

Distinct Epidemiology and Clinical Consequence of Classic Versus Rare EGFR Mutations in Lung Adenocarcinoma

Zoltan Lohinai, MD, *†‡ Mir Alireza Hoda, MD, † Katalin Fabian, MD, § Gyula Ostoros, MD, PhD, *
 Erzsebet Raso, PhD, ||¶ Tamás Barbai, MS, || Jozsef Timar, MD, PhD, DSc, ||¶ Ilona Kovalszky, MD, PhD, #
 Mihaly Cserepes, MS, * Anita Rozsas, MS, *† Viktoria Laszlo, PhD, † Michael Grusch, PhD, **
 Walter Berger, PhD, ** Walter Klepetko, MD, † Judit Moldvay, MD, PhD, §
 Balazs Dome, MD, PhD, *†‡ and Balazs Hegedus, PhD†¶

Introduction: Although classic sensitizing mutations of epidermal growth factor receptor (EGFR) are positive predictive markers for EGFR tyrosine kinase inhibitors (TKIs) in lung adenocarcinoma, there are rare EGFR mutations with unknown epidemiology and influence on prognosis and TKI response.

Methods: Eight hundred and fourteen lung adenocarcinoma patients with KRAS and/or EGFR mutation analyses for TKI therapy indication were identified. Six hundred and forty-five patients were included in the epidemiological analysis. The clinical outcome was analyzed in 419 advanced-stage patients with follow-up data.

Results: Four hundred and eighty (59%) KRAS/EGFR double wild-type, 216 (27%) KRAS mutant, 42 (5%) classic, 49 (6%) rare, and 27 (3%) synonymous EGFR mutant cases were identified. Twenty previously unpublished non-synonymous mutations were found. Rare EGFR mutations were significantly associated with smoking (vs. classic EGFR mutations; $p = 0.0062$). Classic EGFR mutations but not rare ones were independent predictors of increased overall survival (hazard ratios, 0.45; 95% confidence intervals, 0.25–0.82; $p = 0.009$). TKI therapy response rate of patients harboring classic

EGFR mutations was significantly higher (vs. rare EGFR mutations; 71% vs. 37%; $p = 0.039$). Patients with classic or sensitizing rare (G719x and L861Q) EGFR mutations had significantly longer progression-free survival when compared with the remaining rare mutation cases (12 vs. 6.2 months; $p = 0.048$).

Conclusions: The majority of rare EGFR mutations was associated with smoking, shorter overall survival, and decreased TKI response when compared with classic EGFR mutations. However, studies characterizing the TKI sensitizing effect of individual rare mutations are indispensable to prevent the exclusion of patients with sensitizing rare EGFR mutations who may benefit from anti-EGFR therapy.

Key Words: Advanced-stage lung adenocarcinoma, Tyrosine kinase inhibitor therapy, EGFR mutation, Epidemiology.

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Lung cancer is the most frequently diagnosed malignancy worldwide and is a leading cause of mortality.¹ The term lung cancer represents a rather heterogeneous group of diseases including conditions of varying etiology and molecular background.² Nevertheless, the determination of “driver” oncogenic mutations that play a decisive role in tumor development can help to identify targets for therapy. In lung adenocarcinoma, oncogenic mutations of the epidermal growth factor receptor (EGFR) are the most frequent biologically targetable alterations. To date, most of the anti-EGFR drugs introduced are tyrosine kinase inhibitors (TKIs), and the classic mutations of L858R and exon 19 microdeletions can serve as positive predictive biomarkers for response to these agents.³ However, there are other EGFR mutations responsible for primary or acquired resistance against TKI therapy.^{4,5} In addition, many rare EGFR mutations (in our report defined as all nucleotide changes resulting in amino acid sequence change in the tyrosine kinase coding region [exon 18–21] of EGFR excluding exon 19 microdeletions and the L858R point mutation in exon 21) have been described with unknown clinical relevance.^{6–11} Synonymous (silent) EGFR mutations are defined as nucleotide changes without amino acid change in the EGFR protein.¹² EGFR mutations occur almost exclusively in adenocarcinomas; their incidence, however, greatly

*Department of Tumor Biology, National Koranyi Institute of Pulmonology, Budapest, Hungary; †Translational Thoracic Oncology Laboratory, Division of Thoracic Surgery, Department of Surgery, Comprehensive Cancer Center, Medical University of Vienna, Austria; ‡Department of Thoracic Surgery, National Institute of Oncology—Semmelweis University; §Department of Pulmonology, ||Second Department of Pathology, Semmelweis University; ¶Molecular Oncology Research Group, Hungarian Academy of Sciences—Semmelweis University, Budapest, Hungary; #First Department of Pathology, Experimental Cancer Research, Semmelweis University, Budapest, Hungary; and ** Department of Internal Medicine I, Institute of Cancer Research and Comprehensive Cancer Center, Medical University of Vienna, Austria.

