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ORIGINAL ARTICLE – THORACIC ONCOLOGY

# Prognostic Value of Exon 19 Versus 21 *EGFR* Mutations Varies According to Disease Stage in Surgically Resected Non-small Cell Lung Cancer Adenocarcinoma

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## ABSTRACT

**Background.** The prognostic value of exon 19 and 21 *EGFR* mutations in stage IV non-small cell lung cancer (NSCLC) is well established.

**Objective.** We aimed to evaluate the prognostic value of the mutations in surgically resected NSCLC.

**Methods.** We retrospectively reviewed data from 1798 surgically resected NSCLC adenocarcinomas between 2007 and 2017 in three departments of thoracic surgery (Nancy/Strasbourg, France, and Torino, Italy) for whom mutational status was known. Overall survival (OS) was evaluated using log-rank and Cox proportional hazard models.

**Results.** *EGFR* exon 19 deletion was observed in 108 patients (55.1%) and exon 21 L858R mutations were observed in 88 patients (44.9%). In stage I, the median OS was not significantly different between exons 19 and 21 ($p = 0.54$), while, in stage II, the median OS reached 65 months [95% confidence interval (CI) 41.67–88.33] for exon 19 mutations and decreased to 48 months for exon 21 mutations (95% CI 44.21–51.79; $p = 0.027$). In multivariate analysis, exon 19 deletion remained a favorable prognostic factor [hazard ratio (HR) 0.314, 95% CI 0.098–0.997; $p = 0.05$]. In stage III, the median OS reached 66 months (95% CI 44.67–87.32) for exon 19 mutations and decreased to 32 months for exon 21 mutations (95% CI 29.86–34.14; $p = 0.03$). In multivariate analysis, exon 19 deletion remained a significantly favorable prognostic factor (HR 0.165, 95% CI 0.027–0.999; $p = 0.05$).

**Conclusion.** The prognostic value of *EGFR* exon 19 and 21 mutations appears to be different according to disease stage in surgically resected NSCLC.

Lung cancer remains the leading cause of cancer-related deaths worldwide, with non-small cell lung cancer (NSCLC) accounting for nearly 85% of lung cancer cases. The past decades have seen a dramatic increase in the understanding of molecular alterations of NSCLC, particularly with the discovery of oncogenic drivers, allowing clinicians to define prognosis more accurately and adapt therapies in metastatic NSCLC, thus offering new hope to patients.[1] Mutations in the gene encoding the epidermal growth factor receptor (EGFR) have likely been the most extensively studied.[2] *EGFR* mutations occur mainly in NSCLC adenocarcinomas, females, non-smokers, and Asian patients. Overall, 80% of the mutations are represented by exon 19 deletions and exon 21 L858R mutations.

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Prognostic and predictive values of *EGFR* mutations now appear to be clearly defined in the subset of metastatic patients. Indeed, patients harboring *EGFR* mutations exhibit dramatic responses to EGFR-tyrosine kinase inhibitors (EGFR-TKIs) with subsequent improved survival.[3] On the other hand, despite an increasing number of publications on the potential value of *EGFR* mutations as promising prognostic biomarkers in surgically resected NSCLC,[4–10] many questions remain unanswered. In particular, it is now largely accepted that exon 19 deletions exhibit better prognosis than exon 21 L858R mutations following treatment by TKIs in stage IV patients.[11–13] However, few studies, mainly based on small cohorts, have attempted to investigate these different prognosis values in early-stage NSCLC but have failed to conclude any differences.[14–18]

In this study, we therefore aimed to investigate the different prognostic values of *EGFR* exon 19 or 21 mutations in a large Caucasian cohort of surgically resected NSCLC, speculating that differences may exist according to cancer stage because of the phylogenetic evolution of tumors.

## MATERIAL AND METHODS

This study was approved by the Ethics Committee of the French Society of Thoracic and

Cardiovascular Surgeons (Approval Number: 2017-9-28-17-2-13-StRe).

We retrospectively reviewed data from 1798 patients who underwent surgery with curative intent from 2007 to 2017 in three tertiary referral European hospital departments of thoracic surgery (Nancy University Hospital, Nancy, France; Strasbourg University Hospital, Strasbourg, France; and Torino University Hospital, Torino, Italy) and for whom the mutational status of *EGFR* mutations and *V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) mutations was known. We focused on 196 (10.9%) patients harboring *EGFR* mutations. Findings from parts of this cohort have already been published.[4–7]

Preoperative staging was performed as previously described[7] (details on preoperative staging, genotyping technique and other molecular alterations frequencies are provided as electronic supplementary data).

Surgical resections were performed by thoracotomy. For the most recent cases, video-assisted thoracoscopic surgery (VATS) or robot-assisted thoracoscopic surgery (RATS) was performed, and these surgeries included segmentectomies, lobectomies, bi-lobectomies, or pneumonectomies. Adequate lymphadenectomy was performed according to the recommendations of the French Society of Thoracic and Cardiovascular Surgeons.[19] Hence, stations 2R, 4R, 7, 8, 9, 10–12 on the right side and 5, 6, 7, 8, 9, 10–12 on the left side were peroperatively harvested.

