Pathology & Oncology Research (2020) 26:845–851
https://doi.org/10.1007/s12253-019-00628-x

ORIGINAL ARTICLE

# Real-World Data of the Correlation between EGFR Determination by Liquid Biopsy in Non-squamous Non-small Cell Lung Cancer (NSCLC) and the EGFR Profile in Tumor Biopsy

Teresa Soria-Comes[1] · Vicente Palomar-Abril · María Martín Ureste[1] · Mónica Tallón Guerola[2] · Inmaculada Concepción Maestu Maiques[1]

Received: 2 January 2019 / Accepted: 28 February 2019 / Published online: 7 March 2019


## Abstract

EGFR-mutated non-small cell lung cancer (NSCLC) has significant improved outcomes when treated with EGFR-tyrosine kinase inhibitors (TKI). Thus, EGFR-mutational status should be assessed at diagnosis and in the course of treatment with TKI. However, tissue samples are not always evaluable, and molecular profiling has been increasingly performed in cell-free tumor DNA (ctDNA) from blood samples. Our objective is to evaluate the reliability of ctDNA profiling in plasma samples in a real-world setting. We retrospectively analyzed the patients diagnosed with non-squamous NSCLC from May 2016 to December 2017 at Hospital Universitario Doctor Peset who had been tested for EGFR mutations in tissue and plasma samples. Both samples were sent to an external laboratory to perform the analysis by the cobas® EGFR assay. Percentage of agreement and concordance were calculated by kappa statistic. Of 102 patients reviewed, 89 were eligible. The overall EGFR mutation frequency was 18.6% for the evaluable tissue samples and 19.6% for evaluable plasma samples. Mutation status concordance between matched samples was 87.4%. Cohen's kappa index ($\kappa$) = 0.6 (sensitivity 70.6%, specificity 91.7%, positive predictive value 66.7%, negative predictive value 93%). When concordance was stablished only in stage IV tumors $\kappa = 0.7$, suggesting a higher agreement in advanced disease. This real-world data suggest that plasma is a feasible sample for ctDNA EGFR mutation assessment. Results of ctDNA molecular profiling are reliable when using a validated technique such as the cobas® EGFR assay, especially in patients that cannot undergo a tissue biopsy.

**Keywords** EGFR mutation status · Liquid biopsy · ctDNA · Cobas EGFR assay · Non-small cell lung cancer (NSCLC)

Teresa Soria-Comes and Vicente Palomar-Abril contributed equally to this work.

✉ Teresa Soria-Comes
t.soria.comes@gmail.com

Vicente Palomar-Abril
vicente.palomar.abril@gmail.com

María Martín Ureste
martin_marure@gva.es

Mónica Tallón Guerola
motague@gmail.com

Inmaculada Concepción Maestu Maiques
inma.maestu@gmail.com

[1] Department of Medical Oncology, Hospital Universitario Doctor Peset, 46017 Comunitat Valenciana, Valencia, Spain

[2] Department of Medical Oncology, Hospital Universitario Doctor Peset, Comunitat Valenciana, Valencia, Spain

## Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, with approximately 1.6 million deaths in 2012 [1]. Non-small cell lung cancer (NSCLC) accounts for around 85% of all cases, and despite the recent development of new treatments for this disease in advanced setting, the prognosis remains poor and overall survival is only 15% after 5 years of diagnosis [2]. However, that is somehow different for tumors with driver mutations.

One of the recent greatest achievements in expanding knowledge on the natural history of NSCLC was the discovery of oncogenes that work as driver mutations for the origin and development of cancer, because they have shown to be prognostic and predictive factors. Among them, EGFR mutations are found in 10–15% of cases of NSCLC in our environment, especially in

adenocarcinoma histology and in non-smoker patients [3]. Their importance has led to the development of techniques to assess the lung tumor molecular profile.

Determination of EGFR mutations in tissue samples has been classically considered the gold standard. However, this is frequently difficult in lung cancer because of limited sample and due to the need of performing invasive procedures repeatedly during the course of the illness. That is why, in recent years there has been a great interest in the detection of EGFR mutation determinations in blood samples. This procedure has been generically called 'liquid biopsy'. The most commonly used technologies for mutation detection in ctDNA are allele–specific PCR, Scorpion Amplified Refractory Mutation System (ARMS) PCR, droplet digital PCR (ddPCR), and next generation sequencing (NGS) [4].

