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ORIGINAL RESEARCH

Importance of Testing for ROS1 Rearrangements in Non-Small Cell Lung Cancer in the Era of Targeted Therapy in a Latin American Country

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Purpose: Lung cancer is the leading cause of cancer-related deaths worldwide. However, with the optimization of screening strategies and advances in treatment, mortality has been decreasing in recent years. In this study, we describe non-small cell lung cancer patients diagnosed between 2021 and 2022 at a high-complexity hospital in Latin America, as well as the immunohistochemistry techniques used to screen for *ROS1* rearrangements, in the context of the recent approval of crizotinib for the treatment of *ROS1* rearrangements in non-small cell lung cancer in Colombia.

Methods: A descriptive cross-sectional study was conducted. Sociodemographic, clinical, and molecular pathology information from non-small cell lung cancer individuals who underwent immunohistochemistry to detect *ROS1* rearrangements between 2021 and 2022 at Fundación Valle del Lili (Cali, Colombia) was recorded. The clinical outcomes of confirmed *ROS1* rearrangements in non-small cell lung cancer patients were reported.

Results: One hundred and thirty-six patients with non-small cell lung cancer were included. The median age at diagnosis was 69.8 years (interquartile range 61.9–77.7). At diagnosis, 69.8% (n = 95) were at stage IV. *ROS1* immunohistochemistry was performed using the monoclonal D4D6 antibody clone in 54.4% (n = 74) of the cases, while 45.6% (n = 62) were done with the monoclonal SP384 antibody clone. Two patients were confirmed to have *ROS1* rearrangements in non-small cell lung cancer using next-generation sequencing and received crizotinib. On follow-up at months 5.3 and 7.0, one patient had a partial response, and the other had oligo-progression, respectively.

Conclusion: Screening for *ROS1* rearrangements in non-small cell lung cancer is imperative, as multiple prospective studies have shown improved clinical outcomes with tyrosine kinase inhibitors. Given the recent approval of crizotinib in Colombia, public health policies must be oriented toward early detection of driver mutations and prompt treatment. Additionally, future approvals of newly tested tyrosine kinase inhibitors should be anticipated.

Keywords: non-small cell lung cancer, ROS1, proto-oncogene receptor tyrosine kinase, immunohistochemistry, next generation sequencing, tyrosine kinase inhibitor

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. However, due to the optimized screening strategies and advances in treatment, mortality has been decreasing in recent years.[1–3] Within the context of lung cancer, certain genetic alterations, known as driver mutations, confer a selective advantage to oncogenic cells.[4] To date, multiple driver mutations have been identified in non-small cell lung cancer (NSCLC), and some of these mutations predict responses to specific therapies, making molecular diagnosis or biomarking mandatory for treatment selection.[5,6]

The prevalence of proto-oncogene receptor tyrosine kinase (*ROS1*) rearrangements in NSCLC (*ROS1*-NSCLC) has been described in about 2.4% (95% confidence interval (CI) 1.5–3.7), being more frequent in adenocarcinomas than in any other histologic type, representing 2.9% (95% CI: 1.9–4.5) and 0.6% (95% CI: 0.3–1.2), respectively.[7,8] Rarely,

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ROS1 rearrangements coexist with other driver mutations such as epidermal growth factor receptor (*EGFR*) mutations and mesenchymal–epithelial transition factor (*MET*) amplifications, among others.[9–11]

Immunohistochemistry (IHC) is currently accepted as a screening strategy, primarily due to its lower cost compared to molecular tests, which also require more time and technical expertise.[7] The rabbit monoclonal D4D6 antibody clone (Cell Signaling Technology, Danvers, Massachusetts, USA) is the most used technique, with a sensitivity of around 100% and a variable specificity between 92% and 100%, depending on the positivity threshold.[12,13] Recently, a new monoclonal antibody, SP384, has demonstrated a sensitivity of up to 91% and specificity of 100% in the ROSING study.[14]

IHC and molecular tests have demonstrated high sensitivity and concordance when comparing negative results, achieving a concordance rate of nearly 100%, making it possible to rule out *ROS1* rearrangements when IHC is negative. However, in positive cases, the concordance rate can be as low as 79%, indicating the necessity for a confirmation test in cases where IHC is positive. Additionally, immunostaining can sometimes be heterogeneous, leading to the possibility of false-positive results.[7] Confirmation can be made through FISH (fluorescent in situ hybridization), real-time polymerase chain reaction (RT-PCR), or next-generation sequencing (NGS) and Nanostring.[7,15,16]

