateral intrapulmonary or hilar nodes, N2 the involvement of ipsilateral mediastinal or subcarinal nodes, N3 the involvement of contralateral hilar or mediastinal nodes or supraclavicular nodes and NX meaning that the N status was not assessed.[14]

The M component ranges between M0 and M1, with M0 indicating the absence of distant metastases and M1 its presence. M1 can be further divided into M1a, M1b and M1c. M1a means there is malignant pleural/pericardial effusion or malignant/pericardial nodules (M1a Pl Dissem) or separate tumor nodules in a contralateral lobe (M1a Contr Nod). M1b encompasses cases with a single extra thoracic metastasis and M1c the ones with multiple extra thoracic metastases.[14]

All these different parameters can lead to a total of 64 different combinations, that are grouped in a staging system, ranging from IA1 to IVB. Stage I covers patients with primary tumors up to 4 cm, inclusive, without any nodal or metastatic involvement, with IA stage referring to T1 tumors (IA1 for T1a, IA2 for T1b and IA3 for T1c) and IB to T2a tumors. Stage IIA refers to T2b tumors without any nodal or metastatic involvement and stage IIB to T3 tumors, without any nodal or metastatic involvement, and T1 and T2 tumors with N1 nodal involvement. Stage III can be divided in IIIA, IIIB and IIIC: IIIA includes T4 tumors with N0 or N1, T3 tumors with N1 and T1 and T2 tumors with N2; IIIB includes T3 and T4 tumors with N2 and T1 and T2 tumors with N3; and IIIC includes T3 and T4 tumors with N3. As soon as a patient has metastatic disease, it is considered stage IV. Stage IV can be further split into IVA and IVB, with the first comprehending M1a and M1b tumors and the second M1c tumors.[14]

Broadly, contrast-enhance spiral CT of the chest and abdomen is the best exam when it comes to staging lung cancer. CT is the best technique to determine the primary tumor's extension and it is also able to detect nodal involvement and distant metastases, mainly lung ones, with the addition of positron emission tomography (PET) or PET-CT increasing the sensitivity when it comes to N staging and the identification of extra thoracic metastases. Other exams, such as magnetic resonance imaging (MRI), can also be used, but in more particular scenarios, such as cerebral staging.[15]

2. Clinical Case

P. C. B. P., a 45-year-old Caucasian woman, born in Gondomar, went to *Centro Hospital e Universitário do Porto* (CHUP)'s emergency room (ER) due to supraclavicular lymphadenopathy, nocturnal diaphoresis and involuntary weight loss of about 5 kilograms.

Regarding her medical precedents, only of notice a previous surgery in 2011 due to cervix's dysplasia due to lesion caused by human papillomavirus (HPV) and a smoking status (13 pack-years). She denied any history of infectious diseases and was not taking any medication. From her family medical history, a mention of a maternal cousin with an unidentified neoplasm of the airways.

In the ER, she was evaluated by otorhinolaryngology, but no suspicious neoformations of the upper aerodigestive tract were identified. The imaging exams showed cervical and mediastinal nodal formations, compatible with bilateral nodal metastasis, as well as a nodular lesion in the posterior basal region of the left lower lobe, with 35x30 millimeters (mm) of larger diameter, other ipsilateral pulmonary nodules and bilateral hilar nodules. Fine needle aspiration cytology of the right left latero-cervical ganglion and histologic biopsy of the lung were performed, with the first being compatible with carcinoma and the second revealing invasive adenocarcinoma, predominantly acinar, with an immunohistochemical profile compatible with pulmonary origin. Additionally, a focus of hyperactivity left of the uterus was observed in the PET and the CT also showed a 12 mm nodular formation on the left adrenal gland.

The patient was forwarded to a Medical Oncology appointment, where the diagnosis of adenocarcinoma of the left pulmonary lobe, T3N1M1b, was made. At this point, no *EGFR* mutations or *ALK* rearrangements were identified through fluorescence in situ hybridization (FISH).

She was proposed for palliative chemotherapy with cisplatin/pemetrexed, 4 cycles, that she began in March 2019. The treatment was well tolerated, with only asthenia, low back pain relieved with topic anti-inflammatory and nauseas and epigastric pain during the third cycle reported. The chemotherapy ended in May, with the CT showing a partial response. The patient remained on maintenance treatment with

pemetrexed, which was well tolerated, without any complaints suggesting toxicity or disease progression.

Through next-generation sequencing (NGS), a *KIF5B(15)-RET(12)* rearrangement, as well as an *EML4(2)-ALK(20)* rearrangement, is detected.

In October, a CT is done due to an increasing left supraclavicular swelling and nausea, controlled with metoclopramide, and progression of the pulmonary and ganglia disease is confirmed.

Treatment with alectinib was started in November, with complaints of severe cervical pain and facial swelling without congestion or plethora. A CT angiography is performed, with no vascular compression justifying the symptoms being identified.

Despite treatment with alectinib, in February of 2020, disease progression was reported with neck pain limiting the movements of the left arm, dyspnea for moderate physical activity and increasing of the cervical adenopathy. Due to primary resistance to the drug and disagreement between previous tests, new ALK rearrangement search by FISH was requested, coming back negative.

The patient suspended alectinib and began chemotherapy with docetaxel and nintedanib in March, with good tolerance and decreasing of the cervical ganglionic conglomerate. In May/June, however, the patient became more fragilized due to chemotherapy, with weight loss and general worse Eastern Cooperative Oncology Group (ECOG) performance status (ECOG 1), and had developed pain in the right breast with erythema in the left breast, as well as palpable axillar ganglia.

Pulmonary, ganglionic, bone and cerebral disease progression was assessed, with a biopsy of the breast tissue confirming pulmonary carcinoma metastasis.

3. Targeted therapy in lung cancer

3.1. Overview

Lung cancer is a heterogeneous genomic disease, that to this day is still usually diagnosed in later stages, when curative treatments are no longer an option.[11, 13] Despite that, for the longest time, surgical procedures and chemotherapy were the main treatments available for these patients.[11]

That is, until the first mutations with targeting potential by specific therapies were identified, in the beginning of the century.[11] The history of targeted therapy in lung cancer started with the identification of *epidermal growth factor receptor* (*EGFR*) mutations in patients with NSCLC, who later showed clinical response to EGFR tyrosine kinase inhibitor (TKI) gefitinib.[16]

The principle of targeted therapy is strictly related to the concept of oncogene addiction. Among all the genetic alterations we can find in lung cancer, only a minority can promote lung tumorigenesis, with their products being called "oncogenic drivers". Oncogenic addiction is related to these oncogenic drivers – research shows that many cancer cells depend on the continued activity of aberrant driver oncogenes to keep the malignant phenotype.[12, 17]

If we can identify these oncogenic drivers, it means we can target them molecularly with specific drugs.[12]

