



# Routine molecular profiling of cancer: results of a one-year nationwide program of the French Cooperative Thoracic Intergroup (IFCT) for advanced non-small cell lung cancer (NSCLC) patients.

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## Title

**Routine molecular profiling of cancer: results of a one-year nationwide program of the French Cooperative Thoracic Intergroup (IFCT) for advanced non-small cell lung cancer (NSCLC) patients.**

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## ABSTRACT

**Background.** The molecular profiling of advanced non-small cell lung cancer (NSCLC) patients for known oncogenic drivers is currently recommended during routine care. However, nationally, the feasibility and impact of this policy are unknown.

**Methods.** The characteristics, molecular profiles and clinical outcomes of advanced NSCLC patients, who were routinely screened for *EGFR* mutations and *ALK* rearrangements but also *HER2*, *KRAS*, *BRAF*, and *PIK3CA* mutations by certified regional genetics centers in France, were assessed consecutively during a one-year period.

**Results.** Overall, 18,679 molecular analyses of 17,664 NSCLC patients (median age, 64.5 years; male, 64.6%; smokers or former smokers, 81.2%; adenocarcinoma, 76%) were performed. The median interval between the initiation of analysis and the written result was 11 days (interquartile range: 7-16 days). A genetic alteration was found in 49.5% of the analyses: *EGFR*, *HER2*, *KRAS*, *BRAF*, or *PIK3CA* mutations or *ALK* rearrangement in 11.0, 0.8, 28.7, 1.9, 2.3 and 4.8% of the cases, respectively. The presence of a genetic alteration impacted the first-line treatment for 51.3% of the patients and was associated with a significant improvement in the overall response rate for first- (36.5 [95%CI: 34.7-38.2] vs. 32.6% [95%CI: 29.5-35.6], p=0.03) and second-line treatment (16.9 [95%CI: 15.0-18.8] vs. 9.3% [95%CI: 6.7-11.9], p<0.001), first-line progression-free survival (10.0 [95%CI: 9.2-10.7] vs. 7.1 [95%CI: 6.1-7.9] months, p<0.001) and overall survival (16.5 [95%CI: 15.0-18.3] vs. 11.8 months [95%CI: 10.1-13.5], p<0.001).

**Conclusions.** Routine nationwide molecular profiling of advanced NSCLC patients is feasible. The frequency of the genetic alterations, the acceptable turnaround times in obtaining the analysis results and the clinical advantage provided by the detection of a genetic alteration indicates that routine nationwide molecular profiling provides a clinical benefit.

Funding: French National Cancer Institute (INCa).

## RESEARCH IN CONTEXT

Evidence before this study.

Before undertaking this study, we did a systematic review of the scientific literature (English only) published up to December 2010 using PubMed and abstracts from ASCO and ESMO meetings (2007-2010) to identify studies assessing nationwide routine molecular profiling of advanced non small cell lung cancer patients for one or more genetic alterations known (or supposed) to be oncogenic drivers. Using the search terms “non small cell lung cancer”, “advanced” or “metastatic”, and “EGFR” or “ALK” or “BRAF” or “HER2” or “PIK3CA” or “KRAS” or “multiplex” or “sequencing” and “nationwide” or names of various countries around the world, we did not identify any published data.

Added value of this study.

This study demonstrates that a routine nationwide molecular profiling of advanced NSCLC patients is feasible within an acceptable turnaround time in obtaining the results. Even if looking at a limited number of genetic alterations (i.e. presently six genes), the frequency of these genetic alterations allows to potentially consider a targeted therapy (either commercially available for EGFR and ALK or within a clinical trial for the other alterations) for treating these patients. Finally, when a genetic alteration was detected, the outcome was a longer median OS indicating a possible prognostic advantage and/or a major change in the management paradigm for these advanced NSCLC patients.

Implications of all the available evidence.

In the meantime, the LCMC initiative (the largest multi-institutional study in the western world) suggested that molecular profiling helps to orient patients toward targeted therapies

and dedicated trials and individuals with drivers receiving a matched targeted agent lived longer. Our study extends LCMC results to a nation-wide approach and the current evidence indicates that routine nationwide molecular profiling provides a clinical benefit to advanced NSCLC patients.

## INTRODUCTION

Lung cancer is among the most frequent types of cancer in Western countries and, with more than one million expected deaths per year, is the leading cause of cancer deaths.<sup>1</sup> However, the molecular hallmarks of cancer are only currently understood.<sup>2</sup> The treatment of lung cancer has entered a new era due to the discovery of epidermal growth factor receptor (*EGFR*)-activating mutations and anaplastic lymphoma kinase (*ALK*) gene rearrangements, which lead to changes in outcomes of many, but still a minority of, lung cancer patients.<sup>3,4</sup> Moreover, lung cancer displays one of the highest rates of genetic alterations,<sup>5</sup> some of which are actionable via the administration of drugs that have already been approved, are available off-label for other indications (dabrafenib or vemurafenib for *BRAF*,<sup>6</sup> trastuzumab or afatinib for *HER2* mutations,<sup>7</sup> crizotinib for *ROS1* rearrangements<sup>8</sup>) or are under investigation in clinical trials. Therefore, high expectations are placed on this personalized (also referred to as ‘stratified’ or ‘precision’) medicine.

In this context, many medical centers have been organized to provide lung cancer patients with routine assessments of *EGFR* mutations and *ALK* rearrangements. In some of these centers additional molecular alterations are tested, typically by research programs.<sup>9–12</sup> The preliminary data obtained from these previous programs suggest that molecular profiling helps to orient patients toward targeted therapies and dedicated trials. However, the actual impact of broad molecular screening and subsequent personalized medicine has yet to be addressed in a prospective randomized trial.<sup>10</sup> In addition, the characteristics and efficacy results reported by these programs are based on a limited series of selected patients. Therefore, there is a need for a wide overview in an unselected, ‘all-comer’ population to increase our understanding of the actual epidemiology of lung cancer biomarkers and of their potential impact on therapeutic strategies.

The French National Cancer Institute (i.e., INCa) funded a nationwide program for the systematic routine analysis of *EGFR* mutations and *ALK* rearrangements as well as of *HER2*, *KRAS*, *BRAF* and *PIK3CA* mutations in advanced stage non-squamous non-small cell lung cancer (NSCLC) patients in 28 certified molecular genetics centers covering the entire French territory.<sup>13–15</sup> Here, we report the results of the ‘Biomarkers France’ study, which assessed the characteristics, molecular profiles and clinical outcomes of 17,664 consecutive NSCLC patients who were screened during a one-year period by this program.

## PATIENTS AND METHODS

### Patients

All consecutive NSCLC patients who were routinely screened for molecular alterations during a one-year period at one of the 28 certified molecular genetics centers were eligible for this study. The prescription of this routine molecular screening, mandatory for advanced non-squamous NSCLC, was solely the responsibility of the treating physician. Notably, national recommendations for screening for *EGFR* mutations (both activating and T790M), *ALK* rearrangements and four ‘emerging biomarkers’ (*KRAS*, *BRAF*, *HER2* and *PIK3CACA* mutations) have been available since 2010.<sup>16</sup> In addition, NSCLC patients exhibiting a less advanced stage or patients carrying other tumor types (i.e., mixed histology, especially, never-smoker patients, etc) could have been screened upon approval by their local multidisciplinary tumor board (MTB).

