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# Real-world biomarker testing rate and positivity rate in NSCLC in Spain: Prospective Central Lung Cancer Biomarker Testing Registry (LungPath) from the Spanish Society of Pathology (SEAP)

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## ABSTRACT

**Aim** The aim of this study was to describe the testing rate and frequency of molecular alterations observed in the Lung Cancer Biomarker Testing Registry (LungPath).

**Methods** A descriptive study of NSCLC biomarker determinations collected from March 2018 to January 2019, from 38 Spanish hospitals, was carried out. Only adenocarcinoma and not otherwise specified histologies were included for epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*) and programmed death ligand-1 (PD-L1) expression. The testing rate and the positivity rate were calculated. Multivariate logistic regression was used to explore the joint relationship between independent explanatory factors and both testing and positivity rates. Two models were adjusted: one with sample type and histology as independent factors, and the other adding the testing rate or the positivity rate of the other biomarkers.

**Results** 3226 patient samples were analysed, where *EGFR*, *ALK*, *ROS1* and PD-L1 information was collected (a total of 12 904 determinations). Overall, 9118 (71.4%) determinations were finally assessed. *EGFR* (91.4%) and *ALK* (80.1%) were the mainly tested biomarkers. Positivity rates for *EGFR*, *ALK*, *ROS1* and PD-L1 were 13.6%, 3.4%, 2.0% and 49.2%, respectively. Multivariate models showed a lower testing rate for ALK in surgical pieces, fine-needle aspiration or other types of samples versus biopsies.

**Conclusions** Despite the high testing rate in EGFR and ALK in NSCLC, the real-world evidence obtained from the LungPath demonstrates that ROS1 and PD-L1 were not determined in a significant portion of patients. LungPath provides crucial information to improve the coverage in molecular testing in lung cancer, to monitor the positivity rate and the introduction of new biomarker testing in clinical practice.

## INTRODUCTION

Cost-effectiveness will rule oncology practice in the next decade.[1] A key role in establishing cost-effectiveness in clinical cancer management will be played by biomarkers that help screen, detect and diagnose cancers or predict cancer outcomes and influence treatment choice and monitoring.[1]

Among patients with cancer, there exists great variability in tumour biology that determines the response to treatments and clinical outcomes.[2 3] Differences in the biomarker profiles between tumours can explain much of this variability.[3] A putative focused use of expensive cancer treatments on diagnosing patients, together with the fact that biomarkers can provide information about the current status or future risk of a disease, has led to an increased interest in biomarker-driven personalised cancer therapy.[3–5]

Lung cancer (LC) is the leading cause of cancer-related mortality worldwide.[6–8] There are two major types of LC: small cell lung cancer and non-small cell lung cancer (NSCLC).[6] NSCLC represents approximately 85% of all LCs and is classified into several histological subtypes including adenocarcinomas (ACs), squamous cell carcinomas (SCCs), large cell carcinomas and other less frequent subtypes.[8]

The complexity and variability of NSCLC—at least 30%–40% of these cancers have a treatable genomic alteration—and the large number of drugs directed against molecular targets, approved or in clinical development, making it one of the best paradigms of targeted therapies.[8 9]

When choosing a therapeutic option for a patient with LC, the determination of molecular biomarkers plays a key role. According to standardised recommendations[10] and the last consensus of the Spanish Society of Pathology (or Sociedad Española de Anatomía Patológica (SEAP)), molecular determinations for epidermal growth factor receptor (*EGFR*) and *BRAF* mutations, anaplastic lymphoma kinase (*ALK*) and c-ros oncogene 1 (*ROS1*) rearrangements, and programmed death ligand-1 (PD-L1) expression are mandatory to be performed in all patients with advanced NSCLC.[11]

*EGFR* mutations are present in 8%–11% of advanced NSCLCs. Since EGFR-tyrosine kinase inhibitors (TKI) inhibitors improve progression-free survival (PFS) and quality of life in comparison with platinum doublet chemotherapy, TKIs as first-line therapy are the standard in the main clinical guidelines.[12] In patients with an *EGFR* T790M mutation, osimertinib has shown a higher PFS than platinum/pemetrexed regimen (median, 10.1 months vs 4.4 months, respectively) after progression on

first-line treatment with a first-eneration or second-generation EGFR-TKI.[13]

Rearrangements of the *ALK* gene are found in approximately 2%–5% of advanced NSCLC.[14 15] Patients with ALK-positive NSCLC may develop disease resistance and progression, particularly in the central nervous system, resulting in poor prognosis and a negative impact on patients' quality of life.[16] The clinical importance of *ALK* rearrangement and its molecular diagnostic determination has led to the development of new, highly effective ALK inhibitor therapies (crizotinib, ceritinib, alectinib and brigatinib).[17 18] Specific treatment with these therapies has been an important advance in the management of these patients, resulting in response rates of 40%–93.5% and a PFS of up to 8 months.[8]

In approximately 1% of NSCLCs, *ROS1* gene is translocated.[11] For patients with stage IV LC with *ROS1* rearrangement, crizotinib is approved as a first-line or second-line monotherapy.[19–21]

Overexpression of PD-L1 in advanced NSCLC is predictive of clinical benefit with PD-1/PD-L1 inhibitor drugs. In general, there is a correlation between positive testing for the biomarker and efficacy, although this is a marker with a suboptimal negative predictive value.[11]

Due to the importance and clinical consequences of determining molecular biomarkers in LC, SEAP has developed the Lung Cancer Biomarker Testing Registry (LungPath), an online non-profit tool that permits the Pathology Departments to register, monitor and trace the most important NSCLC biomarkers results in clinical practice, enabling as well, data correlation at a national and global level.

