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Michèle Beau-Faller, MD, PhD,[a,b,*] Matthieu Texier, MSc,[c] Hélène Blons, PharmD, PhD,[b,d] Nicolas Richard, PharmD, MSc,[e] Fabienne Escande, PhD,[f] Samia Melaabi, MD,[g] Sarab Lizard, PhD,[h] Florence De Fraipont, PharmD, PhD,[i] Elisabeth Longchampt, MD,[j] Franck Morin, MSc,[b] Gérard Zalcman, MD, PhD,[b,k] Jean-Pierre Pignon, MD, PhD,[c,m] Jacques Cadranel, MD, PhD[b,l]

[a]*Laboratoire de Biologie Moléculaire, Hôpital de Hautepierre, 1 avenue Molière, 67098 Strasbourg, France; Unité INSERM U1113, Streinth Lab, 67200 Strasbourg France*

[b]*Intergroupe Francophone de Cancérologie Thoracique (IFCT), 10 rue de la Grange-Batelière, 75009 Paris, France*

[c]*Service de Biostatistique et d'Epidémiologie, Gustave-Roussy, 114 rue Edouard Vaillant, 94805 Villejuif, France*

[d]*Département de Biologie, Hôpital Européen Georges Pompidou, AP-HP, 75015 Paris, France; Université Paris Descartes, 75006 Paris, France*

[e]*Service de Génétique, Laboratoire de Génétique Moléculaire, CHU de Caen, Caen, France*

[f]*Laboratoire Oncologie et Génétique Moléculaires, Pôle de Biochimie et de Biologie Moléculaire, Centre de Biologie Pathologie, CHRU de Lille, 59037 Lille Cedex, France*

[g]*Service de génétique, unité de pharmacogénomique, Institut Curie, Paris, France*

[h]*Unité de Biologie Moléculaire, Centre GF Leclerc, 1 rue Pr Marion, 21079 Dijon, France*

[i]*UM Biochimie des Cancers et Biothérapies, CHU Grenoble Alpes; Université Grenoble Alpes, 38 000 Grenoble, France*

[j] *Service d'Anatomie Pathologique, Hôpital Foch, Suresnes, France*

[k]*UMR 830 INSERM et Service d'Oncologie thoracique, Assistance Publique Hôpitaux de Paris, Hôpital Bichat-Claude Bernard, Université Paris-Diderot (Paris 7), 46 rue Henri Huchard, 75018 Paris, France*

[l]*Service de Pneumologie, Assistance Publique Hôpitaux de Paris, Hôpital Tenon, GRC-04 Theranoscan, Université Paris VI, 4 rue de la Chine, 75970 Paris Cedex 20, France*

[m] *CESP INSERM U1018, Université Paris-Sud, Villejuif, F-94805, France*

*Corresponding author

Address for correspondence: Michèle Beau-Faller, MD, PhD, Laboratoire de Biologie Moléculaire, Hôpital de Hautepierre, 1 avenue Molière, 67098 Strasbourg, France. Tel.: +33 (0)3 88 12 84 57; Fax: +33 (0)3 88 12 75 39; E-mail: Michele.FALLER@chru-strasbourg.fr

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Microabstract (60)

Detecting driver mutations belongs now to the best practice in advanced/metastatic non-small cell lung cancer (NSCLC). New molecular techniques are highly sensitive. In NSCLC all treated by erlotinib (n=228), we reports that *EGFR* and *KRAS* mutated subclones had a prognostic value, but not minor KRAS mutated sub-clones. Molecular techniques must be sensitive but not under 1% of mutated tumor cells.

ABSTRACT (250)

Introduction: ERMETIC was a prospective study designed to validate the prognostic value of *EGFR/KRAS* mutations in patients with advanced non-small cell lung cancer (NSCLC), all receiving a first generation TKI, erlotinib. ERMETIC2 was an ancillary project evaluating the clinical value of common *EGFR/KRAS*-mutated subclones regarding prognosis using highly sensitive molecular detection methods.

Methods: Tumor samples from 228 NSCLC patients (59% adenocarcinoma, 37% women, and 19% never/former smokers) were available for reanalysis using alternative highly sensitive molecular techniques. A multivariate Cox model was used for prognostic analysis.

Results: Using alternative highly sensitive techniques, 16 *EGFR* and 51 *KRAS* supplementary mutations were newly identified, all still exclusive, leading to an overall rate of 12.3% (n=28) and 33.3% (n=76), respectively. Using real-time polymerase chain reaction (qPCR; hybridization probe), they were significantly associated with progression-free survival (PFS) (p=0.02) and overall survival (OS) (p=0.01), which were better for *EGFR*-mutated patients for PFS (hazard ratio [HR]: 0.46 [95% confidence interval [CI]: 0.28-0.78]) and OS (hazard ratio [HR]: 0.56 [0.31-1]), and worse for *KRAS* mutations and OS (HR: 1.63 [1.09-2.44]). Using the most sensitive technique detection for *KRAS* - clamp PCR -*, KRAS* mutated subclones did not impact OS.