Crizotinib is a tyrosine kinase inhibitor (TKI) that inhibits several enzymes, including anaplastic lymphoma kinase (*ALK*), cellular mesenchymal–epithelial transition factor (*c-MET), and ROS1*. This inhibition is associated with cell cycle arrest in the G1-S phase and the induction of apoptosis in positive cells in vitro and in vivo.[17] Since 2014, the benefit of crizotinib treatment in advanced *ROS1*-NSCLC has been tested,[18] and subsequent studies have confirmed these results.[19,20]

In our hospital in Colombia, both monoclonal antibodies (D4D6 and SP384) have been utilized over the past few years. Once a positive IHC result is obtained, the choice of a confirmatory test depends on the oncologist's criteria and its availability. With the recent approval of crizotinib by the National Institute for Food and Drug Surveillance (INVIMA) in Colombia in October 2021, *ROS1* testing in NSCLC has become especially relevant in determining whether targeted therapy with crizotinib can provide benefits. Therefore, in this study, we describe NSCLC patients diagnosed between 2021 and 2022 in a high-complexity hospital in Latin America, as well as the IHC techniques employed for *ROS1* rearrangement screening and the trend in testing frequency over the past year.

Methods

A descriptive cross-sectional study was conducted, collecting sociodemographic, clinical, and molecular pathology information from individuals with NSCLC who underwent IHC for the detection of *ROS1* rearrangements between 2021 and 2022 at Fundación Valle del Lili (FVL) in Cali, Colombia. Patients older than 18 years at any disease stage were included, excluding those who had a second primary lung tumor.

A descriptive statistical analysis was performed by determining absolute frequencies and proportions for qualitative variables. For the quantitative variables, central tendencies (mean or median) and their corresponding measures of dispersion (standard deviation or interquartile range) were reported, based on the normal distribution. All statistical analyses were performed using RStudio. The study was approved by the FVL Biomedical Research Ethics Committee (trial No. 155–2023).

Results

We included 136 patients diagnosed with NSCLC between 2021 and 2022 who underwent an IHC test for the detection of *ROS1* rearrangements. Among them, 51% (n = 70) were women, 62.5% (n = 85) were aged between 66 and 85 years (median age 69.8; IR 61.9–77.7). The majority of patients had adenocarcinomas (95.6%, n = 130), while the remaining percentage corresponded to squamous cell carcinoma (n = 4), one case of adeno-squamous-cell carcinoma, and one case of lymphoepithelioma-like tumor. At the time of diagnosis, 69.8% (n = 95) were at stage IV. Table 1 summarizes the demographic and clinical characteristics.

A total of 136 IHC tests for *ROS1* were performed within the established timeframe. About 54.4% (n = 74) were performed using the monoclonal D4D6 antibody clone and 45.6% (n = 62) with the monoclonal SP384 antibody clone. Fifty-five tests were carried out in 2021, and 81 tests were done in 2022. The percentage of positive tests with each technique in relation to the year of testing is shown in Table 2. The median time between histological diagnosis and the

Table 1 Demographics and Baseline Characteristics

Characteristics	n = 136 (%)
Sex	
Female	70 (51.5%)
Male	66 (48.5%
Age, years	Mean age:
18–45	5 (3.7%)
46–65	43 (31.6%)
66–85	85 (62.5%)
>85	3 (2.2%)
Comorbidities	
None	22 (16,2%)
Arterial hypertension	64
COPD	19
Diabetes	23
CKD	3
Autoimmune disease	4
Hematologic malignancy	5
Structural heart disease	9
Solid organ tumor (other than lung)	21
Smoking history	
Never	47 (34.6%)
Former	54 (39.7%)
Active smoker	13 (9.6%)
No data	14 (10.3%)
Histological classification	
Adenocarcinoma	130 (95.6%)
Adeno-squamous cell carcinoma	1 (0.7%)
Squamous-cell carcinoma	4 (3%)
Lymphoepithelioma-like	1 (0.7%)
Staging (TNM 8th edition)	
IA	5 (3.7%)
IB	2 (1.5%)
IIA	3 (2.2%)
IIB	3 (2.2%)
IIIA	11 (8.1%)
IIIB	9 (6.6%)
IIIC	4 (2.9%)
IVA	40 (29.4%)
IVB	55 (40.4%)
No data	4 (2.9%)