### Ethics

This study was approved by a national ethics committee for observational studies (Comité d’Evaluation des Protocoles de Recherche Observationnelle, CEPRO) on 09/28/2011, by the French Advisory Committee on Information Processing in Material Research in the Field of Health (Comité Consultatif sur le Traitement de l’Information en Matière de Recherche dans le Domaine de la Santé, CCTIRS) on 09/22/2011 and by the National Commission of Informatics and Liberty (CNIL) on 12/18/2011, according to French laws, and was registered on the ClinicalTrials.gov website (NCT01700582).

Each clinician identified as the prescriber of a molecular analysis between April 2012 and April 2013 received written information describing the study protocol and the process for accessing the database as well as a confidential password to connect to the ‘Biomarkers France’ secured Web CRF.

All NSCLC patients included in this program received information from their institution or referring clinician, as recommended by competent authorities, that specified that, according to French laws, they were allowed to ask for complete access to/removal of their own collected data.

### **Study oversight**

This study was sponsored by the French Cooperative Thoracic Intergroup (IFCT) and was funded by an unrestricted grant from INCa. The steering committee included representatives of the certified molecular genetics centers, INCa, and the IFCT. The feasibility and potential technical issues of this project were initially evaluated by analyzing patients screened at three certified molecular genetics centers over a 3-month period (November 2011 to January 2012); this analysis served as a test for the subsequent nationwide study. As no major difficulty was observed for these initial 346 patients, the steering committee decided to open the one-year national recruitment period in April 2012. The 28 molecular genetics centers had to send their results to the IFCT using a specific datasheet for each patient. Then, the data were recorded and monitored by the IFCT. The authors had full access to the de-identified data and analyses for the current report.

### **Molecular analyses**

The molecular analyses of *EGFR* (NG\_007726.3), *HER2* (NG\_007503.1), *KRAS* (NG\_007524.1), *BRAF* (NG\_007873.3), and *PIK3CACA* (NG\_012113.2) mutations and of *ALK* (NG\_009445.1) rearrangements were performed on a routine basis at 28 certified molecular genetics centers (supplementary Figure 1). The methodology for these analyses<sup>16</sup> and the results of the prospective cross validation quality assessment studies were previously reported.<sup>17-19</sup> Briefly, each molecular genetics center used either the Sanger sequencing method or a more sensitive validated allele-specific technique (generally to be confirmed by Sanger sequencing) to assess *EGFR* (exons 18-21)<sup>17-18</sup>, *HER2* (exon 20), *BRAF* (exon 15),

*KRAS* (exon 2)<sup>17-19</sup> and *PIK3CACA* (exons 9 and 20) mutations (supplementary Table 1). A certified break-apart fluorescence in situ hybridization assay was used to assess *ALK* rearrangements.<sup>20</sup> In addition, each regional genetics center performed either concurrent analysis of all recommended molecular alterations in the six genes or a sequential approach in which the *EGFR* and *ALK* assessments were performed first, and then each of the other molecular alterations were assessed until a mutation was found.

### **Data collection**

The results of the molecular assessments of *EGFR*, *HER2*, *KRAS*, *BRAF*, and *PIK3CA* mutations and *ALK* rearrangements as well as histological typing and the percentage of tumor cells by the referring pathologist and the turnaround time before obtaining the analysis results (from the date of tumor reception to the date of submission of the written molecular report to the clinician) were provided directly to the IFCT by the certified molecular genetics centers. The results regarding the median time until results were obtained were expressed as first and third quartiles (Q1 and Q3) to avoid excessive data dispersion. Simultaneously, the treating physician of each patient (n=3,831) was provided with secure access to her/his own patient's data. Data on sex; ethnicity (Asian versus non-Asian); smoking history (never, former or current smoker); past familial medical history of cancer; ECOG PS (0-1 versus 2 or more); TNM stage, as defined by the seventh edition of the American Joint Committee on Cancer;<sup>21</sup> pathological diagnosis, as defined by the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classification;<sup>22</sup> and the modality of sample's collection (bronchoscopy, thoracic transthoracic biopsy, thoracic surgery or other) were collected. The type of treatment (standard chemotherapy, type of chemotherapy, targeted therapy, or, if applicable, the clinical trial along with the type of treatment), the impact of the molecular results on the treatment decision, and outcomes (overall response rate, assessed – usually by RECIST - by treating

physician; ORR; first-line and second-line, when applicable, date(s) of progression and survival status) were collected and reported per investigator review. The patients were treated on a routine basis after a local MTB and in accordance with national and international guidelines.<sup>23–25</sup> At the time the study was conducted, erlotinib and gefitinib were approved for the treatment of patients with *EGFR* mutations (including first-line) while crizotinib was available only for the second-line treatment of patients with *ALK* rearrangements. *KRAS*, *BRAF*, *HER2* and *PI3K* mutations were targetable by drugs available through clinical trials. The connection to and completion of the database were voluntarily performed by the treating physicians.

## **Objectives**

The primary objective of this study was to describe the frequency of the molecular alterations in six genes that were routinely screened via a nationwide approach in consecutive patients with NSCLC. The secondary objectives were to combine the clinical and biological databases, to document the turnaround time in obtaining the molecular results, to assess the ability of the treating physician to use these data to select an ad hoc therapy (on a standard basis or via inclusion in a clinical trial) and to measure the patients' outcomes (progression-free survival, PFS; and overall survival, OS).

## **Statistics**

Descriptive statistics, including median and range or quartiles for continuous variables or frequencies and percentages for categorical variables, were used. The median follow-up duration was defined as the time from the date of molecular analysis assessment to the closing date of the analysis. First-line PFS was defined as the time from the date of molecular analysis assessment to the date of the first progression or death due to any cause. Second-line PFS was defined as the time from second-line treatment initiation to the date of the second progression or death from any cause. The OS duration was defined as the date of the

molecular analysis assessment to the date of death or final follow-up. Survival curves were estimated globally and for groups of interest using the Kaplan-Meier method. We compared the groups of interest using the two-sided log-rank test. The characteristics (with or without mutation) of each biomarker of the patients were compared using the Chi-square test for qualitative variables or using the nonparametric test for quantitative variables. Univariate Cox models were applied to select the most promising prognostic variables (threshold  $p= 0.20$ ). A multivariate Cox model was then applied to adjust for potential confounders (clinical or molecular characteristics associated with PFS or OS). Adjusted HRs with 95% CIs were calculated. All statistical tests were two-sided, and a P-value of less than 0.05 was considered statistically significant. All analyses were performed using SAS software, version 9.3 (SAS Institute).

#### **Role of the funding source.**

The INCa funded this study. One INCa representative participated in the steering committee of the study. The funding source has no role in study design, data collection, analysis, and interpretation, and preparation of this manuscript.

## RESULTS

### Timelines and flow chart

The study recruitment period was from April 2012 to April 2013, and the database was locked for the current analysis on July 23, 2014. Overall, 19,386 results of routine molecular analyses were recorded in the database. After review, 707 analyses (3.6%) were excluded, primarily because they were related to other types of solid tumors (Figure 1). Finally, 18,679 results, representing 17,664 NSCLC patients, were analyzed.