3.2. Targeted therapy in Lung Adenocarcinoma

Most molecular alterations, particularly the ones that are therapeutically vulnerable, are mostly histologic specific.[2] In the initial articles, adenocarcinoma was the subtype of NSCLC that responded the best to targeted therapy, so the identification of biomarkers has mainly been done in adenocarcinomas.[2, 11]

Lung adenocarcinoma is primarily driven by the above-mentioned driver oncogenes, with genomic alterations involving multiple driver kinase genes, such as *EGFR*, *KRAS proto-oncogene, GTPase* (*KRAS*), *ALK receptor tyrosine kinase* (*ALK*), *ROS proto-oncogene 1, receptor tyrosine kinase* (*ROS1*), *B-Raf proto-oncogene,*

serine/threonine kinase (*BRAF*), *hepatocyte growth factor receptor* (*MET*), *rearranged during transfection* (*RET*), *neurotrophic tyrosine receptor kinase* (*NTRK*), *neuregulin 1* (*NRG1*) and *human epidermal growth factor receptor 2* (*HER2*), each of them with different biologic and epidemiologic characteristics and, more importantly, different prognosis and therapeutic susceptibility.[2, 12]

The frequency of these driver oncogenes can vary with smoking status, sex and ethnicity, and most of them, like *EGFR*, *KRAS*, *BRAF*, *HER2*, *ALK*, *RET* and *ROS1*, are also mutually exclusive.[12]

Among these, *EGFR*, *ALK* and *ROS1* are the most well-established targets.[17] *EGFR* activating mutations are present in around 10-15% of lung adenocarcinomas, with most of them being sensitive to EGFR TKIs, such as gefitinib, erlotinib, afatinib and osimertinib. These drugs have demonstrated higher overall response rates (ORR) and longer progression free survival (PFS), when we compare them to standard platinum-based chemotherapy. *ALK* fusion variants and *ROS1* rearrangements are rarer, occurring respectively in 3-7% and 1-2% of lung NSCLCs. Several multikinase inhibitors, such as crizotinib, ceritinib and alectinib, have been used in patients with *ALK* mutations, with alectinib being currently the standard first-line therapy. ROS1, due to its similarities with ALK, shares some of its therapeutic vulnerabilities, which means that some drugs that are effective in treating lung adenocarcinoma with *ALK* mutations can also be used in patients with *ROS1* fusion variants, with crizotinib having already proved efficacy and other dual inhibitors currently being developed.[2, 18]

In more recent years, research regarding other targets has increased and new drugs that can target other oncogenic drivers, such as *MET*, *RET* and *NTRK*, have started being developed.[17] However, some oncogenic drivers are more difficult to target then others. *KRAS*, for example, is the most frequent driver mutation in lung cancer, being identified in 25-32% of the patients.[16] Despite that, drugs that can target *KRAS* have yet to be developed.[19]

Targeted therapy direct to NSCLC with *RET*-rearrangements, being the theme of this paper, will be separately addressed.

3.3. Advantages and shortcomings of targeted therapy

Targeted therapy is a relatively new treatment approach in lung cancer. However, it has already shown in some studies its ability to improve the patient's response to treatment, provide a better symptomatic control and, particularly drugs against *EGFR*, *ALK* and *ROS1*, increase progression free survival.[11, 20]

However, there are still quite a few shortcomings that we can identify. First of all, there are still known driver mutations that have yet to be paired with an effective targeting drug, like previously mentioned, such as *KRAS*.[11] In some cases, there are drugs that can target these mutations in different neoplasms, but it isn't yet known if they show a similar behavior in lung cancer.[20]

The response to these drugs is also still not ideal. NSCLCs with the same oncogenic driver do not necessarily show the same behavior – they can vary both in their histological appearance and immunohistochemical profile – and this diversity means that the sensibility to targeted therapy can vary.[16]

Besides sensibility, there is also the problem of acquired resistance – patients with lung cancer that initially respond to targeted therapy eventually relapse, most of them within a year, by acquiring resistance mechanisms.[19]

3.4. Today's panorama

Despite all its shortcomings, targeted therapy is a major advantage in the treatment of lung cancer, particularly lung adenocarcinoma. As such, it is recommended that all patients with lung adenocarcinoma, as well as mixed cancer with adenocarcinoma component, large cell neuroendocrine carcinoma and never smokers with squamous cell carcinoma, should be tested at least for *EGFR* mutations, *ALK* rearrangements, *ROS1* rearrangements and *BRAF* mutations, since there are approved targeted therapies for all this genomic alterations.[17, 20] As we will discuss later, anti-RET drugs as well has well have recently been approved.

4. RET

RET is a receptor tyrosine kinase encoded by proto-oncogene *RET*.[21] Located in the chromosome 10 (10q11.2), *RET* was first discovered in 1985, while doing transfection of NIH3T3 cells with human lymphoma deoxyribonucleic acid (DNA), hence its given name.[22]

This tyrosine kinase is essential to the development and maturation of several tissues, including brain, peripheral sympathetic and parasympathetic nervous systems (particularly of the enteric nerve plexus), kidneys, lung, thyroid, and adrenal and pituitary glands. It also plays an important role in maturation of spermatogonia and in the expansion of hematopoietic cells.[20, 22-24]

4.1. RET structure

The tyrosine kinase receptor encoded by *RET* is formed by three different domains: an extracellular domain, a transmembrane domain and an intracellular kinase domain. The extracellular domain is formed by four cadherin like repeats, a calcium binding site and a cysteine-rich region, and it has vital importance in protein structure and ligand interactions. Meanwhile, the intracellular domain, which has a 37% homologous amino acid sequence with ALK kinase domains, is required for autophosphorylation and phosphorylation of the tyrosine residues.[22, 24]

RET is a receptor for neurotrophins from the family of glial-derived neurotrophic factors (GDNF), which include, besides GDNF, neurturin, artemin and persephin.[20] The binding of these ligands to RET is not done directly – unlike other tyrosine kinases, a complex first needs to be formed between them and co-receptors, which are members of the GDNF receptor-alfa protein family. The binding of this complex to RET is what activates the intracellular domain, by inducing dimerization.[25]

This process leads to autophosphorylation of the intracellular tyrosine residues, which allows the binding of proteins carrying SRC homology 2 (SH2) or phosphotyrosine-binding domains. After the binding, RET is recruited into membrane domains – lipid rafts – that act as hubs for the activation of certain pathways – like mitogen-activeted protein kinases (MAPK)/extracellular signal-

regulated kinases (ERK), janus kinase (JAK)/signal transducer and activator of transcription proteins (STAT) and phosphatidylinositol-4,5-biphosphonate 3-kinase (PI3K)/AKT serine/threonine kinase (AKT) – mediated by RET, which are associated with cellular proliferation and differentiation.[2, 18, 20, 22, 24]