ZL and MAH share the first authorship.

BH and BD are cosenior authors of this study.

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Address for Correspondence: Balazs Hegedus, PhD, Division of Thoracic Surgery, Medical University of Vienna, Waehringer Guertel 18–20, A-1090, Vienna, Austria. E-mail: balazs.hegedus@meduniwien.ac.at; Balazs Dome, MD, PhD, Division of Thoracic Surgery, Medical University of Vienna, Waehringer Guertel 18–20, A-1090, Vienna, Austria. E-mail: balazs.dome@meduniwien.ac.at

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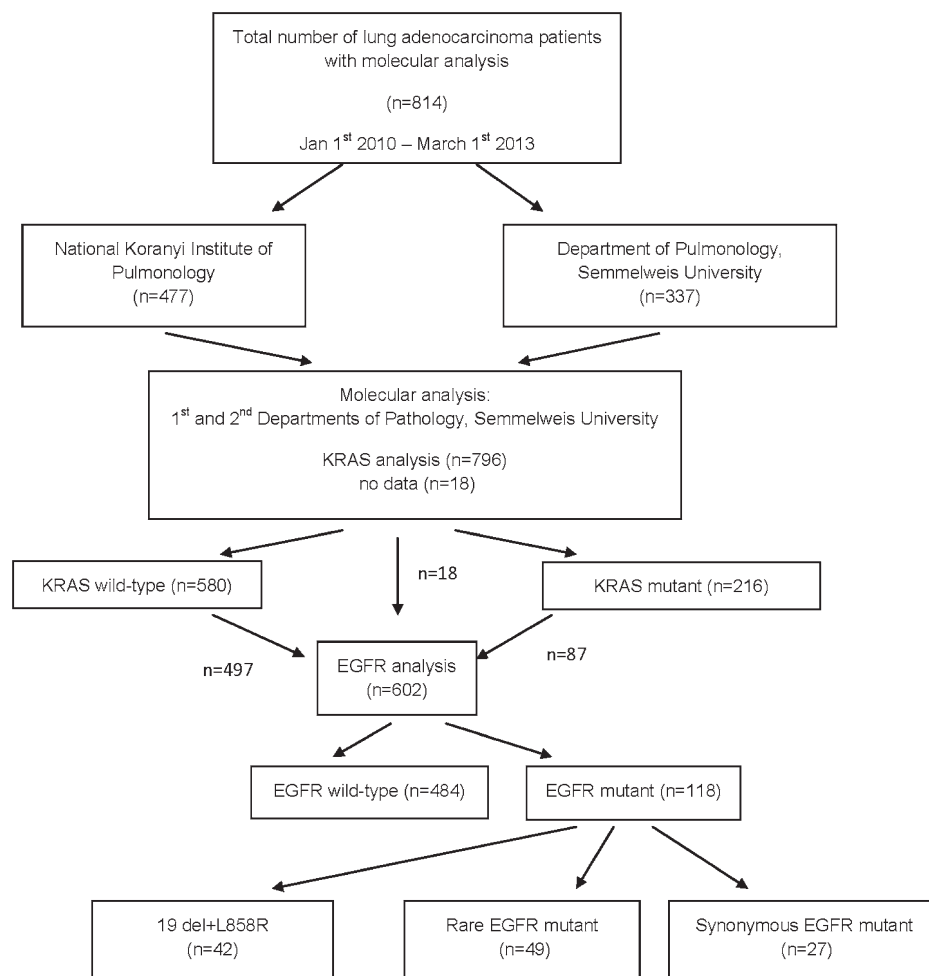


FIGURE 1. Patient cohort and mutational analysis flow chart (n = 814 patients).

varies in different populations, showing the highest frequency among East-Asian nonsmoker women.^{13,14}

The presence of KRAS mutation is a negative predictive factor for anti-EGFR therapy.^{15,16} Massive clinical data also accumulated showing that KRAS and EGFR mutations are mutually exclusive (with rare exceptions).¹⁷ Accordingly, in Hungary, KRAS testing is performed first to exclude KRAS mutant cases from EGFR analysis to optimize testing efficiency.¹⁸ This approach enabled us to compare EGFR mutant, KRAS mutant, and EGFR/KRAS double wild-type (WT) patient cohorts.

In advanced lung adenocarcinoma, the clinical significance of rare EGFR mutations has not yet been clearly established.^{8,19} Therefore, we analyzed one of the largest cohorts of Caucasian patients with known KRAS and EGFR mutational status to compare the epidemiology and clinical consequence of rare and classic EGFR mutations.