Conclusions: *KRAS* and *EGFR* mutations were detected in higher proportions by alternative highly sensitive molecular techniques compared to direct Sanger sequencing. However, minor *KRAS*-mutated subclones offered no prognostic value when representing less than 1% the tumor cells.

Keywords

Driver mutations; Tyrosine kinase inhibitor; NSCLC; molecular techniques; sensitivity

Introduction

EGFR tyrosine kinase inhibitors (EGFR-TKIs) erlotinib, gefitinib, and afatinib are authorized worldwide as first-line treatment for advanced or metastatic non-small cell lung cancer (NSCLC) patients with *EGFR*-activating mutations in their tumor.[1,2,3] Recently, third generation EGFR-TKI osimertinib has been validated as the standard of care for T790M positive NSCLC in whom disease had progressed during first-line EGFR-TKI therapy.[4] And more recently, osimertinib showed efficacy superior to that of standard first/second generation EGFR-TKIs in the first-line treatment of *EGFR* mutation positive advanced NSCLC.[5]

Molecular tumor testing is actually mandatory for selecting first-line treatment in advanced or metastatic non-small cell lung cancer (NSCLC) patients.[6-7-8-9] Yet no *EGFR*-mutation assay is currently specifically recommended by the American Food and Drug Administration (FDA), European Agency for the Evaluation of Medicinal Products (EMEA), or European Society of Medical Oncology (ESMO) to inform treatment decisions.[10-11] Direct sequencing has for many years been considered the gold standard for testing, yet its sensitivity can limit its use to routinely somatic tumor testing, and alternative more sensitive molecular methods - targeted or not - have often since replaced this approach.[12] Furthermore, next-generation sequencing (NGS) or droplet digital PCR (ddPCR) are developped in molecular platforms. All these highly sensitive molecular methods should be able to detect mutated subclones (5% of mutated cells) to minor subclones (<1% of mutated cells), thus raising the question of the prognostic/predictive value of such mutated different subclones.

Although *KRAS* is not a target for therapy, lung cancer molecular analyses often test for *EGFR* as well as *KRAS* mutations. In Caucasian NSCLC population, *EGFR* and *KRAS* mutations are found in 11% and 29% of the patients respectively. [3, 7] These mutations are often mutually exclusive, hence why some authors have even proposed a step-by-step algorithm using *KRAS* mutation testing as first step to rule out the presence of an *EGFR* mutation, in case of using targeted alternative molecular methods. Furthermore, *KRAS* mutations appear to affect an heterogeneous population with different prognostic/predictive values depending on the type of nucleotide base substitution, regarding EGFR-TKI treatment.[13, 14] Finally, several studies have distinguished patients with *EGFR* mutations from those with non-*EGFR* mutated tumors, yet including *KRAS*-mutated tumors. In our study, we evaluated what impact the mutation detection threshold has on prognostic value of erlotinib efficacy.

The ERMETIC (Evaluation of *EGFR* Mutation status for the administration of EGFR-TKIs in non-small cell lung Carcinoma) study, designed and supported by the French Collaborative Thoracic Cancer Intergroup (IFCT), funded by the French NCI (INCa), reported that formaldehyde-fixed paraffin-embedded (FFPE) specimens may be suitable sources for DNA analysis by means of genomic Sanger sequencing, providing rigorous pre-analytical quality control standards are respected.[9, 12] Since that publication, ERMETIC centers have switched to alternative molecular methods. The first step of ERMETIC2 consisted in a nationwide technological evaluation of these new *EGFR/KRAS* testing using NSCLC cell line DNA with various allele proportions. We demonstrated that the best threshold of mutation detection was obtained using allele-specific amplification-based technologies, with cut-off values of 5% and 1% for clamped PCR with peptide nucleic acid (PNA).[15] We report the

prognosis-based clinical impact of this new strategy on 228 ERMETIC patients with available tumor samples reanalyzed using these techniques.

Patients and Methods

Patients and Tumor Samples

The ERMETIC prospective observational study included 522 patients with advanced NSCLC, either newly treated with erlotinib or before erlotinib administration.[6] A preliminary study revealed that *EGFR* and *KRAS* mutations identified using Sanger direct sequencing were independent markers of outcome in this population.[6] A subgroup of 228 patients provided sufficient samples for a second round of common *EGFR* and *KRAS* mutation screening using the alternative molecular methods selected after ERMETIC2 – part 1.[12]

Biological Assessment

The methods used were previously described.[12] Briefly, we used fragment analysis for *EGFR* exon 19 assessment, and targeted molecular techniques based on allele-specific amplification - probe-specific detection, TaqMan assay - for *EGFR* exon 21 L858R mutation, and - hybridization probe (SH assay) - without PNA (peptide Nucleic Acid) (KRAS_SH), or with PNA as clamp-PCR strategy (KRAS_PNA) for *KRAS* mutations.

