

## Identification of ALK, ROS1, RET, MET Mutation in EGFR Wild-Type Non-Small Cell Lung Cancer Patients and Their Associations with Clinicopathological Factors

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### Abstract:

**Objective:** Genetic alterations in non-small cell lung cancer, including ALK, ROS1, RET, and MET, have been discovered, and they play a vital role in the advancement of targeted therapies for lung cancer. This study was conducted to detect the presence of ALK, ROS1, RET, and MET mutations, and to explore the correlation between these mutations and various clinicopathological factors.

**Material and Methods:** A cross-sectional descriptive study was carried out involving 69 patients diagnosed with non-small cell lung cancer at the Vietnam National Cancer Hospital from June to October 2022. Samples were identified through histopathological assessments using the PCR method. Data were analysed by descriptive statistics and Chi-Square tests with SPSS 25.0 software.

**Results:** The study indicated that ALK mutations had the highest prevalence at 21.7%, followed by ROS1 mutations at 8.7%. RET mutations were observed with a prevalence of 4.3%, while MET mutations were the least common at 1.5%. Notably, an association was found between ROS1 mutations and female patients ( $p$ -value=0.005). The observed mutation rates and their associations with some factors differed from the trends reported in numerous international studies ( $p$ -value>0.05).

**Conclusion:** The study identified 4 mutations in samples extracted from non-small cell lung Vietnamese-cohort patients. Female patients had more ROS1 mutations than males. Future studies with larger sample sizes, improved selection criteria, and studies on more parameters are needed in order to demonstrate a more accurate picture of the status of these mutations in Vietnam.

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**Keywords:** ALK, Carcinoma, MET, Non–Small–Cell Lung, RET, ROS1, Vietnam

## Introduction

Lung cancer is widely recognized as a significant form of cancer that profoundly affects both health and the financial well-being of society<sup>1</sup>. In 2018, the International Agency for Research on Cancer reported over 19 million new cases, contributing to 18% of the total 9 million fatalities recorded globally<sup>2</sup>.

Non-small cell lung cancer (NSCLC) is the predominant type of lung cancer, comprising 80–85% of all cases<sup>3</sup>. It is often diagnosed at more advanced stages, specifically stages III and IV<sup>4</sup>. Despite considerable efforts to enhance outcomes for patients with NSCLC, the current 5-year survival rate stands at only 15% for all newly diagnosed cases and drops to less than 4% for those with distant disease. However, recent advances in targeted therapies and immunotherapy have led to significant improvements in survival, particularly among patients with actionable driver mutations such as EGFR, ALK, and ROS1<sup>4,5</sup>.

Numerous genetic alterations have been identified as oncogenic in non-small cell lung cancer (NSCLC), including point mutations, deletions, insertions, and gene fusions<sup>1</sup>. Recent studies indicate that approximately 50% to 60% of NSCLC patients possess at least one identifiable driver mutation. The most prevalent mutations occur in the Kirsten ras gene (24%) and the epidermal growth factor receptor (EGFR) gene (13%–22%), while translocations involving the Anaplastic Lymphoma Kinase (ALK) gene are found in an additional 5% to 6% of cases<sup>6,7</sup>. In Western countries, the most common gene fusions associated with NSCLC involve 3 genes that encode membrane receptors. These include ALK (5% to 7%)<sup>8</sup>, the ROS Proto-Oncogene 1, Receptor Tyrosine Kinase (ROS1), and the ret proto-oncogene (RET), each occurring in approximately 1% to 2% of patients<sup>9,10</sup>.

Additionally, splice variants of the MET Receptor Tyrosine Kinase (MET) are present in about 3% to 4% of NSCLC cases<sup>11</sup>.

