OPEN ACCESS

Biomarker testing strategies in non-small cell lung cancer in the real-world setting: analysis of methods in the Prospective Central Lung Cancer Biomarker Registry (LungPath) from the Spanish Society of Pathology (SEAP)

Javier Martín-López,[1] Federico Rojo,[2,3] Antonio Martínez-Pozo,[3,4] Teresa Hernández-Iglesias,[5] David Carcedo ,[6] Lucía Ruiz de Alda,[7] J Francisco García,[7] Clara Salas[1,3]

[1]Pathology Department, Hospital Universitario Puerta del Hierro Majadahonda, Madrid, Spain
[2]Pathology Department, Hospital Universitario Fundacion Jimenez Diaz-CIBERONC, Madrid, Spain
[3]External Quality Asessment (GCP), Spanish Society of Anatomical Pathology, Madrid, Spain
[4]Pathology Department, Hospital Clinic de Barcelona, Barcelona, Spain
[5]Quality Control Program, Spanish Society of Anatomical Pathology, Madrid, Spain
[6]Hygeia Consulting SL, Madrid, Spain
[7]Roche Farma SA, Madrid, Spain

Correspondence to
David Carcedo, Hygeia Consulting SL, Madrid, Spain; david.carcedo@hygeiaconsulting.com

Received 29 October 2021
Accepted 9 November 2021
Published Online First 13 December 2021

To cite: Martín-López J, Rojo F, Martínez-Pozo A, *et al*. *J Clin Pathol* 2023;**76**:327–332.

ABSTRACT

Aims The aim of this study is to extend the analysis of the Lung Cancer Biomarker Testing Registry (LungPath), by analysing the techniques used in the determination of epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), c-ros oncogene 1 (ROS1) and programmed death ligand-1 (PD-L1) for the diagnostic of patients with advanced non-small-cell lung cancer (NSCLC).

Methods Information of the technique used for the determination of EGFR, ALK, ROS1 and PD-L1 was recorded from March 2018 to January 2019 from 44 centres, but only 34 centres matched with the 38 centres previously analysed, allowing to analyse the techniques used in 8970 matched determinations of EGFR, ALK, ROS1 and PD-L1. Therefore, a by-centre analysis studied the level of implementation of the techniques in the 44 centres, while a by-determination analysis made it possible to assess the overall frequency of the techniques used on the 9134 matched samples.

Results By-centre analysis showed that only 46.5% and 25.6% of the centres used reflex strategies for ALK and ROS1 determination, respectively. By-determination analysis showed that 94.4% of EGFR determinations were performed by PCR, 80.7% of ALK determinations were performed by IHC with clone D5F3, while 55.7% of ROS1 determinations were performed by IHC with clone D4D6. 22C3 were the PD-L1 clone more used (43.5%) followed by SP263 clone (31.1%).

Conclusions The real-world evidence obtained from LungPath shows the effort of Spanish hospitals in performing biomarker determination in NSCLC with different methodologies despite that next-generation sequencing (NGS) utilisation in the year of the analysis was low. Biomarker determination results could be optimised with the incorporation of sequencing methods such as NGS in pathology departments.

INTRODUCTION

Globally, lung cancer (LC) has been the most common cancer and the leading cause of cancer deaths.[1] LC is made up of distinct subtypes, including small-cell lung cancer (SCLC; approx. 15%) and non-SCLC (NSCLC; approx. 85%).[2 3] In NSCLC adenocarcinoma, the most common histological subtype in NSCLC, several oncogenic and actionable drivers have been described.[4]

For therapy decision with targeted drugs in LC patients, the determination of molecular biomarkers is considered indispensable.[5] According to last consensus of the Spanish Society of Pathology (Sociedad Española de Anatomía Patológica, SEAP) and the Spanish Society of Medical Oncology (Sociedad Española de Oncología Médica, SEOM), 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.[6] In biomarker testing era, obtaining enough sample for diagnostic and initial treatment and also in tumour progression is crucial. On the other hand, given that the diagnostic sample is a limited resource, regarding the testing of molecular biomarkers, it is still important to remember two principles: (1) the fewer times paraffin-embedded material (tissue or cytological as cell blocks) is placed in a microtome, the more will be spared and (2) the order of biomarker prioritisation is important, as the tissue can be depleted. To meet the first principle, testing should be always planned in advance for every NSCLC patient.[6]