Statistical Analysis

Endpoint definitions were as previously defined.[6] Survival rates were estimated using the Kaplan-Meier method with 95% Confidence Interval (CI). Impact on survival was quantified using Cox models and hazard ratios with 95%CI. Variables with a p-value

<0.20 in univariate analysis were included into the multivariate analysis. A backwards selection process was undertaken with the final model, including all variables with p-values <0.05.

Results

Patient Characteristics

The population consisted of patients with samples available for both *EGFR* and *KRAS* mutation analyses, excluding patients with insufficient material for simultaneous analysis of both genes or with non-amplifiable samples. Clinical characteristics of the patients (Table 1) did not differ from those with sample not available for this second p art of the study (data not shown).

EGFR/KRAS Status

When tumors (n=228) were tested by Sanger direct sequencing, the overall mutation rate was 16.2% (12 [5.3%] and 25 [11.0%] *EGFR* and *KRAS* mutations, respectively). When the same tumors (n=228) were tested by molecular alternative methods, the overall mutation rate was 45.6% (28 [12.2%] and 76 [33.3%] *EGFR* and *KRAS* mutations, respectively), with 67 new mutations found (16 in *EGFR* and 51 in *KRAS*), including 60.8% (31/51) for *KRAS* mutations identified using the clamp-PCR strategy (Tables 2A and 2B). Details of *EGFR* and *KRAS* mutations by techniques are described in Table 3.

Survival Analysis: PFS and OS

No differences in overall survival (OS) or progression-free survival (PFS) were observed between the initial and re-analyzed ERMETIC populations (data not

shown). Patients with *KRAS*-mutated tumors were categorized according to the mutation detection method, SH assay with or without clamp-PCR strategy (*KRAS*_SH *versus KRAS*_PNA). The median OS was 15 (95%CI: 4.7-28.4), 6.7 (95%CI: 2.1-9.2), 5.3 (95%CI: 3.9-8.1), and 2.7 (95%CI: 2.1-9.2) months for *EGFR*-mutated tumors, *KRAS_PNA*-mutated tumors, *EGFR/KRAS* wild-type (WT) tumors and for *KRAS*_SH-mutated tumors, respectively (p = 0.0018) (Fig. 1a). The median PFS was 9.3 (95%CI:2.6-15.3), 2.8 (95%CI:1.5-3.1), 2.3 (95%CI:2.0-2.6), and 1.6 (95%CI: 0.9-2.5) months for patients with *EGFR*-mutated*, KRAS*_PNA-mutated, WT, and *KRAS*_SH-mutated tumors, respectively (p = 0.0007) (Fig. 1b). The 1-year survival rates [95% CI] were 60.7% [42.4; 76.4], 25.8% [18.9; 34.2], 23.5% [12.4; 40], and 16.7% [8.3; 30.6] for these four groups, respectively. The clinical characteristics significantly associated with prognosis (OS, PFS) were the same as for the initial population (Table 4 and data not shown). After adjusting for clinical factors, multivariate analysis of mutation status remained significantly associated with OS (p=0.01) and PFS (p=0.02) which were better for *EGFR*-mutated patients for PFS (hazard ratio [HR]: 0.46 [95% confidence interval [CI]: 0.28-0.78]) and OS (hazard ratio [HR]: 0.56 [0.31-1]), and worse for *KRAS* mutations for OS (HR: 1.63 [1.09-2.44]) (Table 4). Using the most sensitive technique detection for *KRAS* - clamp PCR -*, KRAS* mutated subclones did not impact OS. *EGFR* mutation significantly decreased the risk or death by 44%, and the risk of progression or death by 54% in patients treated with erlotinib. *KRAS* mutations detected by SH (KRAS_SH) significantly increased the risk of death, by 63%. Conversely, *KRAS* mutations detected by clamp-PCR strategy (KRAS_PNA) did not increase the risk of death.

Type of KRAS Mutation

No prognostic value was related to the alteration type (transition/transversion) or mutation location (codon 12 or 13) among the 76 *KRAS*-mutated patients.

Discussion

The prognostic or predictive value afforded by driver-mutated subclones and minor sub-clones in NSCLC and other cancer types is still open to debate. With the development of high throughput and extremely sensitive methods, such as NGS, clamp-based PCR, or ddPCR, establishing a cut-off is now mandatory. The clinical value of low allele frequency detection needed to be assessed regarding two issues: Can it rescue mutation testing for small biopsies with low tumor-cell content and high stromal component? Does it have any clinical value?