Table 2 IHC for ROS1 According to the Monoclonal Antibody Clone, Year of Testing and Positivity

Clone/Year	2021 (N = 55)	2022 (N = 81)	Total	Positive
D4D6	55 (100%)	19 (23,8%)	74 (54,4%)	9 (12,2%)
SP384	0 (0%)	62 (76,2%)	62 (45,6%)	18 (29%)
Total	55	81	136	27 (19,9%)

IHC result was 11 days (IR 6.0–35.2). Among the 136 patients included, 33.5% (n = 45) had *EGFR* gene mutations, 5.2% (n = 7) had *ALK* rearrangements, and 25.8% (n = 34) had high expression of programmed death-ligand 1 (*PD-L1*) (tumor proportion score >50). Detailed information regarding molecular features, including *EGFR* gene mutations, *ALK* rearrangements, and *PD-L1* expression, can be found in Table 3.

Out of the 27 patients with positive *ROS1* IHC Results, 17 were at stage IV. Among these patients, 6 underwent confirmatory testing, all of which were performed using NGS. The NGS assay utilized in our institution is FoundationOne® CDx. Two cases of *ROS1*-NSCLC were confirmed, and both patients received crizotinib subsequently. Additionally, one patient who tested positive on IHC without a confirmatory test received crizotinib. The remaining 4 confirmatory tests were negative. Patients with *ROS1*-NSCLC who received crizotinib are described in Table 4.

Discussion

Lung cancer treatment has evolved towards targeted therapy over the past decade. The response to treatment depends on the presence of driver mutations. Currently, the National Comprehensive Cancer Network (NCCN) guidelines recommend a panel of genetic tests for NSCLC, including *EGFR, ALK, ROS1, MET*, B-Raf proto-oncogene (*BRAF*), REarranged during transfection (*RET*), erythroblastic oncogene B (*ERBB2*), and Kirsten Rat Sarcoma viral oncogene homolog (*KRAS*).[18,21]

These driver mutations, also known as gene fusions, arise from genomic rearrangement events, including chromosomal inversions, interstitial deletions, duplications, or translocations.[22] The formation of chimeric oncogenic proteins resulting from these processes enables the feasibility of selecting the optimal therapeutic approach based on the identified gene aberration.[23] In the case of *ROS1*, translocations involving this gene are present in only 1–2% of the patient population.[24]

ROS1 rearrangements are more frequent in women (2.42%) than in men (1.57%) (OR = 1.54, 95% CI: 1.02–2.34, P = 0.042), as well as in young people, the non-smoking population compared to smokers (3.65% and 0.90%, respectively, [OR = 3.27, 95% CI: 1.44–7.45, P = 0.005]), and advanced stages.[12] In our cohort, the two patients with *ROS1*-NSCLC were both 65 years or younger, had stage IV disease, had no major comorbidities, and were never smokers.

In this study, IHC tested positive in 12.2% (9/74) using the D4D6 clone, and 29% for the SP384 clone (18/62). Concordance was not evaluated in this study. Although confirmation of *ROS1* rearrangements can be achieved through various techniques, the most commonly used method in our hospital is NGS (n = 6). NGS, along with other innovative methods such as nCounter RNA testing, are considered more promising techniques for concurrently detecting ALK, ROS1, and EGFR alterations in a single analysis.[25]

In this study, the frequency of *ROS1*-NSCLC was 1.4% (n = 2). These results align with previous studies reporting frequencies between 1% and 2%.[8,26–28] Notably, both patients with *ROS1*-NSCLC in this study received immune

Table 3 Molecular Features of NSCLC

Variable Molecular	N = 136 (%)
EGFR mutation (any)	
Yes	45 (33.3%)
No	90 (66.7%)
No data	1
ALK rearrangements	
Yes	7 (5.2%)
No	128 (94.8%)
No data	1
PD-L1 expression	
<1	65 (49.2%)
1–49	33 (25.0%)
≥50	34 (25.8%)
No data	4

Table 4 Clinical, Histological, and Molecular Features of ROS1-NSCLC Patients Treated with Crizotinib