Although the mechanisms by which circulating tumor DNA (ctDNA) is released into the bloodstream remains unclear it has been related to apoptosis and necrosis, mainly in advanced disease [5]. Concordant NSCLC driver gene mutations between ctDNA and primary tumor DNA has been reported by several groups, indicating that liquid biopsy could represent a minimally invasive alternative to tissue biopsy. That is why our main objective is to verify if this correlation is also found in clinical practice in our environment.

## Materials and Methods

### Study Design

We performed a retrospective observational study including patients diagnosed with non-squamous NSCLC in which EGFR mutational status was determined by ctDNA analysis in liquid biopsy and also by DNA study in tissue sample between May 2016 and December 2017 at Hospital Universitario Doctor Peset (Valencia, Spain). Clinical staging was defined according to the guidelines of the tumor-node-metastasis (TNM) staging system of the Union for International Cancer Control (8th Edition) [6].

Patients older than 18 years with a histopathological diagnosis of non-squamous NSCLC were included, unless their clinical or EGFR profiling information was not available. The demographic and clinicopathologic characteristics were recorded and archived in the hospital informatics system.

### Tissue Samples and EGFR Mutation Testing

We retrospectively recorded the results of tumor tissue samples from each patient included. EGFR testing was performed in diagnostic samples obtained by core or fine-needle aspiration biopsy and also in surgical specimens if they were available. They were paraffin embedded and mounted on a microscope slide; eligible samples were defined histopathologically and they had to contain at least 10–20% of tumor tissue. Genotyping of EGFR mutations was conducted by the platform Biomarker Point (Barcelona, Spain), using a validated allele-specific quantitative PCR-based method (the Roche cobas® EGFR assay).

### Plasma Obtainment, Storage and EGFR Mutation Testing

As a part of the diagnostic assessment a blood sample was collected from the patients for DNA extraction, either at the time of diagnosis or when a re-biopsy procedure was performed in the course of the disease. Approximately a 5–8 ml sample was obtained by venipuncture, initially in EDTAK2 tubes and subsequently (from January 2017) in BD Vacutainer® Plasma Preparation Tubes (PPT™) (both recommended by manufacturer). The blood samples were centrifuged at 1800 rpm during 10 min within the first 2 h following the extraction. Then the supernatant plasma was harvested with a sterile Eppendorf Pipette and stored at −20 °C. Samples were also shipped on dry ice to the platform Biomarker Point (Barcelona, Spain) during the following 48–72 h. The study of ctDNA in plasma samples was performed by the validated allele-specific quantitative PCR-based method (the Roche cobas® Mutation Test v2).

### Statistical Analysis

Frequency tabulation and summary statistics were listed to characterize the data distributions. Also, the sensitivity, specificity, positive predictive value (PPV) and negative predicted value (NPV) were calculated for the EGFR mutation testing in plasma samples, using EGFR mutation testing in tumor tissue as a reference. Percentage of concordance between both techniques was determined for the evaluable population (all eligible patients with known tumor and plasma sample mutation status). Furthermore, the inter-test agreement was assessed (also in the evaluable population) using the Cohen's kappa coefficient or kappa statistic ($\kappa$). As recommended [7], kappa statistic was interpreted as follows: $\kappa = 0.01$–$0.2$, slight agreement; $\kappa = 0.21$–$0.4$, fair agreement; $\kappa = 0.41$–$0.6$, moderate agreement; $\kappa = 0.61$–$0.8$, substantial agreement and $\kappa =$ 0.81–0.99, almost perfect agreement.

