Original Article

ALK and PD-L1 Expression in Non-Small Cell Lung Cancer through Immunohistochemical Assays in Indonesia

Elisna Syahruddin[1], Mutia A. Sayekti[2], Sayekti Yuliyanti[2], Hasbullah Thabrany[2], Labbaika Nurmadani[2]

[1]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia, Persahabatan Hospital National Respiratory Center, Jakarta, Indonesia
[2]Kalta Bina Insani Research and Consulting, Jakarta, Indonesia

Abstract

Background: Advanced therapies for non-small cell lung carcinoma (NSCLC), such as targeted therapy and immunotherapy, are available; however, their use depends on biomolecular testing. In the Indonesian context, EGFR testing is covered by the national health insurance (JKN). According to the Indonesian Clinical Guideline for Lung Cancer, if the EGFR test is negative, further testing, including ALK and PD-L1 immunohistochemistry, is required. Nevertheless, the JKN does not currently cover ALK and PD-L1 IHC tests, which restricts access to the appropriate therapies. This study aims to determine the positivity rates of ALK and PD-L1 IHC tests.

Method: This study employs a cross-sectional approach to analyze 2,553 ALK and PD-L1 IHC tests conducted from 2019 to 2023, sourced from four major provinces in Indonesia

Results: The positivity of ALK IHC tests is 8% with a median age of 52 years. The positivity rate for PD-L1 IHC in all patients is 49%, while based on the tumour proportion score (TPS), TPS ≥50% is 17% and TPS 1-49% is 32%. ALK positivity correlates with age and female gender (P<0.001 and P=0.006). Conversely, PD-L1 positivity was significantly associated with cancer type (P=0.008).

Conclusion: ALK positivity in NSCLC in Indonesia is relatively high (8%), with a relatively young median age of 52 years and is predominantly found in females. PD-L1 positivity does not significantly differ by gender and age, but positively correlates with adenocarcinoma cancer type.

Keywords: ALK, biomolecular diagnosis, immunohistochemistry, NSCLC, PD-L1

Corresponding Author:
Elisna Syahruddin | Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia, Persahabatan Hospital National Respiratory Center, Jakarta, Indonesia | elisna2002@gmail.com

Submitted: July 29th, 2024
Accepted: July 21st, 2025
Published: July 30th, 2025

J Respirol Indones. 2025
Vol. 45 No. 3: 161–73
https://doi.org/10.36497/jri.v45i3.794

INTRODUCTION

According to the Global Cancer Observatory (GLOBOCAN) 2022, there were approximately 19 million new cancer cases globally, with the highest cases being lung cancer (12.4%) of the total cancer cases, followed by breast cancer (11.5%).[1] In 2018, the number of lung cancer deaths worldwide was recorded at 1,761,007, making up 18.4% of all cancer deaths. By 2022, this number had risen by 3% to 1,817,469 deaths, underscoring a growing global health crisis. In Indonesia, lung cancer was the second most common cancer in 2022, with 38,904 cases (9.5% of all cancer cases), up from 34,783 cases (8.8%) in 2020. This alarming rise in both global and national cases highlights an urgent need to intensify efforts to combat lung cancer.[2]

Histologically, lung cancer is classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which includes subtypes such as adenocarcinoma, squamous cell carcinoma, and others. Approximately 85% of lung cancer cases in Indonesia are NSCLC, with adenocarcinoma being the most frequently found subtype.[2] In 2018, of all cancer deaths in Indonesia, lung cancer-related deaths reached 30,023 (8.6%) and increased to 34,339 cases (14,1%) in 2022.[1,3]

Lung cancer is the primary cause of cancer-related mortality. It is projected that the number of new cases will increase twofold, from 30,023 in 2018 to 54,983 in 2040.[2] The high mortality rate is primarily attributable to the low survival rate, which is influenced by delayed diagnosis and limited access to therapeutic modalities, including radiotherapy and systemic therapies such as chemotherapy, targeted therapy, and immuno-oncology.[4]

Two principal categories of therapeutic intervention are employed in the treatment of lung cancer: (1) targeted therapy, which impedes tumour

cell proliferation (cytostatic), and (2) chemotherapy, which seeks to destroy tumour cells (cytotoxic). To enhance its capacity to impede the dissemination and proliferation of malignant cells, targeted therapy intervenes at specific molecular targets that are instrumental in the pathogenesis of cancer.[4]

According to meta-analysis results, the efficacy yielded by chemotherapy for lung cancer could provide an average survival time of 11.8 months and an average time to disease progression of 4.2 months.[5] Several targeted therapies and immunotherapies have been developed in the field of medicine, with some of these therapies currently available in Indonesia.[6]

To administer these therapies effectively, it is essential to perform relevant biomolecular tests to identify biomarkers that indicate the progression of lung cancer. These biomarkers may include gene mutations, protein overexpression, or other molecular changes. The results of these tests will inform the decision-making process regarding the administration of the aforementioned therapies.[6]

The biomolecular tests conducted include examinations for oncogenic mutations such as Epidermal Growth Factor Receptor (EGFR), Anaplastic Lymphoma Kinase (ALK), and c-ros oncogene 1 (ROS1). EGFR testing in Indonesia shows a high positivity rate (45%).[6] Chemotherapy combined with anti-EGFR treatment provides a slightly longer survival compared to chemotherapy alone, with an average survival of 7.6 months versus 6.0 months, and a side effect rate of 31%.[7]

Epidermal Growth Factor Receptor testing is covered under the National Health Insurance (JKN) as stipulated in the Regulation of the Minister of Health No. 3 of 2023 concerning the Standard Tariff for Health Services in the implementation of the Health Insurance Program.[8] As recommended by the national guidelines for lung cancer management in Indonesia, further tests such as ALK and programmed death-ligand 1 (PD-L1) Immunohistochemistry (IHC) testing are required if the result of the EGFR test is negative.[9]

Anaplastic Lymphoma Kinase and PD-L1 are known as proteins produced by cancer cells to avoid detection by the immune system. It is essential to have the results of ALK and PD-L1 tests to determine the decisions in administering the first-line treatment, between the anti-ALK targeted therapy and anti-PD-L1 immunotherapy.[10] Therefore, this study was conducted to determine ALK and PD-L1 IHC positivity as essential tests for guiding precision therapy in NSCLC that have a greater impact on reducing lung cancer mortality.