### Patients and results of the molecular analyses

The primary characteristics of the 17,664 patients are summarized in Table 1. The number of samples analyzed per patient was typically one ( $n=16,696$ , 94.5%) but two or more samples were analyzed in 927 (5.2%) and 41 (0.2%) of the patients, respectively. The median interval between tissue specimen collection and the initiation of molecular analysis was 8 days (4-16 days), and the median interval from the initiation of molecular analysis to the final written report of the analysis (the results for EGFR mutation if the analyses were conducted sequentially) was 11 days (7-16 days).

The frequencies of *EGFR*, *HER2*, *KRAS*, *BRAF*, and *PIK3CA* mutations and of *ALK* rearrangements were 11.0, 0.8, 28.7, 1.9, 2.3 and 4.8%, respectively (Table 2 and supplementary Table 2). The frequencies of these molecular alterations in these six genes, overall and for three specific subgroups (i.e., adenocarcinoma, women, and never-smokers), are displayed in Figure 2. The screen failure rates varied from 1.4 to 4.3%. In 173 cases, two ( $n=170$ , 0.9%) or more ( $n=3$ , 0.01%) molecular alterations were identified in the same sample (supplementary Table 3).

### Treatment

For 51.3% of the cases, the results of the routine molecular profiling were considered when making the decision for the first-line therapeutic strategy; however, in 22.6% of the cases, the

turnaround time in obtaining these results motivated the local MTB to decide the treatment strategy without knowing these results. The frequencies of genetic alterations in the patients who were managed without considering the results of the molecular analyses (data not shown) were comparable to that of the overall patient population, except for *EGFR* mutations (11.3 and 13.3%, respectively; p=0.003). The types of first- and second-line treatments are summarized in Table 3. As treatment-resistant *EGFR T790M* mutations were systematically found concomitantly with an activating *EGFR* mutation, the frequencies of these two mutations are jointly reported. Radiotherapy was performed to improve local thoracic control or as a palliative treatment in 10.1 and 25.7% of patients, respectively.

### Clinical outcomes

The median follow-up duration at the time of analysis was 24.9 months (95%CI: 24.8-25.0). The outcomes of the 17,664 patients for which data were available are summarized in Table 4 (and in supplementary Table 4 for advanced stage patients only). The presence of a genetic alteration was associated with a significantly higher ORR for the first-line treatments (36.5% [95%CI: 34.7-38.2] versus in the absence of a genetic alteration (32.6% [95%CI: 29.5-35.6]) (p=0.03) and for the second-line treatments (16.9% [95%CI: 15.0-18.8]) versus in the absence of a genetic alteration (9.3% [95%CI: 6.7-11.9]) (p<0.001). The presence of a genetic alteration was also associated with significantly longer first-line PFS and OS (Figure 3 and supplementary Figure 2). When excluding the patients carrying an *EGFR* mutation, the presence of a genetic alteration (*versus* absence) resulted in a non-significant difference in ORR in first-line (31.4% [95%CI: 29.4-33.5] *versus* 32.6% [95%CI: 29.5-35.7]; p=0.54) or second-line (11% [95%CI: 9.1-12.8] *versus* 9.3% [95%CI: 6.7-11.9]; p=0.34) and OS (13.3 months [95%CI: 12.1-14.3] *versus* 11.8 months [95%CI: 10.1-13.4]; p=0.37). Cox multivariate analysis confirmed that *ALK* rearrangements (HR=0.70 [95%CI: 0.5-0.9]),

*EGFR* (HR=0.53 [95%CI: 0.4-0.6]) and *HER2* mutations (HR=0.60 [95%CI: 0.4-1.0]) had a favorable prognostic impact (supplementary Table 5).

## DISCUSSION

One challenge of personalized medicine is the provision of all cancer patients with an assessment of molecular alterations that are related to the management of their disease. The results reported in this study illustrate the success of the nationwide, INCa-organized program in this setting. The molecular screening performed in the current program, which involved more than 20,000 advanced NSCLC patients per year, enabled the detection with an acceptable turnaround time of at least one potentially actionable molecular alteration in almost 50% of the analyses and impacted the treatment decisions for 51.3% of the included patients. When a genetic alteration was detected, the *primary* outcome was a 4.7 months longer median OS indicating a possible prognostic advantage and/or a major change in the management paradigm for these lung cancer patients.

The successful implementation of molecular profiling of lung cancer patients at a single institution or in a consortium of institutions has been reported previously.<sup>8-11</sup> However, the number of examined patients was frequently small, as the largest number reported previously was 1007 patients. The LCMC initiative was the largest multi-institutional study in the western world. Our study follows LCMC but aims at broadening the number of centers able to provide molecular profiling with a clear ambition to be a nation-wide approach. Considering that 39,000 new lung cancer cases (any stage and histology) are reported each year in France,<sup>26</sup> 18,000 advanced non-squamous NSCLC patients represent the number of patients which is expected to be screened for *EGFR* mutations and *ALK* rearrangements according to the current guidelines.<sup>23-25</sup> With 17,664 patients, the results reported here not only involved the largest sample but also were unlikely to have been influenced by the patients' selection of a specific institution or participation in a given clinical trial or research program.<sup>27</sup>

The frequency of some molecular alterations might appear to be lower than previously reported (11% of *EGFR* mutations compared with 17% for LCMC in the US),<sup>9</sup> but the present results most likely more closely reflect the characteristics of a global population, particularly in Western countries. To the best of our knowledge, no other study at a nationwide level has been published to date. Therefore, these results provide solid evidence for clinical trials or routine programs of molecular screening of lung cancer patients, especially considering the *HER2*, *BRAF* and *PIK3CA* data because very rare genetic alterations (0.8, 1.9 and 2.3%, respectively) could still represent a large population given the high incidence of lung cancer worldwide.

This study attempted to collect data on a common cancer population from daily practice during a one-year period. Based on this objective, a relatively simple case report form was selected and more than 3,800 treating physicians were approached for the study. This design implied the acceptance of some degree of missing data, which was a drawback of this study. Another limitation of the study is related to the molecular alterations that were screened. Several potential actionable targets for lung cancer have been described in recent years, and some of these targets were not included in the program reported here. The molecular alterations screened in this program were selected in 2009 and this strategy was primarily a success, as the data for *BRAF*<sup>6</sup> and *HER2*<sup>7</sup> targeting are now robust. On the other hand, other emerging biomarkers, such as *KRAS* mutations, remained uncertain.<sup>28</sup> *PIK3CA* mutations are no longer routinely assessed, while *ROS1* assessment is now part of the routine molecular testing at certified molecular genetics centers in France.<sup>29</sup> More importantly, the results reported here do not suggest an improvement in the inclusion rate of clinical trials. As only 3% of the patients in the national database have been enrolled in clinical trials while being assessed for molecular alterations that were actionable only using experimental compounds in clinical development, this objective of this national program remains to be met.<sup>29</sup>

In conclusion, this national program broadly (and very exhaustively) screened lung cancer patients for genetic alterations in six genes, including four emerging genetic alterations, to identify actionable targets that improved the survival of approximately 50% of these patients, although at a non-negligible financial cost.<sup>16</sup> Therefore, these results encourage all ongoing worldwide initiatives to provide cancer patients with access to personalized medicine and provide robust information to organize these initiatives.

