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Prognostic Implications of Epidermal Growth Factor Receptor and *KRAS* Gene Mutations and Epidermal Growth Factor Receptor Gene Copy Numbers in Patients with Surgically Resectable Non-small Cell Lung Cancer in Taiwan

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Introduction: The prognostic role of epidermal growth factor receptor (*EGFR*) mutations in patients with surgically resectable non-small cell lung cancer (NSCLC) without EGFR tyrosine kinase inhibitor treatment has not been well established, because the reports are still few.

Materials and Methods: We analyzed the survival data of 164 patients with surgically resectable (stages I to IIIA) NSCLC of two year groups (1996–1998 and 2002–2004), and compared with *EGFR* mutations, *KRAS* mutations, and *EGFR* gene copy numbers.

Results: Comparing the survival of wild-type patients and patients having L858R mutations or exon 19 deletion, the median survival was much longer for patient with *EGFR* mutations (54.7 months) than wild type (34.9 months). The difference was not statistically significant by univariate analysis ($p = 0.1981$) but had borderline significance by multivariate analyses ($p = 0.0506$). In addition, the 3-year survival rates of patients with *EGFR* mutations were also significantly higher than wild type ($p = 0.0232$). After exclusion of 18 patients treated by EGFR-tyrosine kinase inhibitor for tumor recurrence, the trends were still the same. Patients with *KRAS* mutations had shorter median survival (21 months) than wild type (44.4 months). Patients with *EGFR* polysomy (≧copies) also had longer median survival (56.2 months) than wild type (53.4 months). But the survival differences of these two genetic markers were all not significant statistically.

Conclusion: It is intriguing that patients with NSCLC with *EGFR* mutations had better survival than wild type. Such a tumor biology may confound the survival data in a study without the stratification by *EGFR* mutation.

Key Words: *EGFR*, *KRAS*, Lung cancer, Mutation, Survival, Copy number, Chemotherapy.

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Lung cancer, especially non-small cell lung cancer (NSCLC), has become the leading cause of cancer death in most part of the world because of its high mortality rate and poor response to treatment.[1,2] Thus, it has become the leading target for the development of new anticancer drugs. Because conventional chemotherapy (C/T) regimens seemed to have limited efficacy for advanced-staged NSCLC,[3] new therapeutic agents, especially targeted therapy by inhibition of activated oncoprotein kinases, has become a popular approach.[4,5] Among them, the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have achieved rapid and good therapeutic response for the treatment of NSCLC.[6,7] The significantly higher response rate noted in Japanese patients than in a predominantly European-derived population (27.5% versus 10.4%) in two clinical trials, [8,9] has lead to the identification of *EGFR* mutations, which are correlated with the clinical responsiveness, by Lynch et al.[10] and Paez et al.[11] All of these mutations were within exon 18 through 21 of the kinase domain (KD) of *EGFR* and most were identified in adenocarcinoma (ADC).[10–14] These mutations can cause constitutive activation of EGFR protein and confer susceptibility to TKIs.[10–12,15]

For patients with advanced-staged NSCLC with *EGFR* mutations, in addition to the benefit from TKI treatment and longer survival,[16] two phase III clinical trials for patients with NSCLC (TRIBUTE and Iressa Pan-Asia Study (IPASS), the

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former patients from United States, and the latter from Asian countries, respectively), also have shown a higher response rate to C/T for patients with *EGFR* mutation than wild type.[17,18] But the study by Cappuzzo et al.[19] (patients from Italy) did not show such difference. Therefore, the prognostic value of *EGFR* mutations for patients with advanced-staged NSCLC without TKI treatment remained controversial.

The prognostic role of *EGFR* mutations in patients with resectable NSCLC also has not been well established, because the reports are still quite few. Three earlier studies on the overall survival of patients with NSCLC without TKI treatment have reported that there was no significant difference between wild-type and *EGFR* mutations.[14,20,21] The study by Marks et al.[22] was the first to suggest a better prognostic role for *EGFR* mutations. They reported a higher 3-year survival rate for patients with resectable ADC (stages I to IIIa) with *EGFR* mutations than *KRAS* mutations. The latter was well known as a poor prognostic indicator.[23,24] However, due to short follow-up time, they could not demonstrate a significant difference in the overall survival after adjustment with tumor stages. Recently, Kosaka et al.[25] also reported a similar study on the prognostic implications of *EGFR* mutations in a large cohort of Japanese patients with surgically treated lung ADC. Although they also found a significant longer overall survival for patients with *EGFR* mutations compared with wild type by univariate analyses, the difference became insignificant by multivariate analyses.