METHOD

This cross-sectional study aimed to determine the prevalence of ALK and PD-L1 IHC positivity in lung cancer. This approach was selected to demonstrate the prevalence of positive results obtained through ALK and PD-L1 IHC tests in a specific population at a given point in time.

Patient Assistance Program for ALK and PD-L1 IHC Testing in 2019–2023 is the main source of the data sample, collected from nine laboratories in the provinces of DKI Jakarta, West Java, DI Yogyakarta, and East Java. Overall, this data consists of 2,553 specimens retrieved from 2,439 patients. As an initial step, a data cleaning process was carried out, starting with ensuring the completeness of information regarding age, gender, and cancer type for each patient.

The data cleaning process excluded 845 sample data and 792 patient data due to incomplete information, resulting in a ready-to-analyse dataset of 1,708 samples and 1,647 patients. These data were then classified according to the type of test, namely ALK, PD-L1, and both ALK and PD-L1. For descriptive and bivariate analysis, this study used patient data that had undergone further data cleaning by excluding patients who did not have test results of "ALK positive," "ALK negative," "PD-L1 positive strong/high (≥50%)," "PD-L1 positive weak/low (1–49%)," and "PD-L1 negative."

This final data cleaning stage resulted in 1,161 patients undergoing ALK IHC testing, 448 with PD-L1 IHC testing results, and 61 patients who underwent both ALK and PD-L1 tests within the same period.

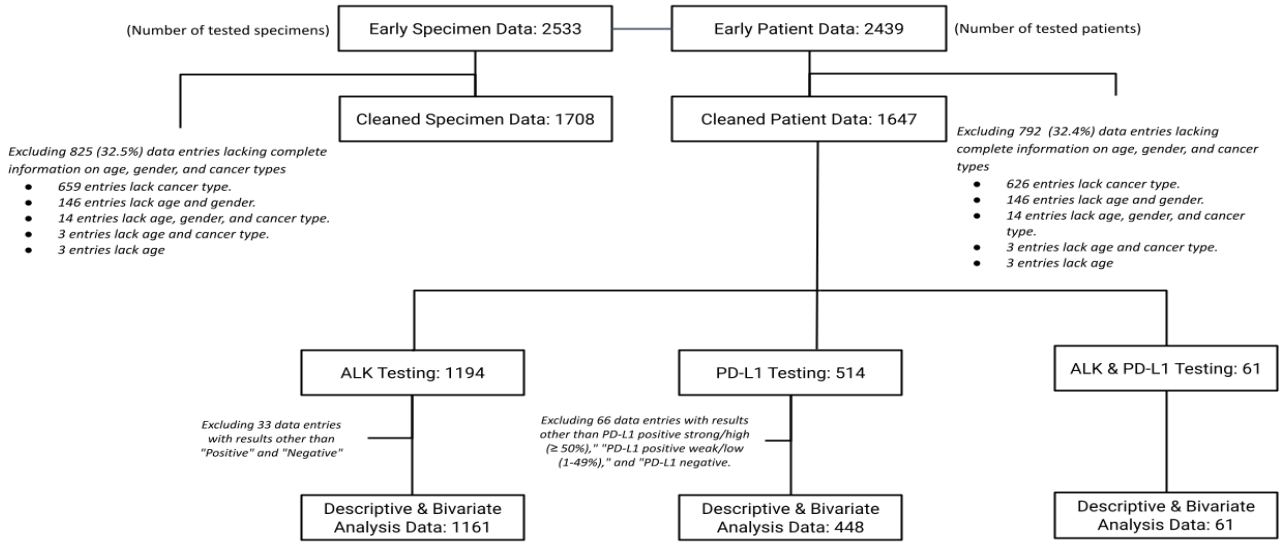

Figure 1. Data Cleaning Process

Table 1. Total samples based on the IHC instrument used in the patient assistance program for ALK and PD-L1 IHC testing 2019-2023

Machine Type	Specimen			Total	Patient			Total
	ALK	PD-L1	ALK & PD-L1		ALK	PD-L1	ALK & PD-L1	
Ventana Ultra	1112 (71%)	450 (29%)	10 (1%)	1572	1112 (71%)	450 (29%)	5 (0,32%)	1567
Ventana GX	19 (51%)	2 (5%)	16 (43%)	37	19 (66%)	2 (7%)	8 (28%)	29
Ventana XT	2 (2%)	1 (1%)	96 (97%)	99	2 (4%)	1 (2%)	48 (94%)	51
Total	1133 (66%)	453 (27%)	122 (7%)	1708	1133 (69%)	453 (28%)	61 (4%)	1647

More detailed results of the data cleaning process are illustrated in Figure 1. Data sampling was also conducted based on the type of machine used in the nine laboratories, as presented in Table 1.

Statistical analysis was conducted using Stata Version 17 (StataCorp). Descriptive analysis was performed to examine the distribution of ALK IHC test results only, PD-L1 IHC test results alone, and the combination of both ALK and PD-L1 test results. Meanwhile, in the bivariate analysis, Pearson's Chi-Square test was used to assess the association between positivity results with variables such as age, gender, cancer type, and type of machine used. To explore the rationale and policy options for expanding ALK and PD-L1 IHC testing services, in-depth interviews were conducted with pulmonologists and other healthcare professionals involved in ALK and PD-L1 IHC testing at the hospital and private laboratory levels.

Data analysis was performed using cleaned patient data. The incompleteness of information in these data is likely due to non-standardized data collection procedures at the respective healthcare facilities. This study has obtained approval from the health research ethics committee of Persahabatan Hospital National Respiratory Center, as stated in the Ethics Review Approval Letter No. 0012/KEPK-RSUPP/01/2024.

RESULTS

The overall ALK positivity rate was 8%. The overall ALK positivity rate was 8%. Among the different machines used, the closest positivity result was observed with the Ventana Ultra. A similar pattern was observed in the IHC test for PD-L1, which demonstrated a 49% positivity rate, with the Ventana Ultra again producing consistent results.

Table 2. ALK and PD-L1 IHC test results by machine type in the Patient Assistance Program (2019–2023)

Machine Type	ALK		PD-L1		
	ALK+	ALK–	PD-L1 (<1%)	PD-L1 (1-49%)	PD-L1 (≥50%)
Ventana Ultra	68	1016	175	137	77
Ventana GX	5	22	7	3	0
Ventana XT	22	28	15	24	10
Total	95	1066	197	164	87

As presented in Table 3, the PD-L1 positivity result is higher among male patients, especially those of older age and diagnosed with non-adenocarcinoma.