In brief, the ultimate goal of this registry is to ensure that patients with NSCLC are properly diagnosed, thus facilitating the choice of treatment and ensuring that each patient receives the best possible care. In the short term, the aim of this study was to describe the testing rate and positivity rate observed in the first analysis of LungPath. The possible factors associated with the testing rate and positivity rate will also be explored.

## METHODS

A descriptive study of NSCLC biomarker determinations collected from March 2018 to January 2019, from 38 Spanish hospitals through the LungPath, was carried out. The LungPath includes samples of patients with advanced or metastatic NSCLC undergoing biomarker determination. Figure 1 represents the flowchart, where first external samples (from another centre) were excluded to avoid double counting. After, other histologies than AC and non-small cell lung cancer– not otherwise specified (NSCLC-NOS) were excluded. Finally, the frequency of EGFR, ALK, ROS1 and PD-L1 were analysed based on testing results according to recommendations of scientific societies.[11]

The LungPath compiles information such as date of registration, source of the sample analysed (it is analysed in the centre itself or in an external reference centre), type of sample analysed and histology of the LC, and variables related with the biomarker (type of biomarker, diagnostic techniques, determination and reason for no determination if appropriate, and test result that can be positive, negative or invalid).

### Statistical analysis and presentation of results

Frequencies and percentages of all study variables (sample type, histology, assessment and result of biomarkers testing) were obtained.

#### Main objective analysis

The testing rate of the selected biomarkers (*EGFR*/*ALK*/*ROS1*/PD-L1) was calculated and, when not determined, the frequencies of the reasons for non-assessment were described. Then, for each biomarker assessed, the positive rate (positivity based on standardised methods) was obtained excluding invalid results. Invalid cases are considered those samples which, due to different circumstances during the testing process, were not finally available to perform the biomarker test or the test results were not conclusive enough for determining if they were positive or negative.

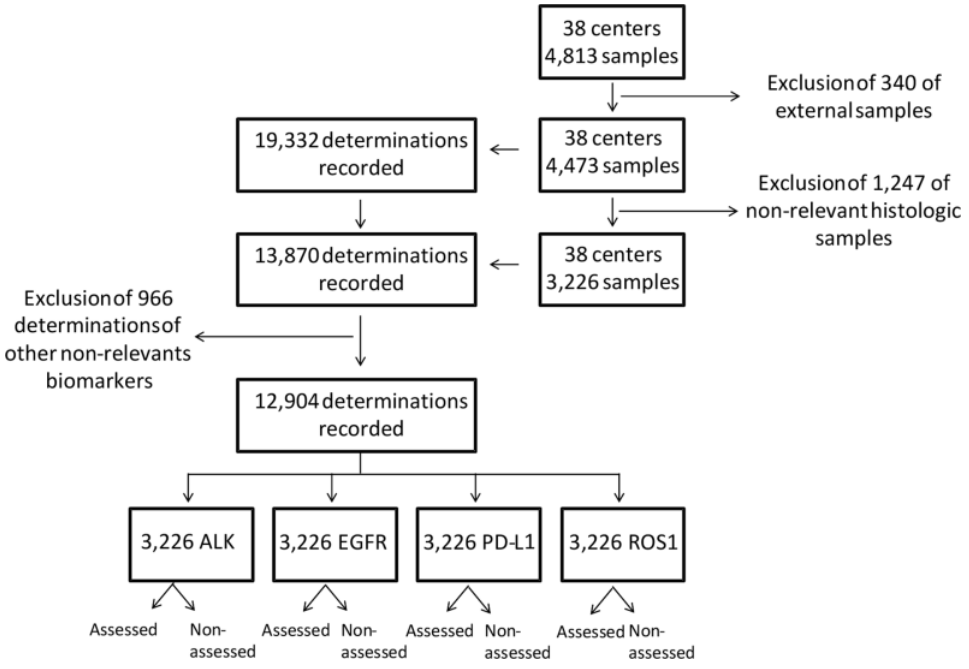


**Figure 1** Flowchart followed during the analysis of the LungPath. ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; LungPath, Lung Cancer Biomarker Testing Registry; PD-L1, programmed death ligand-1; ROS1 c-ros oncogene 1.