4.2. *RET* oncogenic activation

Oncogenic activation of the *RET* can occur by both rearrangements and mutations,[21] with the first being the only mechanism so far described in lung cancer. These rearrangements lead to the formation of chimeric fusion proteins between the RET kinase domain and a partner protein, which contains a dimerization domain. This fusion leads to RET fusion proteins with the ability to auto-dimerize, without any need of ligand binding.[20, 25]

The gain of ligand-independent kinase activation leads to enhanced cellular proliferation and differentiation, which in turn results in neoplastic transformation.[20]

4.3. *RET* as an oncogenic driver in lung cancer

RET is a well-known thyroid cancer driver, with *RET* rearrangements being first discovered in patients with papillary thyroid cancer.[18, 26] *RET* alterations can also occur in sporadic medullary thyroid cancer and multiple endocrine neoplasia type 2 (MEN2) due to activating mutations and, more recently, RET fusions, albeit rare, have been discovered in other cancers, such as Spitzoid tumors, chronic myelomonocytic leukemia, breast, colon, ovarian, salivary gland and inflammatory myofibroblastic tumors.[22, 24]

In lung cancer, *RET* chromosomal rearrangements (which are the only oncogenic activating mechanism so far described in NSCLC) occur in about 1-2% of the cases and in up to 19% of lung cancers pan-negative for other major molecular alterations.[19, 21, 24] These activating rearrangements of the *RET* tend to preserve the tyrosine kinase domain 3' and fuse with upstream 5' partners.[13]

So far, at least 48 fusion partner genes have been identified.[27] The first one to be discovered in lung cancer – and by far the most common one, representing around 75%-90% of all of the cases – is *kinesin family member 5B* (*KIF5B*).[20, 21, 28]

The fusion between *KIF5B* and *RET* originates a chimeric protein, KIF5B-RET, that was first described in 2012.[26] This fusion derives from a 10.6 megabases (Mb) pericentric inversion on chromosome 10, and there are at least 10 fusion variants, with K15;R12 being the most frequent one.[22, 29]

Coiled-coil domain containing 6 (*CCDC6*) is the second most common fusion partner described, representing 10-25% of the cases. Other fusion partner genes already identified include *nuclear receptor coactivator 4* (*NCOA4*), *tripartite motif containing 33* (*TRIM33*), *CAP-Gly domain containing linker protein 1* (*CLIP1*), *myosin VC* (*MYO5C*), *phosphatidylinositol binding clathrin assembly protein* (*PICALM*) and *EMAP like 4* (*EML4*). In the first months of 2020 alone, five new partner genes were described for the first time.[22, 27, 28]

The demographic and histologic groups in which these fusions are more prevalent are similar to some other genetic alterations, such as *ROS1* and *ALK* translocations.[19] *RET* rearrangements are more frequent in lung cancer with adenocarcinoma histology (and can rarely be detected in squamous cell carcinoma), particularly ones with solid (this pattern being specifically associated with *CCDC6-RET*), papillary and lepidic patterns. Patients with these particular alterations are typically younger, with most of them being below 60 years of age, never or lighter smokers (82%), with some articles also mentioning a higher frequency in females.[2, 12, 19, 22, 30, 31]

Lung cancer with *RET* as an oncogenic driver is also associated with smaller primary lesions (<3 cm), but more N2 disease, and has shown to be potentially sensitive to chemotherapy, particularly pemetrexed-based regimens, possibly due to a lower expression of thymidylate synthetase.[21, 22, 32] *RET*-positive NSCLC is also associated with higher prevalence of pleural dissemination and brain metastasis.[32, 33]

RET fusions in lung cancer are considered as mutually exclusive with most of the other well-known oncogenic drivers, such as *EGFR*, *KRAS*, *ALK* and *ROS1*.[20, 22, 29] However, even though this is the case for the majority of patients, there are in the literature cases described of coexistence genetic alterations. Patients with *RET*

rearrangements are also included, with *ALK*, *MET*, *ROS1*, *KRAS* and *EGFR* alterations being reported as concurrent drivers.[34, 35]

Currently, since immunohistochemical methods have low sensitivity and variable specificity when it comes to *RET* alterations, *RET* rearrangements are mainly identified by FISH techniques. When it comes to *RET*-positive lung cancer, FISH is a highly sensitive technique, but with not so optimal specificity, so sometimes it is combined with reverse transcription polymerase chain reaction (RT-PCR), a very specific procedure, that is able to identify specific fusion partners (without, however, being able to detect novel or unknown translocation partners). NGS is an alternative method, that is also accurate and sensitive when it comes to the identification of *RET* alterations, meaning it can be used instead of FISH.[22]

5. Targeted therapy in *RET*-driven lung cancer

5.1. Multi-kinase inhibitors

TKIs are small molecules that are able to pass through cell membranes and inhibit tyrosine kinases, which results in the inhibition of nuclear signal transduction.[36]

Targeted therapy with these drugs was first introduced with imatinib, a drug that targets gene fusions in patients with BCR activator of RhoGEF and GTPase (BCR)-ABL proto-oncogene 1, non-receptor tyrosine kinase (ABL)-positive chronic myeloid leukemia.[22] After that, TKIs were also developed to treat patients with other different malignant tumors, including lung cancer, particularly NSCLC, with currently gefitinib, erlotinib and afatinib being approved in *EGFR*-mutated cancers and crizotinib and ceritinib in *ALK*-rearranged malignant neoplasms.[3]

In regards to *RET* alterations, there are some TKIs approved in other *RET*-driven cancers, specifically cabozantinib and vandetanib in patients with medullary thyroid cancer and lenvatinib and sorafenib in differentiated thyroid cancers.[23] Due to that, several multitarget TKIs' activity is being explored in *RET*-positive NSCLC, with a few clinical trials assessing their potential in clinical trials.[22]

In this section, data regarding multitarget TKIs with potential as targeted therapy against *RET*-rearrangements in lung cancer will be summarized.