This study was designed to reanalyze paraffin-embedded NSCLC tumor samples using alternative molecular techniques currently employed in France and many laboratories worldwide thanks to their cost-efficiency for analysis of recurrent genetic alterations, requiring low amounts of DNA from FFPE samples. We described 16 and 51 new *EGFR* and *KRAS* mutations, respectively, after reanalysis. In the ERMETIC initial population, *EGFR* status impacted both PFS and OS, whereas *KRAS* status only impacted OS.[9, 12] Similar results were observed for *EGFR* mutation in the re-analyzing study. In contrast, though *KRAS* status analyzed by PCR using hybridization probes remained predictive of OS, the more-sensitive clamped PCR method that identified low mutated subclones failed to impact prognosis, raising the question of these minor subclones clinical relevance for patient care.

The number of newly-detected *EGFR* mutations in our study proved relatively small, suggesting that *EGFR* mutations are present in the majority of tumor cells or associated with an amplification of the mutated allele in NSCLC, as previously described.[16, 17] We demonstrated that by using sensitive methods we may be able to reattempt detection of an *EGFR* alteration, an important capability for treatment decisions. All our patients receiving EGFR-TKIs were correctly treated, although the mutation was not identified in the initial ERMETIC study, hence why no modification of *EGFR* prognostic value was observed between initial and re-analyzing ERMETIC studies. In the latest study, testing was performed using methods with a 10-5% detection threshold for fragment analysis of *EGFR* exon 19 deletions and for PCR using TaqMan probes of *EGFR* L858R mutations.[15] Therefore, we deduce that the new *EGFR*-mutated cases were related to the tumor-cell content being low, initially under the level of detecting *EGFR* mutation by direct sequencing. The unpredictable variability in *EGFR* copy number and therefore in *EGFR* wild-type/mutant allelic ratio justifies using sensitive methods to identify patients with *EGFR*-mutated tumors.

The situation is probably more complex for *KRAS* mutations in the setting of NSCLC,[7, 13] with *KRAS*-mutated subclones previously described in NSCLC.[18] All the 25 *KRAS* mutations detected by direct sequencing in the initial population were detected by alternative molecular techniques in the re-analyzing population (internal positive controls). Among the remaining cases, 45 were positive for *KRAS* mutations (19.7%) using PCR with hybridization probes and 76 (33.3%) were detected using more sensitive clamped PCR (no cases with concomitant *EGFR* mutation). However, this increased sensitivity did not detect any minor subclones as having prognostic impact. Our results suggest that patients with *KRAS*-mutated subclones, using 1% as a cut-off (clamp-PCR), behave as with wild-type mutations. Such absence of clinical

relevance of *KRAS*-mutated minor subclones was previously described in advanced colorectal cancer (CRC) treated with anti-EGFR therapy.[19] In NSCLC, it appears that such sensitive methods are not necessarily useful, achieving approximately 1% detection rate for *KRAS* mutations.

Conclusion

Highly sensitive molecular methods increased the number of *EGFR* and *KRAS* mutations in NSCLC tumors. For commun *EGFR* mutations, this increasing is lower and correlated with classical prognostic (OS, PFS) value in first-line EGFR-TKI treated NSCLC patients. For *KRAS* mutation, detection of mutated subclones (5%) is associated with survival (OS) but not the minor subclones (<1%). Our study demonstrated that if more sensitive techniques could detect new mutated cases, it is not necessary to have a too low cut-off for such analysis. Treshold cut-off for mutation analysis must be taking into account for new molecular techniques, as NGS or ddPCR.

Clinical Practice Points

- Detecting driver mutations belongs now to the best practice in advanced/metastatic non-small cell lung cancer (NSCLC). New molecular techniques are highly sensitive.
- Highly sensitive molecular methods increased the number of *EGFR* and *KRAS* mutations in NSCLC tumors. For common *EGFR* mutations, this increasing is lower with classical prognostic (OS, PFS) value. For *KRAS* mutation, detection of mutated subclones is associated with survival (OS) but not the minor subclones.
- Molecular techniques must be sensitive but not under 1% of mutated tumor cells.

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Tables

Table 1. Patients' Characteristics

	Frequency N=228	*Percentage %*
Age (in years)		
<60	84	37
60-69	82	36
>=70	62	27
Sex		
Female	82	36
Male	146	64
Performance status		
0	36	17
1	98	46
2 or 3	77	37
Missing	17	
Histology		
Squamous cell	48	21
Adenocarcinoma	131	57
Others	49	21
Initial disease stage		
I-II-IIIA	48	21
IIIB	32	14
IV	147	65
Missing	1	
Initial number of metastatic sites		
0 or 1	107	47
2	65	29
3 or more	54	24
Missing	2	
Localization of metastasis		
Brain metastais	62	27
Bone metastasis	79	35

Lung metastasis	105	46
Geographical origin		
Two European parents	201	88
Others	27	12
Smoking status		
Never	41	18
Former	150	66
Current	35	15
Missing	2	