The use of neoadjuvant and adjuvant treatments was discussed by a multidisciplinary board in the presence of certified thoracic surgeons, oncologists, pneumologists, and radiation therapists. Because patients were referred from various oncologists to our three referral centers, there was no uniform protocol of perioperative therapy; however, platinum-based chemotherapy, with or without radiation therapy, remains the standard of care. EGFR-TKIs were only used in case of local and/or distant recurrence.

Pathological examinations were performed at each center by senior pathologists familiar with NSCLC. Each patient was staged according to the 7th TNM classification. Molecular analysis of the mutations in *EGFR* exons 19 and 21 was performed as previously described in patients with adenocarcinomas only.[5]

Patients were studied according to their age, sex, type of surgery, perioperative treatment, type of resection, pT/pN, stage (according to the 7th TNM classification), number of N2 stations involved, microscopic N2 disease status,[20] skip N2 disease status,[6] R0/R1/R2 resection type, and use of EGFR-TKIs. The Charlson Comorbidity Index (CCI) was calculated for each patient. We grouped patients into the following established categories according to their total score:[21] 0 (no comorbidity), 1–2 (average), 3–4 (moderate), and $\geq 5$ (severe). Patient smoking status was defined as never smoker, $< 100$ cigarettes in their lifetime, former smoker, quit $> 1$ year before diagnosis, current smoker with an ongoing smoking habit, or quit $< 1$ year before diagnosis.

The primary endpoints of the study were overall survival (OS), which was defined as the time elapsed between surgery and either death or the last follow-up.

### *Statistical Analysis*

Variables were described using position and dispersion statistics as the mean and standard deviation values and as percentages for qualitative variables. Comparisons among groups were performed using Chi-square tests or Fisher's non-parametric test for proportions, depending on theoretical effectiveness. To check for a Gaussian distribution, the Shapiro test for normality was used. Analysis of variance (ANOVA) tests or non-parametric Kruskal–Wallis tests were used to compare numerical data. The association of each factor with the survival of patients was modeled by first using Kaplan–Meier graphs and then associated univariate Cox proportional hazard models. *P*-values were estimated by likelihood type III tests in models. Coefficients were also used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs).

Those variables for which univariate *p*-values were $< 0.20$ were then analyzed using a multivariate Cox regression. A backward stepwise variable selection was

performed to avoid redundant information. The results are presented as HRs fitted from the multivariate final model.

All statistical tests were two-tailed, and $p$-values $< 0.05$ were considered significant. All analyses were performed using IBM SPSS version 25 (IBM Corporation, Armonk, NY, USA).

## RESULTS

### *Clinical and Molecular Characteristics*

Our population was mainly composed of females (63.3%, $n = 124$) and non-smokers (77.6%, $n = 152$). The median age at the time of surgery was 68 years [interquartile range (IQR) 16], and the median number of lymph nodes harvested was 16 (IQR 12). Molecular analysis revealed 108 exon 19 deletions (55.1%) and 88 L858R exon 21 mutations (44.9%). Data are compiled in Table 1.

### *Overall Survival*

With a median follow-up time of 46 months (minimum 1 month, maximum 141 months), the median OS for the whole cohort was 74 months (95% CI 38.69–109.31), with a corresponding 1-, 2-, 3-, 5-, and 10-year OS of 97, 86, 81, 64, and 44%, respectively.

### *Stage I Non-small Cell Lung Cancer (NSCLC)*

The median OS was not reached, with a corresponding 1-, 2-, 3-, 5-, and 10-year OS of 100, 96, 91, 82, and 67%, respectively.

In univariate analysis, median OS for both exon 19 and 21 mutations was neither reached nor significantly different ($p = 0.54$) (Fig. 1a). The presence of angio-invasion ($p = 0.03$), type of resection ($p < 0.0001$), R0 resection (0.028), and use of EGFR-TKIs ($p < 0.0001$) significantly influenced the OS.

In multivariate analysis, no significant independent prognostic factor was identified. Data are compiled in Table 2.

### *Stage II NSCLC*

The median OS was 58 months (95% CI 47.54–68.46), with a corresponding 1-, 2-, 3-, 5-, and 10-year OS of 95%, 91%, 81%, 48%, and 24%, respectively.

In univariate analysis, the median OS was significantly influenced by the type of *EGFR* mutation. Indeed, it reached 65 months (95% CI 41.67–88.33) for exon 19 mutations and decreased to 48 months for exon 21 mutations (95% CI 44.21–51.79; $p = 0.027$) [Fig. 1b]. Otherwise, sex ($p = 0.015$) and angio-invasion ($p = 0.002$) were the only other significant factors in univariate analysis.

In multivariate analysis, the presence of the *EGFR* exon 19 mutation remained the only independent prognostic factor (HR 0.314, 95% CI 0.098–0.997; $p = 0.05$). Data are compiled in Table 3.