5.1.1. Cabozantinib

Cabozantinib is a TKI whose main targets include vascular endothelial growth factor receptor (VEGFR) 2, MET, AXL receptor tyrosine kinase (AXL), KIT proto-oncogene, receptor tyrosine kinase (c-KIT) and RET.[22] It has been, since 2012, approved for patients with medullary thyroid cancer, regardless of RET status.[23, 37]

This TKI initially showed *in vitro* efficacy in suppressing KIF5B-RET cells' growth, with a half maximal inhibitory concentration (IC_{50}) for RET of 5-20 nanomolar (nM).[22]

Cabozantinib was actually the first TKI to have a clinical trial directed to *RET*-positive NSCLC, chosen instead of other TKIs due to its *in vitro* superior inhibition compared to vandetanib, sunitinib and axitinib. In 2013, preliminary data from three patients enrolled in a phase II, single arm study (NCT01639508) determining the effects of cabozantinib in patients with *RET* alterations was published. They were given a daily dose of 60 milligrams (mg) of cabozantinib orally, and the best result obtained was a partial response in two of them, one with a *TRIM33-RET* fusion and the other with *KIF5B-RET*. The third patient had stable disease. Due to adverse effects of the TKI, two of the patients needed a dose reduction, but maintained clinical benefit.[3, 26, 38]

More solid data regarding this trial was later published in 2016. Among the 26 patients enrolled, data from 25 was used for analysis. All the patients had lung cancer of the adenocarcinoma subtype, 62% of which had the KIF5B-RET fusion protein. Notably, half these patients had been previously treated with chemotherapy, but none with a RET inhibitor. In the course of the study, ORR was 28%, with a median PFS of 5,5 months and a median overall survival (OS) of 9,9 months. Interestingly, the ORR was lower (20%) in the group of patients with KIF5B-RET, compared to the group with different fusion proteins. There were no complete responses recorded, but seven of the patients had a partial response to cabozantinib. 96,2% of the patients had toxicity of any grade, with 73% needing a dose reduction and 8% discontinuing treatment. The grade 3 most common adverse events (AE) were lipase elevation (15%), increased alanine aminotransferase (ALT) (8%), increased aspartate aminotransferase (AST) (8%) and decreased platelet count (8%), all of which resolved with dose modifications. There were no grade 4 AEs or deaths related to treatment registered. This trial is still ongoing since there are still patients that remain on active treatment.[13, 22, 28, 39]

CRETA is another phase II Italian study (NCT04131543) exploring the activity of cabozantinib in *RET*-rearranged NSCLC, but so far, no results have been published.[40]

RET-positive lung cancer's response to cabozantinib was also analyzed in the retrospective global multicenter registry (GLORY). There was a total of 165 patients included, with a median age of 61 years. 98% had lung adenocarcinoma, 72% with *KIF5B-RET*, 23% with *CCDC6-RET*, 2% with *NCOA4-RET*, 1% with *EPHA5-RET* and 1% with *PICALM-RET* rearrangements identified. Among these, 21 patients were treated

with cabozantinib. Cabozantinib had an ORR of 33%, with one patient recording a complete response (5%), six having partial responses (32%), five with stable disease (26%) and seven having disease progression (37%). The median PFS was 3.6 months and the median OS was 4.9 months.[31, 41]

5.1.2. Vandetanib

Vandenatib is a TKI targeting mainly VEGFR2, VEGFR3, EGFR and RET. It has been approved since 2011 for the treatment of unresectable, locally advanced or metastatic medullary thyroid cancer, regardless of *RET* status, but with a better median PFS in *RET*-positive patients.[23, 37]

It has shown both *in vitro* and *in vivo* ability to suppress the growth of *KIF5B-RET* and *CCDC6-RET* cells, by competitive binding to the active conformation of the kinase, interrupting downstream signaling, with a IC_{50} of 100 nM.[22, 31]

Vandetanib's efficacy in *RET*-positive NSCLC was first described in two case reports.[42] In 2012, a patient with a *KIF5B-RET* alteration received 300 mg of vandetanib once daily, with a significant improvement of symptoms and disease remission.[43] In 2014, a patient with widely metastatic lung cancer with an identified *CCDC6-RET* fusion, also received vandetanib in the same dose, having demonstrated a 76% decrease of the tumoral mass.[44]

In 2015, a retrospective analysis of *RET* rearrangements in NSCLC was made, by evaluating tumor samples from four previous phase III studies where patients with NSCLC had been treated with vandetanib: ZODIAC (NCT00312377), ZEAL (NCT00418886), ZEPHYR (NCT00404924) and ZEST (NCT00364351). *RET* rearrangements were identified in 7 patients, 3 of which had received vandetanib. These 3 patients all had lung cancer of the adenocarcinoma histology, with *KIF5B* has *RET*'s partner gene. There was tumor shrinkage reported in two of these patients, but no objective response could be confirmed.[22, 31, 45]

There are two main trials assessing vandetanib's efficacy in *RET*-positive NSCLC. The first one is the Japanese phase II, single arm trial LURET (UMIN000010095). This clinical trial enrolled 19 patients, with 17 of them included in the primary analysis, to receive 200 mg daily of vandetanib. All patients had adenocarcinoma,

53% with *KIF5B-RET* fusions and 31% with *CCDC6* as *RET*'s partner gene. In the first published results, the ORR was 53%, with a disease control rate (DCR) of 88% - there were 9 patients with a registered partial response and 6 with stable disease. 17 of the patients with intention to treat achieved disease control. The median PFS was 4.7 months and the median OS was 11.1 months, with 47% OS at 12 months. There were some statistical differences noted between patients with different *RET* partner genes, with better outcomes being reported with the fusion partner *CCDC6* – patients with *CCDC6-RET* had 83% ORR, median PFS of 8.3 months and 12-month OS of 67%, while patients with *KIF5B-RET* had only 20% ORR, with a median PFS of 2.9 months and 12-month OS of 42%. However, due to AEs, 53% of the patients needed a dose reduction and in 21% vandetanib had to be discontinued. The most frequent grade 3-4 AEs reported were hypertension (58%), rash acneiform (16%), diarrhea (11%) and prolonged QT-corrected interval (11%).[13, 22, 28, 31, 46]

The final survival results for the LURET trial were recently published. Vandetanib had an ORR of 47.4%, with 9 partial responses, and a DCR of 89.5%. The median PFS was 6.5 months and the median OS was 13.5 months, with a 12-month OS of 52.6%. The outcomes were better in patients with *CCDC6-RET*: median PFS was 8.9 months and the median OS was not reached, while in patients with *KIF5B-RET* median PFS was 4.2 months and the OS was 10.5 months. There were no differences noted according to previous chemotherapy treatments. All of the patients had at least one AE, with 84.2% having grade 3 or 4 AEs but no treatment related grade 5 AEs being reported. The most common any grade AEs were hypertension (84,2%), diarrhea (78,9%), rash acneiform (63,2%), prolonged QT corrected interval (47,4%) and dry skin (42,1%).[47]

The other main trial is a Korean phase II, single arm study (NCT01823068) that enrolled 18 patients treated with 200 mg of vandetanib daily. In this clinical trial, adenocarcinoma was the predominant histologic subtype and there were three different *RET* partner genes identified: *KIF5B* (28%), *CCDC6* (11%) and *MYO5C* (5%). 72% of the patients had previously been submitted to chemotherapy treatments and 4 had been treated with anti-angiogenic agents. 17 of the 18 patients were evaluated for treatment response. The ORR achieved was 18%, with a DCR of 65% - 18% of the patients had a partial response and 47% stable disease. The median PFS was 4.5 months and the median OS 11.6 months, with a 12-month OS of 33%. Notably, there was no objective response reported in patients with *KIF5B-RET*.