Tables 2. *EGFR* and *KRAS* mutations in the population (n=228)

Table 2A. Categorization of Mutation Status in the population (n=228)

N=228 (%)	Direct Sequencing	New mutations by alternative molecular techniques (including KRAS_SH)	New *KRAS* mutation by Clamped PCR (KRAS_PNA)	Total
EGFR	12 (5.3)	16 (7.0)		28 (12.3)
KRAS	25 (11.0)	20 (8.8)	31 (13.6)	76 (33.3)
Wild Type				124 (54.4)

Table 2B. Number of Mutations by Techniques

Technique	***EGFR*, AT FA (del19)**	***EGFR*, AT TaqMan (L858R)**	Total number of mutations n (%)
***EGFR*-SQC** (n=12)	6	6	12 (5.3)
New *EGFR* mutations (n=16)	9	7	16 (7)
All *EGFR* mutations	15	13	28 (12.2)
Technique	***KRAS*, AT_SH**	***KRAS*, AT_PNA**	Total number of mutations n (%)
***KRAS*-SQC** (n=25)	22	3	25 (11)
New *KRAS* mutations (n=51)	20	31	51 (22.4)
All *KRAS* mutations	42	34	76 (33.3)

EGFR, SQC: Direct Sequencing; *EGFR, AT* FA (del 19): Alternative Technique by Fragment Analysis; EGFR, AT TaqMan (L858R): Alternative Technique by probe-specific detection with TaqMan assay

KRAS, SQC: Direct Sequencing; *KRAS*, AT_SH: Alternative Technique by Hybridization Probe (SH assay); *KRAS*, AT_PNA: Alternative Technique by clamp-PCR (SH+PNA assay)

Table 3. Detail of *EGFR* and *KRAS* mutations detected by molecular methods (n= 104).

EGFR, SQC: Direct Sequencing; *EGFR, AT*: Alternative Technique (Del19: Fragment Analysis; L858R: probe-specific detection by TaqMan assay)

KRAS, SQC: Direct Sequencing; *KRAS*, AT_SH: Alternative Technique by Hybridization Probe (SH assay); *KRAS*,AT_PNA: Alternative Technique by clamp-PCR (SH+PNA assay)

ID-ERMETIC	*EGFR*, SQC	*EGFR*, TA	*KRAS*, SQC	*KRAS*, AT_SH	*KRAS*, AT_PNA
490	WT	WT	**G12A**	**G12A**	**G12A**
130	WT	WT	**G12A**	**G12A**	**G12A**
110	WT	WT	**G12A**	**G12A**	**G12A**
150	WT	WT	**G12C**	**G12C**	**G12C**
320	WT	WT	**G12C**	**G12C**	**G12C**
217	WT	WT	**G12C**	**G12C**	**G12C**
478	WT	WT	**G12C**	**G12C**	**G12C**
75	WT	WT	**G12C**	**G12C**	**G12C**
429	WT	WT	**G12C**	WT	**G12C**
421	WT	WT	**G12D**	**G12D**	**G12D**
282	WT	WT	**G12D**	**G12D**	**G12D**
222	WT	WT	**G12D**	**G12D**	**G12D**
247	WT	WT	**G12V**	**G12V**	**G12V**
203	WT	WT	**G12V**	**G12V**	**G12V**
296	WT	WT	**G12V**	**G12V**	**G12V**
31	WT	WT	**G12V**	**G12V**	**G12V**
415	WT	WT	**G12V**	**G12V**	**G12V**
473	WT	WT	**G12V**	**G12V**	**G12V**
48	WT	WT	**G12V**	**G12V**	**G12V**
215	WT	WT	**G12V**	**G12V**	**G12V**
259	WT	WT	**G12V**	WT	**G12V**
259	WT	WT	**G12V**	WT	**G12V**
475	WT	WT	**M**	**G12F**	**G12F**
96	WT	WT	**M**	**G12C**	**G12C**
388	WT	WT	**M**	**G12F**	**G12F**
226	WT	WT	WT	**G12D**	**G12D**
512	WT	WT	WT	**G12V**	**G12V**
446	WT	WT	WT	**M**	**M**
465	WT	WT	WT	**G12D**	**G12D**
527	WT	WT	WT	**G12D**	**G12D**
522	WT	WT	WT	**G13D**	**G13D**
426	WT	WT	WT	**G12V**	**G12V**
206	WT	WT	WT	**G12C**	**G12C**
245	WT	WT	WT	**G12V**	**G12V**
151	WT	WT	WT	**G12C**	**G12C**
184	WT	WT	WT	**G12V**	**G12V**
335	WT	WT	WT	**G12C**	**G12C**
384	WT	WT	WT	**G13V**	**G13V**