**The Biomarkers France contributors (listed here are the representatives of each regional molecular genetics center and/or the pathologist who collaborated in this project and the treating physicians who provided data for 40 patients or more) not included in the list of authors:**

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FB, JM, DD, FM, BM, QT, JC, JCS et GZ designed the study, all the authors participated in the data acquisition, FB, JM, FM, QT, PM, AL, JC, JCS, and GZ analyzed and interpret the data, FB, JM, FM, QT, PM, AL, JC, JCS, and GZ wrote the manuscript; the final version was approved by all the authors. No writing assistance other than copy editing was provided to the authors.

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**Table 1: Characteristics of the 17,664 patients whose results of routine molecular analyses were available in the database.**

Data availability		N	%
N (%)			
Age, years (median, range)	17,664	64.5 (18-98)	
<b>Sex</b>	17,555	Male	11,346 64.6
		Female	6,209 35.4
<b>Ethnicity</b>	7,350	Asian	96 1.3
		Caucasian or other	7,254 98.7
<b>Smoking history</b>	8,619	Never	1,619 18.8
		Former smoker	3,597 41.7
		Current smoker	3,403 39.5
<b>ECOG PS</b>	7,817	0/1	5,607 71.7
		2	1,423 18.2
		3-4	787 10.1
<b>Previous cancer</b>	7,848	Within family	961 12.2
<b>TNM 2007 stage</b>	8,637	I/II	1,392 16.1
		III/IV/relapse	7,245 83.9
<b>Histology</b>	17,664	Adenocarcinoma	13,425 76.0
		Squamous	877 5.0

		Large cell carcinoma	589	3.3
		NOS or other histology	2,773	15.7
<b>Sample collection</b>	17,664	Bronchoscopy	5,038	28.5
		CT-guided transthoracic biopsy	4,229	23.9
		Surgery	4,712	26.7
		Others/unknown	3,685	20.9
<b>Samples analyzed per patient</b>	17,664	1	16,696	94.5
		>1	968	5.5
<b>Turnaround time, days</b>	18,679*	From sample collection to initiation of analysis	8.0 (4.0-16.0)	-
<b>(median, Q1-Q3)</b>		From initiation of analysis to report of results	11.0 (7.0-16.0)	-

*\*, the turnaround times were reported by analysts and not by patients.*

**Table 2: Results of the 18,679 molecular analyses stratified by clinical characteristics.**

EGFR, N (%)				KRAS, N (%)				BRAF, N (%)			
	M	R (T790M)	WT	UNK	M	WT	UNK	M	WT	UNK	
N (%)	1,786 (9.5)	161 (0.9)	15,759 (84.4)	973 (5.2)	4,894 (26.2)	12,107 (64.8)	1,678 (9.0)	262 (1.4)	13,644 (73.0)	4,773 (25.6)	
<b>Age (median)</b>	68.4 <sup>§</sup>	65.7 <sup>§</sup>	64.5 <sup>§</sup>	65.8 <sup>§</sup>	63.3 <sup>§</sup>	65.4 <sup>§</sup>	66.6 <sup>§</sup>	65.9	64.7	65.7	
<b>Sex*</b>	Male	568 (31.8) <sup>§</sup>	49 (30.4) <sup>§</sup>	10,699 (67.9) <sup>§</sup>	631 (64.9) <sup>§</sup>	3,245 (66.3) <sup>§</sup>	7,698 (63.6) <sup>§</sup>	1,004 (59.8) <sup>§</sup>	160 (61.1)	8,881 (65.1)	2,906 (60.9)
	Female	1,208 (67.6) <sup>§</sup>	111 (68.9) <sup>§</sup>	4,963 (31.5) <sup>§</sup>	339 (34.8) <sup>§</sup>	1,621 (33.1) <sup>§</sup>	4,331 (35.8) <sup>§</sup>	669 (39.9) <sup>§</sup>	101 (38.5)	4,686 (34.3)	1,834 (38.4)
<b>Ethnicity</b>	Asian	49 (5.0) <sup>§</sup>	6 (7.1) <sup>§</sup>	46 (0.7) <sup>§</sup>	7 (1.6) <sup>§</sup>	15 (0.8) <sup>§</sup>	79 (1.5) <sup>§</sup>	14 (1.8) <sup>§</sup>	0	72 (1.2)	36 (1.9)
	Other	938 (95.0) <sup>§</sup>	79 (92.9) <sup>§</sup>	6,365 (99.3) <sup>§</sup>	421 (98.4) <sup>§</sup>	1,954 (99.2) <sup>§</sup>	5,101 (98.5) <sup>§</sup>	748 (98.2) <sup>§</sup>	150 (100)	5,800 (98.8)	1,853 (98.1)
<b>Smoking history</b>	Never	683 (59.9) <sup>§</sup>	53 (57.0) <sup>§</sup>	939 (12.4) <sup>§</sup>	98 (20.4) <sup>§</sup>	138 (5.9) <sup>§</sup>	1,397 (23.0) <sup>§</sup>	238 (28.0) <sup>§</sup>	41 (25.0)	1,229 (18.0)	503 (22.0)
	Former	316 (27.7) <sup>§</sup>	31 (33.3) <sup>§</sup>	3,305 (43.7) <sup>§</sup>	213 (44.3) <sup>§</sup>	1,104 (46.9) <sup>§</sup>	2,410 (39.7) <sup>§</sup>	351 (41.3) <sup>§</sup>	63 (38.4)	2,887 (42.3)	915 (40.1)
	Current	142 (12.4) <sup>§</sup>	9 (9.7) <sup>§</sup>	3,312 (43.8) <sup>§</sup>	170 (35.3) <sup>§</sup>	1,113 (47.3) <sup>§</sup>	2,260 (37.3) <sup>§</sup>	260 (30.6) <sup>§</sup>	60 (36.6)	2,709 (39.7)	864 (37.9)
<b>ECOG PS</b>	0 or 1	784 (76.0) <sup>§</sup>	71 (78.0) <sup>§</sup>	4,916 (71.8) <sup>§</sup>	314 (70.4) <sup>§</sup>	1,526 (71.5)	4,028 (73.2)	531 (68.0)	109 (74.1)	4,538 (72.7)	1,438 (70.8)
	>2	247 (24.0) <sup>§</sup>	20 (22.0) <sup>§</sup>	1,932 (28.2) <sup>§</sup>	132 (29.6) <sup>§</sup>	609 (28.5)	1,472 (26.8)	250 (32.0)	38 (25.9)	1,700 (27.3)	593 (29.2)
<b>Previous cancer</b>	Family	148 (13.8) <sup>§</sup>	23 (24.7) <sup>§</sup>	834 (12.3) <sup>§</sup>	45 (9.8) <sup>§</sup>	267 (12.7)	703 (12.7)	80 (9.9)	19 (12.3)	775 (12.4)	256 (12.6)
<b>Stage (TNM 2007)</b>	I/II	177 (15.4)	13 (13.4)	1,248 (16.5)	61 (12.5)	391 (16.7)	1,015 (16.7)	93 (10.9)	23 (13.9)	1,143 (16.7)	333 (14.6)
	III/IV/relapse	971 (84.6)	84 (86.6)	6,293 (83.5)	428 (87.5)	1,955 (83.3)	5,063 (83.3)	758 (89.1)	143 (86.1)	5,692 (83.3)	1,941 (85.4)
<b>Histology</b>	ADC	1,502 (84.1) <sup>§</sup>	145 (90.1) <sup>§</sup>	11,854 (75.2) <sup>§</sup>	742 (76.3) <sup>§</sup>	4,069 (83.1) <sup>§</sup>	8,845 (73.1) <sup>§</sup>	1,329 (79.2) <sup>§</sup>	228 (87.0) <sup>§</sup>	10,610 (77.8) <sup>§</sup>	3,405 (71.3) <sup>§</sup>
	SCQ	23 (1.3) <sup>§</sup>	1 (0.6) <sup>§</sup>	838 (5.3) <sup>§</sup>	47 (4.8) <sup>§</sup>	47 (1.0) <sup>§</sup>	792 (6.5) <sup>§</sup>	70 (4.2) <sup>§</sup>	1 (0.4) <sup>§</sup>	708 (5.2) <sup>§</sup>	200 (4.2) <sup>§</sup>
	LCC	25 (1.4) <sup>§</sup>	1 (0.6) <sup>§</sup>	557 (3.5) <sup>§</sup>	31 (3.2) <sup>§</sup>	131 (2.7) <sup>§</sup>	432 (3.6) <sup>§</sup>	51 (3.0) <sup>§</sup>	6 (2.3) <sup>§</sup>	471 (3.5) <sup>§</sup>	137 (2.9) <sup>§</sup>
	NOS & Others	236 (13.2) <sup>§</sup>	14 (8.7) <sup>§</sup>	2,510 (15.9) <sup>§</sup>	153 (15.7) <sup>§</sup>	647 (13.2) <sup>§</sup>	2,038 (16.8) <sup>§</sup>	228 (13.6) <sup>§</sup>	27 (10.3) <sup>§</sup>	1,855 (13.6) <sup>§</sup>	1,031 (21.6) <sup>§</sup>
<b>Turnaround time, days</b>	Coll./Lab.	7 (3-15) <sup>§</sup>	9 (3-16) <sup>§</sup>	8 (4-16) <sup>§</sup>	9 (5-27) <sup>§</sup>	8 (4-15)	8 (4-16)	10 (5-25)	9 (6-15)	8 (4-16)	7 (0-15)
<b>(median, Q1-Q3)</b>	Lab./Result	12 (8-17) <sup>§</sup>	13.5 (8-20) <sup>§</sup>	11 (7-16) <sup>§</sup>	12 (7-17) <sup>§</sup>	13 (9-18)	13 (8-18)	14 (10-20)	14 (9-21)	13 (9-19)	15 (10-23)