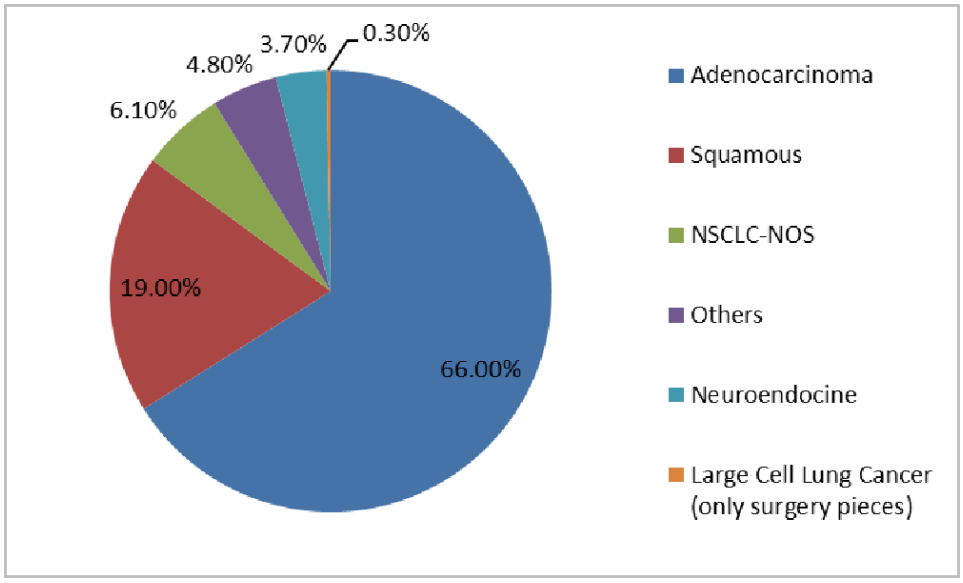


**Figure 2** Type of histology collected in the registry excluding only external samples (all histologies). NSCLC-NOS, non-small cell lung cancer–not otherwise specified.

For each biomarker, possible associations between the testing rate or positivity rate according to histology, sample type and result in other biomarkers were explored using contingency tables and the $\chi^2$ test. Then, multivariate logistic regression was used to explore the joint relationship between independent explanatory factors and both response variables (testing rate and positivity rate). For each outcome, two models were adjusted: one with sample type and histology as independent factors (model 1) and the other one adding the testing rate or the positivity rate of the other biomarkers (model 2). Results are presented as odds-ratio (OR) and 95% CI. Model calibration was assessed using the Hosmer-Lemeshow test. Discrimination ability was measured by means of the area under the receiver operating characteristic curve.

All tests were performed at a significant level of 5%. All analysis was carried out with the R statistical programme.

## RESULTS

Previously to the selection of the histologies of interest, as shown in figure 1, overall frequencies of histological types were analysed. The main histological type collected was AC (2951 out of 4473, 66%), followed far behind by SCC (851 out of 4473, 19%), NSCLC-NOS (275 out of 4473, 6.1%), large cell neuroendocrine carcinoma (166 out of 4473, 3.7%) and large cell carcinoma accounting for 0.3% (15 out of 4473) (figure 2). Over the 3226 patient samples finally analysed (AC and NSCLC-NOS, as defined in the study objective), AC is by far the main histological type (a total of 2951 samples, 91.5%).

Regarding the type of samples obtained, the biopsy was the most commonly used (1859 samples, 57.6%), followed by the surgical resection specimen with 552 samples (17.1%), cell block cytology with 348 samples (10.8%), fine-needle aspiration (FNA) with 103 samples (6.0%), others with 190 samples (5.9%) and peripheral blood with 84 samples (2.6%) (figure 3).

Of these 3226 samples, the four biomarkers selected (*EGFR*/*ALK*/*ROS1*/PD-L1) have been registered in all cases even if the determination was not finally performed, which means a total of 12 904 determination entries (3226 for each biomarker). Of the overall of 12 904 determinations recorded, 9118 (71.4%) were finally assessed, and the remaining 3686 (28.6%) were not finally performed. When determination was not performed, the reason was not recorded in almost half (49%). The absence of requirement/requests (42%) and not enough amount of sample (9%) were the reasons for not determining the biomarkers in the recorded cases.

Figure 4 shows the testing rate for each biomarker. As noted, *EGFR* (91.4%) and *ALK* (80.1%) were the mainly tested biomarkers, following by far by *ROS1* (58.1%) and PD-L1 (56.2%).

Overall, information about techniques performed in 8.934 sample determination were collected. More than half of the determinations were performed by immunohistochemistry (56.3%), followed by fluorescence in situ hybridisation (13.1%) and others techniques (30.6%), such us next-generation sequencing (NGS) or real-time polymerase chain reaction (RT-PCR).

In table 1 are shown the positivity rates of each biomarker after excluding invalid results and the comparison between the two histological types selected (AC and NSCLC-NOS). Statistically significant differences should be noted between the positivity rate of EGFR biomarker in AC or NSCLC-NOS histology (14.6% vs 2.8%, respectively; $p<0.001$).

Bivariate analysis for the testing and positivity rates as outcomes are shown in online supplemental material 1. Statistically significant differences were observed by sample type for the testing rate, but no for tumour histology (AC or NSCLC-NOS).