Real-time Polymerase Chain Reaction (commonly referred to as real-time PCR or qPCR) is a widely utilized laboratory technique for diagnosing various diseases, including lung cancer<sup>12</sup>. This method allows for the monitoring of target DNA molecule amplification throughout the PCR process, distinguishing it from traditional PCR techniques. Following each thermal cycle, the accumulation of amplicons is represented in a line chart. One of the significant advantages of real-time PCR is that the entire process takes place in a closed tube system, which minimizes the risk of sample contamination and reduces overall working time<sup>12</sup>. Another related technique is reverse transcription real-time PCR, which integrates reverse transcription PCR with real-time PCR, beginning the process with RNA as the starting material. RNA-based PCR methods are essential for gene expression profiling, thereby enhancing molecular diagnostics<sup>13</sup>. Numerous studies have highlighted the success and effectiveness of both real-time PCR and reverse transcription real-time PCR in diagnosing non-small cell lung cancer (NSCLC)<sup>14,15</sup>.

In recent years, several studies in Vietnam have focused on mutations such as ALK and ROS1. Notably, a study conducted by AT Dang et al. (2020) classified mutations in a cohort of 350 Vietnamese patients, reporting occurrence rates of 5.4% for ALK and 2.9% for ROS1<sup>16</sup>. In another research effort by Nguyen et al. (2018), the rates of ALK, ROS1, and RET mutations were found to be 2.2% for each, based on a sample of 59 patients<sup>17</sup>. Importantly, there have been no published studies regarding MET mutations in non-small cell lung cancer (NSCLC) patients in Vietnam. The limited number of publications concerning

these 4 types of mutations complicates the understanding of their prevalence in the country. As a result, this study represents the first comprehensive investigation into all 4 mutations, with 2 primary objectives: 1) to ascertain the presence of these mutations in NSCLC patient samples; and 2) to examine the distribution and potential correlations between the mutations and clinicopathological factors.

## Material and Methods

### Patients and tumor sample

Formalin-fixed paraffin-embedded (FFPE) tissue samples were obtained from EGFR wild-type NSCLC patients by the Department of Pathology and Molecular Biology at Vietnam National Cancer Hospital (Tan Trieu) between June 2022 and October 2022. The selection of EGFR wild-type samples was a deliberate, methodological decision to exclude the confounding effects of known EGFR-activating mutations on downstream molecular analyses. EGFR wild-type status was confirmed by real-time PCR analysis targeting common EGFR mutations (exons 18–21), including G719X, exon 19 deletions, L858R, and exon 20 insertions. This molecular testing was performed after initial histopathological diagnosis of NSCLC based on Hematoxylin and Eosin (H&E) staining.

The sample size was derived from the formula for estimating a proportion in quantitative research, based on a reported mutation rate of approximately 20% in similar populations, which was reported in multiple studies<sup>18,19</sup>. There was an absolute error margin ( $d=0.1$ ) and  $\alpha=0.05$ . Applying the formula, a minimum sample size of 61 patients was required. Ultimately, our study yielded 69 samples.

### Extraction of RNA Content

RNA extraction was conducted following the protocol of the Qiagen RNEasy FFPE kit (Qiagen, Germany), which involves a total of 22 steps. The FFPE tissue samples were sliced into thin sections (5–10  $\mu\text{m}$ ), placed onto

microscope slides, and then heated at 70 °C to melt the paraffin. Subsequently, the RNA samples were transferred from the slides into 1.5 mL Eppendorf tubes, where they underwent 18 additional steps involving the addition and removal of reagents, as well as centrifugation, to obtain the final samples.

### RNA quantitation

RNA samples were measured to determine the concentration by BioDrop UV Spectrophotometer (Biodrop Ltd, Cambridge, United Kingdom). Obtained results of A260/280 and A260/230 ratios within the range of 1.8–2.2; they were considered as purified RNA.