### *Stage III NSCLC*

The median OS was 50 months (95% CI 35.74–64.26), with a corresponding 1-, 2-, 3-, 5-, and 10-year OS of 96, 79, 66, 42, and 0%, respectively.

In univariate analysis, the median OS was significantly influenced by the type of *EGFR* mutation. Indeed, median OS reached 66 months (95% CI 44.67–87.32) for exon 19 mutations and decreased to 32 months for exon 21 mutations (95% CI 29.86–34.14; $p = 0.03$) (Fig. 1c). Otherwise, R0/R1 resection ($p < 0.0001$) and use of EGFR-TKIs ($p = 0.002$) were the only other significant factors in univariate analysis.

In multivariate analysis, the presence of the *EGFR* exon 19 mutation remained the only independent prognostic factor (HR 0.165, 95% CI 0.027–0.999; $p = 0.05$). Data are compiled in Table 4.

## DISCUSSION

The prognostic value of *EGFR* mutations in surgically resected NSCLC is still the subject of debate, with contradictory results published. Hence, a recent meta-analysis on the impact of *EGFR* mutations on resected NSCLC in 3337 patients demonstrated no significant impact of *EGFR* mutational status on disease-free survival (DFS) or OS;[22] however, only 6 of the included 22 studies were based on non-Asian patients. Notably, the rates of membranous EGFR (*mEGFR*) were > 25% in 14 studies, not reflecting the expected rate in a Caucasian population (approaching 10%). These populations were also quite heterogeneous and included stages I–IIIA NSCLC with various NSCLC histologies and no uniform exon sequencing. All of these observations limit the interpretation of these previous results. However, in the largest retrospective study to date of 939 Asian patients, 418 patients harbored *EGFR* mutations. Takamochi et al. reported increased OS in surgically resected NSCLC harboring the *EGFR* mutation (HR 0.56, 95% CI 0.38–0.82; $p = 0.003$).[15]

Another question relies on the different prognostic value according to exon 19 versus exon 21 *EGFR* mutations. Indeed, in metastatic NSCLC, it is now known that the *EGFR* exon 19 mutation is associated with a more favorable OS following treatment by EGFR-TKIs in comparison with exon 21 mutations.[11–13] However, published evidence

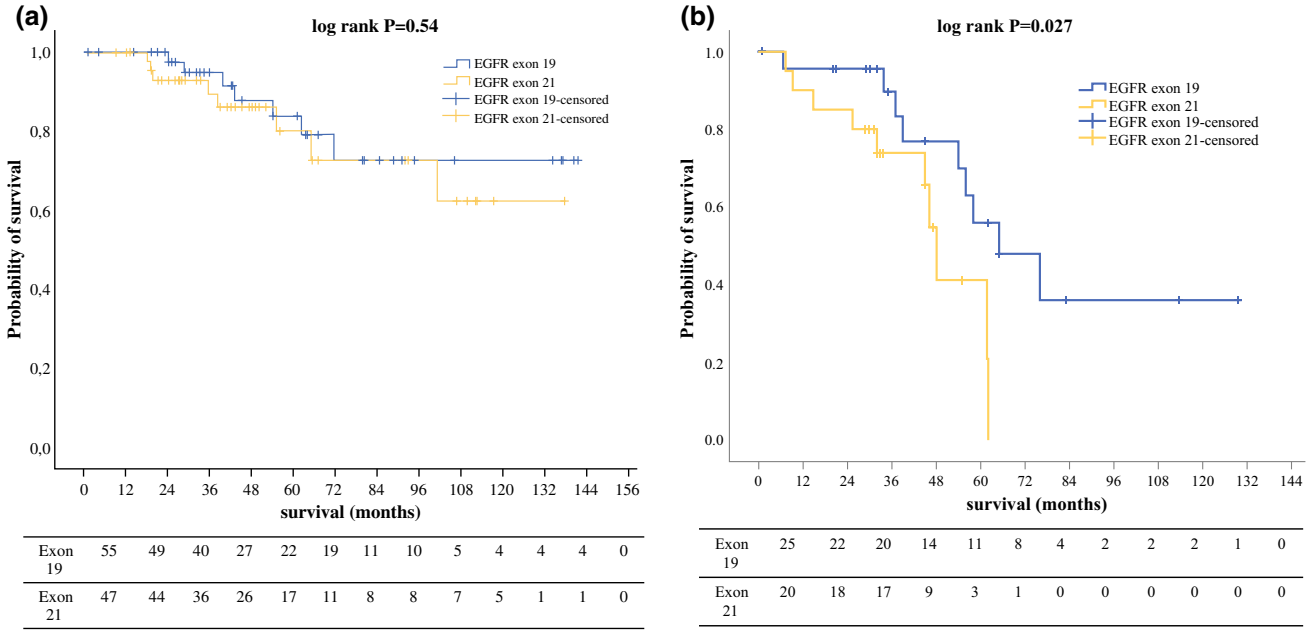
**TABLE 1** Main clinical and molecular characteristics of the study population