456	WT	WT	WT	**G12V**	**G12V**
285	WT	WT	WT	**G12A**	**G12A**
80	WT	WT	WT	**G12S**	**G12S**
101	WT	WT	WT	**G12C**	**G12C**
191	WT	WT	WT	**M**	**M**
244	WT	WT	WT	**G12C**	**G12C**
286	WT	WT	WT	**G12V**	**G12V**
291	WT	WT	WT	WT	**G12D**
303	WT	WT	WT	WT	**G12C**
413	WT	WT	WT	WT	**G12S**
118	WT	WT	WT	WT	**G12C**
205	WT	WT	WT	WT	**G13D**
393	WT	WT	WT	WT	**G13D**
123	WT	WT	WT	WT	**G12D**
391	WT	WT	WT	WT	**G12S**
230	WT	WT	WT	WT	**G12D**
220	WT	WT	WT	WT	**G12D**
234	WT	WT	WT	WT	**G12S**
214	WT	WT	WT	WT	**G12D**
132	WT	WT	WT	WT	**G12C**
453	WT	WT	WT	WT	**G12V**
469	WT	WT	WT	WT	**G12R**
200	WT	WT	WT	WT	**G12R**
254	WT	WT	WT	WT	**G13C**
190	WT	WT	WT	WT	**G12C**
367	WT	WT	WT	WT	**G12C**
34	WT	WT	WT	WT	**G12S**
183	WT	WT	WT	WT	**G12S**
382	WT	WT	WT	WT	**G12D**
401	WT	WT	WT	WT	**G13S**
436	WT	WT	WT	WT	**G12C**
29	WT	WT	WT	WT	**G12V**
443	WT	WT	WT	WT	**G12R**
173	WT	WT	WT	WT	**G12V**
243	WT	WT	WT	WT	**M**
276	WT	WT	WT	WT	**G12D**
368	WT	WT	WT	WT	**G13C**
366	WT	WT	WT	WT	**G12F**
287	**Del19**	**Del19**	WT	WT	WT
375	**Del19**	**Del19**	WT	WT	WT
515	**L858R**	**L858R**	WT	WT	WT
233	**Del19**	**Del19**	WT	WT	WT
319	WT	**L858R**	WT	WT	WT
392	WT	**Del19**	WT	WT	WT
464	WT	**Del19**	WT	WT	WT
545	WT	**Del19**	WT	WT	WT
529	**Del19**	**Del19**	WT	WT	WT
269	WT	**Del19**	WT	WT	WT
78	WT	**Del19**	WT	WT	WT
412	WT	**L858R**	WT	WT	WT

135	**Del19**	**Del19**	WT	WT	WT
364	WT	**Del19**	WT	WT	WT
381	**L858R**	**L858R**	WT	WT	WT
260	WT	**L858R**	WT	WT	WT
344	**Del19**	**Del19**	WT	WT	WT
68	**L858R**	**L858R**	WT	WT	WT
534	**L858R**	**L858R**	WT	WT	WT
162	WT	**L858R**	WT	WT	WT
378	WT	**L858R**	WT	WT	WT
174	WT	**L858R**	WT	WT	WT
87	**L858R**	**L858R**	WT	WT	WT
121	WT	**Del19**	WT	WT	WT
196	WT	**L858R**	WT	WT	WT
242	WT	**Del19**	WT	WT	WT
361	**L858R**	**L858R**	WT	WT	WT
474	WT	**Del19**	WT	WT	WT

WT : Wild-Type

M : Mutation with no precision of the type of mutation

Table 4. Multivariate Cox model for Survival Analysis (n=224).

	Overall Survival			Progression-Free Survival		
	HR	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Mutation						
WT	1*		0.01	1*		0.02
EGFR mutation	0.56	[0.31;1.00]		0.46	[0.28;0.78]	
KRAS mutation (PNA)	1.08	[0.69;1.69]		1.17	[0.77;1.77]	
KRAS mutation (SH)	1.63	[1.09;2.44]		1.10	[0.74;1.65]	
Age						
<60	-	-	-	1*	-	0.07
60-69	-	-	-	0.72	[0.50;1.03]	
>=70	-	-	-	0.66	[0.45;0.97]	
Performance status						
0	1*	-	$<10^{-4}$	1*	-	0.0006
1	1.52	[0.93;2.47]		1.86	[1.17;2.94]	
2 or 3	3.17	[1.90;5.28]		2.68	[1.66;4.33]	
Missing	1.31	[0.62;2.80]		1.75	[0.86;3.56]	
Histology						
Adenocarcinoma	1*	-	0.06	1*	-	0.03
Squamous cell	1.60	[1.07;2.38]		1.10	[0.75;1.62]	
Others	1.31	[0.90;1.90]		1.60	[1.12;2.27]	
Initial number of metastatic sites						
0 or 1	1*	-	$<10^{-4}$	1*	-	0.0014
2	1.47	[1.02;2.13]		1.53	[1.07;2.19]	
3 or more	2.50	[1.67;3.74]		1.93	[1.33;2.80]	
Lung metastasis (2MD)						