PATIENTS AND METHODS

Study Population

In this retrospective analysis, 814 consecutive Caucasian patients with pathologically confirmed lung adenocarcinomas

and treated at the National Koranyi Institute of Pulmonology, Budapest, Hungary or at the Department of Pulmonology, Semmelweis University, Budapest between January 2010 and March 2013 were included (Fig. 1). All patients underwent EGFR and/or KRAS mutation tests required for potential anti-EGFR therapy. All tumor samples were obtained before TKI treatment. Major clinicopathological characteristics including performance status, smoking history, and tumor stage could be collected for 645 patients, and their correlations with mutational status were analyzed for epidemiological purpose. The full clinical follow-up—including overall survival (OS)—was collected for 419 unresected, advanced-stage patients. In this subcohort, 64 EGFR-mutant cases with full clinical follow-up were studied. Tumor, node, metastasis stage was evaluated according to the Union for International Cancer Control (seventh edition)²⁰ at diagnosis. Patients were divided into “never-smokers” (smoked less than 100 cigarettes during their lives), “former smokers” (smoked more than 100 cigarettes but had not smoked for at least a year), and “current smokers.” Smoking status, Eastern Cooperative Oncology Group performance status (ECOG PS), and age were evaluated at the time of diagnosis. Institutional tumor boards in line with

lung cancer therapy guidelines of the participating centers did not allow the use of TKIs in patients with ECOG PS > 2. Accordingly, only patients with initial ECOG PS 0–2 were included into the EGFR mutation analysis.

Mutation Analysis

All mutational analyses were performed at the Second Department of Pathology or at the First Department of Pathology and Experimental Cancer Research, Semmelweis University as previously described.^{18,21} Briefly, regions of tumor samples embedded in paraffin blocks containing the highest concentrations of tumor cells were macrodissected.¹⁸ DNA was extracted using the MasterPure DNA Purification Kit according to the manufacturer's instructions. KRAS mutations were identified by microcapillary-based restriction fragment length analysis as described previously.^{18,21} Polymerase chain reaction amplification of the EGFR exons 18, 19, and 21 in 143 cases (24%) and exons 18, 19, 20, and 21 in 459 patients (76%) was the first step, followed by bidirectional Sanger sequencing. Sensitivity of this methodology is approximately 20% (it is able to detect mutations in specimens with at least 20% cancer cell content); its specificity is nearly 100%.²² In seven cases, the TheraScreen: EGFR29 Mutation Kit (DxS Ltd., UK) was used. This technique has a sensitivity of approximately 1% and a specificity of 100%.²²

Treatment and Response Evaluation

Retrospective clinical data and treatment history were available in the advanced-stage cohort (unresected stages IIIA and IIIB–IV) for 419 lung adenocarcinoma patients. Drug administration was performed according to the Hungarian health care financial regulations for TKI therapy. From January 2010, erlotinib (in second or third line) could be given to advanced lung adenocarcinoma patients with a KRAS WT tumor (orally at a daily dose of 150 mg in second and/or third line). First line gefitinib (orally at a daily dose of 250 mg) became available from March 2012 for patients with an activating EGFR mutation. Thirty-three and 118 patients received gefitinib and erlotinib as monotherapy, respectively. The study and all treatments were conducted in accordance with the current National Comprehensive Cancer Network guidelines, based on the ethical standards prescribed by the Helsinki Declaration of the World Medical Association and with the approval of the national level ethics committee that included a waiver for this retrospective study (52614-4/2013/EKU). According to the national treatment financing scheme, all EGFR TKI-treated patients had to return to the hospital every month for review. The therapeutic efficacy of anti-EGFR TKIs was assessed from contrast-enhanced computed tomography before treatment initiation and then every 3 months. Therapy response was categorized by Response Evaluation Criteria in Solid Tumors 1.1 as stable disease (SD), partial or complete response, or progressive disease. Response rate (RR) was calculated as the number of patients with complete response or partial response divided by the number of patients in a given group. OS was estimated from the time of diagnosis in patients presenting with unresectable advanced-stage disease until death or last available follow-up. Progression-free survival (PFS) was calculated from

the date of initiation of the TKI treatment to the date of detection of progressive disease or death.

Statistical Methods

Categorical parameters of the different mutational groups were analyzed by χ^2 test. Age as a continuous variable was analyzed in the mutational groups by analysis of variance and Tukey's multiple comparison test. Kaplan–Meier curves and two-sided log-rank tests were used for univariate survival analyses. Cox proportional hazards model was used for univariate and multivariate survival analyses to calculate the hazard ratios (HR) and corresponding 95% confidence intervals (CI). For multivariate survival analyses, the Cox regression model was adjusted for age (as a continuous variable), gender (female vs. male), smoking status (never-smoker vs. ever-smoker), ECOG PS (0 vs. 1–2), and stage (IIIA vs. IIIB–IV). The *p* values are two-sided and were considered significant below 0.05. Metric data are shown as median or mean and corresponding range, or, in case of OS and PFS, as median and corresponding 95% CI. All statistical analyses were performed using the PASW Statistics 18.0 package (SPSS Inc., Chicago, IL).