## Results

### Patients

The demographic characteristics of the patients, the tumor type, and mutational EGFR status are summarized in Table 1. The age of the patients ranged from 39 to

**Table 1** Patient demographics and clinical characteristics of patients diagnosed with non-squamous NSCLC

| | Patients ($n = 102$) |
|---|---|
| Age (years) | |
| Median (range) | 65 (39–91) |
| Sex – no. (%) | |
| Male | 68 (66.7) |
| Female | 34 (33.3) |
| Smoking status – no. (%) | |
| Non-smoker | 19 (18.6) |
| Current smoker | 46 (45.1) |
| Ex-smoker | 37 (36.3) |
| ECOG performance status score – no. (%) | |
| 0 | 21 (20.6) |
| 1 | 50 (49) |
| 2 | 25 (24.5) |
| 3 | 6 (5.9) |
| Histology – no. (%) | |
| Adenocarcinoma | 96 (94.1) |
| Other | 6 (5.9) |
| Tissue EGFR status – no. (%) | |
| Wild-type | 76 (74.5) |
| Mutated | 19 (18.6) |
| Unknown/Invalid | 7 (6.9) |
| Liquid biopsy EGFR status – no. (%) | |
| Wild-type | 76 (74.5) |
| Mutated | 20 (19.6) |
| Invalid | 6 (5.9) |
| Clinical stage – no. (%) | |
| I | 4 (3.9) |
| II | 6 (5.9) |
| III | 13 (12.7) |
| IV | 79 (77.5) |

91 years and 66.7% patients were female. Most patients had a previous or current history of smoking habit (81.4%). The rate of EGFR mutations was around 18–20% in our study population.

### Invalid Test Rate

Of the 102 specimens that were evaluated for EGFR mutational status in both tissue and plasma samples 6 gave invalid test results at plasma ctDNA, resulting in an invalid test rate for plasma detection of 5.9% (6/102). In the case of the detection of EGFR mutations in tissue samples, 7 results were not valid (invalid test rate 6.9% (7/102)), 3 of which were obtained from cytologic samples and 4 from biopsy samples.

### EGFR Mutational Status

The overall EGFR mutation frequency was 18.6% for the evaluable biopsy/cytologic samples and 19.6% for evaluable plasma samples. There were 89 patients for whom we could gather the information on both tissue biopsy/cytology and plasma EGFR mutation status and this was considered the evaluable population. A summary of the mutational status distribution is shown in Tables 2 and 3. All of the mutations detected were found in exons 19 (deletions in exon 19) and 21 (mutation L858R in exon 21).

### Agreement Analysis

Of the 89 patients that were considered evaluable the result was consistent in 87.4% cases (Fig. 1). Plasma ctDNA evaluation detected 12/17 EGFR mutations from EGFR mutant tissue samples, resulting in a sensitivity of 70.6%. However, in 66/72 patients, mutation was not detected in plasma samples, resulting in a specificity of 91.7%. PPV was 66.7% and NPV was 93% (Table 4).

We established concordance between both techniques calculating the Cohen's kappa index, resulting in $\kappa = 0.6$, which indicates a moderate agreement between plasma ctDNA and tissue DNA observations. Furthermore, the same test was completed considering only patients diagnosed with stage IV non-squamous NSCLC ($n = 68$), with a result of $\kappa = 0.7$, which indicates a substantial agreement, suggesting that in patients with more advanced disease ctDNA is more easily detected.

## Discussion

Advances in molecular profiling of NSCLC have led to the classification of lung cancers into different biological subtypes that have prognostic implications and predict response to specific treatments [8]. Therefore, outcome in patients harboring an EGFR mutation with EGFR-TKI (tyrosine kinase inhibitors) directed therapies is one of the most favorable that can be seen in lung cancer patients.

**Table 2** Summary of the EGFR mutational status from non-squamous NSCLC patients

| | | TISSUE | | | |
|---|---|---|---|---|---|
| EGFR status | | Positive | Wild-type | Invalid | *Total* |
| PLASMA | Positive | 12 | 6 | 2 | *20* |
| | Wild-type | 5 | 66 | 5 | *76* |
| | Invalid | 2 | 4 | 0 | *6* |
| | *Total* | *19* | *76* | *7* | *102* |