The determination of molecular biomarkers in NSCLC requires the analysis of biological molecules (DNA, RNA, proteins), and therefore, the involvement of different analytical techniques such as fluorescent in situ hybridisation (FISH), immunohistochemistry (IHC), PCR, sequencing techniques such as Sanger and new molecular techniques as next-generation sequencing (NGS).[7 8] The choice of technique depends on criteria such as the type of molecular alteration (mutation, translocation or amplification), personnel requirements, available tissue, analytical parameters (sensitivity, specificity), associated costs and others.[9 10] For example, considering the complexity of EGFR mutations and the different platforms used between different laboratories, these new molecular techniques may

increase the detection of rare variants, critical to clinical decision.[11] In relation to the increasing use of NGS, the SEOM has established a framework of useful recommendations for planned and controlled implementation of NGS in the context of hereditary cancer.[12] The ESMO has also issued recommendations for the use of NGS panels in patients with metastatic cancers, including the routine use of NGS on tumour samples in advanced NSCLC.[13]

Recently, the 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, comparison of their data with the overall national data with the aim to improve quality diagnostic procedures. A previous analysis of the Lungpath database has already been published, describing in detail the methodology for statistical analysis and the results of the testing and positivity rate observed in the registry, exploring also the possible factors associated with both testing and positivity rates.[14]

The aim of this study is to extend the information reported in the published analysis of the LungPath database,[14] in particular by analysing the techniques used in the determination of EGFR, ALK, ROS1 and PD-L1 for the diagnostic of patients with advanced NSCLC.

METHODS

The methods used for the analysis of the LungPath database have been described in detail in the previous publication by Salas *et al*.[14]

To summarise, from March 2018 to January 2019, information of biomarker determinations from samples of patients with advanced or metastatic NSCLC was collected from 38 Spanish hospitals. After the inclusion and exclusion criteria described in Salas *et al*, 12 904 determination of *EGFR, ALK, ROS1 and* PD-L1 from 3226 patient samples were finally analysed.[14]

For the present analysis of the techniques used in the biomarker determination, information of the variable 'name of diagnostic test' was recorded from 44 centres in LungPath database, but only 34 of them matched with the 38 hospitals of the main analysis, allowing cross-checking the biomarker determination data from the main analysis with the data from the techniques used. Therefore, of the 12 904 determinations analysed in Salas *et al*,[14] the technique used was recorded in only 8970 samples that were finally determined out of a total of 12 128 samples, as shown in figure 1.

Given the availability of this information, two types of analysis were conducted:

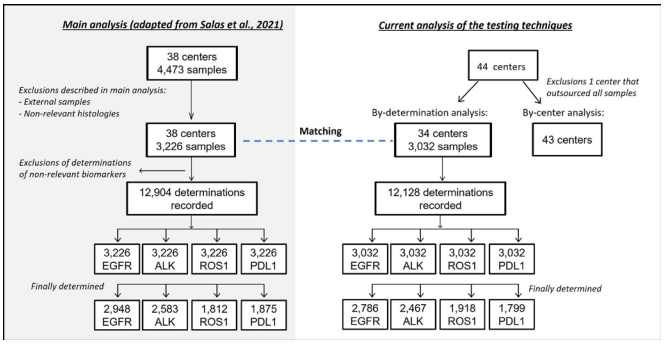

Figure 1 Flow chart. ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; PD-L1, programmed death ligand-1; ROS1, c-ros oncogene 1.

- By-centre analysis: The biomarker determination protocol established in the 44 centres mentioned above was considered to estimate the level of implementation of the techniques in the centres. That is, which determination techniques are used in each hospital, without analysing the number of determinations performed in each one (not matching available).
- By-determination analysis: The overall frequency of the techniques used in the 8970 matched determinations is analysed (figure 1). In other words, the number of determinations performed is analysed regardless of the centre from which they originate.

Through these two approaches, we obtain information on the frequency of use of the techniques both by hospital and by determination.