Table 3. PD-L1 IHC test results by gender, age group, and cancer type (n=448)

Factor	PD-L1			*P*
	Positive High (≥50%) (n=87)	Positive Low (1–49%) (n=164)	Negative (<1%) (n=197)	
Gender				
Male	55 (19.4%)	108 (38.1%)	120 (42.4%)	0.63[a]
Female	32 (19.3%)	56 (33.9%)	77 (46.6%)	
Age group				
<59 years	38 (18.0%)	72 (34.1%)	101 (47.8%)	0.29[a]
≥59 years	49 (20.6%)	92 (38.8%)	96 (40.5%)	
Type of cancer				
Adenocarcinoma	69 (17.9%)	135 (35.1%)	180 (46.8%)	0.008[a]*
Non-adenocarcinoma	18 (28.1%)	29 (45.3%)	17 (26.5%)	

Note: [a]using Pearson's Chi-Square Statistical Analysis; [b]Fisher's exact if expected frequency of machine <5; *significant by statistic if <0.05

Table 4. ALK IHC test results by gender, age group, and cancer type (n=1,161)

Factor	ALK		*P*
	Negative (n=1,066)	Positive (n=95)	
Gender			
Male	711 (93.4%)	50 (6.5%)	0.006[a]
Female	355 (88.7%)	45 (11.2%)	
Age group			
≥59 years	582 (95.2%)	29 (4.7%)	<0.001[b]
<59 years	484 (88.0%)	66 (12.0%)	
Type of cancer			
Adenocarcinoma	875 (91.1%)	85 (8.8%)	0.068[a]
Non-adenocarcinoma	191 (95.0%)	10 (4.9%)	

Note: [a]using Pearson's Chi-Square Statistical Analysis;
[b]Fisher's exact if expected frequency of machine <5;
*significant by statistic if <0.05

As shown in Table 4, the ALK IHC test result demonstrated a higher positivity rate in females compared to males, particularly in the younger age group and those diagnosed with adenocarcinoma.

Table 5. Bivariate analysis of ALK and PD-L1 positivity based on IHC testing by sex, age and cancer type (n=61)

Factor	ALK & PD-L1		*P*
	Positive (n=17)	Other (n=44)	
Gender			0.61[a]
Male	12 (30.0)	28 (70.0)	0.77[b]
Female	5 (23.8)	16 (76.1)	
Age group			0.44[a]
<59 years	10 (32.2)	21 (67.7)	0.55[b]
≥59 years	7 (23.3)	23 (76.6)	
Type of cancer			0.53[a]
Adenocarcinoma	15 (26.7)	41 (73.2)	0.61[b]
Non-adenocarcinoma	2 (40.0)	3 (60.0)	

Note: [a]using Pearson's Chi-Square Statistical Analysis;
[b]Fisher's exact if expected frequency of machine <5;
*significant by statistic if <0.05

As indicated in Table 5, the positivity rates for specimens tested in parallel for ALK and PD-L1 were slightly higher among male patients, particularly those younger age with non-adenocarcinoma cancer type. The findings suggest that a positive test for both biomarkers is more likely due to PD-L1 expression. This distinction may be relevant for treatment decisions.

DISCUSSION

This is the first study in Indonesia examining the positivity rate of ALK expression in NSCLC using a large sample size and broad subject range. Although not statistically representative, the data reflect national use patterns of Ventana-type IHC machines. However, the single-center design and non-concurrent sample collection may affect the findings, particularly in molecular research where results can be affected by the variability of reagents or kits used.

Anaplastic Lymphoma Kinase is a receptor of tyrosine kinase that belongs to the superfamily of insulin receptors.[2] Anaplastic Lymphoma Kinase IHC testing aims to detect the arrangement of the ALK gene specifically on cancer cells and tissues and to determine the likelihood of ALK inhibitor responses. The decision to conduct ALK IHC testing in Indonesia is based on negative EGFR test results.[9,11]

Of the total ALK IHC tests performed on 1,194 patients, the number of samples that could be analysed was 1,161. A total of 33 samples were not analysed further due to a lack of definitive results, with details of 4 cancelled, 6 no result and 23 requesting new samples. The results of ALK IHC test are highly likely to be positive in the Asian population, such as 5% in the United Arab Emirates, 9.4% in China, 9.6% in Saudi Arabia, 10% in Morocco, and

19.6% in Egypt in where these countries use the Ventana IHC instrument.[12–14]

Meanwhile, in the United States, ALK positivity rates are relatively low at 2.8–3.8% using the FISH method.[15,16] The low positivity rates are also evident in the population of the United Kingdom at approximately 4%, 2,1% in Norway, 3.5% in Slovenia, 3.5% to 4.8% in the Czech Republic, and 4.1% in Malaysia.[12,17–19] The ALK IHC positivity analysed in this study shows that the results tend to be higher than in other countries, at 8%.

Positive ALK IHC results from these 95 patients were obtained using different types of instruments: 68 patients were tested on the Ventana Ultra, 22 patients on the Ventana XT, and 5 patients on the Ventana GX. Bivariate analysis showed a significant association between ALK IHC positivity and instrument type (P=0.041). Anaplastic Lymphoma Kinase IHC testing using Ventana's BenchMark XT or BenchMark ULTRA machines is known to be used as a diagnostic modality to guide the administration of anti-ALK therapies such as crizotinib, ceritinib, and alectinib in the United States and Japan.[20]

The modality of the diagnosis method and the antibodies used in the testing may influence the positivity of the ALK IHC test. The recommended method for ALK testing is IHC with sensitivity and specificity of 100% and 96%, respectively, which are higher compared to other methods such as FISH, RT-PCR, and NGS (Next Generation Sequencing) technology.[21]

Furthermore, the more complex techniques and/or higher costs associated with FISH, RT-PCR, and NGS are other factors contributing to the increasing use of IHC as an ALK testing method.[17] The ALK antibody (D5F3), which contains a recombinant rabbit monoclonal antibody, is used to detect ALK expression in FFPE (formalin-fixed paraffin-embedded) cancer tissue. Testing results using a dichotomous system indicate positivity or negativity based on staining in the granular cytoplasm.