**Table 3** Distribution of EGFR mutation types

| | TISSUE | PLASMA |
|---|---|---|
| Del19 | 11 | 16 |
| Mut21 | 8 | 4 |

Del19: deletions in exon 19; Mut21: mutation L858R in exon 21

**Table 4** Concordance of EGFR mutation status between matched tissue/cytologic and plasma samples in the evaluable population

| Characteristic | % | 95% CI |
|---|---|---|
| Sensitivity | 70.6 | 48.9–92.2 |
| Specificity | 91.7 | 85.8–97.5 |
| Positive predictive value | 66.7 | 43.7–83.7 |
| Negative predictive value | 93.0 | 84.6–97.0 |

Unfortunately, patients that are initially sensitive to first and second-generation EGFR-TKIs acquire resistance in the course of treatment, with a median PFS reported to be around 9–11 months [9, 10] and about half of these resistances are due to the presence of the T790 M mutation in EGFR [11]. In recent years, third-generation molecules have been developed to inhibit both EGFR sensitizing mutations and T790 M resistance mutation [12]. Consequently, it is important to re-assess EGFR mutation status when a progressive disease is observed in order to be able to choose the most appropriate therapy. However, assessing the EGFR mutation status in tissue sample is not always possible because re-biopsing the tumor is an invasive procedure with risk of complications, and even when it can be performed, obtaining sufficient sample to perform molecular testing is a challenge. That is why analysis of ctDNA is gaining relevance as a minimally invasive alternative method for the detection of EGFR mutations [13].

Several technologies have been evaluated for the detection of EGFR mutations using plasma ctDNA. In the present study we analyzed the correlation between EGFR mutation status in plasma and tissue samples using the Roche cobas® EGFR assay in a real-world setting.

The cobas® EGFR assay is a validated allele-specific quantitative PCR-based method which uses selective oligonucleotide probes for targeted mutations located in exons 18, 19, 20 and 21 labeled with a fluorescent reporter [14]. Its workflow is based on two major procedures: firstly, manually extracting genome DNA from tissue or plasma samples and secondly, PCR amplification to detect mutations in the EGFR gene by measuring fluorescence. This has proved to be a simple and fast method for detecting the most frequent clinically significative EGFR mutations and that is why it was implemented in our hospital, being performed by an external laboratory.

In randomized clinical trials, the cobas® EGFR mutation test in plasma samples has shown concordance rates higher than 90% compared with tissue samples and a sensitivity ranging from 85 to 100% [14, 15]. However, it is uncertain if these promising results are alike in a real-world setting. We are aware that this series is limited by its retrospective nature and being based on a single center experience. Nevertheless, there is a need to confirm the reported findings reported in controlled clinical trials [16, 17] and in larger centers experience to improve local practice and to make us able to provide our patients with the most accurate diagnosis and personalized treatment options. Moreover, real-world data studies have shown differences between results in ctDNA testing depending on the geographic region [18], which demonstrates that ctDNA assessment methodology needs reassurance.

The mutation status concordance observed in our population (87.4%) and the assessed inter-test agreement ($\kappa = 0.6$) suggest that plasma is a feasible sample for real-world EGFR mutation analysis even though tissue sample analysis is still considered the gold standard [13]. Furthermore, the results are even more encouraging if we only consider stage IV NSCLC, where $\kappa = 0{,}7$, indicating a substantial agreement between tumor and plasma EGFR mutation status [19].

Notwithstanding these promising results, we analyzed possible reasons for the low positive predictive value obtained (PPV = 66.7%). Firstly, in almost 20% of patients, EGFR