Regarding AEs, the most common all-grade were hypertension (89%), diarrhea (44%) and acne (28%). 5 patients had grade 3 events, with 4 of them needing a dose reduction to 100 mg. There was no grade 4 or 5 toxicity reported.[22, 28, 31, 48]

Vandetanib is also being studied in a phase I study in *RET*-rearranged NSCLC, combined with mTOR inhibitor everolimus. 13 patients participated in the study, and an ORR of 54% was achieved, with a median PFS of 4.4 months. The most frequent AEs reported were diarrhea (21%), thrombocytopenia (16%), QT prolongation (5%) and rash (5%).[31]

Vandetanib's efficacy was also evaluated in the retrospective GLORY registry. 11 patients received vandetanib, with a partial response in 2 patients (18%) being the best result achieved. 27% of the patients had stable disease and 55% disease progression. The median PFS reached was 2.9 months and the median OS 10.2 months.[31, 41]

5.1.3. Lenvatinib

Lenvatinib is a multitarget TKI with affinity got VEGFR1-3, fibroblast growth receptor (FGFR) 1-4, platelet derived growth factor receptor (PDGFR) alpha, c-KIT and RET.[22] It was approved in 2015 for the treatment of current, radioactive iodine-refractory differentiated thyroid cancers (papillary and follicular), regardless of *RET* status.[23, 37]

Lenvatinib has demonstrated *in vitro* and *in vivo* ability to suppress the growth of *KIF5B-RET* and *CCDC6-RET* cells, with a IC_{50} of 1.5 nM. [22, 28]

Lenvatinib's possible efficacy in *RET*-positive NSCLC was assessed in a phase II Japanese/US trial (NCT01877083), that enrolled 25 patients with lung cancer driven by *RET* rearrangements. The two most common fusion partners identified were *KIF5B* (52%) and *CCDC6* (48%). Among the enrolled patients, only 8% were treatment-naïve, with 28% having already received a different anti-RET therapy. They were treated with 24 mg of lenvatinib daily and at the time of the primary data cutoff, ORR was 16%, with a DCR of 76% and no complete responses registered. ORR was not significantly different in patients with previous anti-RET treatment (14%) or between different fusion partner genes (15.4% for *KIF5B* and

16.7 in *CCDC6*), DCR was superior in patients with *CCDC6-RET* (91.7%, compared to 61.5% in patients with *KIF5B-RET*). Median PFS was 7.3 months and it was also different according to the partner gene: 9.1 months for *CCDC6* and 3.6 for *KIF5B*. The median OS was not reached in this trial, but 12-month OS rate was 40% for patients with *KIF5B-RET* and 67% for patients with *CCDC6-RET*. All of the patients had any-grade AEs, with 92% having grade 3 or superior AEs, most commonly hypertension (56%) and hyponatremia (20%). Serious AEs were registered in 52% of the patients, but only one death being related to lenvatinib. 64% required a dose reduction and 24% discontinued lenvatinib due to AEs. At the time of data cutoff, only 20% remain in treatment.[22, 28, 31, 36, 49]

In the GLORY registry, 2 patients were treated with lenvatinib, with one of them having partial response and the other disease progression.[41]

5.1.4. Alectinib

Alectinib's main targets are ALK, fms related receptor tyrosine kinase 3 (FLT3), leukocyte receptor tyrosine kinase (LTK), checkpoint kinase 2 (CHEK2) and RET and it is already used in the treatment of lung cancer, specifically for *ALK*-positive NSCLC.[22, 23]

Just like the previous drugs, alectinib has shown pre-clinical evidence of growth suppression of *KIF5B* and *CCDC6* cells, with an IC_{50} of 4.8 nM.[22, 31] Its main advantage, when compared with the above-mentioned drugs, is the fact that alectinib does not block VGFR2, which may translate in fewer antiangiogenic side effects.[22, 31, 50]

A four-patient case report of alectinib being used off-label to treat *RET*-positive NSCLC was published in 2016. Patient 1 was given 600 mg twice daily initially and the dose was later increased to 900 mg, due to the presence of brain metastasis. There was no toxicity reported and the patient showed improvement both regarding the intracranial and extracranial disease. A partial response was objectified, but alectinib was eventually discontinued. Patient 2 had a *KIF5B-RET*-positive NSCLC, with both lung and liver metastasis. He had previously been treated with cabozantinib and was now given 600 mg of alectinib. The disease remained stable, but he had to eventually discontinue treatment due to grade 3 AEs. Patient

3 had been previously heavily treated, including with anti-RET therapy (cabozantinib), due to NSCLC positive for a *CCDC6-RET* rearrangement. He improved under alectinib in both energy and respiratory symptoms, but the partial response could not be confirmed. The last patient also had *KIF5B* has *RET*'s partner gene and was treated with 600 mg of alectinib. However, there was still disease progression.[51]

A small phase I/II study was conducted in the US (NCT03131206) with alectinib. 4 out of the 5 patients enrolled had NSCLC with *RET*-rearrangements. Despite phase I having ended without enough participants to calculate the maximum tolerable dose, some secondary outcomes were still evaluated. ORR and OS were 20% and no serious AEs were reported, with fatigue, hypokalemia and dyspnea being the most frequent AEs described.[52]

A European phase II, single arm trial (NCT03445000) trying to evaluate the activity of alectinib in pre-treated patients with *RET*-positive NSCLC (ALERT-lung) enrolled 14 patients and has recently terminated. However, no results have been published so far.[53]

In the GLORY retrospective registry, 2 patients were treated with alectinib, with both of them, however, shown disease progression.[41]

5.1.5. Ponatinib

Multikinase inhibitor ponatinib's main targets are BCR-ABL, FLT3, c-KIT, FGFR, SRC proto-oncogene, non-receptor tyrosine kinase (SRC), VEGFR, PDGFR and RET.[22] This drug, approved by US's Food and Drug Administration (FDA) for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia and chronic myelogenous leukemia, has shown pre-clinical activity against *KIF5B* and *CCDC6* cells, with an IC_{50} of 25.8 nM. [22, 23, 31, 54]

A phase II, open-label study (NCT01813734) was conducted to understand if ponatinib was effective and safe in treating *RET*-positive NSCLC. The study enrolled 9 patients, that were given 30 mg daily of ponatinib. The ORR in this study was 0%, with a DCR of 55.6%. The median PFS was 3.8 months and 12-month OS was 55.6%. All patients reported AEs, with constipation, diarrhea, non-cardiac chest pain and

hypertension being the most frequent ones. Two patients (22.22%) reported more serious AEs, specifically dry skin and maculo-papular rash.[55]

In the GLORY retrospective study, 2 patients were given ponatinib, both maintaining with stable disease.[31, 41]

5.1.6. Sunitinib

Sunitinib, a c-KIT, VEGFR1-3, PDGFRbeta, FLT3 and RET inhibitor, is currently approved for the treatment of renal cell carcinoma, gastrointestinal stromal tumors and pancreatic neuroendocrine tumors.[23, 31]

Regarding *RET*-positive NSCLC, a 2015 case report has shown sunitinib's potential in the treatment of these patients. A 63-year old Asian woman with metastatic lung adenocarcinoma, with metastasis of lung, bone and brain, suffering from respiratory disease, was given sunitinib and showed improvement of respiratory function since day 3 of treatment and significant control disease for 10 weeks until discontinuation.[20, 56]

10 patients were given sunitinib in the GLORY retrospective registry, with two of them (22%) achieving a partial response, 3 patients having stable disease and another 3 disease progression. Median PFS was 2.2 months and median OS 6.8 months.[31, 41]

There are a few clinical trials assessing sunitinib's efficacy in the treatment of NSCLC, but none of them is specific to patients with RET rearrangements.