### Multivariate

Tables 2 and 3 show the logistic regression models for the testing rate for each of the four biomarkers of interest. Regarding *ALK*, both models capture a lower testing rate in FNA (model 1: OR 0.16, 95% CI 0.12 to 0.22; model 2: OR 0.29, 95% CI 0.19 to 0.44), surgical piece (model 1: OR 0.66, 95% CI 0.52 to 0.85; model 2: OR 0.58, 95% CI 0.41 to 0.82) and other sample types (model 1: OR 0.20, 95% CI 0.15 to 0.26; model 2: OR: 0.23, 95% CI 0.16 to 0.33) compared with biopsies as reference sample type. On the contrary, for *EGFR* testing rate, both models showed a higher testing rate in FNA (model 1: OR 2.89, 95% CI 1.44 to 6.88; model 2: OR 3.83, 95% CI 1.74 to 9.69) and other sample types (model 1: OR 2.2, 95% CI 1.28 to 4.10; model 2: OR 2.78, 95% CI 1.50 to 5.55) compared with

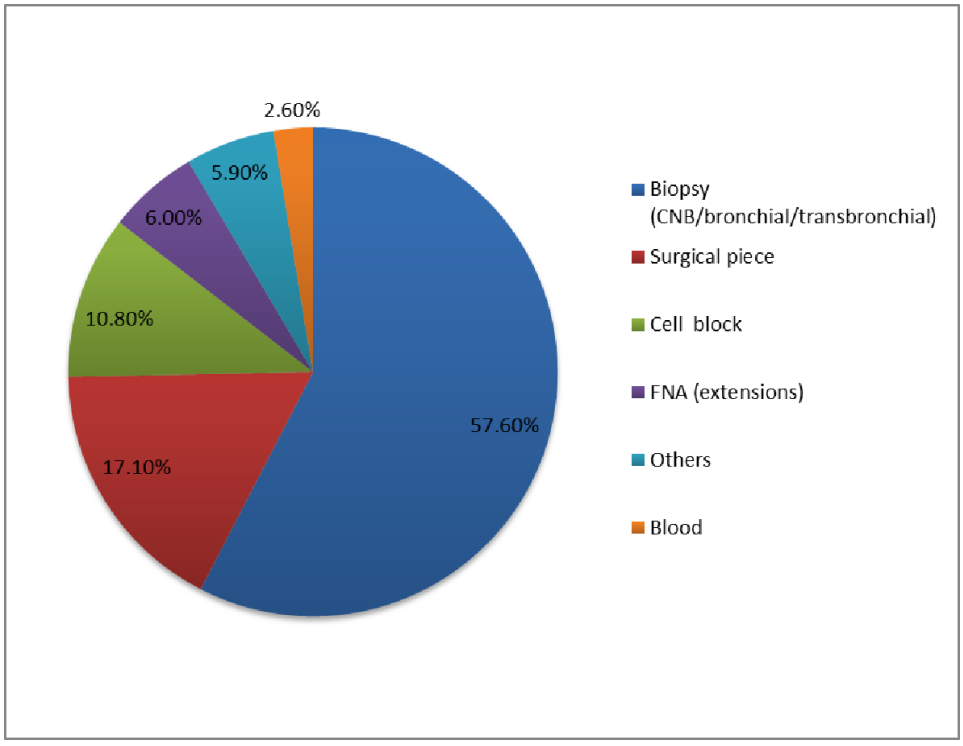


**Figure 3** Origin of the samples analysed. CNB, core needle biopsy; FNA, fine-needle aspiration.

biopsies as reference. Regarding the testing rate for PD-L1, all sample types showed a considerably lower testing rate compared with biopsies with both models. Finally, for *ROS1*, similarly to *ALK*, model 1 shown a lower testing rate in cell block (OR 0.77, 95% CI 0.61 to 0.97), FNA (OR 0.24, 95% CI 0.17 to 0.33) and other sample types (OR 0.33, 95% CI 0.26 to 0.44) compared with biopsies, while model 2 does not capture statistically significant differences.

Model 2 captures a strong association between the testing rate of every biomarker and the testing rate of other biomarkers; this is logical since in most cases the determinations are performed in parallel (*ALK*, *EGFR*, *ROS1* and PD-L1 are determined simultaneously).

Tables 4 and 5 show the logistic regression models for the positivity rate for the selected biomarkers, except for *ROS1*, since both models presented stability problems due to the lack of data. Concerning *ALK*, both models show no association with histology and sample type. The second model shows a significant reduction in *ALK* positivity for samples with positive *EGFR* result (OR 0.10, 95% CI 0.01 to 0.45), and this relationship is captured in turn by model 2 for *EGFR* positivity (OR 0.10, 95% CI 0.01 to 0.47). Model 2 for *EGFR* positivity also showed a higher positivity in FNA (OR 0.58, 95% CI 0.35 to 0.93) and blood samples (OR 0.42, 95% CI 0.22 to 0.76) compared with biopsies as reference. As described previously, both models showed a statistically significant higher positivity rate of *EGFR* in AC compared with NSCLC-NOS as reference histology (model 1: OR 0.16, 95% CI 0.07 to 0.33; model 2: OR 0.15, 95% CI 0.06 to 0.31). Lastly, model 2 for PD-L1 revealed a slightly