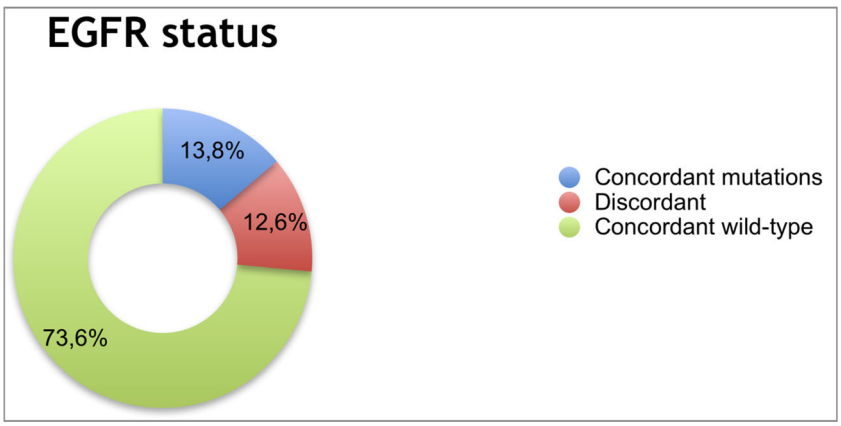


**Fig. 1** Percentage of concordant (wild-type and mutations) and discordant mutations in matched tissue and plasma mutations

mutational tissue status was determined in cytologic samples, which may not be representative of the disease; and the percentage was higher in discordant results (42.9%). Also, some of the differences observed among tissue and plasma samples could be due to the different timing of the diagnostic procedures. Therefore, the low PPV could be due to false-negative results in tissue samples, that can occur because of tumor heterogeneity or initial inexperience in the workflow procedure, rather than because of false-positive results in plasma samples.

Our results were more consistent with other studies of real-world basis [18, 20]. They reported values of sensitivity around 50% and specificity around 95%. PPV was 82% in the Asian and Russian study and 78% in the Japanese and European study (specifically, 70% in the European cohort), and NPV was shown to be around 80–90%.

This study however, has limitations. Besides its retrospective nature and the small sample size, not all of the patients diagnosed with non-squamous NSCLC in our hospital during 2016 and 2017 were tested for EGFR mutations in plasma, and patients that did not have both determinations (tissue and plasma) were not included in our analysis, and these may explain why the prevalence of EGFR mutation is higher in our series than in previous publications [21]. Molecular profiling in ctDNA was not initially included in our diagnostic protocol, and it was performed according to the physician's criteria, but it was added subsequently.

Finally, as our samples are externally studied, our major concern is not he DNA extraction, but the pre-analytical workflow. In our case, all these procedures are performed by the same trained personnel following manufacturer instructions, which reduces the possibility of compromising the correct workflow. Importantly, in 2017 we started using BD Vacutainer® Plasma Preparation Tubes (PPT™) which allow a longer interval between the extraction of the blood sample and its processing. Shipping is also carefully scheduled, and plasma samples are transported on dry ice. Although shipping is also a source of possible mismanagement of the samples, we did not record any problems in this area during the time of collecting the data.

More sensitive techniques such as next generation sequencing (NGS) are being used in clinical trials and in large hospitals to evaluate the mutational status in patients diagnosed with NSCLC [22–24]. Nevertheless, these systems are not widely available, and therefore, we have to make an effort to offer our patients the optimal mutation analysis of both tissue and plasma samples that is accessible in our centers. With validated allele-specific quantitative PCR-based methods like the Roche cobas® mutation test that can be performed also in ctDNA [14] we are given the potential of real-time monitoring of tumor mutation status using a minimally invasive procedure.

## Conclusions

Taken together, our results suggest that assessing the mutational status of the EGFR gene in plasma ctDNA is an equally robust method in detecting the most common EGFR mutations as performing the same technique in tumor DNA, which is considered the gold standard. Furthermore, it is a feasible technique highly useful in patients in which an invasive procedure such as a tissue biopsy cannot be performed because of anatomic difficulties, impaired performance status or comorbidities.

**Acknowledgements** The authors would like to express our gratitude to Joanna Gołąb for her contribution providing language help for the manuscript.

**Author Contributions** Concept and design of the study: Teresa Soria-Comes, Vicente Palomar-Abril, Inmaculada Concepción Maestu Maiques.

Data collection: Teresa Soria-Comes, Mónica Tallón Guerola, María Martín Ureste.

Analysis and interpretation of data: Teresa Soria-Comes, Vicente Palomar-Abril.

Draft manuscript: Teresa Soria-Comes, Vicente Palomar-Abril.

Critical review of the manuscript: All authors.

Final approval of the manuscript: All authors.

## Compliance with Ethical Standards

**Informed Consent** The study was conducted in accordance with the Declaration of Helsinki, written informed consent was exempted because of the retrospective nature of the study.