*M, mutated (i.e. activating mutation); R, resistant mutation; WT, wild type; UNK, unknown (not contributive or not done analysis); ECOG PS, ECOG performance status; ADC, adenocarcinoma; SCQ, squamous cell carcinoma; LCC, large cell carcinoma; NOS, not otherwise specified; Full WT, patients with an established molecular profile without an EGFR, KRAS, BRAF, HER2, or PIK3CA mutation or ALK rearrangement; \*, in 109 cases, the sex was not specified in the report (0.6%); §, comparison between the population with the considered molecular alteration and the population with unknown or full WT is significantly different, p<0.05. See also supplementary Table 2 for percentage presented per row.*

**Table 2 (cont): Results of the 18,679 molecular analyses stratified by clinical characteristics.**

		HER2, N (%)			PIK3CA, N (%)			ALK, N (%)		Full WT, N (%)	
		M	WT	UNK	M	WT	UNK	Rearranged	WT	UNK	
N (%)	98 (0.5)	11,625 (62.3)	6,956 (37.2)	252 (1.4)	10,426 (55.8)	8,001 (42.8)	388 (2.1)	7,746 (41.5)	10,545 (56.4)	2,833 (15.2)	
Age (median)	66.2	64.7	65.3	67.9 <sup>§</sup>	64.6 <sup>§</sup>	65.3 <sup>§</sup>	61.2 <sup>§</sup>	65.0 <sup>§</sup>	65.1 <sup>§</sup>	64.8	
Sex	Male	40 (40.8) <sup>§</sup>	7,577 (65.2) <sup>§</sup>	4,330 (62.2) <sup>§</sup>	154 (61.1)	6,812 (65.3)	4,981 (62.3)	206 (53.1) <sup>§</sup>	5,016 (64.8) <sup>§</sup>	6,725 (63.8) <sup>§</sup>	2,033 (71.8)
	Female	58 (59.2) <sup>§</sup>	3,971 (34.2) <sup>§</sup>	2,592 (37.3) <sup>§</sup>	98 (38.9)	3,549 (34.0)	2,974 (37.2)	180 (46.4) <sup>§</sup>	2,675 (34.5) <sup>§</sup>	3,766 (35.7) <sup>§</sup>	775 (27.4)
Ethnicity	Asian	0	64 (1.3)	44 (1.6)	1 (1.0)	63 (1.3)	44 (1.4)	5 (2.1)	38 (1.1)	65 (1.5)	8 (0.7)
	Other	63 (100)	5,054 (98.7)	2,686 (98.4)	101 (99.0)	4,634 (98.7)	3,068 (98.6)	238 (97.9)	3,304 (98.9)	4,261 (98.5)	1,141 (99.3)
Smoking history	Never	42 (63.6) <sup>§</sup>	1,065 (17.6) <sup>§</sup>	666 (21.0) <sup>§</sup>	38 (30.9) <sup>§</sup>	987 (18.1) <sup>§</sup>	748 (20.3) <sup>§</sup>	116 (43.3) <sup>§</sup>	697 (18.6) <sup>§</sup>	960 (18.3) <sup>§</sup>	167 (13.1)
	Former	16 (24.2) <sup>§</sup>	2,553 (42.3) <sup>§</sup>	1,296 (40.9) <sup>§</sup>	49 (39.8) <sup>§</sup>	2,292 (42.0) <sup>§</sup>	1,524 (41.3) <sup>§</sup>	92 (34.3) <sup>§</sup>	1,638 (43.6) <sup>§</sup>	2,135 (40.7) <sup>§</sup>	574 (45.1)
	Current	8 (12.1) <sup>§</sup>	2,419 (40.1) <sup>§</sup>	1,206 (38.1) <sup>§</sup>	36 (29.3) <sup>§</sup>	2,178 (39.9) <sup>§</sup>	1,419 (38.4) <sup>§</sup>	60 (22.4) <sup>§</sup>	1,422 (37.8) <sup>§</sup>	2,151 (41.0) <sup>§</sup>	532 (41.8)
ECOG PS	0/1	51 (81.0)	3,921 (72.2)	2,113 (72.2)	80 (70.2)	3,610 (73.0)	2,395 (71.3)	205 (80.7) <sup>§</sup>	2,476 (71.2) <sup>§</sup>	3,404 (72.7) <sup>§</sup>	817 (69.2)
	>2	12 (19.0)	1,506 (27.8)	813 (27.8)	34 (29.8)	1,334 (27.0)	963 (28.7)	49 (19.3) <sup>§</sup>	1,003 (28.8) <sup>§</sup>	1,279 (27.3) <sup>§</sup>	364 (30.8)
Previous cancer	Family	8 (11.8)	731 (13.3)	311 (10.9)	14 (12.5)	633 (12.6)	403 (12.2)	28 (11.0)	457 (13.2)	565 (12.0)	156 (13.2)
Stage	I/II	5 (7.2) <sup>§</sup>	1,086 (18.0) <sup>§</sup>	408 (12.8) <sup>§</sup>	25 (19.8)	980 (17.9)	494 (13.4)	34 (12.5)	527 (14.0)	938 (17.9)	215 (16.8)
	III/IV/relapse	64 (92.8) <sup>§</sup>	4,931 (82.0) <sup>§</sup>	2,781 (87.2) <sup>§</sup>	101 (80.2)	4,480 (82.1)	3,195 (86.6)	238 (87.5)	3,228 (86.0)	4,310 (82.1)	1,061 (83.2)
Histology	ADC	90 (91.8) <sup>§</sup>	8,959 (77.1) <sup>§</sup>	5,194 (74.7) <sup>§</sup>	161 (63.9) <sup>§</sup>	8,079 (77.5) <sup>§</sup>	6,003 (75.0) <sup>§</sup>	331 (85.3) <sup>§</sup>	6,218 (80.3) <sup>§</sup>	7,694 (73.0) <sup>§</sup>	2,084 (73.6)
	SCQ	0 <sup>§</sup>	656 (5.6) <sup>§</sup>	253 (3.6) <sup>§</sup>	45 (17.9) <sup>§</sup>	577 (5.5) <sup>§</sup>	287 (3.6) <sup>§</sup>	4 (1.0) <sup>§</sup>	346 (4.5) <sup>§</sup>	559 (5.3) <sup>§</sup>	232 (8.2)
	LCC	0 <sup>§</sup>	436 (3.8) <sup>§</sup>	178 (2.6) <sup>§</sup>	8 (3.2) <sup>§</sup>	405 (3.9) <sup>§</sup>	201 (2.5) <sup>§</sup>	12 (3.1) <sup>§</sup>	262 (3.4) <sup>§</sup>	340 (3.2) <sup>§</sup>	140 (4.9)
	NOS or other	8 (8.2) <sup>§</sup>	1,574 (13.5) <sup>§</sup>	1,331 (19.1) <sup>§</sup>	38 (15.1) <sup>§</sup>	1,365 (13.1) <sup>§</sup>	1,510 (18.9) <sup>§</sup>	41 (10.6) <sup>§</sup>	920 (11.9) <sup>§</sup>	1,952 (18.5) <sup>§</sup>	377 (13.3)
Turnaround time, days	Coll. to Lab.	9 (5-23)	8 (5-16)	7 (1-15)	9 (5-17)	8 (5-16)	7 (1-16)	7 (3-13) <sup>§</sup>	7 (3-15) <sup>§</sup>	9 (5-17) <sup>§</sup>	7 (3-14)