The D5F3 antibody is recommended for ALK IHC testing as it can recognize the carboxyl terminus of the human ALK protein and has been approved by the United States Food and Drug Administration.[17] The use of different antibodies in ALK IHC testing can produce varying staining concordance results. This is consistent with a study by Shen et al that examined 60 FFPE tissue biopsies using the D5F3 (Ventana), D5F3 (CST), 1A4/1H7 (OriGene Tech.), and 5A4 (Abcam) clones, resulting in staining concordance of 96.7%, 91.7%, 96.7%, and 76.7% respectively (compared to the D5F3, Ventana clone).[22]

This study shows a higher positivity rate in 45 female patients in which 11.2% compared to the positivity rate in 50 male patients (6.5%). This finding is consistent with the results of two studies conducted by Liang et al and Poh et al, which also reported higher ALK positivity in female patients.[12,13]

Table 3 shows a significant association between gender and ALK IHC positivity (P=0.006). The higher proportion of females (11%) with positive ALK results compared to males (6.5%) in this study is consistent with the study by Liang et al, who found that ALK positivity tends to occur in females aged ≤60 years without a history of smoking (P<0.001).[13] Similar results were also shown by Chang et al, who reported that female lung cancer patients tend to have positive ALK IHC results (P=0.005).[23]

The results of the ALK IHC test in this study revealed that 95 (8%) patients with positive ALK results had a median age of 52 years (age range: 25–72 years). Bivariate analysis indicated no significant correlation between age and ALK IHC test positivity. Although not statistically correlated, the median age of patients associated with positive ALK results in this study is similar to Liu's study and Kim et al study, which reported a median age of 53 years (P<0.001), suggesting a tendency for ALK positivity in patients of this age group.[24,25]

Among the 95 patients with positive ALK results, 85 (89.5%) had adenocarcinoma and 10 (9.5%) had non-adenocarcinoma cancers. This study shows that adenocarcinoma is the most frequently tested cancer type for the ALK IHC test. This finding is consistent with the study by Chang et al, which reported that adenocarcinoma is the most commonly tested cancer type for the ALK IHC test (90.6%) with

a positivity rate of 9.9%.[23] Bivariate analysis indicated no significant correlation between positivity and cancer type. Although not statistically correlated, the higher proportion of adenocarcinoma aligns with the study by Uruga, which showed a tendency for patients with adenocarcinoma (female, under 50 years old, non-smokers) to have positive ALK results.[20]

According to the 2020 edition (printed in 2024) of the Indonesian Society of Respirology's Guidelines for the Diagnosis and Management of Lung Cancer in Indonesia, a positive ALK test result is used as a reference for administering anti-ALK therapy as first-line treatment.[26] The Overall Response Rate (ORR) for anti-ALK therapy is known to contribute to a survival increase of 11 months in about 88% of lung cancer patients undergoing ALK IHC testing in China, Japan, and South Korea.[27] The ALK treatments approved by the U.S. Food and Drug Administration (FDA oncology approval) include Crizotinib, Alectinib, Brigatinib, Lorlatinib, and Ceritinib.[28]

Crizotinib is the first-generation anti-ALK therapy approved by the FDA since 2011 and is included in first-line treatment. However, resistance to first-generation anti-ALK therapy has developed, leading to the approval of Ceritinib in 2014 as a second-generation anti-ALK therapy for patients after first-line treatment. The development of second-generation anti-ALK therapies continued with the introduction of Alectinib, which can overcome resistance to Ceritinib. Currently, a third-generation anti-ALK therapy, Lorlatinib is also available.

In addition to ALK IHC testing, PD-L1 IHC testing is needed to detect protein expression that inhibits T cells in the detection of cancer cells.[29] Immunohistochemistry testing has been proven to be a widely available, practical, and economical method for assessing PD-L1 protein expression in cancer and is useful in identifying appropriate therapy for patients.[30]

Programmed death-ligand 1 expression testing is also necessary to support the administration of anti-PD-L1 immunotherapy, which works by blocking the interaction between PD-L1 and receptors on T cells, allowing the immune system to more effectively attack and destroy lung cancer cells. NSCLC with EGFR mutations tends to have lower PD-L1 response, and PD-L1 response correlates more strongly in NSCLC with negative EGFR mutations.[23]

Unlike ALK IHC positivity, PD-L1 expression is graded based on the tumor proportion score (TPS): high positivity is indicated by a TPS of ≥50% (strong=high positive) and low positivity by a TPS of 1–49% (weak=low positive). PD-L1 testing results are specifically obtained from tissue samples with a minimum of 100 tumor cells.[31] One study noted a tendency for PD-L1 IHC positivity with a TPS of ≥50% in a small proportion of patients (11.3%) with EGFR or ALK mutations.[32]

Programmed Death Ligand 1 IHC test results form the basis for the administration of immunotherapy, with the level of positivity based on TPS determining the line of therapy. According to the 2020 edition (printed in 2024) of the Indonesian Society of Respirology's Guidelines for the Diagnosis and Management of Lung Cancer in Indonesia, patients with high positivity (TPS ≥50%) receive first-line immunotherapy, while patients with low positivity (TPS 1–49%) receive second-line immunotherapy.

Out of a total of 514 patients tested for PD-L1 IHC, 448 patient samples were analyzable. A total of 66 samples were not further analyzed due to inconclusive results (no result). The PD-L1 IHC positivity rate analyzed in 251 patients was 49%. The patient population associated with PD-L1 IHC positivity had a median age of 60 years (age range 23–83 years), comprising 163 male patients and 88 female patients, with 204 patients having adenocarcinoma and 47 patients having non-adenocarcinoma cancers.

This positivity rate is lower than the previous study conducted by Syahruddin et al, which reported a positivity rate of 53%.[33] Based on the tumor proportion score (TPS), out of 251 patients with positive PD-L1 IHC results, 87 (17%) had a TPS of ≥50%, and 164 (32%) had a TPS of 1–49%. There are variations in PD-L1 positivity across different countries. The 2019 multicenter study by Dietel et al found variations in PD-L1 IHC positivity rates: 52% in

Europe (Austria, Denmark, Germany, Italy, Spain, Sweden, and the Netherlands), 53% in the Asia Pacific (Japan, Hong Kong, Korea, Singapore, and Taiwan), and 47% in United States, Argentina, Canada, and Columbia.