RESULTS

Epidemiology of EGFR and KRAS Mutations in Lung Adenocarcinoma

Five hundred and eighty patients of the full cohort of 814 cases were identified as KRAS WT (71%) and 216 (27%) as KRAS mutant (in 18 cases [2%], no KRAS mutation analysis was performed, Fig. 1). There were 42 (5%) classic EGFR mutant (four patients with concomitant KRAS mutation), 49 (6%) rare EGFR mutant (non-classic mutation where amino acid change occurs; including three patients with concomitant KRAS mutation), and 27 (3%) patients with synonymous (silent) EGFR mutations (non-classic mutations without amino acid change in EGFR; including nine patients with concomitant KRAS mutation), and 480 (59%) of the cases was classified as KRAS/EGFR double WT (Fig. 2A). Of note, in five patients, the G719x or L861Q rare sensitizing mutation was identified.²³ All rare and synonymous EGFR mutations are listed in Supplemental Table 1 (Supplemental Digital Content 1, <http://links.lww.com/JTO/A803>). On the basis of the COSMIC database (retrieved at July 28, 2014), we found synonymous and rare EGFR gene mutations already published in lung cancer (N = 33 mutations) or in malignancies of other organs (N = 20 mutations).²⁴ In addition, 45 previously unpublished novel mutations were identified. The T790M mutation was not detected in any patients. Interestingly, in 16 patients, we identified a complex mutation pattern, with at least two different EGFR mutations found within a single sample (total of 39 mutations identified for the 16 patients).

The major clinicopathological characteristics could be collected for 645 patients and are presented for the various mutational statuses in Supplemental Table 2 (Supplemental Digital Content 1, <http://links.lww.com/JTO/A803>). Significant association of gender and mutational status was not detected

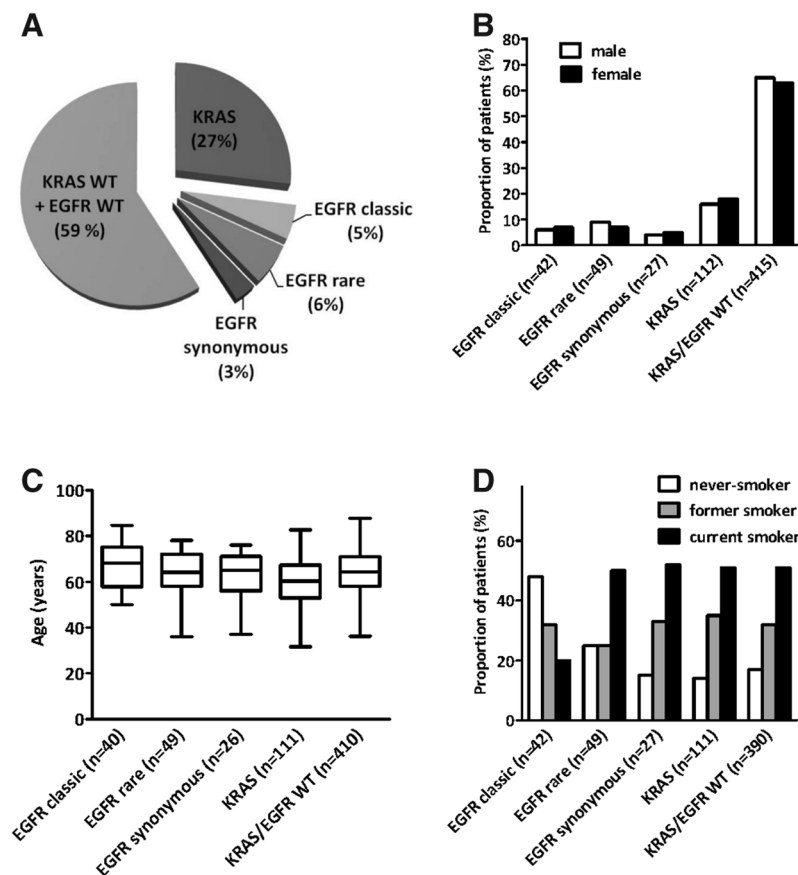


FIGURE 2. Distribution and epidemiology of KRAS and EGFR mutations in lung adenocarcinoma patients. *A*, Mutational status in the full cohort ($n = 814$). *B*, There was no significant association between mutational status and gender. *C*, Patients with KRAS mutation were significantly younger than those with classic EGFR mutations or with EGFR/KRAS double WT tumors ($p = 0.0002$). *D*, In contrast to classic EGFR mutations, rare EGFR mutations were significantly associated with smoking ($p = 0.0062$). EGFR, epidermal growth factor receptor; WT, wild-type.

(Fig. 2*B*; $p = 0.780$). Patients with KRAS mutations were significantly younger than those with classic EGFR mutations or with EGFR/KRAS double WT tumors (Fig. 2*C*; $p = 0.0002$). Classic EGFR mutation was significantly associated with never-smoker status when compared with all other mutational statuses (Fig. 2*D*; $p < 0.0001$). Rare EGFR mutations were found to be associated with smoking (vs. classic EGFR mutations; Fig. 2*D*; $p = 0.0062$).