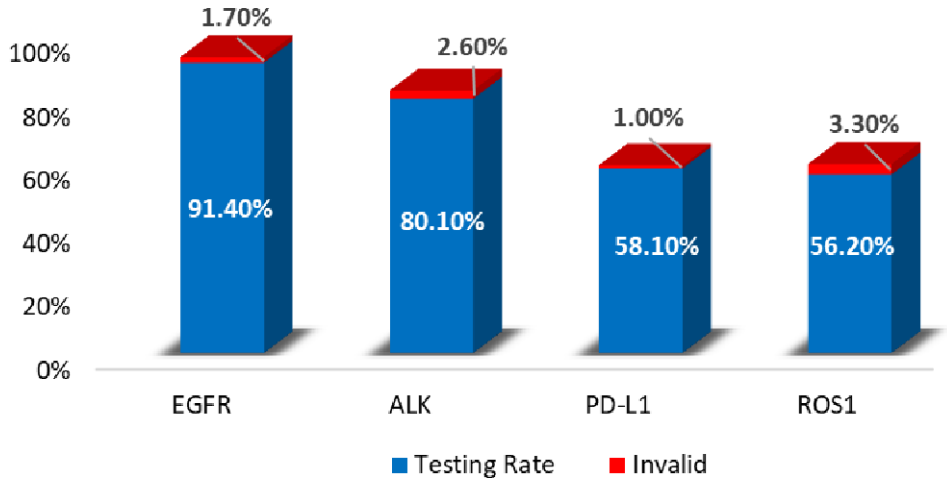


**Figure 4** Testing rate and invalid samples in adenocarcinoma and NSCLC-NOS histology cases. ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; NSCLC-NOS, non-small cell lung cancer–not otherwise specified; PD-L1, programmed death ligand-1; ROS1, c-ros oncogene 1.

**Table 1** Positivity rate of both AC and NSCLC-NOS histology cases

| | | AC | | NSCLC-NOS | | | AC+NSCLC-NOS | |
|---|---|---|---|---|---|---|---|---|
| **Biomarker** | **Result** | **n** | **%** | **n** | **%** | **P value*** | **n** | **%** |
| ALK | Positive | 79 | 3.4 | 6 | 2.9 | 0.854 | 85 | 3.4 |
| | Negative | 2232 | 96.6 | 200 | 97.1 | | 2432 | 96.6 |
| EGFR | Positive | 387 | 14.6 | 7 | 2.8 | <0.001 | 394 | 13.6 |
| | Negative | 2261 | 85.4 | 244 | 97.2 | | 2505 | 86.4 |
| PD-L1 | Positive | 822 | 48.7 | 91 | 54.2 | 0.204 | 913 | 49.2 |
| | Negative | 866 | 51.3 | 77 | 45.8 | | 943 | 50.8 |
| ROS1 | Positive | 33 | 2.0 | 2 | 1.5 | 0.909 | 35 | 2.0 |
| | Negative | 1585 | 98.0 | 132 | 98.5 | | 1717 | 98.0 |

*P value determined by the $\chi^2$ test.
AC, adenocarcinoma; ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; NSCLC-NOS, non-small cell lung cancer–not otherwise specified; PD-L1, programmed death ligand-1; ROS1, c-ros oncogene 1.

lower positivity rate if the result of *EGFR* biomarker is positive (OR 0.70, 95% CI 0.51 to 0.97).

## DISCUSSION

Biomarkers are becoming an essential requirement to properly treat patients with NSCLC.[22] This, coupled with recent advances in genomic and proteomic technologies plus bioinformatic tools that have allowed the discovery of several new biomarkers, has led to the fast and substantial accumulation of new biomarker-related data.[1 23] Nevertheless, while the research of new biomarkers is ongoing, the number of biomarkers for which a recommendation for testing is included in clinical guidelines is limited, as many of these promising biomarkers reported require reliable determination processes, validation and correlation to clinical outcomes.[24–26] In this context, registries may play a key role ensuring a more balanced evaluation of proposed biomarkers, a harmonisation and comparability of the collected data, and enabling the translation of the scientific literature to biomarker analysis of tumour tissues to assist biomarker–drug association evidence useful in clinical decision making.[25 27 28]

The main objective of this study was to analyse all the information recorded in the largest multicentre, prospective registry in NSCLC in Spain, the LungPath. In line with the literature, the descriptive analysis underscored that AC was the main histological type collected (66% of all histology types). Indeed, as previously mentioned, more than 85% of LC cases are currently classified as NSCLC, with AC being the predominant NSCLC histological phenotype (~50%).[29] Lung biopsies accounted for 57.6% of sample types, a figure that falls far from the other sample types, such as surgical resection, cell block FNA and blood. The analysis of some molecular biomarkers requires the extraction of nucleic acids from different samples (tumour tissue or cells, and/or blood samples).[30] The fact that the quality of nucleic acids in blood samples, in particular RNA, can vary and that FNA could not be effective for small lesions as small nodules as it cannot provide enough tissue for an accurate diagnosis may be at the root of the preference for lung biopsies that are currently the gold standard.[30] It is important to highlight that, although its use is currently not widespread, in the future, an increasing number of patients with NSCLC could benefit from liquid biopsy to identify their disease mutation instead of tissue samples, as recently reported in the Blood First Assay Screening Trial (BFAST) that demonstrated the clinical utility of blood-based NGS as a method to inform clinical decision-making in *ALK*+NSCLC.[31]

Regarding biomarker determinations, even though the mandatory test for each patient with advanced NSCLC are *EGFR* and BRAF mutations, *ALK* and *ROS1* rearrangements and PD-L1 expression, the analysis of LungPath focused only in four main biomarkers (*EGFR*/*ALK*/*ROS1*/PD-L1).[10 11] One aspect to be highlighted from the analysis of the registry is that the determination of these biomarkers was not always performed, probably because the determination in some laboratories is sequential, and there is not enough sample material or samples were of poor quality containing insufficient tumour cell percentage to determine all biomarkers.