The distribution of PD-L1 IHC positivity by instrument type was as follows: 214 patients tested on the Ventana Ultra, 34 patients tested on the Ventana XT and 3 patients tested on the Ventana GX. Bivariate analysis showed no significant correlation between PD-L1 IHC positivity and the type of instrument used. One study mentions that PD-L1 IHC tests used to assess protein expression in cancer cells include PD-L1 IHC 28-8 pharmDx (28-8), PD-L1 IHC 22C3 pharmDx (22C3), Ventana PD-L1 SP142 (SP142), and Ventana PD-L1 SP263 (SP263).[34] Differences in antibody types may produce varying assessments of specimen prevalence.[35–37]

Programmed death-ligand 1 IHC testing with antibodies SP263, 22C3, and 28-8 shows relatively high results in PD-L1 membrane staining, while PD-L1 protein expression is lower when using the SP142 antibody.[31,32,38] Other studies have demonstrated that IHC testing with 22C3 and SP263 antibodies shows high correlation results in determining first- and second-line targeted therapy for lung cancer.[39]

According to the patient's gender, PD-L1 IHC positivity involves 163 males and 88 females, with a slightly higher positivity in the male group at 49% compared to 48% in the female group. This aligns with the study by Fu et al, which mentioned a higher percentage of PD-L1 positivity in NSCLC cancer in males compared to females (P<0.001), with a TPS 1–49% proportion of 26% in males and 18.3% in females, and a TPS ≥50% proportion of 17% in males and only 5.5% in females.[40] Similarly, the study by Wu et al reported that PD-L1 expression is more commonly found in males than in females (P=0.019).[41] In contrast, the study by Rangachari et al stated that there is no significant relationship between gender and PD-L1 test results (P=0.0743).[42]

The PD-L1 IHC positivity in this study was observed in 251 patients with a median age of 60 years (age range 23–83 years). Bivariate analysis showed that PD-L1 IHC positivity did not correlate with age. This finding aligns with several studies that also found no significant correlation between PD-L1 IHC test results and patient age.[43–46] However, in the study by Fu et al, age was reported to have a significant relationship with PD-L1 IHC positivity, with a median age of 64 years (P<0.001).[40]

Of the 251 (49%) patients with positive PD-L1 results, 204 (81.3%) had adenocarcinoma, and 47 (18.7%) had non-adenocarcinoma cancer. PD-L1 is frequently found in adenocarcinoma, especially in males.[37] A positive correlation between PD-L1 IHC positivity and cancer type was shown by bivariate analysis with P=0.008. This finding is similar to two other studies: Lin stated that lung cancer histology with adenocarcinoma significantly influences PD-L1 test results (P<0.001), and Takada et al also showed a positive correlation with P=0.0166.[47,48]

In clinical practice, PD-L1 expression is the most widely used predictive biomarker for the use of immune checkpoint inhibitors (ICI) in NSCLC. Patients with PD-L1 TPS ≥50% typically receive PD-1 inhibitor monotherapy. Conversely, patients with PD-L1 expression below 50% generally receive a combination of chemotherapy and PD-L1 inhibitors.[49] According to the 2020 edition (printed in 2024) of the Indonesian Society of Respirology's Guidelines for the Diagnosis and Management of Lung Cancer in Indonesia, a PD-L1 positivity result with a proportion of ≥50% is used as a reference for administering immunotherapy as a first-line treatment.

Immunotherapy is the most commonly used treatment for lung cancer and aims to block the interaction between PD-1 and PD-L1. PD-1 is a receptor commonly found on the surface of immune cells, while PD-L1 is a protein found more widely, including on tumour cells.[50] Five immunotherapies that have been approved by the US Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA) include pembrolizumab and nivolumab, which are classified as anti-PD-1 therapies, and atezolizumab, avelumab, and durvalumab, which are classified as anti-PD-L1 therapies.[28]

Pembrolizumab and nivolumab were approved by the US FDA in 2015 as second-line treatments for

NSCLC cases, followed by atezolizumab in 2016. In 2016, pembrolizumab was further recognized as a first-line immunotherapy, followed by atezolizumab in 2020 for patients with PD-L1 TPS ≥50%.[51] Meanwhile, in Indonesia, in June 2017, *Badan Pengawas Obat dan Makanan* (BPOM) approved pembrolizumab as the first available immunotherapy drug in Indonesia for lung cancer treatment and in 2019, another immunotherapy option, atezolizumab, became available.[52] According to experts from a pulmonologist at one of the vertical hospitals in Indonesia, immunotherapy for advanced-stage lung cancer is administered as first-line treatment, and if it fails, it will be given as second-line treatment.

As an attempt to diagnose lung cancer through biomolecular testing, the term "reflex testing" is known, defined as rapid tests that standardize the ordering of biomarker tests to ensure more patients are tested and these tests must be regulated by protocols established by a multidisciplinary team (MDT).[53] Up to this point, reflex testing standards are not found in international guidelines due to variations in resources, challenges, and reimbursement in various countries. However, several international guidelines have mentioned EGFR, ALK, and PD-L1 testing as part of the main predictive biomarker tests needed.

The EGFR and ALK biomolecular testing for lung cancer diagnosis is mentioned in five international guidelines: Pan-Asian, European Society for Medical Oncology (ESMO), National Comprehensive Cancer Network (NCCN), College of American Pathologists (CAP)/International Association for the Study of Lung Cancer (IASLC)/Association for Molecular Pathology (AMP), and American Society of Clinical Oncology (ASCO). Meanwhile, PD-L1 testing for lung cancer is mentioned in three guidelines: ESMO, NCCN, and Pan-Asian.[54]

Nationally, the Indonesian Clinical Guideline for Lung Cancer mentions the need for molecular testing as a predictive factor in determining the administration of targeted therapy and immunotherapy. The first recommended tests are EGFR, ALK, and ROS-1, including PD-L1 testing that can be done as indicated. It also mentions additional tests such as RET, KRAS, PIK3CA, BRAFV600E, MAP2K1, MET, ERBB2, NRAS, TP53, NTRK mutations, and others, as indicated and depending on the availability of facilities. In the context of the availability of targeted therapy and immunotherapy in Indonesia at present, ALK and PD-L1 testing are recommended to be performed after EGFR is negative.