### PCR process

The PCR process was executed according to the manual handbook provided with the EasyPGX Ready ALK/ROS1/RET/MET Diatech kit (Diatech Pharmacogenetics, Italy). To prevent contamination during the procedure, all laboratory equipment was sterilized prior to use. Each single 8-well strip included in the kit is designed for a single RNA sample. The strips were briefly vortexed before proceeding. The positive control assay supplied in the kit was centrifuged for 10 seconds and then resuspended with 800  $\mu\text{L}$  of the provided water, and subsequently stored at 2–8 °C until use. Strips were carefully labeled with a marker to prevent any mix-up. Each well requires 25  $\mu\text{L}$  of sample, necessitating a minimum total volume of 200  $\mu\text{L}$  for a single RNA sample using one 8-well strip. RNA samples were diluted according to their quantitation data. Samples, along with the positive and negative controls (Biological Water, Corning, USA), were added to the previously marked strips. All strips were briefly vortexed again before being placed in the EasyPGX qPCR 96 machine (Diatech Pharmacogenetics, Italy). The PCR machine was configured for the thermal cycling process. This kit employs the technique of Reverse Transcription Real-Time PCR and is capable of detecting

ALK, ROS1, RET fusions, and MET 14 skipping. This assay specifically detects:

ALK gene fusions (including common partners like EML4),

ROS1 fusions (e.g., CD74, SLC34A2),

RET fusions (e.g., KIF5B, CCDC6), and

MET exon 14 skipping mutations.

### Data analysis

Statistical analysis was conducted using SPSS Statistics Version 25.0. Data pertaining to mutation results and patient characteristics were gathered and analyzed using frequency counts, as well as by calculating the mean (M) and standard deviation (S.D.). The correlation between variables was evaluated using the Chi-Square test and Fisher's exact test. A p-value of  $\leq 0.05$  was deemed statistically significant.

### Ethical considerations

The study was approved by the Biomedical Research Ethics Committee of Vietnam National Cancer Hospital (Tan Trieu) with number 58/GCN-BVKHN dated 03 March 2022 before being conducted.

## Results

### Participants' characteristics

In a study of 69 patients, the average age was 59.4 years (S.D.=8.9), with 50 patients (72.5%) being male. Regarding tumor location, 46 cases (66.7%) were found at the primary site (Table 1).

### Prevalence of ALK, ROS1, RET, MET mutations in NSCLC patients

#### RNA quantitation and dilution results

RNA samples extracted from 69 NSCLC patients

were analyzed using a BioDrop UV Spectrophotometer (Biodrop Ltd, Cambridge, United Kingdom). The A260/280 (absorbance ratio at 260 nm and 280 nm) and A260/230 (absorbance ratio at 260 nm and 230 nm) ratios for most of the samples fell within the acceptable range of 1.8 to 2.2, indicating good purity. The concentration of the samples ranged from 15 to 620 ng/ $\mu$ L.

### Mutational analysis results with reverse transcription real-time PCR

Among 69 EGFR NSCLC patients, the prevalence of ALK mutation was the greatest, with 15 (21.7%) cases being identified, with a 95% confidence interval (CI) ranging from 13.6% to 32.8%. Following that was the prevalence of ROS1 mutation in 8.7% (6/69; 95% CI: 4.0% to 17.7%) and RET mutation in 4.3% (3/69; 95% CI: 1.5% to 12.0%). In terms of specific alterations identified within each mutation, for ROS1, all 6 cases were identified as ROS1 Exon 32. For RET, all 3 cases are identified as RET Exon 8–11. Finally, for MET, the single case was identified as MET Exon 14 Skipping (METex 14), in 1.5% (1/69; 95% CI: 0.3% to 7.8%). No patient was observed to have 2 out of 4 mutations at the same time (Table 2).

**Table 1** Participants' characteristics

Contents	Number (n)	Percentage (%)
Age	M=59.4; S.D.=8.9	
<60	29	42.0
$\geq 60$	40	58.0
Gender		
Male	50	72.5
Female	19	27.5
Tumor site		
Primary	46	66.7
Metastatic	23	33.3

S.D.=standard deviation

### Association of ALK, ROS1, RET, and MET

#### mutations with clinicopathological factors

Table 4 illustrates the correlation between ROS1 mutation and gender features ( $p$ -value=0.005). The prevalence of ROS1 mutations in female patients was 26.3% (5/19), while the rate was 2% (1/50) in males. Consequently, as observed in this study, the ROS1 mutation occurred more commonly in female patients than in males. The remaining clinicopathological factors showed no significant association with any of the 4 mutations ( $p$ -value>0.05) (Tables 3–5).