Distinct Effect of Classic Versus Rare EGFR Mutations on Overall Survival in Lung Adenocarcinoma

Clinical follow-up including OS could be collected in the advanced-stage cohort (unresected stages IIIA and IIIB–IV) for 419 patients (Table 1). Gender, age, ECOG PS, disease stage, smoking status, and mutational status were tested for predicting OS. Male patients had significantly shorter OS (vs. females; HR, 1.32; 95% CI, 1.04–1.66; $p = 0.0195$). Patients with ECOG PS 0 had significantly better OS than those presenting with ECOG PS 1–2 (HR, 2.07; 95% CI, 1.63–2.62; $p < 0.0001$; Fig. 3*A*). Patients with stage IIIB or IV tumors

had significantly shorter OS than those with unresected stage IIIA (HR, 0.637; 95% CI, 0.478–0.850; $p = 0.002$; Fig. 3*B*). We found no difference in OS between stages IIIB and IV patients (data not shown). Furthermore, we found significantly increased OS among never-smokers when compared with ever-smoker patients (HR, 0.666; 95% CI, 0.497–0.892; $p = 0.0063$; Fig. 3*C*). Classic EGFR mutation conferred a significant benefit for OS when compared with WT (HR, 0.58; 95% CI, 0.37–0.91; $p = 0.02$) or KRAS mutation (HR, 0.52; 95% CI, 0.31–0.89; $p = 0.0167$). In contrast, there was no significant difference in the OS of rare EGFR mutation positive patients compared with patients with WT EGFR or with mutant KRAS. Of note, there was no effect of KRAS mutational status on OS.

Multivariate survival analyses (Supplemental Table 3*A*, Supplemental Digital Content 1, <http://links.lww.com/JTO/A803>) showed that—besides ECOG and stage—classic EGFR mutation was an independent survival predictor (HR, 0.45; 95% CI, 0.25–0.82; $p = 0.009$). Importantly, rare EGFR mutation was not a significant independent predictor of OS (Supplemental Table 3*B*, Supplemental Digital Content 1, <http://links.lww.com/JTO/A803>).

TABLE 1. Major Clinicopathological Characteristics of the Advanced-Stage Lung Adenocarcinoma Patient Cohort with Full Clinical Follow-up (n = 419)

	Total	Classic EGFR Mutation	Rare EGFR Mutation	Synonymous EGFR Mutation	KRAS mutation	KRAS and EGFR wild-type	P value
Total	419 ^a (100)	22 ^c (5)	26 ^d (6)	16 ^e (4)	80 ^b (20)	271 ^e (65)	
Age (mean ± SD)	63.9±10.3	67.4±10.4	63.5±9.8	64.3±8.80	59.9±10.9	64.6±9.8	0.003
Gender							
Male	203 (48)	7 (32)	13 (50)	7 (44)	37 (46)	135 (50)	0.571
Female	216 (52)	15 (68)	13 (50)	9 (56)	43 (54)	136 (50)	
ECOG status							
0	185 (45)	13 (59)	13 (50)	8 (50)	35 (44)	116 (45)	0.709
1–2	223 (55)	9 (41)	13 (50)	8 (50)	45 (56)	144 (55)	
Unknown data	11	0	0	0	0	11	
Smoking status							
Never-smoker	70 (17)	9 (43)	6 (23)	3 (19)	8 (10)	44 (17)	0.028
Former smoker	134 (33)	7 (33)	6 (23)	6 (37)	34 (43)	81 (32)	
Current smoker	198 (50)	5 (24)	14 (54)	7 (44)	37 (47)	131 (51)	
Unknown data	17	1	0	0	1	15	
Tumor Stage							
Unresected IIIA	68 (16)	2 (9)	5 (19)	10 (63)	16 (20)	36 (13)	<0.001
IIIB–IV	351 (84)	20 (91)	21 (81)	6 (37)	64 (80)	235 (87)	

Data shown in parentheses are column percentages. Categorical parameters of the different mutational groups were analyzed by χ^2 . Age as a continuous variable was analysed in the mutational groups by analysis of variance and Tukey's multiple comparison test.

^a KRAS molecular analysis was not done in four cases.

^b EGFR molecular analysis was not done in 54 cases.

^c Two concomitant KRAS mutations were identified.

^d Two concomitant KRAS mutations were identified, and KRAS analysis was not done in two cases.

ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor.