As reflected in the guidelines,[6] for several biomarkers such as ALK or ROS1, screening is performed by immunohistochemistry, and while for ALK determination, confirmation by a second technique is advisable only in cases that are inconclusive, in the case of ROS1, the positive results obtained should be always confirmed by another orthogonal method (cytogenetic or molecular) such as FISH or PCR (defined as reflex). Therefore, for the determination of ALK and ROS1, some centres use (and register in LungPath) two techniques, an IHC and a FISH usually. Therefore, in LungPath several hospitals register the use of 2 techniques (an IHC and a FISH) for the determination of ALK and ROS1, interpreting that they perform a reflex strategy to FISH.

Thus, in the by-centre analysis those centres that records an IHC and a FISH for the determination of ALK and ROS1 are categorised as reflex centres.

In the quantitative analysis, the matched determinations corresponding to reflex centres should be assigned to IHC and/or FISH. It is assumed that IHC is performed in all the samples (screening), and FISH would be performed only in positive results obtained by IHC. From the main analysis of LungPath[14] we know the positivity rate of ALK and ROS1, and we can assume that both techniques (IHC and FISH) have been used in the reflex centres, but the exact number of FISH determinations is unknown, since it depends on the specificity of IHC. For example, considering for the IHC a specificity close to 100%, there would be very few false positives so that the number of confirmatory determinations performed by FISH would be very similar to the number of positives recorded in LungPath. By decreasing the specificity of IHC, more positive results would be obtained in the screening that would have to be confirmed by FISH. The influence of the specificity of IHC in the overall frequency of the techniques is evaluated in the sensitivity analysis.

RESULTS

By-centre analysis

Of the 44 hospitals included in the by-centre analysis, 1 centre outsourced to a referral hospital all its samples for analysis, so it did not collect information from the techniques. Therefore, information on the biomarker determination protocols of 43 centres were finally analysed.

EGFR was main determined by PCR. Specifically, 37 out of 43 centres (86.0%) registered a PCR method for the determination for EGFR, with the Cobas 4800 EGFR Mutation Test (Roche) being the most widely used commercial kit (30 out of 37 centres). Three centres (7.0%) used sequencing methods such us Sanger (one centre) and NGS (two centres), while the remaining three centres (7.0%) outsourced the EGFR determination (figure 2).

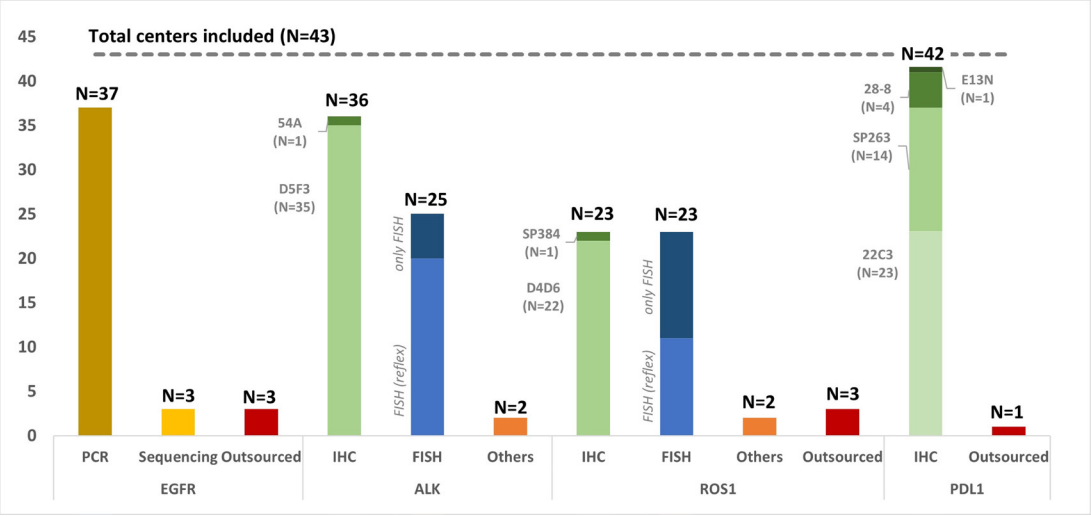

Figure 2 Summary of the results of the by-centre analysis (N=centres). ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridisation; IHC, immunohistochemistry; PD-L1, programmed death ligand-1.