### Discussion

#### Prevalence of ALK, ROS1, RET, and MET in NSCLC

In the present study, we found that the prevalence of ALK mutations was the highest at 21.7%. This frequency aligns with recent studies<sup>20,21</sup>, yet it is significantly higher compared to other reports<sup>22,23</sup>, where the frequency of ALK mutations typically hovers around 5%. The prevalence observed in our study is markedly elevated in comparison to the usual rates for ALK mutations. Additionally, the prevalence of ROS1 mutations in our study was recorded at 8.7%, which exceeds the findings reported by Gupta et al. (2017).

**Table 2** Distribution of specific genetic alterations in ALK, ROS1, RET, MET

Mutation	Genetic alteration	Number (n)	Percentage (%)	95% CI
MET	METex14	1	1.5	0.3–7.8
RET	RET Exon 8–11	3	4.3	1.5–12
ROS1	ROS1 Exon 32	6	8.7	4–17.7
ALK		15	21.7	13.6–32.8
Negative		44	63.8	52–74.1
Total		69	100	

ALK=anaplastic lymphoma kinase, ROS1=ROS Proto-Oncogene 1, RET=RET proto-oncogene, MET=receptor tyrosine kinase, CI=confidence interval

**Table 3** Association of ALK, ROS1, RET, MET mutations with patient's age

Gene		Patient's age		OR; 95% CI	p-value
		<60 (n,%)	≥60 (n,%)		
ALK mutation (n=15)	Po.	9 (31.0)	6 (15.0)	2.55 (0.79–8.23)	0.33
	Ne.	20 (69.0)	34 (85.0)		
ROS1 mutation (n=6)	Po.	4 (13.8)	2 (2.0)	3.04 (0.52–17.86)	0.23
	Ne.	25 (86.2)	38 (98.0)		
RET mutation (n=3)	Po.	0 (0)	3 (7.5)	0.00 (0.01–4.41)	0.26
	Ne.	29 (100)	37 (92.5)		
MET mutation (n=1)	Po.	0 (0.0)	1 (2.5)	0.00 (0.02–20.73)	1.0
	Ne.	29 (100)	39 (97.5)		

ALK=anaplastic lymphoma kinase, ROS1=ROS Proto-Oncogene 1, RET=RET proto-oncogene, MET=receptor tyrosine kinase, Po.=positive, Ne.=negative, OR=odd ratio, CI=confidence interval

**Table 4** Association of ALK, ROS1, RET, MET mutations with patient's gender

Gene		Patient's gender		OR; 95% CI	p-value
		Male (n,%)	Female (n,%)		
ALK mutation (n=15)	Po.	9 (18.0)	6 (36.1)	0.48 (0.14–1.59)	0.11
	Ne.	41 (82.0)	13 (68.4)		
ROS1 mutation (n=6)	Po.	1 (2.0)	5 (26.3)	0.06 (0.01–0.53)	0.00
	Ne.	49 (98.0)	14 (73.7)		
RET mutation (n=3)	Po.	2 (2.0)	1 (5.3)	0.75 (0.06–8.79)	1.0
	Ne.	48 (98.0)	18 (94.7)		
MET mutation (n=1)	Po.	1 (2.0)	0 (0.0)	– (0.02–24.09)	1.0
	Ne.	49 (98.0)	19 (100)		

ALK=anaplastic lymphoma kinase, ROS1=ROS Proto-Oncogene 1, RET=RET proto-oncogene, MET=receptor tyrosine kinase, Po.=positive, Ne.=negative, OR=odd ratio, CI=confidence interval