This manuscript has not been published and is not under consideration for publication elsewhere.

**Conflict of Interest** The authors declare that they have no conflicts of interest.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References

1. Cancer Incidence IARC (2012) Mortality and prevalence worldwide. In: GLOBOCAN
2. Malvezzi M, Carioli G, Bertuccio P, Boffetta P, Levi F, La Vecchia C et al (2017) European cancer mortality predictions for the year 2017, with focus on lung cancer. Ann Oncol 28:1117–1123. https://doi.org/10.1093/annonc/mdx033
3. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, Majem M, Lopez-Vivanco G, Isla D, Provencio M, Insa A, Massuti B, Gonzalez-Larriba JL, Paz-Ares L, Bover I, Garcia-Campelo R, Moreno MA, Catot S, Rolfo C, Reguart N, Palmero R, Sánchez JM, Bastus R, Mayo C, Bertran-Alamillo J, Molina MA, Sanchez JJ, Taron M (2009) Screening for epidermal growth factor receptor mutations in lung Cancer. N Engl J Med 361:958–967. https://doi.org/10.1056/NEJMoa0904554
4. Janku F, Angenendt P, Tsimberidou AM, Fu S, Naing A, Falchook GS, Hong DS, Holley VR, Cabrilo G, Wheler JJ, Piha-Paul SA, Zinner RG, Bedikian AY, Overman MJ, Kee

BK, Kim KB, Kopetz SE, Luthra R, Diehl F, Meric-Bernstam F, Kurzrock R (2015) Actionable mutations in plasma cell-free DNA in patients with advanced cancers referred for experimental targeted therapies. Oncotarget 6: 12809–12821. https://doi.org/10.18632/oncotarget.3373

5. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao Y, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SKN, Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih M, Oba-Shinjo SM, Siena S, Theodorescu D, Tie J, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing D, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Diaz LA (2014) Detection of circulating tumor DNA in early and late stage human malignancies. Sci Transl Med 6:224ra24. https://doi.org/10.1126/scitranslmed.3007094
6. Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WEE, Nicholson AG, Groome P, Mitchell A, Bolejack V, Goldstraw P, Rami-Porta R, Asamura H, Ball D, Beer DG, Beyruti R, Bolejack V, Chansky K, Crowley J, Detterbeck F, Erich Eberhardt WE, Edwards J, Galateau-Sallé F, Giroux D, Gleeson F, Groome P, Huang J, Kennedy C, Kim J, Kim YT, Kingsbury L, Kondo H, Krasnik M, Kubota K, Lerut A, Lyons G, Marino M, Marom EM, van Meerbeeck J, Mitchell A, Nakano T, Nicholson AG, Nowak A, Peake M, Rice T, Rosenzweig K, Ruffini E, Rusch V, Saijo N, van Schil P, Sculier JP, Shemanski L, Stratton K, Suzuki K, Tachimori Y, Thomas CF Jr, Travis W, Tsao MS, Turrisi A, Vansteenkiste J, Watanabe H, Wu YL, Baas P, Erasmus J, Hasegawa S, Inai K, Kernstine K, Kindler H, Krug L, Nackaerts K, Pass H, Rice D, Falkson C, Filosso PL, Giaccone G, Kondo K, Lucchi M, Okumura M, Blackstone E, Abad Cavaco F, Ansótegui Barrera E, Abal Arca J, Parente Lamelas I, Arnau Obrer A, Guijarro Jorge R, Ball D, Bascom GK, Blanco Orozco AI, González Castro MA, Blum MG, Chimondeguy D, Cvijanovic V, Defranchi S, de Olaiz Navarro B, Escobar Campuzano I, Macía Vidueira I, Fernández Araujo E, Andreo García F, Fong KM, Francisco Corral G, Cerezo González S, Freixinet Gilart J, García Arangüena L, García Barajas S, Girard P, Goksel T, González Budiño MT, González Casaurrán G, Gullón Blanco JA, Hernández Hernández J, Hernández Rodríguez H, Herrero Collantes J, Iglesias Heras M, Izquierdo Elena JM, Jakobsen E, Kostas S, León Atance P, Núñez Ares A, Liao M, Losanovscky M, Lyons G, Magaroles R, de Esteban Júlvez L, Mariñán Gorospe M, McCaughan B, Kennedy C, Melchor Íñiguez R, Miravet Sorribes L, Naranjo Gozalo S, Álvarez de Arriba C, Núñez Delgado M, Padilla Alarcón J, Peñalver Cuesta JC, Park JS, Pass H, Pavón Fernández MJ, Rosenberg M, Ruffini E, Rusch V, Sánchez de Cos Escuín J, Saura Vinuesa A, Serra Mitjans M, Strand TE, Subotic D, Swisher S, Terra R, Thomas C, Tournoy K, van Schil P, Velasquez M, Wu YL, Yokoi K (2016) The IASLC lung cancer staging project: proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for lung cancer. J Thorac Oncol 11:39–51. https://doi.org/10.1016/j.jtho.2015.09.009
7. Viera AJ, Garrett JM (2005) Understanding interobserver agreement: the kappa statistic. Fam Med 37:360–363
8. Planchard D, Popat S, Kerr K, Novello S, Smit E, Faivre-Finn C et al (2018) Metastatic non-small-cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. Ann Oncol 27:v192–v237. https://doi.org/10.1093/annonc/mdw326.
9. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, Porta R, Cobo M, Garrido P, Longo F, Moran T, Insa A, de Marinis F, Corre R, Bover I, Illiano A, Dansin E, de Castro J, Milella M, Reguart N, Altavilla G, Jimenez U, Provencio M, Moreno MA, Terrasa J, Muñoz-Langa J, Valdivia J, Isla D, Domine M, Molinier O, Mazieres J, Baize N, Garcia-Campelo R, Robinet G, Rodriguez-Abreu D, Lopez-Vivanco G, Gebbia V, Ferrera-Delgado L, Bombaron P, Bernabe R, Bearz A, Artal A, Cortesi E, Rolfo C, Sanchez-Ronco M, Drozdowskyj A, Queralt C, de Aguirre I, Ramirez JL, Sanchez JJ, Molina MA, Taron M, Paz-Ares L (2012) Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 13:239–246. https://doi.org/10.1016/S1470-2045(11)70393-X
10. Sequist LV, Yang JC-H, Yamamoto N, O'Byrne K, Hirsh V, Mok T et al (2013) Phase III study of Afatinib or cisplatin plus Pemetrexed in patients with metastatic lung adenocarcinoma with *EGFR* mutations. J Clin Oncol 31:3327–3334. https://doi.org/10.1200/JCO.2012.44.2806
11. Oxnard GR, Arcila ME, Sima CS, Riely GJ, Chmielecki J, Kris MG, Pao W, Ladanyi M, Miller VA (2011) Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. Clin Cancer Res 17:1616–1622. https://doi.org/10.1158/1078-0432.CCR-10-2692
12. Goss G, Tsai CM, Shepherd FA, Bazhenova L, Lee JS, Chang GC, Crino L, Satouchi M, Chu Q, Hida T, Han JY, Juan O, Dunphy F, Nishio M, Kang JH, Majem M, Mann H, Cantarini M, Ghiorghiu S, Mitsudomi T (2016) Osimertinib for pretreated EGFR Thr790Met-positive advanced non-small-cell lung cancer (AURA2): a multicentre, open-label, single-arm, phase 2 study. Lancet Oncol 17:1643–1652. https://doi.org/10.1016/S1470-2045(16)30508-3
13. Rolfo C, Mack PC, Scagliotti GV, Baas P, Barlesi F, Bivona TG, Herbst RS, Mok TS, Peled N, Pirker R, Raez LE, Reck M, Riess JW, Sequist LV, Shepherd FA, Sholl LM, Tan DSW, Wakelee HA, Wistuba II, Wynes MW, Carbone DP, Hirsch FR, Gandara DR (2018) Liquid biopsy for advanced non-small cell lung Cancer (NSCLC): a statement paper from the IASLC. J Thorac Oncol 13: 1248–1268. https://doi.org/10.1016/j.jtho.2018.05.030
14. Malapelle U, Sirera R, Jantus-Lewintre E, Reclusa P, Calabuig-Fariñas S, Blasco A, Pisapia P, Rolfo C, Camps C (2017) Profile of the Roche cobas® EGFR mutation test v2 for non-small cell lung cancer. Expert Rev Mol Diagn 17:209–215. https://doi.org/10.1080/14737159.2017.1288568
15. Reckamp KL, Melnikova VO, Karlovich C, Sequist LV, Camidge DR, Wakelee H, Perol M, Oxnard GR, Kosco K, Croucher P, Samuelsz E, Vibat CR, Guerrero S, Geis J, Berz D, Mann E, Matheny S, Rolfe L, Raponi M, Erlander MG, Gadgeel S (2016) A highly sensitive and quantitative test platform for detection of NSCLC EGFR mutations in urine and plasma. J Thorac Oncol 11:1690–1700. https://doi.org/10.1016/j.jtho.2016.05.035
16. Douillard JY, Ostoros G, Cobo M, Ciuleanu T, Cole R, McWalter G, Walker J, Dearden S, Webster A, Milenkova T, McCormack R (2014) Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. J Thorac Oncol 9:1345–1353. https://doi.org/10.1097/JTO.0000000000000263
17. Soria J-C, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH et al (2018) Osimertinib in untreated *EGFR* -mutated advanced non–small-cell lung Cancer. N Engl J Med 2017:NEJMoa1713137. https://doi.org/10.1056/NEJMoa1713137
18. Han B, Tjulandin S, Hagiwara K, Normanno N, Wulandari L, Laktionov K, Hudoyo A, He Y, Zhang YP, Wang MZ, Liu CY,