(median, Q1-Q3)	Lab. to result	17 (10.5-29) <sup>§</sup>	14 (9-20) <sup>§</sup>	16 (10-23) <sup>§</sup>	15 (10-21)	14 (9-22)	16 (11-23)	21 (12-35.5) <sup>§</sup>	16 (8-28) <sup>§</sup>	26 (13-48) <sup>§</sup>	24 (14-40)
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**Table 3: Patient treatment stratified by the line of therapy and by the results of routine molecular analyses.**

N overall	Global	EGFRm		KRASm		BRAFm		HER2m		PIK3CAM		ALKrearr.		Full WT
	17,664	1,787	All	Adapted <sup>\$</sup>	All	Adapted <sup>\$</sup>	All	Adapted <sup>\$</sup>	All	Adapted <sup>\$</sup>	All	Adapted <sup>\$</sup>	All	2,769
<b>First-line Trt.</b>														
N (%), with data	8,448 (47.8)	1,128 (63.1)	662 (37.0)	2,085 (45.4)	979 (21.3)	146 (63.5)	64 (27.8)	62 (67.4)	28 (30.4)	73 (46.5)	29 (18.5)	236 (69.4)	120 (35.3)	1214 (43.8)
<b>PEM-based Rx</b>	2,747 (32.5)	188 (16.7)	57 (8.6)	792 (38.0)	525 (53.6)	51 (34.9)	34 (53.1)	31 (50.0)	18 (64.3)	17 (23.3)	11 (37.9)	111 (47.0)	55 (45.8)	401 (33.0)
<b>VNR-based Rx</b>	504 (6.0)	39 (3.5)	9 (1.4)	128 (6.1)	68 (6.9)	5 (3.4)	2 (3.1)	0	0	7 (9.6)	3 (10.3)	13 (5.5)	9 (7.5)	80 (6.6)
<b>TAX-based Rx</b>	1,064 (12.6)	60 (5.3)	18 (2.7)	261 (12.5)	166 (17.0)	20 (13.7)	12 (18.8)	8 (12.9)	4 (14.3)	11 (15.1)	7 (24.1)	17 (7.2)	11 (9.2)	188 (15.5)
<b>EGFR-TKI</b>	684 (8.1)	543 (48.1)	520 (78.5)	26 (1.2)*	9 (0.9)*	3 (2.1)*	2 (3.1)*	0	0	1 (1.4)*	1 (3.4)*	4 (1.7)*	2 (1.7)*	17 (1.4)
<b>Crizotinib</b>	18 (0.2)	0	0	0	0	0	0	0	0	0	0	18 (7.6)	18 (15.0)	0
<b>Trial<sup>£</sup></b>	253 (3.0)	36 (3.2)	31 (4.7)	63 (3.0)	48 (4.9)	8 (5.5)	5 (7.8)	3 (4.8)	1 (3.6)	0	0	16 (6.8)	12 (10.0)	36 (3.0)
<b>Other<sup>\$</sup></b>	709 (8.4)	27 (2.4)	9 (1.4)	171 (8.2)	77 (7.9)	11 (7.5)	3 (4.7)	5 (8.1)	3 (10.7)	10 (13.7)	5 (17.2)	6 (2.5)	3 (3.5)	131 (10.8)
<b>BSC only</b>	2,469 (29.2)	235 (20.8)	18 (2.7)	644 (30.9)	86 (8.8)	48 (32.9)	6 (9.4)	15 (24.2)	2 (7.1)	27 (37.0)	2 (6.9)	51 (21.6)	10 (8.3)	361 (29.7)
<b>Second-line Trt.</b>														
N (%with data)	5,518 (31.2)	698 (39.1)	381 (21.3)	1,358 (29.6)	566 (12.3)	106 (46.1)	37 (16.1)	43 (46.7)	22 (23.9)	48 (30.6)	12 (7.6)	157 (46.2)	102 (30.0)	797 (28.8)
<b>Taxane</b>	782 (14.2)	46 (6.6)	34 (8.9)	236 (17.4)	203 (35.9)	16 (15.1)	8 (21.6)	6 (14.0)	4 (18.2)	5 (10.4)	2 (16.7)	5 (3.2)	4 (3.9)	119 (14.9)
<b>Pemetrexed</b>	612 (11.1)	125 (17.9)	97 (25.5)	136 (10.0)	105 (18.6)	8 (7.5)	6 (16.2)	5 (11.6)	4 (18.2)	4 (8.3)	2 (16.7)	13 (8.3)	10 (9.8)	81 (10.2)
<b>Erlotinib</b>	776 (14.1)	231 (33.1)	218 (57.2)	125 (9.2)	94 (16.6)	9 (8.5)	4 (10.8)	5 (11.6)	4 (18.2)	2 (4.2)	2 (16.7)	10 (6.4)	6 (5.9)	96 (12.0)
<b>Crizotinib</b>	73 (1.4)	0	0	0	0	0	0	0	0	0	0	73 (46.5)	73 (71.6)	0
<b>Trial<sup>£</sup></b>	116 (2.1)	8 (1.1)	7 (1.8)	33 (2.4)	27 (4.8)	5 (4.7)	5 (13.5)	3 (7.0)	2 (9.1)	2 (4.2)	1 (8.3)	4 (2.5)	4 (3.9)	25 (3.1)
<b>Other<sup>\$</sup></b>	442 (8.0)	10 (1.4)	6 (1.6)	90 (6.6)	60 (10.6)	8 (7.5)	7 (18.9)	8 (18.6)	8 (36.4)	2 (4.2)	2 (16.7)	5 (3.2)	3 (2.9)	79 (9.9)
<b>BSC only</b>	2,711 (49.1)	272 (39.0)	15 (3.9)	738 (54.3)	77 (13.6)	60 (56.6)	7 (18.9)	16 (37.2)	0	33 (68.8)	3 (25.0)	47 (29.9)	2 (2.0)	397 (49.8)