A concern raised by this study is the large proportion of non-assessment of biomarkers in samples from patients with AC and

**Table 2** Logistic regression model 1 for the testing rate

| | ALK | | EGFR | | PD-L1 | | ROS1 | |
|---|---|---|---|---|---|---|---|---|
| | **OR (95% CI)** | **P value** | **OR (95% CI)** | **P value** | **OR (95% CI)** | **P value** | **OR (95% CI)** | **P value** |
| Sample type* (ref. biopsy) | | | | | | | | |
| Cell block | 0.84 (0.62 to 1.17) | 0.291 | 1.41 (0.93 to 2.23) | 0.121 | 0.44 (0.35 to 0.56) | <0.001 | 0.77 (0.61 to 0.97) | 0.026 |
| FNA | 0.16 (0.12 to 0.22) | <0.001 | 2.89 (1.44 to 6.88) | 0.007 | 0.03 (0.02 to 0.05) | <0.001 | 0.24 (0.17 to 0.33) | <0.001 |
| Surgical piece | 0.66 (0.52 to 0.85) | 0.001 | 1.10 (0.80 to 1.55) | 0.568 | 0.62 (0.51 to 0.75) | <0.001 | 0.97 (0.79 to 1.18) | 0.726 |
| Others | 0.20 (0.15 to 0.26) | <0.001 | 2.2 (1.28 to 4.10) | 0.008 | 0.17 (0.13 to 0.22) | <0.001 | 0.33 (0.26 to 0.44) | <0.001 |
| NSCLC-NOS (ref. adenocarcinoma) | 0.93 (0.68 to 1.29) | 0.666 | 1.06 (0.68 to 1.72) | 0.806 | 1.31 (0.99 to 1.75) | 0.062 | 0.87 (0.68 to 1.13) | 0.296 |
| Hosmer-Lemeshow, p value | *>0.999* | | *0.957* | | *0.968* | | *0.998* | |
| AUROC (95% CI) | *0.65 (0.62 to 0.67)* | | *0.559 (0.530 to 0.589)* | | *0.673 (0.655 to 0.691)* | | *0.588 (0.569 to 0.606)* | |

The results of the Hosmer-Lemeshow test and the AUROC are in italics to differentiate them from the ORs.
*Category 'others' includes blood samples.
ALK, anaplastic lymphoma kinase; AUROC, area under receiver operating characteristic curve; EGFR, epidermal growth factor receptor; FNA, fine-needle aspiration; NSCLC-NOS, non-small cell lung cancer–not otherwise specified; PD-L1, programmed death ligand-1; ref., reference; ROS1, c-ros oncogene 1.

**Table 3** Logistic regression model 2 for the testing rate

| | ALK | | EGFR | | PD-L1 | | ROS1 | |
|---|---|---|---|---|---|---|---|---|
| | OR (95% CI) | P value | OR (95% CI) | P value | OR (95% CI) | P value | OR (95% CI) | P value |
| Sample type* (ref. biopsy) | | | | | | | | |
| Cell block | 1.20 (0.79 to 1.83) | 0.393 | 0.94 (0.57 to 1.60) | 0.807 | 0.47 (0.37 to 0.60) | <0.001 | 0.85 (0.65 to 1.12) | 0.242 |
| FNA | 0.29 (0.19 to 0.44) | <0.001 | 3.83 (1.74 to 9.69) | 0.002 | 0.05 (0.03 to 0.09) | <0.001 | 0.71 (0.47 to 1.08) | 0.112 |
| Surgical piece | 0.58 (0.41 to 0.82) | 0.002 | 1.02 (0.68 to 1.55) | 0.909 | 0.65 (0.52 to 0.80) | <0.001 | 1.27 (1.00 to 1.63) | 0.051 |
| Others | 0.23 (0.16 to 0.33) | <0.001 | 2.78 (1.50 to 5.55) | 0.002 | 0.27 (0.20 to 0.37) | <0.001 | 0.80 (056 to 1.13) | 0.198 |
| NSCLC-NOS (ref. adenocarcinoma) | 0.86 (0.57 to 1.31) | 0.477 | 1.13 (0.67 to 2.00) | 0.660 | 1.40 (1.04 to 1.90) | 0.029 | 0.79 (0.59 to 1.07) | 0.128 |
| Testing rate ALK (yes vs no) | – | – | 20.61 (13.96 to 31.01) | <0.001 | 3.61 (2.71 to 4.87) | <0.001 | 61.50 (36.97 to 111.36) | <0.001 |
| Testing rate EGFR (yes vs no) | 24.9 (16.8 to 37.4) | <0.001 | – | – | 0.12 (0.08 to 0.17) | <0.001 | 3.07 (1.99 to 4.76) | <0.001 |
| Testing rate PD-L1 (yes vs no) | 4.16 (3.07 to 5.69) | <0.001 | 0.12 (0.08 to 0.17) | <0.001 | – | – | 2.34 (1.95 to 2.80) | <0.001 |
| Testing rate ROS1 (yes vs no) | 72.3 (42.7 to 133.3) | <0.001 | 3.56 (2.29 to 5.54) | <0.001 | 2.41 (2.01 to 2.90) | <0.001 | – | – |
| Hosmer-Lemeshow, p value | *0.313* | | *<0.001* | | *<0.001* | | *0.234* | |
| AUROC (95% CI) | *0.93 (0.92 to 0.94)* | | *0.854 (0.825 to 0.884)* | | *0.744 (0.727 to 0.762)* | | *0.793 (0.777 to 0.810)* | |