Patient	Clinical Features	Histology	Confirmatory Test for *ROS1*	Molecular Features	Staging and Imaging	Follow-up
Patient #1	Male 43 y/o Never- smoker, obesity	Adenocarcinoma	Yes, confirmed with NGS	*EGFR* negative *ALK* negative *PD-L1* TPS 5% *ROS1* done by SP384	T4N3M1a, Stage IVA 6-cm right mediastinal mass with pleural infiltration, no extra thoracic disease Complications: superior vena cava thrombosis at month 3 of diagnosis	Time: 5.3 months Partial response TKI toxicity: none
Patient #2	Female 64 y/o Never-smoker, mood disorder (anxiety)	Adenocarcinoma	Yes, confirmed with NGS	*EGFR* negative *ALK* negative *PD-L1* TPS 90% *ROS1* done by SP384	T4N2M1a, Stage IVA 4.6-cm mass in right lower lobe, pleural and rib infiltration, no CNS disease Complications: deep vein thrombosis of the lower extremity at month 2 of diagnosis	Time: 7.0 months Oligoprogressive disease (pulmonary nodule) TKI toxicity: skin rash (grade I)
Patient #3	Male 54 y/o Former-smoker	Adenocarcinoma	No	*EGFR* negative *ALK* negative *PD-L1* TPS 70% *ROS1* done by D4D6	T1cN3M1c, Stage IVB Dense nodular images of 8 and 10 mm in the precarinal, parahilar, and supracarinal region. The largest of these measures 22×17 mm in relation to adenopathies Complications: Cerebellar ischemic stroke, pulmonary thromboembolism	Time: 0.3 months No response TKI toxicity: none

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chemotherapy while awaiting *ROS1* confirmation since confirmation test results in Colombia are obtained several weeks after histological diagnosis, and the risk of disease progression is a concern.

Over the past decade, multiple trials have evaluated TKIs in advanced *ROS1*-NSCLC. The Food and Drug Administration (FDA) has approved crizotinib, entrectinib,[29] ceritinib,[30] and lorlatinib[31,32] for the treatment of advanced *ROS1*-NSCLC. Repotrectinib is currently under FDA review for approval, based on the results of the Phase 1/2 TRIDENT-1 trial.[33] In Colombia, crizotinib was the first TKI to be approved by the National Institute for Food and Drug Surveillance, making it the only available TKI for *ROS1*-NSCLC.

Crizotinib efficacy studies in *ROS1*-NSCLC have involved relatively few patients given its low prevalence. In the 62.6-month analysis of the PROFILE 1001 trial, the objective response rate (ORR) and progression-free survival (PFS) of 53 enrolled patients was 72% (95% CI, 58% to 83%) and 19.3 months (95% CI, 15.2–39.1), with 15% of patients being monitored for progression at that time.[20] In a Phase II study conducted in East Asia, the ORR among 127 patients was 72% (95% CI, 63% to 79%), the median PFS was 15.9 months (95% CI, 12.9–24.0), and the median duration of response (DOR) was 19.7 months (95% CI, 14.1 to NR).[34] In a smaller phase II study in France, the ORR among 37 patients was 69.4% (95% CI, 53–82). The median PFS in this study was 5.5 months (95% CI, 4.6–9.1), with an overall survival (OS) of 17.2 months (95% CI 6.8–32.8).[35] Variations in PFS among these three trials may be influenced by the relatively small samples.

Patients who benefit from crizotinib therapy for *ROS1*-NSCLC are generally those with advanced NSCLC.[19,20,36] In our hospital, IHC screening is performed on all patients with NSCLC regardless of staging. However, the request for a confirmatory test is made based on the oncologist's criteria and the clinical context, although a confirmatory test is always preferred. In our case, 11 patients did not undergo a confirmatory test due to the co-occurrence of *ROS1* and *EGFR* mutations (*EGFR::ROS1*) present in 8 cases, in which therapeutic priority was given to this mutation. One patient was in an advanced disease stage and compromised condition, prompting a decision to prioritize the initiation of treatment with crizotinib, even in the absence of a confirmatory test. The 2 remaining cases had an Eastern Cooperative Oncology Group (ECOG) > 2, and due to their clinical condition, palliative care was indicated.