Ratcliffe M, McCormack R, Reck M (2017) EGFR mutation prevalence in Asia-Pacific and Russian patients with advanced NSCLC of adenocarcinoma and non-adenocarcinoma histology: the IGNITE study. Lung Cancer 113:37–44. https://doi.org/10.1016/j.lungcan.2017.08.021

19. Wu Y-L, Sequist LV, Hu C-P, Feng J, Lu S, Huang Y, Li W, Hou M, Schuler M, Mok T, Yamamoto N, O'Byrne K, Hirsh V, Gibson N, Massey D, Kim M, Yang JCH (2016) EGFR mutation detection in circulating cell-free DNA of lung adenocarcinoma patients: analysis of LUX-lung 3 and 6. Br J Cancer 116:175–185. https://doi.org/10.1038/bjc.2016.420
20. Reck M, Hagiwara K, Han B, Tjulandin S, Grohé C, Yokoi T, Morabito A, Novello S, Arriola E, Molinier O, McCormack R, Ratcliffe M, Normanno N (2016) CtDNA determination of EGFR mutation status in European and Japanese patients with advanced NSCLC: the ASSESS study. J Thorac Oncol 11:1682–1689. https://doi.org/10.1016/j.jtho.2016.05.036
21. Esteban E, Majem M, Martinez Aguillo M, Martinez Banaclocha N, Dómine M, Gómez Aldaravi L, Juan O, Cajal R, Gonzalez Arenas MC, Provencio M (2015) Prevalence of EGFR mutations in newly diagnosed locally advanced or metastatic non-small cell lung cancer Spanish patients and its association with histological subtypes and clinical features: the Spanish REASON study. Cancer Epidemiol 39:291–297. https://doi.org/10.1016/j.canep.2015.02.003
22. Normanno N, Denis MG, Thress KS, Ratcliffe M, Reck M (2017) Guide to detecting epidermal growth factor receptor (EGFR) mutations in ctDNA of patients with advanced non-small-cell lung cancer. Oncotarget 8:12501–12516. https://doi.org/10.18632/oncotarget.13915
23. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A (2013) Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol 10:472–484. https://doi.org/10.1038/nrclinonc.2013.110
24. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Liquid biopsies: genotyping circulating tumor DNA. Nat Med 2014;4:224ra24-224ra24. https://doi.org/10.1158/2159-8290.CD-13-1014.Blood.