We have previously reported a high *EGFR* mutation rate (38.6%) and very low *KRAS* mutation rate (3.8%) in surgically treated patients with NSCLC in Taiwan.[13,26] We were also quite interested in the prognostic significance of *EGFR* mutation in these patients. Because all of the patients with surgical resectable NSCLC (stages I to IIIa) in our published study series received operations >5 years ago,[13,26,27] the follow-up time was long enough for evaluation of their overall survival. Thus, we have performed a retrospective study of the prognostic roles of three molecular markers, including *EGFR* mutation, *KRAS* mutation, and *EGFR* gene copy number, on the survival of our patients with surgically resectable NSCLC.

PATIENTS AND METHODS

Patient Samples

Fresh-frozen tumor specimens from patients received surgical resection and with signed informed consent at Chang-Gung Memorial Hospital for NSCLC from January 1996 to December 1998, and from May 2002 to May 2004, were obtained from the tissue bank of Chang-Gung Memorial Hospital. All specimens were snap frozen soon after resection and stored at −80°C. The patients from May 2002 to May 2004 were the patient group of our first *EGFR* mutation study.[13] The specimens from 1996 to 1998 were a retrospective application for the earliest fresh-frozen tumor tissue of the tissue bank, mainly to study the genetic markers and survival of NSCLC of earlier years for comparison. We did not apply more surgical specimens from January 1999 to April 2002 from the tissue bank because of limitation of research manpower. The study protocol had been reviewed and approved by the Institutional Review Board of Chang-Gung Memorial Hospital and National Health Research Institutes. Patients of stage IIIb or IV disease were excluded for this study. Thus, totally 168 patients were included (70 patients operated from January 1996 to December 1998, and 98 patients operated from May 2002 to May 2004). The therapeutic protocol and the surgical procedures of these patients were all similar. More than 90% of the patients were operated by the same surgeon (H-P.L.). For stage I patients, no adjuvant therapy was given. Most stage II and stage IIIa patients received postoperative radiotherapy (R/T). A few patients received neoadjuvant or adjuvant C/T. All clinicopathological information was obtained from the medical records. The stage data listed is pathology stage according to American Joint Committee on Cancer guidelines. Smoking status is defined as nonsmokers (never smoke or <100 lifetime cigarettes) and ever smokers. Date of death was obtained from the cancer registration data of Chang-Gung Memorial Hospital or the government's records from Department of Health of Taiwan up to year 2007. Overall survival was from the date of surgery until death.

EGFR and *KRAS* Mutation Analyses and *EGFR* Gene Copy Number Detection

DNA extraction from fresh-frozen tumor tissue was performed according to the protocol as mentioned previously.[13] For *EGFR* mutation, coding sequences from exons 18 to 21 were amplified and subjected to direct sequencing. For analysis of the *KRAS* mutation, coding sequences of exons 2 and 3 were amplified and subjected to direct sequencing. Determination of *EGFR* gene copy number was performed by chromogenic in situ hybridization (CISH) on paraffin sections of the tumor tissue. The tumor was considered to be CISH positive if the *EGFR* gene copy number was ≥5 signals per nucleus in ≥40% of tumor cells. The methodology of the above three studies were the same as mentioned previously.[13,26,27] The *EGFR* mutation and *KRAS* mutation analyses were performed and successful in 158 and 156 patients, respectively. The *EGFR* gene copy number by CISH was performed in 134 patients and successful in 124 patients (CISH study failed in 10 cases. One from the patients received surgery during 2002–2004, and nine from the patients received surgery in 1996, mainly due to degradation of the DNA in the paraffin block). The data of *EGFR* mutation, *KRAS* mutation, and *EGFR* gene copy number of these patients have been included in our three published study series, respectively.[13,26,27]