In this study, there were 61 patients who underwent two biomolecular tests, ALK and PD-L1, with a positivity rate of 28% (17 patients). This positivity was determined using the Ventana XT machine, involving 12 male and 5 female patients, with a median age of 54 years. Among these, 15 patients had adenocarcinoma, and 2 had non-adenocarcinoma. Bivariate analysis only showed a significant correlation between positivity and the type of machine used (P<0.041), while other variables such as gender, age, and cancer type did not show statistical significance. Studies focusing on simultaneous ALK and PD-L1 testing in lung cancer are still quite limited, which may be influenced by differences in guidelines, availability of facilities, and accessible therapies.[55–57]

The NSCLC can be classified as a heterogeneous disease, characterized by genetic alterations in oncogene drivers or key mediators that regulate the proliferation, growth, and survival of cancer cells.[58] The success of NSCLC therapy, which has seen many innovations, is influenced by the various genetic mutations and immunogenicity of each patient.

According to the Indonesian Lung Cancer Diagnosis and Management Guidelines, 2020 edition (printed in 2024) by the Indonesian Pulmonologist Association (PDPI), if both ALK and PD-L1 tests are positive, the prioritized therapy is anti-ALK. This aligns with the analysis by Ota et al, which stated that ALK mutation positivity, as an oncogene driver in lung cancer, can be immunogenic; ALK inhibitors can reduce PD-L1 expression, potentially affecting the cancer's ability to evade the immune system.[59] Targeted therapy is recommended as a priority, especially if there are limitations in conducting

molecular genotyping for patients with oncogene driver mutations (e.g., non-smoking patients).

The technique used in clinical practice in Indonesia to detect ALK and PD-L1 is immunohistochemistry (IHC) because it requires less time and has high sensitivity and specificity. However, IHC testing for ALK and PD-L1 is still limited as it is not yet covered under the benefits package of the National Health Insurance or *Jaminan Kesehatan Nasional* (JKN). Data related to IHC testing for ALK and PD-L1 in Indonesia is also limited, posing a challenge in formulating evidence-based policy.

LIMITATION

This study has several limitations that may affect the analysis results. The primary limitation is the unknown data collection or recording techniques of the Patient Assistance Program for ALK and PD-L1 IHC testing from 2019–2023. The data is also limited in terms of demographic and racial data, smoking history, and EGFR mutation results for each patient. If the smoking status can be identified, a positive result can be more robustly explained in smokers and non-smokers, specifically in guiding the decision-making process regarding treatment options for patients with lung cancer.[60] If a patient has a positive EGFR mutation, it will be related to the selection of therapy with a positive ALK or PD-L1 result.

In the event of a positive EGFR mutation despite the ALK-positive or strong PD-L1 result, the prioritized therapy for patients is anti-EGFR. In addition, it limits the ability to explore their associations with ALK expression and the potential mutual exclusivity between EGFR mutations and ALK IHC positivity.[61] Another limitation is the use of different IHC testing instruments, which may cause the sensitivity and specificity of the tests to vary between instruments. This may result in different positivity rates between patient groups using different machines, which may affect the interpretation of study results.

CONCLUSION

The ALK positivity in NSCLC patients in Indonesia is relatively high, with a median age of 52 years and a predominance of female patients. PD-L1 positivity shows no significant association with gender or age but is associated with adenocarcinoma.

To support this empirical evidence, a comprehensive analysis of ALK and PD-L1 IHC test data in conjunction with EGFR test results, smoking history, and personal and family history of cancer is needed to study the NSCLC patients in Indonesia who require detailed biomolecular profiling. In addition, a cost analysis of the benefits of ALK and PD-L1 IHC testing is needed to estimate the unit cost of each test, which will help determine the investment required by the government to provide comprehensive lung cancer diagnostic services.

ACKNOWLEDGMENTS

Our gratitude to the Indonesian Association for the Study of Thoracic Oncology (IASTO) for the invaluable support in the form of the provision of Patient Assistance Program Data. The authors have no competing interests to declare. We acknowledge the financial support from Roche Indonesia.

REFERENCES

1. International Agency for Research in Cancer. Global cancer observatory [Internet]. International Agency for Research in Cancer. 2022 [cited 2022 Aug 30]. Available from: https://gco.iarc.fr/
2. Nirmawati R, Zuraidah E, Dwina Billianti Y. Deteksi anaplastic lymphoma kinase gene rearrangement (ALK Gene Rearrangement) pada adenokarsinoma paru sebagai molekul target pengobatan pada kanker paru jenis karsinoma bukan sel kecil. Pratista Patologi. 2019;6:41–54.