Regarding the determination of ALK and ROS1, 20 (46.5%) and 11 (25.6%) centres up of 43 used reflex strategies (confirmation by FISH of positive results obtained by IHC), respectively. For the determination of ALK, IHC was used in 36 centres (83.7%), FISH was used in 25 centres (58.1%) among which FISH is the only method used in 5 centres (11.6%), while in 20 centres (46.5%) FISH is used as a confirmatory assay, and a PCR and NGS methods were only used in 1 centre each. D5F3 was the most used ALK-clone by far (35 centres, 81.4%). For the determination of ROS1, IHC was used in 23 centres (53.5%) and FISH was used also in 23 centres (53.5%) among which FISH is the only method used in 12 centres (27.9%), while in 11 centres (25.6%) FISH is used in a confirmatory manner. D4D6 was the most used ROS1-clone (22 centres, 51.2%). For both ALK and ROS1, the most commonly used commercial FISH assay was the Break Apart FISH Probe Kit (Vysis/Abbott) (17 out of 25 for ALK and 13 out of 23 for ROS1). NGS and Nanostring were used only in one centre each (figure 2).

Finally, for the determination of PD-L1, IHC was used in the 42 centres (97.7%). The four PD-L1 clones used were 22C3 (23 centres, 53.5%), SP263 (14 centres, 32.6%), 28–8 (4 centres, 9.3%) and E1L3N (1 centre, 2.3%).

Figure 2 shows the results of the qualitative analysis for the four biomarkers, showing the grouping by type of technique used in the centres, also differentiating the clones used in the case of IHC.

By-determination analysis

In addition to the by-centre analysis data shown in figure 2, the by-determination analysis shows the data from the techniques used in the determination of 8970 samples (table 1). As described in methods, for ROS1 and ALK two techniques are frequently used in the determination of a sample (IHC for screening and FISH for confirmation of positives), so the number of determinations shown in table 1 is greater than the number of samples. Therefore, as can be seen in table 1, the total number of determinations exceeds 100% for ALK and ROS1. In the FISH frequencies shown in the table, it was assumed that there are no false positives in the previous determination by IHC, that means that only 3.4% of ALK and 2% of ROS1 positives are confirmed by FISH.

Sensitivity analysis

As described in methods section, due to the centres that perform reflex for the determination of ALK and ROS1, the exact number of confirmations by FISH is unknown, since it depends on the specificity of IHC.

As a sensitivity analysis, table 2 shows how the number of FISH confirmations shown in the previous table would be increased by modifying the specificity of IHC.

Table 1 Results of the quantitative analysis (n=determinations)

Biomarker	Grouping by technique type	Determinations n	Determinations (%)
	PCR	2631	94.4
	Sequencing	110	3.90
	Outsourced	45	1.60
	Total EGFR determinations	2786	100
ALK	IHC—clone D5F3	1990	80.70
	IHC—clone 54A	210	8.50
	FISH	320	13.00
	Other (PCR)	3	0.10
	Total ALK determinations	2523	102.3*
ROS1	IHC—clone D4D6	1069	55.70
	IHC—clone *SP384*	0	–
	FISH	846	44.10
	Outsourced	16	0.80
	Total ROS1 determinations	1931	100.7*
PD-L1	IHC—clone 22C3	782	43.50
	IHC—clone *SP263*	559	31.10
	IHC—clone 28–8	393	21.80
	IHC—clone *E1L3N*	31	1.70
	Outsourced	34	1.90
	Total PD-L1 determinations	1799	100.00

*IHC and FISH are mutually non-exclusive. 3,4% and 2% of positive results obtained by IHC screening and confirmed by FISH for ALK and ROS1, respectively.
ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridisation; IHC, immunohistochemistry; PD-L1, programmed death ligand-1; ROS1, c-ros oncogene 1.