5.1.7. Sorafenib

Sorafenib is a drug that was approved in 2013 for the treatment of differentiated thyroid cancers, regardless of RET status.[23, 37] It is a RAF proto-oncogene serine/threonine-protein kinase (c-RAF,) BRAF, c-KIT, FLT3, VEGFR and RET inhibitor.[31]

Pre-clinical studies have shown sorafenib's activity against *KIF5B* cells.[31] A phase II clinical trial (UMIN 000007515) attempted to understand sorafenib's usefulness in *RET*-positive NSCLC. 3 patients were enrolled in this study. Patient 1 had stage IV unclassified NSCLC with a *KIF5B-RET* fusion. She had been previously treated with chemotherapy regimens and, despite institution of sorafenib, her disease progressed, with the identification of a fracture due to bone metastases and liver metastases. Patient 2 had adenocarcinoma with an unidentified *RET* rearrangement and he had also been previously treated with chemotherapy. The target lesions decreased minimally with sorafenib, however, the patient also had pleural effusion that continued increasing. The last patient had adenocarcinoma with *CCDC6* has *RET*'s partner gene. She had been previously treated with docetaxel monotherapy and there was a slight decrease of tumor size and improvement of disease-related pain with sorafenib. The response to the drug was maintained for 12 months. However, due to grade 3 palmar-plantar erythrodysesthesia syndrome and infection, dose reduction was needed more than once.[57]

5.1.8. Agerafenib

Agerafenib, previously known as RXDX-105, is a VEGFR-sparing multikinase inhibitor that has demonstrated potent preclinical activity against RET.[31, 58]

A phase I/Ib study ((NCT01877811) was conducted to appraise agerafenib's safety and antitumor activity in *RET*-altered neoplasms. Among the 152 patients enrolled in the study, 40 had *RET*-positive NSCLC. 31 of these patients had previously been treated with a multikinase inhibitor and most of them harbored a *KIF5B-RET* rearrangement (65%). The remaining 9 patients were TKI-naïve.[31, 58]

Among the cohort with NSCLC that had been previously treated, the best response achieved was stable disease, in 33% of the patients, with 45% eventually having disease progression. The ORR was 0%. Better results were achieved in the TKI-naïve cohort – 19% of the patients achieved partial response, with 39% having stable disease and 32% eventually progressing. The ORR was 19%. Notably, responses were only attained in patients that did not harbor *KIF5B-RET* rearrangements.[31, 58]

The most common AEs in reported in this study were fatigue (25%), diarrhea (24%), hypophosphatemia (18%) and non and maculopapular rash (17% and 18%,

respectively), with hypophosphatemia (9%), elevated ALT (8%), maculopapular rash (7%), elevated AST (5%) and diarrhea (5%) being the most common grade 3 and higher AEs described. Hypertension and proteinuria, some of the AEs commonly associated with inhibition of VEGFR, were rare. Due to AEs, dose reduction was necessary in 31% of the patients being treated with 275 mg of agerafenib in the phase Ib of the study.[31, 58]

5.1.9 Other TKIs

The potential of multikinase inhibitors in *RET*-positive NSCLC doesn't end with the above-mentioned drugs. Several other multitarget TKIs, such as dovitinib and AD80, have shown *in vitro* potential in the treatment of *RET* rearranged neoplasms.[22]

Others, like apatinib, a VEGFR2, PDGFRbeta, SRC, c-KIT and RET inhibitor, do not show *in vitro* potential in suppressing cell viability, but instead have potential in the inhibition of cancer cell migration and invasiveness, which translates in an interesting anti metastatic potential.[22, 59]

In the GLORY retrospective registry, two other TKIs had been used off-label in patients with NSCLC with *RET* rearrangements. 2 patients were treated with nintedanib, with one of them having a complete response and the other stable disease, and 2 other were treated with regorafenib, with the one patient whose information the investigators were able to retrieve having had disease progression.[41]

5.1.10. Multitarget TKIs' efficacy

Despite the many different available drugs, *RET*-positive NSCLC's response to multitarget TKIs is limited and short lived.[13]

When we look at the clinical activity against *RET* rearrangements, the results are not as promising as in targeted therapy directed at other oncogenic drivers. ORR is much lower in the studies with *RET*-positive patients, varying between 16% and 57%,

as well as PFS, which ranges between 2.3 and 7.3 months; while in *EGFR*, *ALK* and *ROS1*-positive NSCLC, ORR values vary from 56% to 85%, 60% to 90% and 65% to 85% and median PFS of 9.2 to 13.7 months, 8 to 11 months and 9.1 to 19.3 months can be achieved, respectively.[22, 23, 25]

There are several possible explanations for multitarget TKIs' limited efficacy when it comes to *RET*-positive NSCLC. The first one relates to off-target activity. Being multitarget agents, the above mentioned TKIs, besides the RET kinase, also inhibit non-RET kinases and non-kinase targets, which may diminish their efficacy (although, this is not a consensual statement for all authors). Besides clinical activity, there are also AEs related to off-target inhibition, most importantly as hypertension and proteinuria, attributed to VEGFR2 inhibition, QT prolongation due to potassium voltage-gated channel subfamily H member 2 (hERG) inhibition and diarrhea as a result of EGFR inhibition. In the above mentioned studies, toxicity limited greatly the potential of TKIs, with 23% to 79% of the patients needing dose reductions and 6% to 21% having to discontinue treatment.[60]

Another explanation relates to intrinsic resistance, particularly in regards to *KIF5B-RET*. Despite being the most common gene fusion, *KIF5B-RET* seems to be harder to target than other *RET* fusion genes. One possible reason for this difficulty seems to be *KIF5B*'s correlation to higher levels of *RET* expression. When compared to other upstream partners, like *CCDC6*, *KIF5B-RET* rearrangements result in much higher levels of *RET* expression, which may translate in higher levels of chimeric RET oncoproteins and, thus, less efficacy from targeted therapy. Signaling and functional differences between *RET* fusions with different upstream partners, shown in *Drosophila* models, can also explain TKIs' different efficacy according to the partner gene.[25, 58, 60]