3. Andarini S, Syahruddin E, Aditya N, Zaini J, Kurniawan FD, Ermayanti S, et al. Indonesian Society of Respirology (ISR) consensus statement on lung cancer screening and early detection in Indonesia. Jurnal Respirologi Indonesia. 2023;43:144–50.
4. Ngurah Rai I. Manajemen kelainan respirasi dengan fokus unit layanan primer. Denpasar: Pendidikan Kedokteran Berkelanjutan Program Studi Ilmu Penyakit Paru Fakultas Kedokteran Universitas Udayana; 2017.
5. Shimizu T, Yokoi T, Tamaki T, Kibata K, Inagaki N, Nomura S. Comparative analysis of carboplatin and paclitaxel combination chemotherapy schedules in previously untreated patients with advanced non-small cell lung cancer. Oncol Lett. 2013;5:761–7.
6. Syahruddin E, Wulandari L, Muktiati NS, Rima A, Soeroso N, Ermayanti S, et al. Uncommon EGFR mutations in cytological specimens of 1,874 newly diagnosed indonesian lung cancer patients. Lung Cancer: Targets and Therapy. 2018;9:25–34.
7. Wu YL, Lee JS, Thongprasert S, Yu CJ, Zhang L, Ladrera G, et al. Intercalated combination of chemotherapy and erlotinib for patients with advanced stage non-small-cell lung cancer (FASTACT-2): A randomised, double-blind trial. Lancet Oncol. 2013;14:777–86.
8. Kementerian Kesehatan Republik Indonesia. Standar tarif pelayanan kesehatan dalam penyelenggaraan program jaminan kesehatan. Permenkes No. 3 Tahun 2023 Indoensia; 2023.
9. Kementerian Kesehatan Republik Indonesia. Pedoman nasional pelayanan kedokteran (PNPK) tata laksana kanker paru tahun 2023. Kementerian Kesehatan Republik Indonesia, NOMOR HK.01.07/MENKES/1438/2023 Indonesia; 2023.
10. Inamura K. Lung cancer: understanding its molecular pathology and the 2015 wHO classification. Front Oncol. 2017;7:193.
11. Testing. ALK mutation (gene rearrangement) [Internet]. 2021 [cited 2024 Feb 27]. Available from: https://www.testing.com/tests/alk-mutation-gene-rearrangement/
12. Poh ME, How SH, Ho GF, Pang YK, Hasbullah HH, Tho LM, et al. Real-world treatment and outcomes of ALK-positive metastatic non–small cell lung cancer in a Southeast Asian Country. Cancer Manag Res. 2023;15:31–41.
13. Liang H, Song X, Zhang Y, Zhang S, Li F, Fang J, et al. Real-world data on EGFR/ALK gene status and first-line targeted therapy rate in newly diagnosed advanced non-small cell lung cancer patients in Northern China: A prospective observational study. Thorac Cancer. 2019;10:1521–32.
14. Jazieh AR, Gaafar R, Errihani H, Jaafar H, Al Dayel F, Bahnassy AA, et al. Real-world data on the prevalence of anaplastic lymphoma kinase–positive non–small-cell lung cancer in the Middle East and North Africa. JCO Glob Oncol. 2021;7:1556–63.
15. Illei PB, Wong W, Wu N, Chu L, Gupta R, Schulze K, et al. ALK testing trends and patterns among community practices in the United States. JCO Precis Oncol. 2018;2:1–11.
16. Lin HM, Wu Y, Yin Y, Niu H, Curran EA, Lovly CM, et al. Real-world ALK testing trends in patients with advanced non–small-cell lung cancer in the United States. Clin Lung Cancer. 2023;24:e39–49.
17. Adizie JB, Tweedie J, Khakwani A, Peach E, Hubbard R, Wood N, et al. Biomarker testing for people with advanced lung cancer in England. JTO Clin Res Rep. 2021;2:100176.
18. Eide IJZ, Nilssen Y, Stensland EM, Brustugun OT. Real-world data on EGFR and ALK testing and TKI usage in Norway—A Nation-wide population study. Cancers (Basel). 2023;15:1505.
19. Ryska A, Berzinec P, Brcic L, Cufer T, Dziadziuszko R, Gottfried M, et al. NSCLC molecular testing in Central and Eastern European countries. BMC Cancer. 2018;18:269.
20. Uruga H, Mino-Kenudson M. ALK (D5F3) CDx: An immunohistochemistry assay to identify alk-

positive NSCLC patients. Pharmgenomics Pers Med. 2018;11:147–55.
21. Nathany S, Sharma M, Batra U. Testing modalities for ALK-driven lung cancer: A narrative review. Cancer Research, Statistics, and Treatment. 2023;6:432–9.
22. Shen Q, Wang X, Yu B, Shi S, Liu B, Wang Y, et al. Comparing four different ALK antibodies with manual immunohistochemistry (IHC) to screen for ALK-rearranged non-small cell lung cancer (NSCLC). Lung Cancer. 2015;90:492–8.
23. Chang GC, Yang TY, Chen KC, Hsu KH, Huang YH, Su KY, et al. ALK variants, PD-L1 expression, and their association with outcomes in ALK-positive NSCLC patients. Sci Rep. 2020;10:21063.
24. Kim D, Ahn M, Shi Y, De Pas T, Yang P, Riely G, et al. Results of a global phase II study with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC). Clinical Advances in Hematology and Oncology. 2012;23:xi32-3.
25. Liu Y, Ye X, Yu Y, Lu S. Prognostic significance of anaplastic lymphoma kinase rearrangement in patients with completely resected lung adenocarcinoma. J Thorac Dis. 2019;11:4258–70.
26. Perhimpunan Dokter Paru Indonesia. Kanker paru: Pedoman diagnosis & penatalaksanaan di Indonesia. 2020th ed. Jakarta: Perhimpunan Dokter Paru Indonesia; 2024.
27. Zhou F, Zhou C. Lung cancer in never smokers-the East Asian experience. Transl Lung Cancer Res. 2018;7:450–63.
28. American Cancer Society. Targeted drug therapy for non-small cell lung cancer [Internet]. 2023 [cited 2024 Mar 1]. Available from: https://www.cancer.org/cancer/types/lung-cancer/treating-non-small-cell/targeted-therapies.html
29. National Cancer Institute. Definition of immune checkpoint inhibitor [Internet]. [cited 2024 Feb 28]. Available from: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/immune-checkpoint-inhibitor
30. Akhtar M, Rashid S, Al-Bozom IA. PD−L1 immunostaining: What pathologists need to know. Diagn Pathol. 2021;16:94.
31. Wang S, Hao J, Wang H, Fang Y, Tan L. Efficacy and safety of immune checkpoint inhibitors in non-small cell lung cancer. Oncoimmunology. 2018;7:e1457600.
32. Yoneshima Y, Ijichi K, Anai S, Ota K, Otsubo K, Iwama E, et al. PD-L1 expression in lung adenocarcinoma harboring EGFR mutations or ALK rearrangements. Lung Cancer. 2018;118:36–40.
33. Syahruddin E. Overview of advanced NSCLC focus on ALK rearrangement. In: Seminar Konferensi Nasional Perhimpunan Dokter Paru Indonesia. Lampung; 2023.
34. Buttner R, Gosney JR, Skov BG, Adam J, Motoi N, Bloom KJ, et al. Programmed death-ligand 1 immunohistochemistry testing: A review of analytical assays and clinical implementation in non-small-cell lung cancer. Journal of Clinical Oncology. 2017;35:3867–76.
35. Kim H, Chung JH. PD-L1 testing in non-small cell lung cancer: Past, present, and future. J Pathol Transl Med. 2019;53:199–206.
36. Yu H, Boyle TA, Zhou C, Rimm DL, Hirsch FR. PD-L1 Expression in Lung Cancer. J Thorac Oncol. 2016;11:964–75.
37. Wang L, Liu S. Discordant genomic correlates of PD-L1 expression in lung adenocarcinoma among multiple cohorts using dissimilar PD-L1 testing techniques. Journal of Clinical Oncology. 2022;40:e20526.
38. Marchetti A, Barberis M, Franco R, De Luca G, Pace MV, Staibano S, et al. Multicenter comparison of 22C3 PharmDx (Agilent) and SP263 (Ventana) assays to test PD-L1 expression for NSCLC patients to be treated with immune checkpoint inhibitors. Journal of Thoracic Oncology. 2017;12:1654–63.
39. Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Jansson M, Kulangara K, et al. PD-L1 immunohistochemistry assays for lung cancer:

Results from phase 1 of the blueprint PD-L1 IHC assay comparison project. Journal of Thoracic Oncology. 2017;12:208–22.