Table 2 Results of sensitivity analysis of the quantitative results for ALK and ROS1

	ALK*				ROS1†			
	IHC		FISH		IHC		FISH	
	n	(%)	n	(%)	n	(%)	n	(%)
Base case (100% specificity)	2200	89.20	320	13.00	1069	55.70	846	44.10
95% IHC specificity	2200	89.20	399	16.20	1069	55.7	879	45.80
90% IHC specificity	2200	89.20	479	19.40	1069	55.70	912	47.50
85% IHC specificity	2200	89.20	558	22.60	1069	55.70	945	49.30
80% IHC specificity	2200	89.20	638	25.90	1069	55.70	975	51.00
75% IHC specificity	2200	89.20	717	29.10	1069	55.70	1010	52.70
70% IHC specificity	2200	89.20	797	32.30	1069	55.70	1043	54.40

*IHC and FISH are mutually non-exclusive. The samples analysed by PCR (N=3; 0,1%) are not shown in the table as they do not affect the sensitivity analysis.
†IHC and FISH are mutually non-exclusive. The samples outsourced to a referral hospital (N=16; 0.8%) are not shown in the table as they do not affect the sensitivity analysis.
ALK, fluorescent in situ hybridisation; FISH, fluorescent in situ hybridisation; IHC, immunohistochemistry; ROS1, c-ros oncogene 1.

DISCUSSION

It is now a reality that in patients with advanced NSCLC, not only pathological information but also predictive biomarker results are needed to enable treatment selection in some patient subgroups.[15] As a result, accurate molecular diagnosis in patients with NSCLC is becoming increasingly important in routine clinical practice.[10] In this clinical context, registries allow control and monitoring of predictive biomarkers and play an important role in standardisation and quality of clinical practice, which will ultimately have a clear benefit for patients (Lungpath database).[14 16]

This study aims to extend the information provided by the analysis *LungPath* registry previously carried out by Salas *et al*,[14] in particular, by analysing the techniques used in the determination of EGFR, ALK, ROS1 and PD-L1 for the diagnostic of patients with advanced NSCLC.[14] It should be noted that even though the mandatory test for each patient with advanced NSCLC are EGFR and BRAF mutations, ALK and ROS1 rearrangements and PD-L1 expression, this analysis of LungPath (in line with previous one) focused only in four main biomarkers (EGFR/ALK/ROS1/PD-L1).[6 14]

As previously discussed in the analysis by Salas *et al*,[14] no other similar registries of biomarkers testing in patients with LC have been found in Spain. In the European context and focusing on the techniques used in the determination of biomarkers in NSCLC, Ryska *et al*[1] conducted a questionnaire about molecular testing and NSCLC management to relevant specialists in nine Central and Eastern Europe countries in 2014. According to this questionnaire, IHC, followed by FISH confirmation in positive cases, had been widely adopted for ALK testing. According to the authors, this could be explained by the fact that IHC is a quick, less expensive and technically easier method, in addition that the Food and Drug Administration's acceptance of *Ventana ALK D5F3* IHC as an adjunctive test to identify patients for crizotinib treatment provides additional support for the routine use of IHC.[1] It should be noted that the data from Ryska *et al*[1] reflect the status quo in 2014, and molecular testing is evolving fast with changes in testing methods being implemented. Therefore, it could be considered that results of the Ryska *et al*[1] study are in line with the results of our analysis, according to which, IHC for ALK determination is the technique used in most Spanish centres (83.7% of the centres) followed by FISH (58.1% of the centres) used in most of the centres in a confirmatory manner, also called reflex strategy (of all samples analysed by IHC, 3.4% were confirmed by FISH). It is possible that the higher representation of the use of IHC compared with FISH is due to the fact that, in addition to be an equivalent alternative to FISH, it is a quick and cost-effective technique that can be applied to different biological specimens, such as biopsies or cytological samples.[6] In addition, ALK testing with IHC is well established and there is ample evidence to support its use.[17] On the other hand, the use of the reflex strategy shown in the analysis is also advised by SEAP, especially in inconclusive IHC cases.[6]

Relative ROS1 determination and according to the present analysis, IHC and FISH were used in the same number of centres (53.5% each) although in roughly half of the centres where FISH was used, it was assumed that it was used as a reflex strategy (of all samples analysed by IHC, 2% were confirmed by FISH). NGS and Nanostring were the least used techniques (2,3% of centres, each one). This is not completely in line with international and national guidelines that, among the possible methodological approaches (IHC, FISH, PCR and NGS), recommend IHC as a screening method and confirmation of positive cases with another orthogenetic method (cytogenetic or molecular).[6 17] According to the results of our analysis, ALK IHC was more widespread in clinical practice than ROS1 IHC, for whose determination, FISH testing was still widely used. This could be due to ALK has a higher reliability than ROS1-IHC for which there is still no real-world evidence (although there are two commercially available antibodies).[6] Moreover, it is important to consider for the understanding of these results, that ROS1 expression without underlying rearrangement (false positives) has been described in nearly a third of tumours.[6]