**Table 5** Association of ALK, ROS1, RET, MET mutations with patient's tumor site

Gene		Patient's tumor site		OR; 95% CI	p-value
		Primary (n,%)	Metastatic (n,%)		
ALK mutation (n=15)	Po.	8 (17.4)	7 (30.4)	0.48 (0.15–1.55)	0.22
	Ne.	38 (82.6)	16 (69.6)		
ROS1 mutation (n=6)	Po.	3 (6.5)	3 (13.0)	0.47 (0.09–2.51)	0.39
	Ne.	43 (93.5)	20 (87.0)		
RET mutation (n=3)	Po.	3 (6.5)	0 (0.0)	– (0.15–66.85)	0.55
	Ne.	43 (93.5)	23 (100)		
MET mutation (n=1)	Po.	1 (21.7)	0 (0.0)	– (0.03–31.62)	1.0
	Ne.	45 (78.3)	23 (100)		

ALK=anaplastic lymphoma kinase, ROS1=ROS Proto-Oncogene 1, RET=RET proto-oncogene, MET=receptor tyrosine kinase, Po.=positive, Ne.=negative, OR=odd ratio, CI=confidence interval

Global prevalence figures for ROS1 mutations indicate a rate of 2%, with the rates for Asians and non-Asians being 2.2% and 1.9%, respectively<sup>24</sup>. One potential explanation for the high prevalence of ALK and ROS1 mutations in our findings could be the sample selection process. The NSCLC samples chosen for this study were initially screened to be negative for EGFR (epidermal growth factor receptor) mutations. Given that EGFR is one of the most common oncogenic drivers in non-small cell lung cancer and has the second highest prevalence after KRAS (Kirsten rat

sarcoma virus), this approach may have inadvertently excluded a significant number of NSCLC patients from the study. This exclusion could account for the increased frequency of the observed mutations. Besides, technical bias cannot be excluded; while the EasyPGX RT-qPCR kit used is a validated tool, its sensitivity may differ from that of next-generation sequencing or FISH, potentially leading to overestimation under certain conditions (e.g., nonspecific amplification or detection of rare variants). Therefore, to confirm these findings, additional testing using orthogonal

methods such as FISH, IHC, or RNA-based NGS panels is recommended. Larger, population-based cohorts would also help determine whether the observed frequency reflects a true epidemiological signal or study-specific limitations.

The RET mutation was the third most prevalent in this study, occurring in 4.3% of cases. This finding is twice the rate reported for RET mutations in NSCLC, which is approximately 2% according to some studies<sup>25,26</sup>. In contrast, the MET mutation exhibited the lowest prevalence at only 1.5%, falling short of the approximately 3% observed in other reports<sup>27,28</sup>. While this may reflect true epidemiological differences, the small number of cases limits the ability to draw definitive conclusions about MET mutation prevalence in the Vietnamese population. Despite its rarity in this cohort, MET exon 14 skipping mutations remain clinically relevant, as MET inhibitors such as capmatinib and tepotinib have demonstrated therapeutic efficacy in MET-altered NSCLC. Therefore, even infrequent detection supports the inclusion of MET testing in comprehensive molecular profiling panels. Further studies with larger sample sizes are needed to better define the epidemiological burden and therapeutic implications of MET mutations in Vietnam.

From a clinical perspective, the high frequency of ALK and ROS1 mutations among EGFR-negative NSCLC patients suggests that routine molecular screening for these alterations should be considered in this subpopulation, particularly in Vietnam. As EGFR-negative status often prompts further testing for alternative oncogenic drivers, broader profiling for ALK and ROS1 fusions is clinically justified. Given the availability of effective targeted therapies, under-screening may lead to missed treatment opportunities. These findings support the need for policy-level consideration of multigene testing and confirmatory methods such as FISH, IHC, or RNA-based NGS to ensure diagnostic accuracy. Larger, multicenter studies are also needed to validate these results and guide national screening strategies.