Different Response to TKI Therapy in Advanced Lung Adenocarcinoma Patients with Classic Versus Rare EGFR Mutations

Next, we evaluated the therapy response and PFS of TKI-treated advanced lung adenocarcinoma patients with

classic and rare EGFR mutations (Table 2 and Supplemental Table 4, Supplemental Digital Content 1, <http://links.lww.com/JTO/A803>). Irrespective of treatment line, there was a significantly increased RR among patients with classic EGFR mutations compared with those with rare EGFR mutations

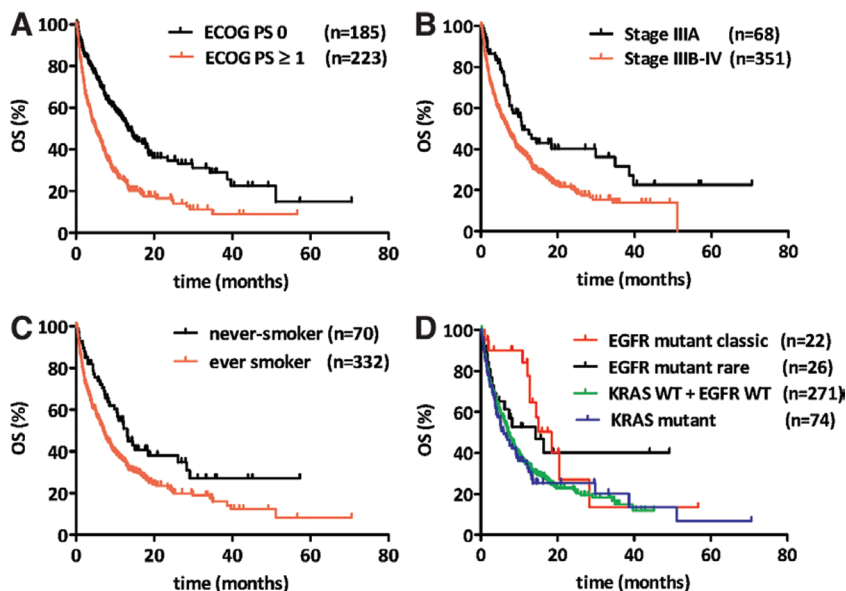


FIGURE 3. Kaplan–Meier curves for the OS of advanced lung adenocarcinoma patients (n = 419). A, ECOG PS 1–2 (vs. ECOG PS 0; $p < 0.0001$), (B) stages IIIB–IV (vs. stage IIIA; $p = 0.002$), and (C) smoking (vs. never-smoking; $p = 0.006$) were significant prognostic factors for reduced OS. D, Moreover, patients with tumors harboring classic EGFR mutations had a significantly better OS than those with EGFR/KRAS double WT ($p = 0.02$) or with KRAS mutant tumors ($p = 0.002$). Importantly, EGFR classic mutation was not associated with benefit in OS if these patients were compared with the rare EGFR mutant cohort ($p = 0.529$). OS, overall survival; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; WT, wild-type.

TABLE 2. Distribution of EGFR Mutation Status in TKI-Treated Patients

	Total	TKI Therapy	
		First line	Second and Third Line
Total	151 (100)	30 (20)	121 (80)
WT for KRAS and EGFR	98	0	98 (100)
Synonymous EGFR mutation	9	2 (22)	7 (78)
Non-synonymous EGFR mutation	Total	28 (64)	16 (36)
	Classic	17 ^a (71)	7 ^a (29)
	Rare	11 ^b (55)	9 (45)

Data shown in parentheses are row percentages.

^aIn one patient concomitant KRAS mutation was identified.

^bIn two patients concomitant KRAS mutation was identified.

TKI, tyrosine kinase inhibitor; WT, wild-type; EGFR, epidermal growth factor receptor.

(RR 71% vs. 37%, respectively; $p = 0.039$, Supplemental Fig. 1A, Supplemental Digital Content 2, <http://links.lww.com/JTO/A804>). This translated into a statistically not significant but clinically notable longer PFS: the median PFS values were 12 and 6.2 months in the classic and rare EGFR mutation cohorts, respectively; ($p = 0.076$; Supplemental Fig. 1B, Supplemental Digital Content 2, <http://links.lww.com/JTO/A804>). We found no significant difference in the OS in the above-mentioned subgroup of patients ($p = 0.212$; Supplemental Fig. 1C, Supplemental Digital Content 2, <http://links.lww.com/JTO/A804>). Importantly, when classic EGFR mutation positive patients were pooled together with patients harboring TKI-sensitizing rare EGFR mutations (G719 and L861)²³ and compared with the remaining rare mutation cases, the difference in RR remained significant (RR 70% vs. 36%, respectively; $p = 0.044$, Fig. 4A), and the effect on PFS reached statistical significance ($p = 0.048$; Fig. 4B). Importantly, there was a significant difference in the OS in the latter comparison ($p = 0.01$; Fig. 4C).