For both ALK and ROS1 by-determination analysis, there is some uncertainty about the exact number of confirmations performed by FISH after a positive result detected by IHC. This is due to a limitation of the database analysis, for which individualised information was not available in case the same sample was analysed by two different techniques. Therefore, to evaluate this uncertainty associated with the number of FISH determinations for both ALK and ROS1, a sensitivity analysis was performed. This analysis shows that in the most extreme cases where a low specificity of IHC would lead to more false positives, the number of FISH needed to confirm those positives results increases considerably.

In terms of EGFR determination and according to the present analysis, the most commonly used technique was PCR by far. These results are also in line with the Ryska's *et al* study[1] (previously described) in which it is reflected that the PCR was used in all countries included and direct sequencing in five of nine countries included. PCR and sequencing techniques (Sanger and NGS) are the methods described for EGFR testing in the

national consensus of the SEAP and SEOM.[6] However, if sufficient expertise is available, and if the extended biomarker panel is to be tested, the SEAP and other international societies recommends to determine the EGFR mutation with targeted NGS panels.[6] This is because, beyond the most common mutations such as deletions in exon 19 and point mutations in exon 21 (accounting for approximately 90% of cases), NGS utilisation enable the identification of rare variants, usually missed by available commercial kits that detect only a limited number of EGFR mutations. Some of these uncommon EGFR-mutations include insertions and/or point mutations in the exon 20, substitutions in the exon 18, complex mutations, exon 19 insertions or rare variant deletions, and less common mutations in the exon 21.[18]

Finally, as for PD-L1 determination, IHC was the only technique used according to the present analysis, which is explained by the fact that it is the only technique that detects protein overexpression, given that the westernblot could be a more accurate alternative but is not used in pathological anatomy.

As our results show, the clone most commonly used for PD-L1 determination in clinical practice is clone 22C3 by Agilent/Dako, which share the Autostainer LINK 48 diagnostic platform by Dako. Also, clone SP263 by Spring/Bioscience/Ventana shares the Ventana BenchMark diagnostic platform, and it is the second most used clone according to the results of the by-determination analysis. It is noteworthy that in the year of analysis the determination of PD-L1 expression by IHC was rapidly adopted by pathology laboratories, reaching testing rates higher than those of ROS1.[14] However, the results of the present analysis show how in the same year the use of NGS is almost marginal, so future efforts in biomarker diagnosis should focus on the implementation of NGS in hospital pathology departments.

Following the discovery of new low-frequency abnormalities and due to the limited resources of the diagnostic samples, there is a need to change the approach to testing.[6] The analysis of Lungpath registry carried out by Salas *et al*[14] shows that the determination of some mandatory biomarkers (EGFR/ALK/ROS1/PD-L1) was not always performed, and list among the probable causes, the sequential determination in some laboratories, and the lack of sample material or the poor quality of the sample containing insufficient tumour cell percentage to determine all biomarkers.[14] NGS is a multigene testing technique that allows sample optimisation and that it is capable of detecting not only point mutations or insertions/deletions but also rearrangements and copy number variations.[6] As the results of the present analysis for the different biomarkers show, NGS is not a widely used technique in clinical practice in Spain, however, the needs previously discussed and also the financial burden of molecular testing warrants the establishment of a routine and more comprehensive molecular assessment with targeted NGS.[1 6] In this respect, the creation of multidisciplinary committees for analysing the molecular diagnoses will facilitate and optimise the diagnosis of the NSCLC patient.[6] Also, more studies assessing the cost-effectiveness of implementing NGS in anatomic pathology laboratories are needed. The results of our additional analysis of the LungPath database show that the use of NGS should be encouraged in the coming years.

In summary, LungPath database contains the largest amount of real-world data on biomarker testing in Spain, and it allows a better understanding of national diagnostic practices in LC biomarkers, represents a useful tool to analyse variations between different centres and an advance in protocol standardisation, the quality assurance and the implementation of future biomarkers.