Statistical Analysis

To examine the differences in the major clinicopathological features and molecular markers, frequencies and proportions are compared by conventional χ^2 association test or Fisher's exact test (when there is at least a cell frequency <5). For specific subgroups defined by different covariates, we report the median survival estimates with corresponding 95% confidence intervals. The differences in overall survivals are checked by log-rank tests. If the survival comparison focused on a fixed time point (such as 3 year- or 5-year

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survivals), a method offered in Klein et al.[28] is used. The test statistic denoted X_2^2 therein is:

$$\frac{\hat{S}_1^2(t)\hat{S}_2^2(t)\{\log \hat{S}_1(t) - \log \hat{S}_2(t)\}^2}{\hat{S}_2^2(t)\hat{\sigma}^2_{\hat{S}_1} + \hat{S}_1^2(t)\hat{\sigma}^2_{\hat{S}_2}}.$$

The X_2^2 statistic is distributed as a χ^2 distribution on one degree of freedom. This is in particular meaningful when the overall survival of a group is not apparently superior to that of the other group, but benefit has appeared at an earlier time period in the therapeutic process. Multivariate analysis of overall survival was performed using Cox's regression model and use stratified analysis to deal with the stratification effect.

Multiple-comparison step-down Holm-Bonferroni adjustments were also made.

RESULTS

Of the 168 patients originally included in this study, two patients died of surgical complications and two patients died of other causes were excluded for survival analysis. Thus, a total of 164 patients were included for this study. Because the nonsurgical treatments for NSCLC have much improvement in recent years, we have divided these patients in two year groups: year group 1 (69 patients for 1996–1998) and year group 2 (95 patients for 2002 –2004), so that we could also evaluate the impact of treatment improvement. All patients, except one, were belonged to functional class 1. The follow-up period ranged from 27 days to 158 months. The clinicopathological features are shown in Table 1. For year group 1, 12 patients had received postoperative R/T. None received adjuvant C/T. For year group 2, 21 patients had received postoperative R/T, three received R/T + C/T. The C/T regimens included gemcitabine + cisplatin (one patient) and bleomycin + cisplatin (one patient). The third one was unknown because the therapy was performed in another hospital. Two patients received neoadjuvant C/T (all were Taxol + cisplatin), and five received postoperative C/T. The regimens included: Taxol + cisplatin (two patients), vinorelbine + cisplatin (one patient), gemcitabine + cisplatin (one patient), and gemcitabine + cisplatin followed by 5FU + cisplatin (one patient). Totally, there were 10 patients received adjuvant or neoadjuvant C/T, and three of the 10 patients had EGFR mutations. For the treatment of TKI, in year group 1, only one patient with squamous cell carcinoma (SCC) and no *EGFR* mutation had received TKI (gefitinib) treatments for tumor recurrence. For year group 2, 17 patients, including 14 with ADC, two with SCC, and one with large cell carcinoma, had received TKI (gefitinib or erlotinib) for tumor recurrence. Among them, 11 patients (all were ADC) had *EGFR* mutations (six were L858R, four were exon 19 deletion, and one was L858R+ E709G), and one had no EGFR mutation data. The responsiveness to TKI treatment of these patients was not evaluated because of complex treatment courses.

The clinicopathological features and genetic markers of the two year groups of patients were compared (Table 1). Several demographic variables exhibited significant distributional difference between the two groups. There were more male ($p = 0.0094$), more SCC ($p = 0.0031$), and lower *EGFR* mutation rate ($p = 0.0457$) in year group 1. The clinicopathological characteristics associated with *EGFR* mutations is also analyzed (Table 2). The *EGFR* mutations were significantly associated with female, ADC, nonsmokers, and CISH (+) tumor. *EGFR* mutations were also associated with younger age (65 years and younger) in this study series ($p = 0.0095$). We have further divided the patients with *EGFR* mutations into three groups: (1) L858R mutation, (2) exon 19 deletions, and (3) other mutations (including all double mutations), because the associated genetic features and the TKI sensitivity of these mutations were more diverse than single L858R mutations or exon 19 deletion in our previous studies and other reports.[16,25,29–31] The patterns of all *EGFR* mutations are shown in Supplement Table 1 (Supplemental Digital Content 1, http://links.lww.com/JTO/A25).