| | Stage I ($n = 102$) | Stage II ($n = 45$) | Stage III ($n = 49$) | $p$-Value |
|---|---|---|---|---|
| EGFR exon 19 | 55 (50.9) | 25 (23.1) | 28 (25.9) | 0.93 |
| EGFR exon 21 | 47 (52) | 20 (23) | 21 (25) | |
| Sex | 36 (50) | 18 (25) | 18 (25) | 0.86 |
| Male | 66 (53.2) | 27 (21.8) | 31 (25) | |
| Female | | | | |
| Age, years | 54 (49.5) | 24 (22) | 31 (28.4) | 0.46 |
| < 70 | 48 (55.2) | 21 (24.1) | 18 (20.7) | |
| ≥70 | | | | |
| Smoking habit | 79 (52) | 36 (23.7) | 37 (24.3) | 0.82 |
| Non-smoker | 5 (71.4) | 1 (14.3) | 1 (14.3) | |
| Active smoker | 18 (48.6) | 8 (21.6) | 11 (29.7) | |
| Former smoker | | | | |
| pT | 46 (68.7) | 9 (13.4) | 12 (17.9) | **<0.0001** |
| 1 | 56 (63.6) | 15 (17) | 17 (19.3) | |
| 2 | 0 | 21 (60) | 14 (40) | |
| 3 | 0 | 0 | 6 (100) | |
| 4 | | | | |
| pN+ | 0 | 24 (26.3) | 44 (63.7) | **<0.0001** |
| Angio-invasion (yes) | 19 (31.1) | 17 (27.9) | 25 (41) | **<0.0001** |
| Neoadjuvant treatment (yes) | 0 | 15 (44.2) | 19 (55.8) | **<0.0001** |
| Adjuvant treatment (yes) | 10 (15.4) | 18 (27.7) | 37 (56.9) | **<0.0001** |
| Use of EGFR-TKIs | 15 (26.8) | 20 (35.7) | 21 (37.5) | **<0.0001** |
| CCI | 5 (55.6) | 1 (11.1) | 3 (33.3) | 0.87 |
| 0 | 16 (44.4) | 10 (27.8) | 10 (27.8) | |
| 1 | 8 (42.1) | 7 (36.8) | 4 (21.1) | |
| 2 | 9 (52.9) | 4 (23.5) | 4 (23.5) | |
| 3 | | | | |
| Type of resection | 81 (49.4) | 38 (23.2) | 45 (27.4) | 0.14 |
| Lobectomy | 4 (40) | 3 (30) | 3 (30) | |
| Pneumonectomy | 3 (60) | 2 (40) | 0 | |
| Bi-lobectomy | 14 (82.4) | 2 (11.8) | 1 (5.9) | |
| Typical segmentectomy | | | | |
| Skip N2 (yes) | 0 | 0 | 13 (100) | 0.15 |
| Microscopic N (yes) | 0 | 4 (28.6) | 10 (71.4) | 0.79 |
| Number of N2 stations | 0 | 0 | 13 (100) | |
| 1 | 0 | 0 | 6 (100) | |
| 2 | 0 | 0 | 3 (100) | |
| 3 | | | | |
| R0 | 75 (51) | 36 (24.5) | 36 (24.5) | 0.85 |
| R1 | 3 (50) | 2 (33.3) | 1 (16.7) | |

Data are expressed as $n$ (%)

*CCI* Charlson Comorbidity Index, *TKIs* tyrosine kinase inhibitors, *EGFR* epidermal growth factor receptor

in surgically resected NSCLC does not allow an unequivocal conclusion to be reached, mainly due to the small sample size, retrospective nature of the research, and grouped analysis of several disease stages. Furthermore, all published studies were based on Asian populations, limiting extrapolation of results to Caucasian patients. Hence, in a study of 104 patients harboring an *EGFR* mutation, Jin et al. did not report any difference in DFS between exon 19 and 21 mutations.[16] In line with these results, Takamochi et al. analyzed 418 patients and did not



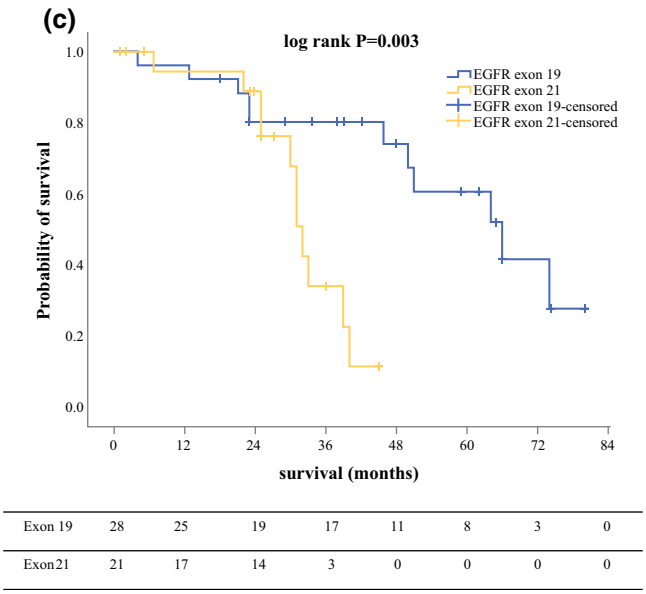




**FIG. 1** Schematic representation of Kaplan–Meier overall survival according to (**a**) stage I NSCLC, (**b**) stage II NSCLC, and (**c**) stage III NSCLC. *NSCLC* non-small cell lung cancer