Given the clinical and economic impact of the determination of biomarkers for the correct treatment of patients with NSCLC, the information presented both in the previous analysis[14] and in this analysis, and future studies in this field are of special interest for pathologists, for the scientific society and for the national health system.[19 20]

Take home messages

- ⇒ Lung Cancer Biomarker Testing Registry (LungPath) contains one of the largest amounts of real-world data on biomarker NSCLC testing.
- ⇒ At the time of analysis, despite the effort of Spanish hospitals in performing biomarker determination in NSCLC, there is still room for improvement, thus optimising the diagnosis of these patients.
- ⇒ The way of optimising the biomarker determination in NSCLC could be the implementation of next-generation sequencing in clinical practice for molecular characterisation in patients with cancer.

Handling editor Runjan Chetty.

Acknowledgements The coauthors thank the SEAP pulmonary pathology working group and the following hospitals for the data registration: 'Hospital Universitario Fundación Jiménez Díaz', 'Complejo Hospitalario Universitario Insular', 'Hospital Clínico Universitario de Santiago de Compostela', 'Hospital Arnau de Vilanova de Lleida', 'Hospital San Pedro de Logroño', 'Hospital Son Espases', 'Instituto Valenciano Oncológico', 'Hospital Galdakao-Usansolo', 'Hospital Dr. Josep Trueta', 'Hospital Universitario de Burgos', 'Hospital Nuestra Señora de Candelaria', 'Hospital Lozano Blesa', 'Hospital General Universitario de Alicante', 'Hospital Universitario Ramón y Cajal', 'Hospital Clínic de Barcelona', 'Hospital Sant Pau i Santa Tecla', 'Hospital Universitario Mutua de Terrassa', 'Hospital UniversitarioCentral de Asturias', 'Hospital de Basurto', 'Hospital Universitario Dr Peset', 'Hospital Universitario de Cruces', 'Hospital Universitario de Canarias', 'Hospital Vall d'Hebron', 'Hospital del Mar', 'Consorci Sanitari Integral', 'Hospital Puerta del Mar', 'Hospital Universitario Puerta de Hierro', 'Hospital Universitario Virgen de la Victoria', 'Hospital Universitario Campus de la Salud', 'Hospital Universitario La Paz', 'Hospital Clínico San Carlos', 'Hospital Reina Sofía', 'Hospital de Cabueñes', 'Hospital Universitari Sant Joan de Reus', 'Hospital Virgen de la Salud', 'Hospital Universitario de Cartagena', 'Hospital Universitario La Fé', 'Hospital General San Jorge', 'Onkologikoa', 'Hospital Universitario de Gran Canaria Dr. Negrín', 'Hospital Universitario Joan XXIII', 'Llebalust Patología SL', 'Hospital Ernest Lluch Martin', 'Hospital Universitario General de Castellón'.

Contributors All authors contributed substantially to the development of the study. CS acts as guarantor. DC developed the analysis and wrote the first draft of the manuscript. All the authors read and approved the final manuscript.

Funding This study was funded by Roche Farma S.A.

Competing interests CS has no conflicts of interest to declare outside the submitted work. JM-L has received personal fees from Bristol-Myers Squibb, Pfizer and Roche and research grants from Roche. AM-P has no conflicts of interest to declare outside the submitted work. TH-I has received support from ROCHE for teaching several courses on quality control. JFG received research grant support from Pfizer and Roche and received speaker or consulting fees from Roche, BMS, MSD, Merck, Novartis, Pfizer, AstraZeneca, Genomic Health, Bayer. LRdA and JFG are employees of Roche. DC is employee of Hygeia Consulting which received funding from Roche to conduct the analysis.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement Data are available on reasonable request. All data relevant to the study are included in the article. Qualified researchers may request access to individual patient level data through the clinical study data request platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available here (https://vivli.org/members/ourmembers/). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm).