To our knowledge, this is the first study to evaluate the prevalence of *ROS1* rearrangements in Colombian NSCLC patients and to describe the clinical outcomes with TKI treatment. At the six-month follow-up, one patient showed a partial response, while the other patient had oligoprogressive disease (a pulmonary nodule). The patient who received crizotinib without a confirmatory test was already in an advanced stage of their disease. Unfortunately, they could only be followed up for 10 days from the day of their first dose due to the deterioration of their condition, ultimately leading to their death. In this case, Conclusions regarding the efficacy of TKI treatment cannot be drawn, highlighting the importance of confirmatory testing among TKI-eligible patients. It is worth noting that both *ROS1*-NSCLC patients had deep venous thrombosis, consistent with the increased incidence of venous thromboembolism (including pulmonary embolism, deep venous thrombosis, renal vein thrombosis, internal jugular thrombosis, and peripheral inserted central catheter-related) reported in *ROS1*-NSCLC in recent studies.[37,38]

Despite the small sample of *ROS1*-NSCLC patients in this study and our emerging experience in treatment with TKI treatment in this context, the clinical outcomes have been optimistic. In this regard, this study reinforces the importance of *ROS1* rearrangement screening in a Latin American country where crizotinib was recently approved for *ROS1*-NSCLC (October 2022). More investigation is warranted to establish better evidence regarding clinical outcomes in our population, which may differ from previous cohorts mainly comprising white and Asian individuals, as well as to assess the concordance of emerging IHC techniques in our population.

Conclusions

Screening for *ROS1* rearrangements in NSCLC is imperative, as multiple prospective studies have demonstrated improved clinical outcomes with TKIs. Given the recent approval of crizotinib in Colombia, public health policies must be oriented to focus on early detection of driver mutations and prompt treatment. Additionally, efforts should be directed towards the potential approval of newly tested TKIs in the future.

Abbreviations

NSCLC, Non-Small Cell Lung Cancer; ROS1, Proto-Oncogene Receptor Tyrosine Kinase; ROS1-NSCLC, ROS1 Rearrangements in NSCLC; CI, Confidence Interval; EGFR, Epidermal Growth Factor Receptor; MET,

Mesenchymal–Epithelial Transition Factor; IHC, Immunohistochemistry; FISH, Fluorescent In Situ Hybridization; RT-PCR, Real-Time Polymerase Chain Reaction; NGS, Next-Generation Sequencing; TKI, Tyrosine Kinase Inhibitor; ALK, Anaplastic Lymphoma Kinase; c-MET, cellular mesenchymal–epithelial transition factor; INVIMA, National Institute for Food and Drug Surveillance; FVL, Fundación Valle del Lili; PD-L1, Programmed Death-Ligand 1; NCCN, National Comprehensive Cancer Network; BRAF, B-Raf Proto-Oncogene; RET, REarranged during Transfection; ERBB2, Erythroblastic oncogene B; KRAS, Kirsten Rat Sarcoma viral oncogene homolog; FDA, Food and Drug Administration; ORR, Objective Response Rate; PFS, Progression-Free Survival; DOR, Duration of Response; OS, Overall Survival; EGFR ROS1, ROS1 and EGFR mutations; ECOG, Eastern Cooperative Oncology Group.

Data Sharing Statement

Datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This manuscript was written in compliance with the ethical standards of the institutional ethics committee and with the 1964 Helsinki Declaration. We have the approval of the Ethics Committee in Biomedical Research from Fundación Valle del Lili. This is supported in letter No. 155 of 2023, which is available with the Corresponding Author if needed. According to Colombian regulations for research involving human subjects, since this is a retrospective observational study, informed consent signatures are not required; this was approved by the ethics committee.

Consent for Publication

According to Colombian regulations for research involving human subjects, since this is a retrospective observational study, informed consent signatures are not required; this was approved by the ethics committee. The authors declare that all sensitive and confidential patient data were handled with confidentiality in compliance with the requirements of the ethics committee.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

Dr Alvaro Osorio reports personal fees from Fundación Valle de lili, personal fees from Universidad Icesi, personal fees from Pfizer, personal fees from Roche, personal fees from MSD, personal fees from Bristol, personal fees from AstraZeneca, personal fees from Ipsen, personal fees from Astellas, personal fees from Tecnofarma, outside the submitted work. The authors declare that they have no other competing interests.

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