The results of the Hosmer-Lemeshow test and the AUROC are in italics to differentiate them from the ORs.
*Category 'others' includes blood samples.
ALK, anaplastic lymphoma kinase; AUROC, area under receiver operating characteristic curve; EGFR, epidermal growth factor receptor; FNA, fine-needle aspiration; NSCLC-NOS, non-small cell lung cancer–not otherwise specified; PD-L1, programmed death ligand-1; ref., reference; ROS1, c-ros oncogene 1.

NSCLC-NOS (28.6%), despite guidelines regarding the mandatory molecular analysis of *EGFR*/*ALK*/*ROS1*/PD-L1 biomarkers for these patients. It should be also noted that in 42% of cases, the absence of biomarker determination was due to the lack of request, which suggests the need to create more awareness on the benefits that biomarker determinations add to the patients, and also increase multidisciplinary collaboration to improve molecular diagnosis.[32 33]

When analysing the positivity rate, significantly more EGFR mutations were found in the AC (14.6%) versus NSCLC-NOS subgroup (2.85%). This statistically significant difference seen in *EGFR* biomarker for the positivity comparison between the two selected histological types is similar to previously published data.[34]

As previously described in the literature, the results of the bivariate and multivariate analyses for the testing rate of each biomarker show the importance of the type of sample in the realisation or non-realisation of the biomarker determinations.[35 36] This can be observed in the multivariate analysis, where a lower testing rate for the *ALK* biomarker is evident when the samples are surgical pieces, FNA or other types of samples versus biopsies as reference sample type. This lower rate of ALK assessment observed in FNA-derived samples could be due to the fact that the use of the method for detecting *ALK* gene rearrangement in cytology smears is quite controversial[11] to recent studies, however, have proven the suitability of the method.[37] Nevertheless, these results may be subjected to change in the future due to an upward trend towards minimally invasive sampling procedures, such as liquid biopsies.[31]

In line with several previous literature reports showing that *ALK* rearrangement tend to be mutually exclusive with mutations in *EGFR*, an absence of positive ALK results in EGFR-positive samples was observed in the logistic regression model 2 for ALK with the positivity as an outcome.[38–40]

In summary, the LungPath has allowed obtainment and analyses, for the first time in Spain, of the largest amount of real-world data of biomarker determinations, thus aiding a better understanding of the national diagnostic practices in LC biomarkers. It should be noted, however, that LungPath is an online-based data entry registry which can lead to missing data, inaccuracies or human errors in data entry.

To date, no other similar registries about real diagnostic practice and performance of biomarkers test in a sample this large of patients with LC have been described in Spain. There is, however, a Thoracic Tumours Registry in Spain, created by the Spanish Lung Cancer Group, in its commitment to improving the prognosis and the treatment of NSCLC and other thoracic tumours, which included data about biomarkers tested (from

**Table 4** Logistic regression model 1 for the positivity rate

| | ALK | | EGFR | | PD-L1 | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | P value | OR (95% CI) | P value | OR (95% CI) | P value |
| Sample type* (ref. biopsy) | | | | | | |
| Cell block | 0.77 (0.31 to 1.60) | 0.513 | 0.95 (0.66 to 1.36) | 0.802 | 1.00 (0.72 to 1.37) | 0.984 |
| FNA | 1.72 (0.59 to 4.04) | 0.261 | 1.20 (0.76 to 1.84) | 0.417 | 0.86 (0.28 to 2.60) | 0.785 |
| Surgical piece | 1.13 (0.62 to 1.96) | 0.682 | 0.94 (0.69 to 1.25) | 0.661 | 0.80 (0.63 to 1.03) | 0.081 |
| Blood | – | – | 1.54 (0.85 to 2.65) | 0.134 | – | – |
| Others | 1.48 (0.60 to 3.12) | 0.341 | 1.47 (0.96 to 2.19) | 0.068 | 1.17 (0.73 to 1.88) | 0.515 |
| NSCLC-NOS (ref. adenocarcinoma) | 0.85 (0.33 to 1.83) | 0.707 | 0.16 (0.07 to 0.33) | <0.001 | 1.20 (0.87 to 1.66) | 0.256 |
| Hosmer-Lemeshow, p value | *0.997* | | *0.995* | | *0.997* | |
| AUROC (95% CI) | *0.543 (0.486 to 0,601)* | | *0.565 (0.538 to 0.592)* | | *0.526 (0.503 to 0.549)* | |

The results of the Hosmer-Lemeshow test and the AUROC are in italics to differentiate them from the ORs.
*Category 'others' includes blood samples for ALK and PD-L1 models.
ALK, anaplastic lymphoma kinase; AUROC, area under receiver operating characteristic curve; EGFR, epidermal growth factor receptor; FNA, fine-needle aspiration; NSCLC-NOS, non-small cell lung cancer–not otherwise specified; PD-L1, programmed death ligand-1; ref., reference.