### Association of ALK, ROS1, RET, and MET with clinicopathological factors

Our results found that the incidence of ALK mutation was higher in female patients compared to males, though this difference was not statistically significant ( $p$ -value>0.05). This finding aligns with a study by Zhang et al. (2013), who also reported no significant association between ALK status and gender ( $p$ -value=0.727), despite a slightly higher prevalence in male patients (4.5% vs 3.9%)<sup>29</sup>. Conversely, other studies, such as Zhou et al. (2019) and Vidal et al. (2014), identified a significant association, noting a higher frequency of ALK mutations in female patients ( $p$ -value=0.039 and  $p$ -value<0.001, respectively)<sup>30,31</sup>. While the current data do not confirm a robust association, the recurrence of this pattern across diverse cohorts suggests it may not be incidental. Therefore, future research with larger, stratified sample sizes may help uncover whether this gender disparity reflects a true biological trend or remains a product of sampling variability. Clinically, awareness of such patterns could inform the development of more personalized approaches in screening and targeted therapy selection.

Similarly, although a higher frequency of ALK mutations was observed among younger patients in this study, the association was not statistically significant. This contrasts with previous research by Wen et al. (2015) and Gitlitz et al. (2015), which demonstrated a strong association between younger age and ALK positivity ( $p$ -value<0.001)<sup>32,33</sup>. Moreover, while the current study observed more ALK mutations in patients with metastatic tumors, no significant correlation was found ( $p$ -value>0.05). This pattern was also noted in a study by Wang et al. (2018), which reported a similar trend without statistical significance<sup>34</sup>. This finding may indicate that ALK mutations are not exclusively linked to the extent of tumor spread at diagnosis. Further studies with larger cohorts are warranted to clarify these potential associations and better understand the clinical implications of ALK mutations in relation to age and metastatic status.



Among the 6 ROS1 mutation cases, more cases were observed in females than male patients ( $p$ -value<0.01). The same trend was also reported in studies by Cai et al. (2012) (Male ROS1+: 37.5%; Female ROS1+:62.5%)<sup>23</sup> and Marchetti et al. (2017) (Male ROS1+: 6.9%; Female ROS1+:93.1%), but only Marchetti's study found a correlation ( $p$ -value<0.001)<sup>35</sup>. Besides, our study could not find an association between ROS1 mutation and age, despite a greater incidence occurring in younger patients compared to older patients. In contrast to this finding, patients who suffered ROS1 mutation in the study of Zhu et al. (2019) were revealed to be older than the patients who did not, but no association was found ( $p$ -value>0.05)<sup>26</sup>. This study also showed that ROS1 mutation was detected more in metastatic sites than in primary sites ( $p$ -value>0.05). Reports completed by Joshi et al. (2019) on Indian patients and Zhu et al. (2019) on Chinese patients also showed the same trend. Both studies showed a correlation ( $p$ -value<0.001)<sup>26</sup>. These findings suggest that although trends in ROS1 mutation patterns exist, especially by sex and tumor site, larger and more representative studies are needed to validate their clinical relevance.

The status of the RET mutation was found to occur more in female patients compared to males, but no association was found ( $p$ -value>0.05). RET status analysis from Tsuta et al. (2014) also shared a similar finding<sup>36</sup>. Conversely, a meta-analysis from Lin et al. (2015) showed that in Asian patients, RET fusion occurred more frequently in females, and this difference compared with male patients was statistically significant ( $p$ -value=0.017)<sup>37</sup>. For the age group, all 3 RET mutation cases were recorded in the older patient group and the primary tumor site ( $p$ -value>0.05). This finding contrasts with the findings of Tsuta et al. (2014), which implied that the RET mutation tends to occur in younger patients ( $p$ -value=0.038) and primary sites ( $p$ -value=0.189)<sup>36</sup>. Vice versa, Cai et al. (2013) demonstrated no such association, despite more

RET mutation cases being observed in younger patients than older patients ( $p$ -value=0.784)<sup>23</sup>.