observe any difference between exon 19 and 21 mutations in terms of both DFS and OS.[15] On the other hand, Liu et al. analyzed 58 patients with the *EGFR* mutation and reported longer DFS and significantly better OS in patients harboring the exon 19 mutation in comparison with exon 21.[18] In contrast, Okamoto et al. analyzed 72 patients with the *EGFR* mutation and reported a significantly better DFS with exon 21 compared with exon 19 mutations.[17] Finally, Lee et al. analyzed 48 patients and reported a significantly higher risk of recurrence following surgery with exon 19 compared with exon 21 mutations.[12]

We decided to investigate the differences between exons 19 and 21 according to disease stages, hypothesizing that differences between surgical and metastatic cases were linked to the phylogenetic evolution of *EGFR*-mutated tumors. Indeed, in the Tracking Non-Small Cell Lung Cancer Evolution through Therapy (TRACERx) prospective multicenter trial, Jamal-Hanjani et al. performed a multi-region whole-exome sequencing of surgically resected stages IA through IIIA NSCLC.[23] They identified the early occurrence of alterations in some cancer gene alterations, such as *EGFR* mutations, suggesting their

**TABLE 2** Univariate and multivariate analyses on overall survival in stage I patients

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Median OS (95% CI) | *p*-Value | HR (95% CI) | *p*-Value |
| EGFR exon 19 | Not reached | 0.54 | – | – |
| EGFR exon 21 | Not reached | | | |
| Sex | Not reached | 0.78 | – | – |
| Male | Not reached | | | |
| Female | | | | |
| Age, years | Not reached | 0.08 | – | 0.36 |
| < 70 | Not reached | | | |
| ≥70 | | | | |
| Smoking habit | Not reached | 0.32 | – | – |
| Never smoker | 39.44 | | | |
| Active smoker | 101.09 (33.09–168.49) | | | |
| Former smoker | | | | |
| pT | Not reached | 0.64 | – | – |
| 1 | Not reached | | | |
| 2 | | | | |
| Angio-invasion | Not reached | **0.03** | – | 0.27 |
| No | 101 (36.32–165.86) | | | |
| Yes | | | | |
| Adjuvant treatment | Not reached | 0.99 | – | – |
| Yes | Not reached | | | |
| No | | | | |
| EGFR-TKIs | 62 (26.29–97.71) | **<0.0001** | – | 0.12 |
| Yes | Not reached | | | |
| No | | | | |
| CCI | Not reached | 0.35 | – | – |
| 0 | Not reached | | | |
| 1 | Not reached | | | |
| 2 | Not reached | | | |
| 3 | | | | |
| Type of resection | Not reached | **<0.0001** | 1 | 0.008 |
| Lobectomy | 39.22 | | 0.21 (0.067–0.661) | 0.96 |
| Pneumonectomy | Not reached | | 0.933 (0.068–12.711) | 0.99 |
| Bi-lobectomy | 57.23 | | 0 (0) | |
| Typical segmentectomy | | | | |
| R0 resection | Not reached | **0.028** | – | 0.15 |
| R1 resection | Not reached | | | |

*OS* overall survival, *CI* confidence interval, *HR* hazard ratio, *CCI* Charlson Comorbidity Index, *TKIs* tyrosine kinase inhibitors, *EGFR* epidermal growth factor receptor

involvement in tumor initiation. Moreover, they observed that more than half of the mutations affecting chromatin remodeling, histone methylation, or DNA damage response and repair were late events appearing during tumor evolution. It does not seem unrealistic to hypothesize that the co-existence of oncogenic driver mutations, such as *EGFR* mutations, with these late mutations may be responsible for modifications of cancer cell behavior. Indeed, in *KRAS*-mutated NSCLC, three major subgroups of *KRAS*-mutant NSCLC adenocarcinomas are defined by the coexistence of other genetic alterations: *STK11/LKB1*, *TP53* and *CDKN2A*/B.[24] These additional genetic alterations appear to be predictive of the response to anti-PD1/PDL1 immunotherapy in *KRAS*-mutated NSCLC because the immune tumor microenvironment differs according to these co-mutations.[25,26] Otherwise, different downstream