A third explanation centers around acquired resistance, which may happen through mutations (however, this has only been identified in pre-clinical models) or concurrent genomic alterations, such as *EGFR*.[60]

5.2. Highly specific RET inhibitors

Highly specific RET inhibitors started being evaluated to overcome the limitations identified in clinical trials with multikinase inhibitors.[13] In 2020, FDA approved for the first time a RET-specific inhibitor for patients with NSCLC, selpercatinib, and then later praseltinib as well.[61, 62]

Both drugs are already in the newest American Socity of Clinical Oncology (ASCO)/ Ontario Health (OH) joint guideline. So far, standard treatment has been doublet chemotherapy, sometimes with immunotherapy. But as off 2021, patients with stage IV *RET*-rearranged untreated NSCLC, with performance status of 2 or below, may be offered selpercatinib or pralsetinib as standard therapy. Both drugs can also be offered to patients previously treated with *RET*-targeted therapy, such as multitarget TKIs.[62]

5.2.1. Selpercatinib

Selpercatinib, formerly known as LOXO-292, is an adenosine triphosphate (ATP) competitive, highly selective inhibitor of the RET kinase, with potency against *RET* rearrangements in pre-clinical models, while sparing other targets.[24, 33]

LIBRETTO-001 is a phase I/II study (NCT03157128) investigating the safety, tolerability and pharmacokinetics of selpercatinib in patients with *RET*-positive NSCLC.[31] In phase I, patients were given selpercatinib in doses ranging from 20 mg once daily to 240 mg twice a day. In phase II, all patients started by receiving 160 mg twice daily. Among the 144 patients enrolled in the study, 105 had previously been treated with platinum chemotherapy and 29 were treatment naïve. In the previously treated cohort, 48% had been treated with a multitarget TKI. The majority of patients had lung adenocarcinoma and 45 had documented brain metastases at the time of enrollment. *KIF5B-RET* was the most common genetic alteration, identified in 85 patients, followed by *CCDC6-RET*, in 32 patients.[33, 61]

ORR was calculated for each cohort – 64% for patients that had previously received treatment, with 2 complete responses (2%) and 65 partial responses (62%) identified, and 85% for untreated patients, with 33 partial responses (85%)

documented. 12-month PFS was 66% for previously treated patients and 75% for the treatment-naïve cohort.[33, 61]

Selpercatinib also showed great intracranial activity. Among the 11 patients with baseline measurable metastases, 3 complete responses and 7 partial responses were obtained, with 91% objective intracranial response.[33]

The most common grade 3 and 4 events reported were hypertension (14%), increased ALT (13%), increased AST (10%), hyponatremia (6%) and lymphopenia (6%), most of them reversible with dose modification. There were 6 patients with grade 5 events, but all were considered to be unrelated to selpercatinib.[33]

Selpercatinib's results in the LIBRETTO-001 trial led to its approval by FDA in May 2020.[61, 63]

There are currently other phase II studies assessing the response rate associated with selpercatinib in patients with *RET*-rearranged NSCLC ongoing (NCT04268550 and NCT04280081). A phase III study, LIBRETTO-431 (NCT04194944), is a randomized, open-lable trial, with the intention to compare selpercatinib's efficacy in treatment-naïve patients with non-squamous *RET*-positive NSCLC, against carboplatin or cisplatin and pemetrexed chemotherapy.[64]

5.2.2. Pralsetinib

Pralsetinib, formerly known as BLU-667, is a *RET*-selective inhibitor that has shown *in vitro* potential in targeting RET-altered malignant neoplasms, including NSCLC.[31]

The efficacy and safety of pralsetinib is currently being evaluated in phase I/II study ARROW (NCT03037385). The first results showed pertained two patients, a 37-year-old with metastatic NSCLC and a 72-year-old with locally advanced *KIF5B-RET* NSCLC. The first one had previously been treated with cisplatin, pemetrexed and bevacizumab, but the disease eventually progressed. After initiating 200 mg daily of pralsetinib, tumor reduction was verified after 8 weeks of treatment, with partial response after 16 weeks. Pralsetinib was well-tolerated, with only grade 1 AEs (constipation, dry skin, rash and leukopenia). The second patient had previously been enrolled in a clinical trial testing vandetanib's efficacy in combination with

everolimus. The patient initially showed partial response, but eventually the disease progressed, with increased dyspnea and worsening performance status. After 16 weeks of 300 mg daily of pralsetinib, the patient showed partial response with improved of the symptoms. The drug was well-tolerated, without any AEs.[28, 65]

In 2019, results regarding 79 patients enrolled in the study were shared. Among them, 44 patients had a *KIF5B-RET* fusion identified and 16 a *CCDC6-RET* rearrangement. Most of the patients were not treatment-naïve: 76% had previously been treated with chemotherapy, 41% with immunotherapy and 27% with multitarget TKIs. 39% had baseline brain metastases. ORR among the 57 patients evaluated was 56%, with 32 partial responses, 20 patients with stable disease and only 5 had disease progression. DCR was 91% and at the time of data cut-off, 91% of the patients remained in treatment. Response to pralsetinib occurred regardless of prior treatment or *RET* fusion genotypes. Pralsetinib was well-tolerated, with only 3% of the patients discontinuing treatment due to AEs. AEs identified were mainly low grade and reversible, with 28% having grade 3 or above AEs. The most common AEs reported were increased AST (22%), hypertension (18%), increased ALT (17%), constipation (17%), fatigue (15%) and decreased neutrophils (15%).[66]

More recent results were shared in 2020. 116 were participating in the study, 72% with *KIF5B-RET* and 16% with *CCDC6-RET* rearrangements. 80 patients had previously been treated with platinum-based chemotherapy and 26 never had any previous systemic treatment. Overall ORR was 65%, with 7 complete responses (6%) and without major differences between previously treated and treatment naïve patients (61% and 73%, respectively). ORR was also similar regardless of the fusion partner and central nervous system involvement. DCR was overall 93%, with 96% of the patients having experienced tumor size reduction. Median time to response was 1.8 months and median duration of response was not reached.[67]

Thanks to these results, pralsetinib was approved by FDA for *RET*-rearranged NSCLC in September 2020.[62] A phase 3 study (NCT04222972) to compare praseltinib's efficacy in RET-rearranged NSCLC compared to platinum-based chemotherapy is currently ongoing.[68]

6. Discussion

The clinical case introduces us a young patient diagnosed initially with stage IVA lung cancer, in the context of supraclavicular lymphadenopathy and weight loss, with later analyses revealing a *KIF5B-RET* rearrangement as its oncogenic driver.