ORCID iD
David Carcedo http://orcid.org/0000-0002-2809-7399

REFERENCES

1 Ryska A, Berzinec P, Brcic L, *et al*. Nsclc molecular testing in central and eastern European countries. *BMC Cancer* 2018;18:269.
2 Howlader N, Forjaz G, Mooradian MJ, *et al*. The effect of advances in lung-cancer treatment on population mortality. *N Engl J Med* 2020;383:640–9.
3 Hynds RE, Frese KK, Pearce DR, *et al*. Progress towards non-small-cell lung cancer models that represent clinical evolutionary trajectories. *Open Biol* 2021;11:200247.
4 Remon J, Besse B. Brain metastases in Oncogene-Addicted non-small cell lung cancer patients: incidence and treatment. *Front Oncol* 2018;8:88.
5 Sampsonas F, Ryan D, McPhillips D, *et al*. Molecular testing and personalized treatment of lung cancer. *Curr Mol Pharmacol* 2014;7:22–32.
6 Garrido P, Conde E, de Castro J, *et al*. Updated guidelines for predictive biomarker testing in advanced non-small-cell lung cancer: a national consensus of the Spanish Society of pathology and the Spanish Society of medical oncology. *Clin Transl Oncol* 2020;22:989–1003.
7 Imyanitov EN, Iyevleva AG, Levchenko EV. Molecular testing and targeted therapy for non-small cell lung cancer: current status and perspectives. *Crit Rev Oncol Hematol* 2021;157:103194.
8 Lin C, Shi X, Yang S, *et al*. Comparison of ALK detection by fish, IHC and NGS to predict benefit from crizotinib in advanced non-small-cell lung cancer. *Lung Cancer* 2019;131:62–8.
9 Letovanec I, Finn S, Zygoura P, *et al*. Evaluation of NGS and RT-PCR methods for ALK rearrangement in European NSCLC patients: results from the European thoracic oncology platform Lungscape project. *J Thorac Oncol* 2018;13:413–25.
10 Doshi S, Ray D, Stein K, *et al*. Economic analysis of alternative strategies for detection of ALK rearrangements in non small cell lung cancer. *Diagnostics* 2016;6. doi:10.3390/diagnostics6010004. [Epub ahead of print: 06 01 2016].
11 Passaro A, Mok T, Peters S, *et al*. Recent advances on the role of EGFR tyrosine kinase inhibitors in the management of NSCLC with uncommon, non exon 20 insertions, EGFR mutations. *J Thorac Oncol* 2021;16:764–73.
12 Soto JL, Blanco I, Díez O, *et al*. Consensus document on the implementation of next generation sequencing in the genetic diagnosis of hereditary cancer. *Med Clin* 2018;151:80.e1–80.e10.
13 Mosele F, Remon J, Mateo J, *et al*. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO precision medicine Working group. *Ann Oncol* 2020;31:1491–505.
14 Salas C, Martín-López J, Martínez-Pozo A. 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). *J Clin Pathol* 2021;0:1–8.
15 López-Ríos F, de Castro J. Actualización de las recomendaciones para La determinación de biomarcadores en El carcinoma de pulmón avanzado de célula no pequeña. Consenso Nacional de la Sociedad Española de Anatomía Patológica Y de la Sociedad Española de Oncología Médica. *Rev Esp Patol* 2015;48:80–9.
16 Gómez JJ, De Castro J. Recomendaciones para La determinación de biomarcadores en El carcinoma de pulmón no microcítico avanzado. Consenso nacional de la Sociedad Española de Anatomía Patológica Y de la Sociedad Española de Oncología Médica. *Rev Esp Patol* 2012;45:14–28.
17 Lindeman NI, Cagle PT, Aisner DL, *et al*. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *J Thorac Oncol* 2018;13:323–58.
18 Russo A, Franchina T, Ricciardi G, *et al*. Heterogeneous responses to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in patients with uncommon EGFR mutations: new insights and future perspectives in this complex clinical scenario. *Int J Mol Sci* 2019;20. doi:10.3390/ijms20061431. [Epub ahead of print: 21 Mar 2019].
19 Sociedad Española de Anatomía Patológica International Academy of Pathology (SEAP-IAP). Libro Blanco 2019 de la Anatomía Patológica en España. In: *Edición Con carácter no venal ©Sociedad Española de Anatomía Patológica*, 2019: 75–126.
20 Ong FS, Das K, Wang J, *et al*. Personalized medicine and pharmacogenetic biomarkers: progress in molecular oncology testing. *Expert Rev Mol Diagn* 2012;12:593–602.