**TABLE 3** Univariate and multivariate analyses on overall survival in stage II patients

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Median OS (95% CI) | *p*-Value | HR (95% CI) | *p*-Value |
| EGFR exon 19 | 65 (41.67–88.33) | **0.027** | 0.314 (0.098–0.997) | **0.05** |
| EGFR exon 21 | 48 (44.21–51.79) | | 1 | |
| Sex | 56 (29.57–82.43) | **0.015** | – | 0.18 |
| Male | 65 | | | |
| Female | | | | |
| Age, years | 58 (32.59–83.41) | 0.13 | – | 0.79 |
| < 70 | 48 (25.89–70.11) | | | |
| ≥70 | | | | |
| Smoking habit | 61.72 (51.48–71.96) | 0.69 | – | – |
| Never smoker | 48 | | | |
| Active smoker | 54 (26.55–81.45) | | | |
| Former smoker | | | | |
| pT | 62 (61.54–62.46) | 0.8 | – | – |
| 1 | 48 (24.82–71.18) | | | |
| 2 | 58 (40.16–75.84) | | | |
| 3 | | | | |
| pN− | 58 (40.16–75.84) | 0.92 | – | – |
| pN+ | 61.72 (40.85–82.57) | | | |
| Microscopic N | 62 (17.08–106.9) | 0.69 | – | – |
| Yes | 48 | | | |
| No | | | | |
| Angio-invasion | 76 (53.06–98.94) | **0.002** | – | 0.42 |
| No | 46 (28.74–63.26) | | | |
| Yes | | | | |
| Neoadjuvant treatment | 58 (42.33–73.68) | 0.75 | – | – |
| Yes | 61.72 (41.25–82.19) | | | |
| No | | | | |
| Adjuvant treatment | 58 (49.06–66.94) | 0.45 | – | – |
| Yes | 61.72 (38.84–84.6) | | | |
| No | | | | |
| EGFR-TKIs | 54 (40.87–67.13) | 0.056 | – | 0.13 |
| Yes | Not reached | | | |
| No | | | | |
| CCI | Not reached | 0.57 | – | – |
| 0 | 58 | | | |
| 1 | 50.11 | | | |
| 2 | 57.22 | | | |
| 3 | | | | |
| Type of resection | 54.8 | 0.52 | – | – |
| Lobectomy | Not reached | | | |
| Pneumonectomy | 57.75 | | | |
| Bi-lobectomy | Not reached | | | |
| Typical segmentectomy | | | | |
| R0 resection | 61.72 (44.28–79.15) | 0.25 | – | – |
| R1 resection | 33.92 | | | |

*OS* overall survival, *CI* confidence interval, *HR* hazard ratio, *CCI* Charlson Comorbidity Index, *TKIs* tyrosine kinase inhibitors, *EGFR* epidermal growth factor receptor

**TABLE 4** Univariate and multivariate analyses on overall survival in stage III patients

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Median OS (95% CI) | *p*-Value | HR (95% CI) | *p*-Value |
| EGFR exon 19 | 66 (44.67–87.32) | **0.03** | 0.165 (0.027–0.999) | **0.05** |
| EGFR exon 21 | 32 (29.86–34.14) | | 1 | |
| Sex | 40 (11.15–68.85) | 0.24 | – | – |
| Male | 64 (25.45–102.55) | | | |
| Female | | | | |
| Age, years | 50.97 (25.42–76.57) | 0.9 | – | – |
| < 70 | 45.85 (35.98–55.72) | | | |
| ≥70 | | | | |
| Smoking habit | 50 (37–62.99) | 0.26 | – | – |
| Never smoker | 66 | | | |
| Active smoker | Not reached | | | |
| Former smoker | | | | |
| pT | 50.98 (31.29–70.66) | 0.96 | – | – |
| 1 | Not reached | | | |
| 2 | 40 (4.48–75.52) | | | |
| 3 | 66 (18.62–102.5) | | | |
| 4 | | | | |
| pN− | 66 (40.39–91.61) | 0.33 | – | – |
| pN+ | 45.85 (31.43–60.27) | | | |
| Microscopic N | 64 (29.08–98.92) | 0.13 | 1 | 0.07 |
| Yes | 31 (26.84–35.16) | | 4.266 (0.864–21.07) | |
| No | | | | |
| Skip N | Not reached | 0.71 | – | – |
| Yes | 45.85 (28.31–63.39) | | | |
| No | | | | |
| Number of N2 stations | 77 | 0.99 | – | – |
| 1 | Not reached | | | |
| 2 | Not reached | | | |
| 3 | | | | |
| Angio-invasion | 45.85 (23.21–68.5) | 0.45 | – | 0.42 |
| No | 64 (36.3–91.7) | | | |
| Yes | | | | |
| Neoadjuvant treatment | 50 (21.46–78.55) | 0.64 | – | – |
| Yes | 50.98 (39.59–62.36) | | | |
| No | | | | |
| Adjuvant treatment | 50.97 (27.29–74.65) | 0.85 | – | – |
| Yes | 50 (0–104.19) | | | |
| No | | | | |
| EGFR-TKIs | 33 (23.63–42.37) | **0.002** | – | 0.27 |
| Yes | 66 | | | |
| No | | | | |
| CCI | 33 (16.99–49) | 0.58 | – | – |
| 0 | 66 (26.76–105.24) | | | |
| 1 | 39 | | | |
| 2 | 64 | | | |
| 3 | | | | |