RET-positive NSCLC, as described above, is associated with some demographic characteristics, some of which can be identified in this patient. They tend to be associated with younger patients, like in this case, and to be less common in heavy smokers. Despite there being history of tobacco consumption, and thus not being a never smoker, the smoking history described isn't particularly heavy.

RET rearrangements are also associated with characteristics of the disease itself. It is an oncogenic driver associated with NSCLC, predominantly being identified in adenocarcinomas. The primary lesion identified in the imaging exams was not particularly large, even though it is slightly bigger than the average for *RET*-positive lung cancer. There is also an association with more local nodal disease, that is present here, but still without involvement of ipsilateral mediastinal or subcarinal nodes.

The patient was initially treated with platinum-based chemotherapy, which is standard therapy in advanced NSCLC, associated with pemetrexed. *RET*-positive NSCLC in particular, as described, has a high sensitivity to this chemotherapy agent.

One of the most particular aspects of this clinical case is the identification of both an *ALK* and a *RET* rearrangement, since they are considered mutually exclusive oncogenic drivers. There is more than one explanation possible for this fact. The discrepancy may be, first and foremost, due to inaccuracy of the techniques used. No technique is 100% sensitive and specific, false-positives may happen. However, as previously stated, even though very rare, there is some literature published regarding coexistence of oncogenic drivers, with at least one patient with concurrent *ALK* and *RET* rearrangements. For the patient, this may mean that we have present two different genetic alterations that we can target.

And, since at the time no anti-RET drugs were approved, she was treated with alectinib, a multi-target TKI that is first-line therapy for *ALK*-positive NSCLC.

However, the patient showed no response to this treatment. Considering the identification of the *ALK* rearrangement alone, this could either be due to a false-positive identification, or due to primary resistance to the drug. Nevertheless, we can also look at alectinib considering its anti-RET potential, since it is a multi-target agent.

According to the literature, alectinib showed pre-clinical efficacy when it came to suppressing the growth of *KIF5B-RET* cells, as did many other TKIs. However, while cabozantinib, vandetanib and lenvatinib have somewhat robust studies assessing their role in the treatment of *RET*-positive NSCLC, when it comes to alectinib there are only a few case reports and a small US study. While some patients are known to have had stable disease or even a partial response, the results obtained in the US study were not overwhelming. It is true that the number of participants was very limited, but both ORR and OS reported were of only 20%.

As such, considering solely the *RET* rearrangement, progression disease under alectinib is not unexpected, at least when comparing to what we would anticipate in an *ALK*-positive NSCLC.

This encompasses what we described as the limitations of the multi-target TKIs in general when it comes to *RET*-driven NSCLC. Despite the efficacy and ability to suppress cell-growth reported in pre-clinical studies, the results achieved in clinical trials aren't as good as what would be expected, with significant difference in terms of ORR and OS, when comparing other oncogenic drivers.

This is even more true when we talk about *RET* rearrangements with *KIF5B* as a fusion partner. Of all the most common *RET* rearrangements, *KIF5B-RET* seems to be one particularly difficult to target, as stated, due to a larger expression of the altered RET protein. Being the genetic alteration identified in the patient, the probabilities of a good response to multi-target TKIs becomes even lower.

Since disease progression was assessed, the patient started being treated with a combination of docetaxel and nintedanib, a second-line chemotherapy regimen approved for patients with advanced NSCLC, with this association having proved to have better outcomes than docetaxel alone.

Nintedanib, just like alectinib, is a multi-target TKI, so we can also appraise its RET-targeting potential. In *RET*-positive lung cancer specifically, however, nintedanib is

even less studied than alectinib. Still, there is reference in the GLORY registry to a complete response from a patient, with the only other one who received this drug having disease progression.

Summing up, the *RET* targeting potential itself available at the time was very limited, with no approved anti-RET drugs and underwhelming results from the ones being tested.

The disease progression under alectinib can lead the question of what and if other targeting therapies could have been used in this particular case. As already mentioned, other TKIs besides alectinib have anti-RET potential, with some of them actually having been more researched in clinical trials. We do know from the results of these trials, however, that ORR an OS across those studies were far from ideal, not being that much different than the one we mentioned for alectinib.

Nevertheless, there are cases of even partial responses from patients after switching to a different TKI, even though disease progression had previously occurred, and at least one study compared ORR in treatment-naïve and previously treated patients, with no differences found. Which means, that if able to enroll in a clinical trial, a different multi-target TKI could still be tried with some hope of a good response and outcome.

Even though this is true, we now have a much better option. Since then, FDA-approved highly specific *RET* inhibitors have emerged, with a greater potential in this context.

At the time of alectinib discontinuation from the patient, neither selpercatinib or pralsetinib were approved. If they had been, though, either of them could have been an option, with both being already mentioned in guidelines as first-line options for the treatment of *RET*-positive NSCLC. Selpercatinib, in particular, due to the more advanced clinical trial's results and thus slightly stronger evidence, could have been offered to the patient, admitting she maintained an acceptable performance status (ECOG $\leq$ 2).

When comparing to multi-target TKIs, both drugs currently in clinical trials have already shown greater results than any other TKI studied so far, with results comparable to what we obtain in NSCLC with different oncogenic drivers. The ORRs

achieved, in particular, were much higher, even when looking at patients previously treated with anti-RET therapies.

Another great advantage of highly selective *RET*-inhibitors is their potential in NSCLC with *KIF5B-RET* rearrangements, which is particularly encouraging. While multi-target TKIs had in general shown even worse results with this *KIF5B-RET* in particular, both selpercatinib and pralsetinib seem to be have no differences according to the fusion partner gene.

These drugs are also, in general, well tolerated, which is also an important point both for the comfort of the patient and its efficacy. Being well tolerated, it's much easier to use them without the need of dose reduction or even discontinuation.

Finally, these more recent developments also mean change to the current panorama of targeted therapy in NSCLC. With the existence of proper, effective anti-RET options, patients with lung adenocarcinoma should also start being routinely tested for *RET* rearrangements.

7. Conclusion

Ever since *RET* rearrangements were first discovered in lung cancer, a lot of achievements were reached in this area. Multi-target TKIs, already being studied for different oncogenic drivers, were the first *RET*-directed therapy to be tested – cabozantinib and vandetanib were just a few of the drugs that showed promise. However, even though some patients responded to them, their efficacy wasn't as great as initially though, specially comparing to their results in NSCLC with different drivers.

With multi-target TKIs performing below expectations, there was a need for a different alternative, one that could circumvent their limitations. Highly selective *RET* inhibitors started, then, being developed – selpercatinib and pralsetinib were both studied in phase II clinical trials with great outcomes, which lead to their approval by FDA, and phase III studies are already planned.

These two drugs are changing the way we approach *RET*-positive patients and the ongoing and future clinical trials will give us an even better understanding of all their potential and the future role they will have in the treatment of *RET*-positive NSCLC.

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