No	1*	-	0.10	-	-	-
Yes	0.76	[0.54;1.06]		-	-	-
Geographical origin						
Two European parents	1*	-	0 09	-	-	-
Others	0.62	[0.36;1.08]		-	-	-
Smoking status(2MD)						
Never	1*	-	0.15	1*	-	0.07
Former	1.27	[0.80;2.03]		1.68	[1.08;2.62]	
Current	1.76	[0 99;3 13]		1.73	[0.98;3.05]	

* reference classe

HR: Hazard Ratio; CI: Confidence Interval

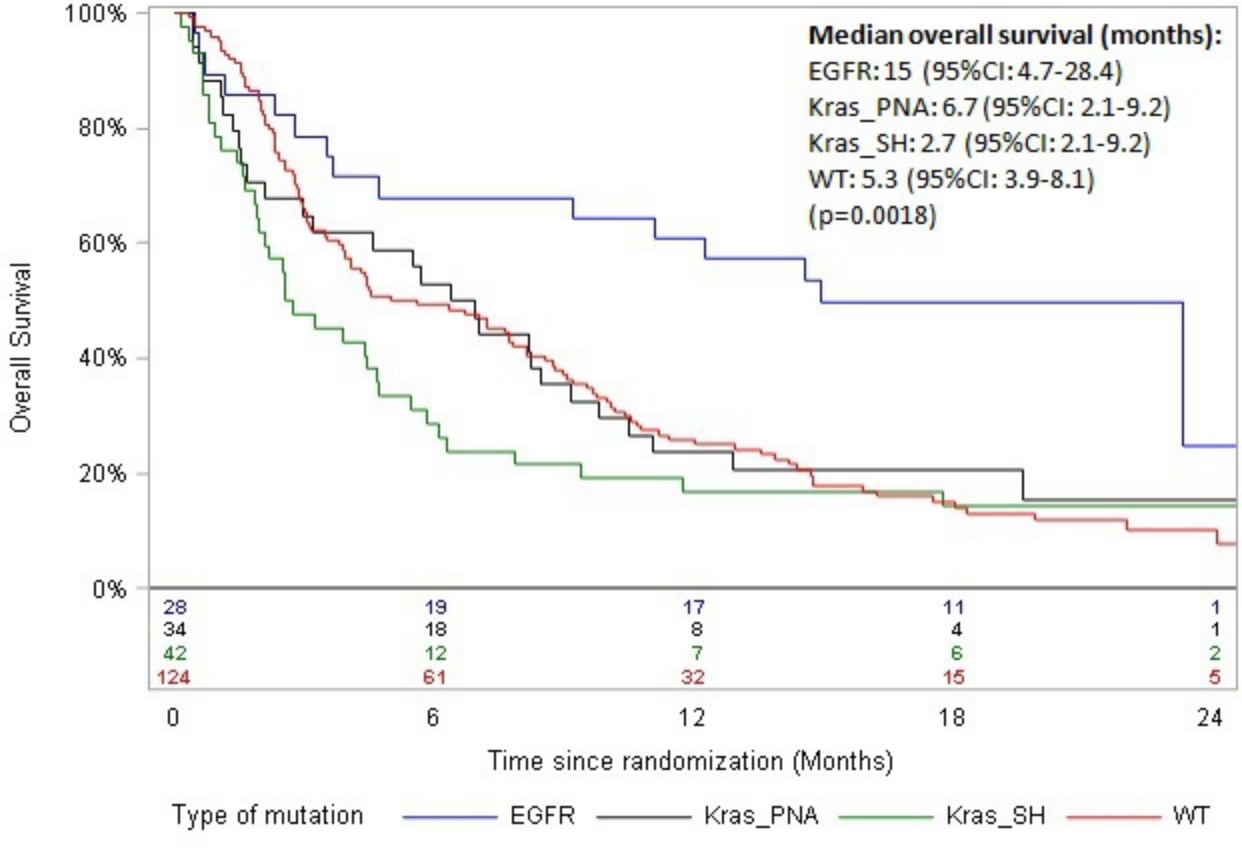
EGFR: fragment analysis (exon 19) and TaqMan assay for L858R; KRAS_SH: real-time polymerase chain reaction (qPCR) with hybridization probe; KRAS_PNA: clamped PCR with peptide nucleic acid (PNA); WT: wild-type EGFR and wild-type KRAS.