**TABLE 4** continued

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Median OS (95% CI) | *p*-Value | HR (95% CI) | *p*-Value |
| Type of resection | 48.8 | 0.67 | – | – |
| Lobectomy | Not reached | | | |
| Pneumonectomy | Not reached | | | |
| Typical segmentectomy | | | | |
| R0 resection | 50 (36.95–63.05) | **<0.0001** | 1 | 0.12 |
| R1 resection | 12.69 | | 2.23 (0.723–17.608) | |

*OS* overall survival, *CI* confidence interval, *HR* hazard ratio, *CCI* Charlson Comorbidity Index, *TKIs* tyrosine kinase inhibitors, *EGFR* epidermal growth factor receptor

signaling pathways are activated according to *KRAS* amino-acid substitution. Hence, both *KRAS* G12C and G12V mutations exhibited activated Ral signaling and decreased growth factor-dependent Akt activation, while the G12D mutation exhibited activated PI3K and MEK signaling.[27] Consequently, it would not be surprising that the co-existence of different *EGFR* mutations with different genetic alterations during cancer progression may change the behavior and then the prognosis of cancer cells. This finding might partially explain why we observed such differences in our cohort according to cancer stages. Indeed, we did not observe any difference between exons 19 and 21 in stage I NSCLC, most likely because of the absence of co-mutations. This result is in accordance with the findings of Takamochi et al.,[15] whose cohort was largely based on stage I NSCLC (84% stage I). On the other hand, the differences in OS between exons 19 and 21 increased with the stage of the disease, most likely because of the occurrence of other genetic alterations. However, we were not able to compare these findings to previous published series because these papers included very heterogeneous populations, including stage I–IIIA NSCLC.

Our results must be interpreted with caution because of a few limitations. First, the present investigation was a retrospective cohort study, and the sample size was relatively small. However, the expected rate of *EGFR* mutations in Caucasian populations ranges between 10 and 15%. To the best of our knowledge, the present study is the largest cohort study to date on a surgically resected Caucasian population focusing on differences between *EGFR* exon 19 and 21 mutations and taking into account disease stage. Finally, because of the small sample size ($n$ = 15 patients in stage I, 20 patients in stage II, 21 patients in stage III), we were not able to perform a statistical analysis and to clearly evaluate the prognostic value of these exons on time to recurrence. Hence, one can speculate that the better OS related to exon 19 mutations is linked to the use of EGFR-TKIs in case of recurrence, with a better-known response in exon 19 deletions. However, in our cohort, even though the use of EGFR-TKIs was related to a better OS in univariate analysis in stages I and III, it was not a significant factor in multivariate analysis, likely limiting its impact on OS.

## CONCLUSIONS

In this largest cohort study of Caucasian surgically resected NSCLC adenocarcinomas, we have shown that prognosis of exon 19 and 21 mutations differs according to cancer stages. This result is likely related to the occurrence of new genetic alterations during cancer cell evolution that could impact their behavior, with different responses occurring according to the type of interactions (i.e. exon 19 or 21 and co-mutations). Oncogenic alterations were again shown to be promising biomarkers. Further studies are needed to properly understand the molecular behavior of *EGFR* mutations in surgically resected NSCLC, along with their place in decision making after surgery.

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## REFERENCES

1. Inamura K. Lung cancer: understanding its molecular pathology and the 2015 WHO classification. *Front Oncol.* 2017;7:193.
2. Passiglia F, Listi A, Castiglia M, et al. EGFR inhibition in NSCLC: new findings…. and opened questions? *Crit Rev Oncol Hematol.* 2017;112:126-135.
3. Lee CK, Davies L, Wu YL, et al. Gefitinib or erlotinib vs chemotherapy for EGFR mutation-positive lung cancer: individual patient data meta-analysis of overall survival. *J Natl Cancer Inst.* 2017. https://doi.org/10.1093/jnci/djw279
4. Seitlinger J, Renaud S, Falcoz PE, et al. Epidermal growth factor receptor and v-Ki-ras2 Kirsten rat sarcoma viral oncogen