40. Fu F, Deng C, Sun W, Zheng Q, Jin Y, Li Y, et al. Distribution and concordance of PD-L1 expression by routine 22C3 assays in East-Asian patients with non-small cell lung cancer. Respir Res. 2022;23:302.
41. Wu S, Shi X, Sun J, Liu Y, Luo Y, Liang Z, et al. The significance of programmed cell death ligand 1 expression in resected lung adenocarcinoma. Oncotarget. 2017;8:16421–9.
42. Rangachari D, VanderLaan PA, Shea M, Le X, Huberman MS, Kobayashi SS, et al. Correlation between classic driver oncogene mutations in EGFR, ALK, or ROS1 and 22C3–PD-L1 ≥50% expression in lung adenocarcinoma. Journal of Thoracic Oncology. 2017;12:878–83.
43. Rubio-Viqueira B, Tarruella MM, Lázaro M, Estévez SV, Córdoba-Ortega JF, Maiques IM, et al. PD-L1 testing and clinical management of newly diagnosed metastatic non-small cell lung cancer in Spain: MOREL study. Lung Cancer Manag. 2021;10:LMT53.
44. Yang L, Xue R, Pan C. Prognostic and clinicopathological value of PD-L1 in colorectal cancer: A systematic review and meta-analysis. Onco Targets Ther. 2019;12:3671–82.
45. Jain E, Sharma S, Aggarwal A, Bhardwaj N, Dewan A, Kumar A, et al. PD-L1 expression and its clinicopathologic and genomic correlation in the non-small cell lung carcinoma patients: An Indian perspective. Pathol Res Pract. 2021;228:153497.
46. Kilaru S, Panda SS, Moharana L, Mohapatra D, Mohapatra SSG, Panda A, et al. PD-L1 expression and its significance in advanced NSCLC: Real-world experience from a tertiary care center. J Egypt Natl Canc Inst. 2024;36:3.
47. Lin G, Fan X, Zhu W, Huang C, Zhuang W, Xu H, et al. Prognostic significance of PD-L1 expression and tumor infiltrating lymphocyte in surgically resectable non-small cell lung cancer. Oncotarget. 2017;8:83986–94.
48. Takada K, Okamoto T, Shoji F, Shimokawa M, Akamine T, Takamori S, et al. Clinical significance of PD-L1 protein expression in surgically resected primary lung adenocarcinoma. Journal of Thoracic Oncology. 2016;11:1879–90.
49. Gavralidis A, Gainor JF. Immunotherapy in EGFR-mutant and ALK-positive lung cancer: Implications for oncogene-driven lung cancer. Cancer Journal (United States). 2020;26:517–24.
50. American Lung Association. PD-L1, PD1,TMB and Lung Cancer [Internet]. 2024 [cited 2024 Mar 28]. Available from: https://www.lung.org/lung-health-diseases/lung-disease-lookup/lung-cancer/symptoms-diagnosis/biomarker-testing/pdl1-pd1-tmb
51. Zhang X, Declue RW, Herms L, Yang M, Pawar V, Masters ET, et al. Real-world treatment patterns and outcomes in PD-L1-positive non-small cell lung cancer. Immunotherapy. 2021;13:1521–33.
52. Putri AW. Indonesia siap akses imunoterapi, harapan baru pasien kanker [Internet]. tirto.id. [cited 2024 Jun 5]. Available from: https://tirto.id/indonesia-siap-akses-imunoterapi-harapan-baru-pasien-kanker-eosz
53. Gosney JR, Paz-Ares L, Jänne P, Kerr KM, Leighl NB, Lozano MD, et al. Pathologist-initiated reflex testing for biomarkers in non-small-cell lung cancer: Expert consensus on the rationale and considerations for implementation. ESMO Open. 2023;8:101587.
54. Kerr KM, Bibeau F, Thunnissen E, Botling J, Ryška A, Wolf J, et al. The evolving landscape of biomarker testing for non-small cell lung cancer in Europe. Lung Cancer. 2021;154:161–75.
55. Gainor JF, Shaw AT, Sequist L V., Fu X, Azzoli CG, Piotrowska Z, et al. EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: A retrospective

analysis. Clinical Cancer Research. 2016;22:4585–93.

56. Mazieres J, Drilon A, Lusque A, Mhanna L, Cortot AB, Mezquita L, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: Results from the IMMUNOTARGET registry. Annals of Oncology. 2019;30:1321–8.
57. Oya Y, Kuroda H, Nakada T, Takahashi Y, Sakakura N, Hida T. Efficacy of immune checkpoint inhibitor monotherapy for advanced non-small-cell lung cancer with ALK rearrangement. Int J Mol Sci. 2020;21:2623.
58. Arbour KC, Riely GJ. Systemic therapy for locally advanced and metastatic non-small cell lung cancer: A review. JAMA - Journal of the American Medical Association. 2019;322:764–74.
59. Ota K, Azuma K, Iwama E, Harada T, Matsumoto K, Takamori S, et al. Induction of PD-L1 expression by the EML4-ALK oncoprotein and downstream signaling pathways in non–small cell lung cancer. Annals of Oncology. 2015;21:4014–21.
60. Corke LK, Li JJN, Leighl NB, Eng L. Tobacco use and response to immune checkpoint inhibitor therapy in non-small cell lung cancer. Current Oncology. 2022;29:6260–76.
61. Hu H, Tan S, Xie M, Guo P, Yu Q, Xiao J, et al. Case report: Concomitant EGFR mutation and ALK rearrangement in non-small cell lung cancer. Front Pharmacol. 2023;14:1167959.