,\$, the treatment was selected considering the results of the molecular analyses (i.e., targeted therapy if an actionable alteration had been identified, chemotherapy for WT patients, etc); Trt, treatment; Rx, Regimen; PEM, pemetrexed; VNR, vinorelbine; TAX, taxanes; £, usually based on targeted agents; BSC, best supportive care; §, including, but not limited to, another type of chemotherapy, crizotinib via an expanded access program (ATU) before its registration, off-label targeted therapy, a non-registered combination of therapies, etc; \*, patients with tumor displaying two molecular alterations including EGFR mutation.

**Table 4: Global outcomes and outcomes stratified by line of therapy and by molecular alteration.**

	<b>Global</b>	<b>EGFRm</b>	<b>KRASm</b>	<b>BRAFm</b>	<b>HER2m</b>	<b>PIK3Cam</b>	<b>ALK rearr.</b>	<b>UNK</b>	<b>Full WT</b>
<b>First-line treatment</b>									
<b>ORR (Available Data)</b>	6319	896	1499	109	50	54	191	2546	896
<b>ORR, %</b>	34.1	47.5	30.0	22.9	32.0	46.3	41.4	32.0	32.6
<b>95%CI</b>	32.9-35.3	44.3-50.8	27.6-32.3	15.0-30.8	19.1-44.9	33.0-59.6	34.4-48.3	30.2-33.8	29.5-35.7
<b>PFS (Available Data)</b>	7821	1017	1966	132	56	72	214	3131	1137
<b>PFS, median</b>	8.3	15.4	7.3	7.5	7.3	13.7	14.5	7.5	7.1
<b>95%CI</b>	8.0-8.7	13.7-17.6	6.5-8.0	5.6-12.3	4.9-21.2	8.3-NR	11.0-16.7	7.0-8.0	6.1-7.9
<b>6-month PFS, %</b>	59.0	75.8	55.0	56.7	57.8	70.9	67.3	56.9	54.0
<b>95%CI</b>	57.8-60.2	73.0-78.6	52.7-57.4	47.8-65.7	44.3-71.3	59.4-82.3	60.7-73.8	55.0-58.8	50.8-57.2
<b>12-month PFS, %</b>	41.5	56.1	38.9	41.6	44.5	54.3	54.1	38.2	37.9
<b>95%CI</b>	40.3-42.8	52.6-59.5	36.4-41.5	32.3-50.9	30.5-58.5	40.2-68.4	46.8-61.3	36.2-40.2	34.6-41.3
<b>Second-line treatment</b>									
<b>ORR (Available Data)</b>	3325	441	762	59	34	26	115	1361	482
<b>ORR, %</b>	12.7	30.8	7.7	8.5	11.8	3.8	34.8	9.4	9.3
<b>95%CI</b>	11.6-13.8	26.5-35.1	5.8-9.6	1.4-15.6	0.9-22.6	0-11.2	26.1-43.5	7.8-10.9	6.7-11.9
<b>PFS (Available Data)</b>	4029	518	1017	71	35	30	125	1585	598
<b>PFS, median</b>	3.1	5.6	2.5	3.1	4.5	4.6	9.3	2.9	3.0
<b>95%CI</b>	3.0-3.3	4.3-6.6	2.3-2.9	1.4-6.1	2.4-6.6	1.5-9.0	6.7-12.0	2.7-3.2	2.8-3.6
<b>6-month PFS, %</b>	36.4	48.3	32.7	41.3	42.5	36.0	59.7	33.5	33.6
<b>95%CI</b>	34.7-38.0	43.5-53.1	29.5-36.0	28.7-53.9	24.6-60.4	15.6-56.4	50.4-69.0	30.9-36.1	29.4-37.9
<b>12-month PFS, %</b>	23.8	32.6	24.6	18.2	22.7	23.1	41.1	19.8	23.4
<b>95%CI</b>	22.1-25.5	27.4-37.8	21.3-27.9	6.2-30.1	5.3-40.0	3.3-42.9	30.2-51.9	17.2-22.4	19.1-27.8
<b>OS (Available Data)</b>	7821	1017	1966	132	56	72	214	3131	1137
<b>OS, median</b>	13.8	NR	11.7	13.8	NR	13.7	20.7	12.2	11.8

<b>95%CI</b>	13.3-14.4	10.6-13.1	8.5-21.9	8.7-NR	17.0-NR	11.5-13.0	10.1-13.5
<b>6-month OS, %</b>	70.0	84.2	64.5	67.8	80.6	74.0	80.2
<b>95%CI</b>	68.9-71.0	81.9-86.6	62.2-66.7	59.5-76.2	69.7-91.5	62.9-85.0	74.7-85.7
<b>12-month OS, %</b>	54.0	72.9	49.3	52.0	61.4	57.4	70.2
<b>95%CI</b>	52.7-55.3	69.8-75.9	46.6-51.9	42.4-61.6	47.0-75.7	43.4-71.4	63.6-76.8