Only 1 case of MET was recorded in male patients, not at the metastatic site, and it belonged to the older patient group. Unfortunately, no association was identified ( $p$ -value>0.05). Similarly, the study of Zheng et al. (2016) showed no correlation between this mutation and the gender factor, even though more MET mutation cases were observed in females than males ( $p$ -value=0.823)<sup>38</sup>. In contrast, Kim et al. (2018) showed that MET mutation was associated statistically with male patients ( $p$ -value<0.001)<sup>39</sup>. For the study of age in relation to MET mutation, Kim et al. (2018) found that this mutation tends to occur more in older patients ( $p$ -value<0.001)<sup>39</sup>. In a study of tumor site and MET mutation, Alex et al. (2020) showed that the incidence of MET mutation was higher in stage I–II patients than in stage III–IV patients, even though no correlation was found ( $p$ -value>0.05)<sup>40</sup>. Clinical observation on the status of MET mutation in relation to tumors showed that this mutation tends to occur more in patients at an advanced stage<sup>40</sup>.

Although the research results did not find many correlations, the research is considered pioneering in Vietnam, showing that there is a need for more in-depth, large-scale studies with the participation of many subjects to find other related factors, thereby helping clinicians understand the disease and shape treatment strategies for patients in a more personalized and effective way.

### Limitations

The study had some limitations. First, the study collected data on 69 patients, which was a sample of the population. Therefore, the results obtained are quite modest. Future studies need to be conducted on larger sample sizes to evaluate the mutation rates more clearly. Secondly, the study only selected NSCLC samples that were first identified to be negative for EGFR (epidermal growth factor receptor) mutation. Because of this action, a potentially large number



of NSCLC patients were missing from the study, leading to an increase in the frequency of this mutation. Third, the study did not include identification of fusion partners or use of confirmatory tests, such as fluorescence in situ hybridization (FISH) or immunohistochemistry (IHC), which are important for confirming and characterizing genetic alterations. Future studies should therefore incorporate comprehensive molecular profiling techniques and minimize false-positive or negative results. Finally, the study lacked longitudinal or survival data, which limits the ability to assess the clinical impact and prognostic significance of the mutation findings. This limitation is inherent in the cross-sectional design of the study, which focused on the molecular characteristics of EGFR-negative NSCLC patients at a single point in time. Therefore, longitudinal data were not available to assess the prognostic or predictive value of these alterations. However, the study provides important baseline data on the prevalence of ALK and ROS1 fusions in this population, which may inform the design of future prospective studies that integrate both molecular and clinical outcome data to more comprehensively assess the therapeutic relevance of these findings.

## Conclusion

The study demonstrated that reverse real-time PCR effectively identified 4 mutations in samples from 69 Vietnamese patients with non-small cell lung cancer (NSCLC). Among these mutations, the ALK mutation exhibited the highest prevalence at 21.7%, followed by ROS1 mutations at 8.7%. The prevalence of the RET mutation was recorded at 4.3%, while the MET mutation was found in 1.5% of the samples. From a clinical perspective, the high frequency of ALK and ROS1 mutations in EGFR-negative NSCLC patients in Vietnam highlights the urgent need for routine molecular screening of these alterations. Broader testing is essential, as effective targeted therapies are

available and can significantly improve patient outcomes, while under-screening risks missing these treatment opportunities. Additionally, the study identified a significant association between ROS1 mutations and female patients. To gain a clearer understanding of the prevalence of these mutations in Vietnam, further research is recommended. This finding strongly supports implementing multigene testing with confirmatory methods like FISH, IHC, or RNA-based NGS in national protocols. Larger multicenter studies are crucial to validate these results and shape effective screening and treatment strategies in Vietnam, unlocking the full potential of targeted therapies for patients.

## Conflict of interest

The authors have no conflicts of interest associated with the material presented in this paper.

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