**Table 5** Logistic regression model 2 for the positivity rate

| | ALK | | EGFR | | PD-L1 | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | P value | OR (95% CI) | P value | OR (95% CI) | P value |
| Sample type* (ref. biopsy) | | | | | | |
| Cell block | 0.74 (0.30 to 1.56) | 0.474 | 0.86 (0.58 to 1.23) | 0.417 | 1.01 (0.74 to 1.40) | 0.931 |
| FNA | 1.82 (0.60 to 4.52) | 0.237 | 0.58 (0.35 to 0.93) | 0.029 | 0.78 (0.25 to 2.38) | 0.665 |
| Surgical piece | 1.15 (0.63 to 2.01) | 0.633 | 0.80 (0.58 to 1.08) | 0.156 | 0.80 (0.62 to 1.02) | 0.075 |
| Blood | – | – | 0.42 (0.22 to 0.76) | 0.005 | – | – |
| Others | 1.52 (0.62 to 3.25) | 0.313 | 1.22 (0.78 to 1.87) | 0.366 | 1.20 (0.75 to 1.93) | 0.457 |
| NSCLC-NOS (ref. adenocarcinoma) | 0.77 (0.29 to 1,66) | 0.547 | 0.15 (0.06 to 0.31) | <0.001 | 1.19 (0.86 to 1.65) | 0.289 |
| Result ALK: positive | – | – | 0.10 (0.01 to 0.47) | 0.025 | 1.63 (0.94 to 2.86) | 0.084 |
| Result ALK: missing | – | – | 4.12 (2.97 to 5.72) | 0.000 | 1.21 (0.83 to 1.77) | 0.318 |
| Result EGFR: positive | 0.10 (0.01 to 0.45) | 0.022 | – | – | 0.70 (0.51 to 0.97) | 0.035 |
| Result EGFR: missing | 0.76 (0.18 to 2.10) | 0.652 | – | – | 1.38 (0.95 to 2.01) | 0.089 |
| Result PD-L1: positive | 1.61 (0.94 to 2.83) | 0.088 | 0.71 (0.51 to 0.98) | 0.039 | – | – |
| Result PD-L1: missing | 1.22 (0.69 to 2.19) | 0.501 | 1.07 (0.80 to 1.43) | 0.651 | – | – |
| Result ROS1: positive | – | – | 2.03 (0.80 to 4.54) | 0.103 | 0.97 (0.41 to 2.29) | 0.952 |
| Result ROS1: missing | – | – | 0.91 (0.68 to 1.20) | 0.498 | 0.88 (0.70 to 1.11) | 0.274 |
| Hosmer-Lemeshow, p value | *0.68* | | *0.904* | | *0.929* | |
| AUROC (95% CI) | *0.613 (0.555 to 0.672)* | | *0.679 (0.650 to 0.708)* | | *0.562 (0.536 to 0.587)* | |

The results of the Hosmer-Lemeshow test and the AUROC are in italics to differentiate them from the ORs.
*Category 'others' includes blood samples for ALK and PD-L1 models.
ALK, anaplastic lymphoma kinase; AUROC, area under receiver operating characteristic curve; EGFR, epidermal growth factor receptor; FNPA, fine-needle aspiration; NSCLC-NOS, non-smallcell lung cancer–not otherwise specified; PD-L1, programmed death ligand-1; ref., reference; ROS1, c-ros oncogene 1.

2012 to 2018) considering all stages of LC and whose data are aligned with the data obtained in LungPath.[41] In Europe, there are differences between different countries in the availability of diagnoses of molecular alterations in NSCLC,[42] so in this context, LungPath can be a useful online tool to monitor the availability and the incorporation of new biomarkers testing in LC. Other biomarker-based registries have been developed, such as the Caris Registry promoted by Caris Life Sciences, and intended to become a robust library of tumour biomarker results, covering not only NSCLC but also breast, ovary, colon, endometrium and other cancers.[43] Thus, LungPath represents a great step forward to ensure the quality of biomarker determination and results homogenisation, provided by SEAP. Development of more central biomarker databases, such as LungPath, or promoting the use and content expansion of LungPath in the future, could be useful to monitor, correlate results between different centres at a national and international level, and improve the available knowledge regarding biomarkers in NSCLC.

Given the incremental importance of biomarkers in guiding the treatment of patients with NSCLC and the cost saving that optimal biomarker determination could mean in drug spending-related health expenditure, having this information available could be also essential to elaborate future analysis in this area, being therefore,specifically interesting to the pathology departments and, generally, to the scientific society.[9 44]

## Take home messages

- For first time, Lung Cancer Biomarker Testing Registry (LungPath) allows obtainment and analysis of the large amount of real-world data of biomarker determinations in Spain.
- The real-world evidence obtained from LungPath demonstrates that epidermal growth factor receptor (*EGFR*) (91.4%) and anaplastic lymphoma kinase (*ALK*) (80.1%) were the mainly tested biomarkers, while c-ros oncogene 1 (*ROS1*) (58.1%) and PD-L1 (56.2%) were not determined in almost half of the patients.
- Multivariate models explored the different associations between the response variables (testing and positivity rates) and the different explanatory factors such as sample type or histology.
- This study aids to a better understanding of the national diagnostic practices in lung cancer biomarkers and to continue with future analysis in this area.

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