homologue-specific amino acid substitutions are associated with different histopathological prognostic factors in resected non-small-cell lung cancer. *Interact Cardiovasc Thorac Surg.* 2016;23(6):902–907.
5. Renaud S, Seitlinger J, Falcoz PE, et al. Specific KRAS amino acid substitutions and EGFR mutations predict site-specific recurrence and metastasis following non-small-cell lung cancer surgery. *Br J Cancer.* 2016;115(3):346–353.
6. Guerrera F, Renaud S, Tabbo F, et al. Epidermal growth factor receptor mutations are linked to skip N2 lymph node metastasis in resected non-small-cell lung cancer adenocarcinomas. *Eur J Cardiothorac Surg.* 2017;51(4):680–688.
7. Renaud S, Falcoz PE, Schaeffer M, et al. Prognostic value of the KRAS G12V mutation in 841 surgically resected Caucasian lung adenocarcinoma cases. *Br J Cancer.* 2015;113(8):1206–1215.
8. Marks JL, Broderick S, Zhou Q, et al. Prognostic and therapeutic implications of EGFR and KRAS mutations in resected lung adenocarcinoma. *J Thorac Oncol.* 2008;3(2):111–116.
9. D'Angelo SP, Janjigian YY, Ahye N, et al. Distinct clinical course of EGFR-mutant resected lung cancers: results of testing of 1118 surgical specimens and effects of adjuvant gefitinib and erlotinib. *J Thorac Oncol.* 2012;7(12):1815–1822.
10. Izar B, Zhou H, Heist RS, et al. The prognostic impact of KRAS, its codon and amino acid specific mutations, on survival in resected stage I lung adenocarcinoma. *J Thorac Oncol.* 2014;9(9):1363–1369.
11. Sheng M, Wang F, Zhao Y, et al. Comparison of clinical outcomes of patients with non-small-cell lung cancer harbouring epidermal growth factor receptor exon 19 or exon 21 mutations after tyrosine kinase inhibitors treatment: a meta-analysis. *Eur J Clin Pharmacol.* 2016;72(1):1–11.
12. Lee CK, Wu YL, Ding PN, et al. Impact of specific epidermal growth factor receptor (EGFR) mutations and clinical characteristics on outcomes after treatment with EGFR tyrosine kinase inhibitors versus chemotherapy in EGFR-mutant lung cancer: a meta-analysis. *J Clin Oncol.* 2015;33(17):1958–1965.
13. Kuan FC, Kuo LT, Chen MC, et al. Overall survival benefits of first-line EGFR tyrosine kinase inhibitors in EGFR-mutated non-small-cell lung cancers: a systematic review and meta-analysis. *Br J Cancer.* 2015;113(10):1519–1528.
14. Lee YJ, Park IK, Park MS, et al. Activating mutations within the EGFR kinase domain: a molecular predictor of disease-free survival in resected pulmonary adenocarcinoma. *J Cancer Res Clin Oncol.* 2009;135(12):1647–1654.
15. Takamochi K, Oh S, Matsunaga T, Suzuki K. Prognostic impacts of EGFR mutation status and subtype in patients with surgically resected lung adenocarcinoma. *J Thorac Cardiovasc Surg.* 2017;154(5):1768–1774.e1.
16. Jin Y, Chen M, Yu X. Differences among lesions with exon 19, exon 21 EGFR mutations and wild types in surgically resected non-small cell lung cancer. *Sci Rep.* 2016;6:31636.
17. Okamoto T, Kitahara H, Shimamatsu S, et al. Prognostic impact of EGFR driver mutations on postoperative disease recurrence in lung adenocarcinoma. *Anticancer Res.* 2016;36(6):3057–3063.
18. Liu WS, Zhao LJ, Pang QS, Yuan ZY, Li B, Wang P. Prognostic value of epidermal growth factor receptor mutations in resected lung adenocarcinomas. *Med Oncol.* 2014;31(1):771.
19. Thomas P, Dahan M, Riquet M, et al. Practical issues in the surgical treatment of non-small cell lung cancer. Recommendations from the French Society of thoracic and cardiovascular surgery [in French]. *Rev Mal Respir.* 2008;25(8):1031–1036.
20. Garelli E, Renaud S, Falcoz PE, et al. Microscopic N2 disease exhibits a better prognosis in resected non-small-cell lung cancer. *Eur J Cardiothorac Surg.* 2016;50(2):322–328.
21. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis.* 1987;40(5):373–383.
22. Zhang Z, Wang T, Zhang J, et al. Prognostic value of epidermal growth factor receptor mutations in resected non-small cell lung cancer: a systematic review with meta-analysis. *PLoS One.* 2014;9(8):e106053.
23. Jamal-Hanjani M, Wilson GA, McGranahan N, et al. Tracking the evolution of non-small-cell lung cancer. *N Engl J Med.* 2017;376(22):2109–2121.
24. Skoulidis F, Byers LA, Diao L, et al. Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. *Cancer Discov.* 2015;5(8):860–877.
25. Dong ZY, Zhong WZ, Zhang XC, et al. Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. *Clin Cancer Res.* 2017;23(12):3012–3024.
26. Calles A, Liao X, Sholl LM, et al. Expression of PD-1 and Its Ligands, PD-L1 and PD-L2, in smokers and never smokers with KRAS-mutant lung cancer. *J Thorac Oncol.* 2015;10(12):1726–1735.
27. Ihle NT, Byers LA, Kim ES, et al. Effect of KRAS oncogene substitutions on protein behavior: implications for signaling and clinical outcome. *J Natl Cancer Inst.* 2012;104(3):228–239.