DISCUSSION

Precise definition of the tumor type, including comprehensive histological classification and description of clinically

relevant molecular pathological characteristics, is crucial for precision or individualized (lung) cancer therapy.²⁵ On the basis of the classic activating mutations of EGFR, a number of patients can now be successfully treated with selective EGFR TKIs. However, there are several rare mutations in the EGFR gene with unknown epidemiology and influence on TKI response. Importantly, the incidence of these mutations varies in different ethnic groups and is also influenced by environmental factors and smoking habit.²⁶ For this very reason, we compared the epidemiology and clinical consequence of classic and rare EGFR mutations in a Hungarian cohort of lung cancer patients.²⁷

In the current study, 5% of patients carried classic EGFR mutation. In a very recent survey from Germany, the incidence of confirmed activating EGFR mutation among adenocarcinoma patients was reported to be 6%.^{27,28} The incidence of rare nonsynonymous EGFR mutations in our cohort was 6% and therefore is higher than in similar Caucasian studies (1.9–2.7%)^{28,29} or in a mixed US study population (4%),⁹ but similar to East-Asian studies where the incidence of rare mutations is ranging from 7% to 8%.^{7,23,30} The higher proportion of rare mutations in our Caucasian cohort is likely due to the facts that exon 20 sequencing was also performed in

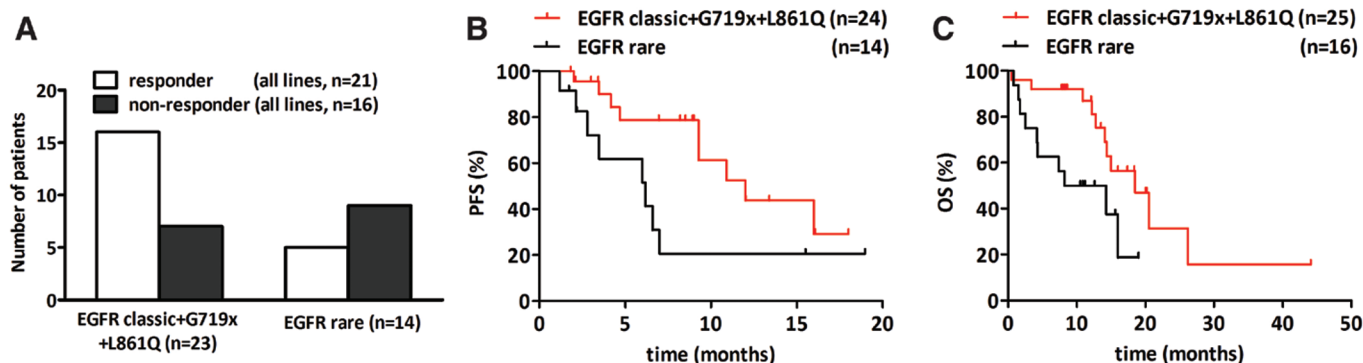


FIGURE 4. Anti-EGFR tyrosine kinase inhibitor treatment in advanced lung adenocarcinoma patients with confirmed sensitizing (classic EGFR mutations pooled together with patients with sensitizing rare EGFR mutations [G719x and L861Q]) versus all other rare EGFR mutations. A, Irrespective of treatment line, patients with sensitizing EGFR mutations responded significantly better to TKI therapy (data presented as number of patients; $p = 0.047$). Furthermore, these patients had significantly longer PFS (B) and OS (C) than those with other rare EGFR mutations ($p = 0.043$ and 0.01 , respectively). EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PFS, progression-free survival; OS, overall survival.

76% of the patients and, moreover, that 40% of all KRAS mutant cases underwent EGFR analysis as well. Of note, in seven patients, concomitant KRAS and classic or rare EGFR mutations were identified in our study. These patients represent 0.9% (7 of 814) of the whole cohort and 1.2% (7 of 584) of the group of patients with both KRAS and EGFR mutation analyses. This ratio is in line with previously published data.^{6,31} Of note, 2% of our patients carried complex mutation pattern (at least two different EGFR mutations in one sample), meanwhile an East-Asian study published 7.3%.⁷ To our knowledge, no Caucasian population-based study reported the incidence of complex EGFR mutations so far.

We did not detect the resistance associated T790M mutation in our patient cohort. This is in line with its very low incidence (0–0.9%) in previous studies in patients before TKI therapy using methods without increased sensitivity toward mutant alleles.^{32,33} In contrast, studies enriching for mutant alleles using a peptide-nucleic acid to inhibit the amplification of WT allele found much higher incidence of pretreatment T790M mutations (35–65%).^{34,35}

Significant associations between gender and rare EGFR mutational status were not detected in our cohort in line with a very recent—and to date the only similar—Caucasian study.¹⁰ We found no significant association either between age and rare EGFR mutations in accordance with previous reports from Asia or with a mixed US cohort studying only EGFR exon 20 insertions.^{9,23} According to our knowledge, our study is the first to compare the age among classic and rare EGFR mutants in a Caucasian cohort. In the current cohort, patients with classic EGFR mutations tended to be older (mean age: 67±9.6 years) than those with rare EGFR mutations (mean age, 64.2±9.2 years) and, moreover, were significantly older than patients carrying KRAS mutations (mean age: 60±10.4 years). Accordingly, the above-mentioned recent German study also found an almost significant trend between patients with KRAS (mean age, 65.3±9.8 years) and EGFR mutations (mean age, 70.3±11.4 years).²⁸