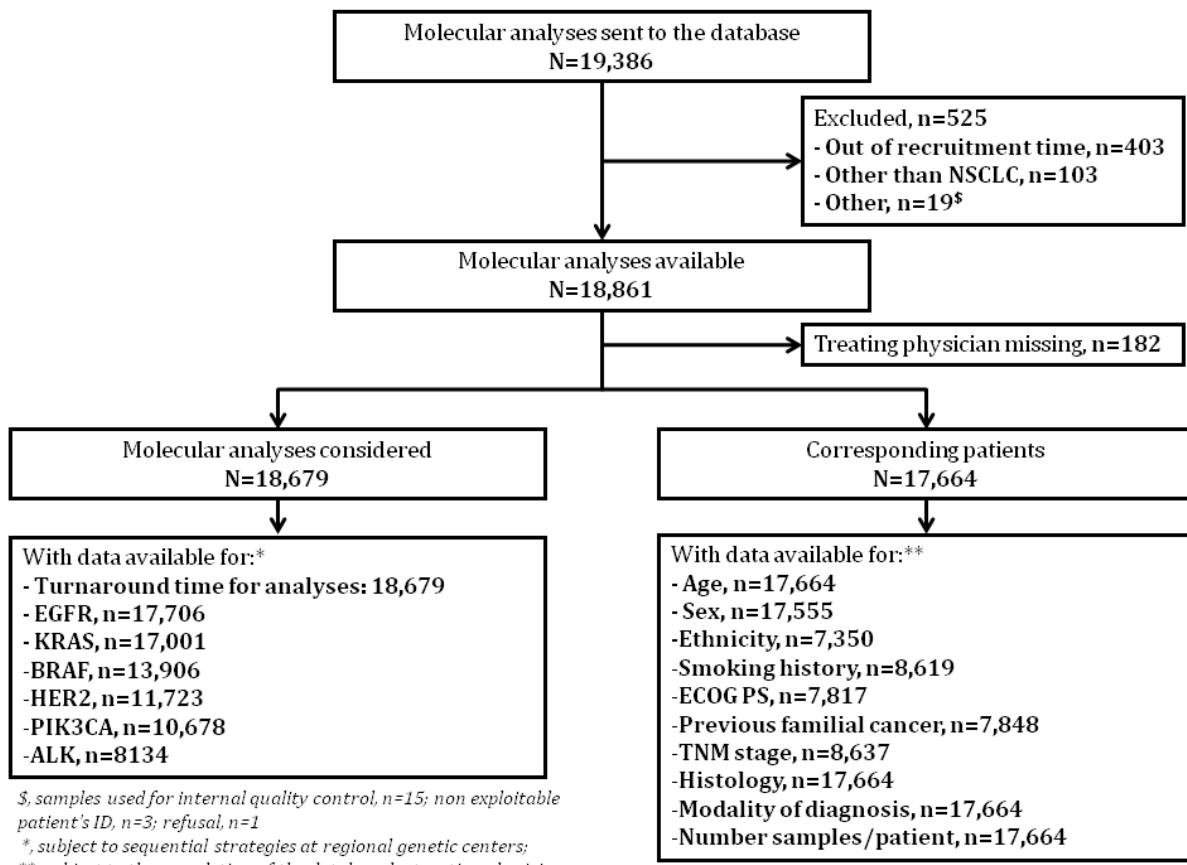
*ORR, overall response rate; PFS, progression free survival; OS, overall survival; UNK, Unknown representing the cases with at least one unknown result after the assessment of the six genes status, NR, not reached.*

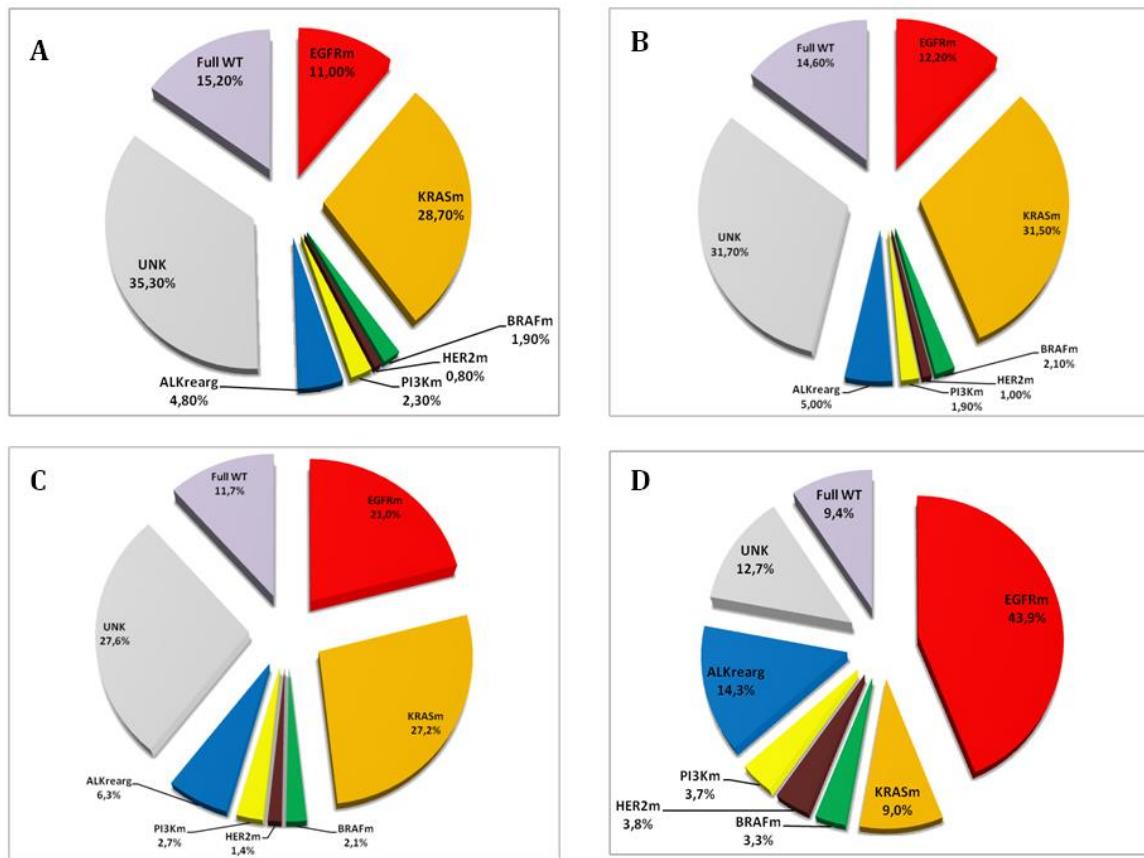
**FIGURE LEGENDS**

Figure 1: Flow-chart of the study (patients and molecular analyses performed).

Figure 2: Frequency of the genetic alterations in the six genes in the 18,679 analyzed samples (expressed as the percentage of positive samples for each molecular alteration relative to the number of available analyses, with UNK representing the cases with at least one unknown result after the assessment of the six genes status), global results (panel A), adenocarcinoma only (panel B), women only (panel C), and never-smokers only (panel D).

Figure 3: Outcomes of the 17,664 patients undergoing molecular analyses: first-line PFS for patients with and without genetic alteration (panel A); first-line PFS stratified by molecular profile (panel B); second-line PFS for patients with and without a genetic alteration (panel C); second-line PFS stratified by molecular profile (panel D); OS of patients with and without a genetic molecular alteration (panel E); and OS stratified by molecular profile (panel F). UNK in panel B, D and F represents the cases with at least one unknown result after the assessment of the six genes status.

**Figure 1**

**Figure 2**

**Figure 3**