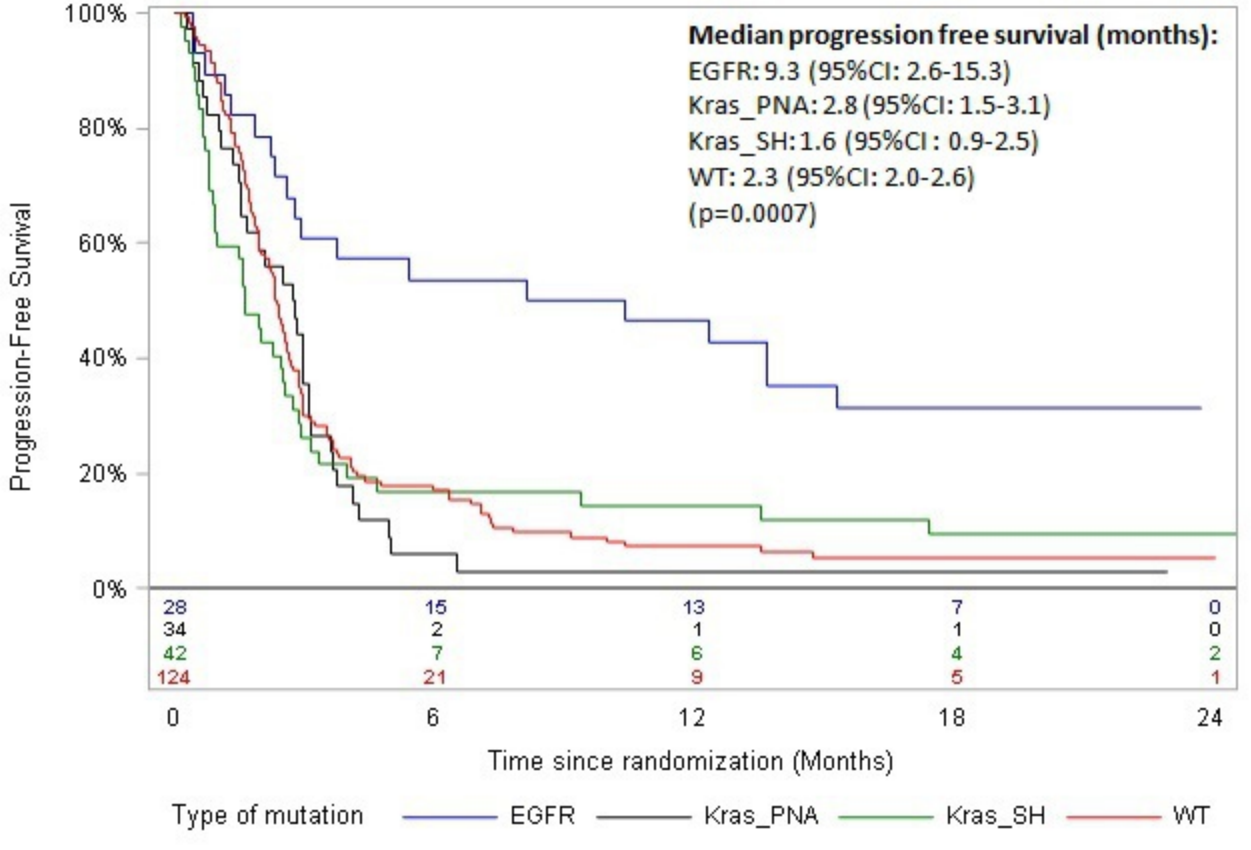
Figure Caption

Figure 1. Survival curves for patients in the ERMETIC re-analyzing models according to detection technique: 1A) Overall survival; 1B) Progression-free survival. *EGFR*. *EGFR* status: fragment analysis for *EGFR* exon 19 and TaqMan assay for *EGFR* L858R analysis.

KRAS status: real-time polymerase chain reaction (qPCR) with hybridization probe (KRAS_SH) or clamped PCR with peptide nucleic acid (PNA) (KRAS_PNA).

WT: wild-type for *EGFR* and *KRAS* mutations.


Median overall survival (months):
EGFR: 15 (95%CI: 4.7-28.4)
Kras_PNA: 6.7 (95%CI: 2.1-9.2)
Kras_SH: 2.7 (95%CI: 2.1-9.2)
WT: 5.3 (95%CI: 3.9-8.1)
(p=0.0018)
100%
80%
60%
40%
20%
0%
Overall Survival
28 19 17 11 1
34 18 8 4 1
42 12 7 6 2
124 61 32 15 5
0
6
12
18
24
Time since randomization (Months)
Type of mutation
EGFR
Kras_PNA
Kras_SH
WT


Median progression free survival (months):
EGFR: 9.3 (95%CI: 2.6-15.3)
Kras_PNA: 2.8 (95%CI: 1.5-3.1)
Kras_SH: 1.6 (95%CI : 0.9-2.5)
WT: 2.3 (95%CI: 2.0-2.6)
(p=0.0007)
Progression-Free Survival
100%
80%
60%
40%
20%
0%
28 15 13 7 0
34 2 1 1 0
42 7 6 4 2
124 21 9 5 1
0
6
12
18
24
Time since randomization (Months)
Type of mutation
EGFR
Kras_PNA
Kras_SH
WT