In our cohort, rare—in contrast to classic—EGFR mutations appeared to be associated with smoking status. This is in line with a Greek study in which Pallis et al.²⁹ demonstrated that among patients with rare mutations smokers are more frequent, albeit not significantly, when compared with never-smokers: 20.8% versus 8%. In a mixed population study, among EGFR exon 20 insertion mutant patients the proportion of smokers was higher than among classic EGFR mutant patients.⁹ In contrast, East-Asian data show that rare EGFR mutations together with complex rare mutations are associated with never-smoker status,⁷ and that uncommon mutations are higher among never-smokers.²³

To our knowledge, this is the first Caucasian population-based advanced-stage disease cohort with RRs for first-generation TKI-treated rare EGFR mutation positive patients. In our cohort, RRs were 71% and 37% among patients with classic and rare EGFR mutations, respectively. This finding is in accordance with studies from East-Asia where RRs were found to be 74–75% and 28–48%, respectively.^{7,23} Interestingly, in the LUX-Lung 2 phase II trial of the second-generation covalent TKI inhibitor afatinib, similar results were found as well (RRs

in classic and rare EGFR mutant cohorts were 66% and 39%, respectively).³⁰ Of note, the 12-month median PFS among classic EGFR mutant patients in our cohort is rather similar to previously published data (9.4–11.9 months) from other studies.^{7,36,37} Patients in our cohort with rare EGFR mutations demonstrated a shorter median PFS of 6.2 months. This is comparable to the 5-month median PFS of rare EGFR mutant patients in a recent East-Asian study performed by Wu et al.²³ Importantly, when patients with classic EGFR mutations were pooled with patients with rare activating EGFR mutations (G719 and L861), and then this cohort was compared with the remaining rare mutation harboring population, the effect on PFS reached significance. Interestingly, a similar robust difference was found in the recently published LUX-Lung 2 clinical trial.³⁰ Of note, the RR and PFS in our patient cohort with rare EGFR mutations (PFS: 7.4 months; RR: 31%) are comparable to that of the cisplatin–pemetrexed combination arm in the LUX-Lung 2 clinical trial (PFS, 6.9 months; RR, 23%), which is now considered the most effective chemotherapy regimen in lung adenocarcinoma.³⁸

Classic EGFR mutations were associated with a significantly better median OS when compared with rare EGFR mutations (20.5 vs. 7.4 months) in the current study. This finding is in line with the results of other studies (19.3–20 months) on classic EGFR mutation positive cohorts but differs in the case of rare mutations (9–17 months), possibly due to the different types and proportion of rare EGFR mutations.^{7,23,29}

With regard to factors associated with OS in lung adenocarcinoma, we confirmed the prognostic significance of gender, ECOG PS, smoking status, disease stage, and EGFR mutations similar to the findings of Johnson et al.³⁹ In multivariate analysis of standard prognostic parameters, only ECOG PS 0 proved to be an independent prognostic factor associated with longer OS. Furthermore, in line with the study of Johnson et al.,³⁹ the presence of classic EGFR mutations had a statistically significant effect. Similar to the majority of previous publications,¹⁵ we were not able to confirm the prognostic effect of KRAS mutations.

Our study has several limitations. Despite the fact that the initial cohort was large—as expected—the final number of patients with classic or rare EGFR mutations was relatively small. Nevertheless, our cohort—one of the largest in this setting—provided the opportunity to draw some conclusions that evidently need to be validated in additional studies. Because of the study's retrospective nature, some of our results need to be confirmed in a prospective setting. Furthermore, it remains unclear whether classic EGFR mutation itself confers a more benign behavior or the increased RR and median PFS of the classic mutant cohort translate to this better prognosis. An important potential confounding factor is smoking status as several studies have demonstrated that never-smokers have improved OS.^{40,41} In our cohort, we found a significant OS advantage for never-smokers. However, at the same time, classic EGFR mutations were significantly more frequent among never-smokers than rare EGFR mutations. Thus, it is likely that this increased OS is owing to the overall better performance and the lack of smoking-related comorbidities.^{40–43}

Altogether, classic EGFR mutations were associated with never-smoker status, older age, longer OS, and increased TKI response when compared with rare EGFR mutations in this retrospective study. Molecular methods focusing only on classic EGFR mutations may prevent a number of patients with certain rare mutations to benefit from anti-EGFR therapy. Our study suggests that the predictive and prognostic values of rare EGFR mutations with regard to TKI therapy need to be evaluated in wide multinational studies for each individual mutation to optimize EGFR-targeted therapy in lung adenocarcinoma and, moreover, to define optimal screening strategy that can significantly reduce the costs of treatment with expensive therapeutic agents.

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