TABLE 1. Comparison of the Clinical Characteristics of the 164 Patients of the Two Year Groups

Variables	Year Group 1 (1996–1998) Patients No. (%)	Year Group 2 (2002–2004) Patients No. (%)	*p*
Gender			
Female	15 (21.7)	39 (41.1	0.0094
Male	54 (78.3	56 (59.0)	
Age (yr)			
<65	32 (46.4)	48 (50.5)	0.5997
≥65	37 (53.6)	47 (49.5)	
Diagnosis			
ADC	30 (43.5)	67 (70.5)	0.0031[a]
SCC	33 (47.8)	22 (23.2)	
ADSC	2 (2.9)	4 (4.2)	
others	4 (5.8)	2 (2.1)	
Stage[b]			
I	41 (59.4	48 (50.5)	0.5288
II	9 (13.0)	15 (15.8)	
IIIA	19 (27.5)	32 (33.7)	
Smoking status			
(−)	31 (56.4)	65 (68.4)	0.1382
(+)	24 (43.6)	30 (31.6)	
CISH			
(−)	21 (70.0)	51 (54.3)	0.1281
(+)	9 (30.0)	43 (45.7)	
KRAS mutations			
(−)	65 (97.0)	84 (94.4)	0.6995[a]
(+)	2 (3.0)	5 (5.6	
EGFR Mutations			
(−)	53 (76.8)	53 (59.6)	0.0457
(+)	16 (23.2)	36 (40.4)	

[a] *p* value from the Fisher's exact test. The other *p* values were by χ^2 tests.
[b] Pathological stage according to the AJCC staging criteria, sixth edition.
ADC, adenocarcinoma; SCC, squamous cell carcinoma; CISH, chromogenic in situ hybridization; *EGFR,* epidermal growth factor receptor; AJCC, American Joint Committee on Cancer; ADSC, adenosquamous carcinoma.

Survival of Different Year Groups and Pathology

The major difference in the associated clinicopathological characteristics of the two year groups was a decrease of

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TABLE 2. Clinical Characteristics of *EGFR* Mutation Analyses in 158 Patients

Variables	EGFR Mutation (−) (%)	EGFR Mutation (+) (%)	p^a
Gender			
Female	24 (46.2)	28 (53.9)	<0.0001
Male	82 (77.4)	24 (22.6)	
Age			
<65	44 (57.1)	33 (42.9)	0.0095
≥65	62 (76.5)	19 (23.5)	
Pathology diagnosis			
ADC	43 (46.7)	49 (53.3)	<0.0001[a]
SCC	52 (96.3)	2 (3.7)	
ADSC	5 (83.3)	1 (16.7)	
others	6 (100)	0 (0)	
Stage			
I	55 (63.9)	31 (36.1)	0.1967[a]
II	20 (83.3)	4 (16.7)	
IIIA	31 (64.6)	17 (35.4)	
Smoking status			
(−)	47 (51.1)	45 (48.9)	<0.0001[a]
(+)	47 (90.4)	5 (9.6)	
CISH			
(−)	61 (88.4)	8 (11.6)	<0.0001
(+)	13 (26.5)	36 (73.5)	
KRAS mutations			
(−)	97 (65.1)	52 (34.9)	0.0961[a]
(+)	7 (100)	0 (0)	

[a] p value from Fisher's exact test, the others from χ^2 test.

ADC, adenocarcinoma; SCC, squamous cell carcinoma; CISH, chromogenic in situ hybridization; *EGFR,* epidermal growth factor receptor; ADSC, adenosquamous carcinoma.

SCC and increase of ADC from year group 1 to year group 2 (Table 1). This difference might be largely related to the sampling procedures (patients agreement or specimen availability), but it also reflected the trend of a gradual decrease of SCC and increase of ADC in recent years.[32] Because SCC was more common in male gender and rarely associated with *EGFR* mutations, it would result in the statistical differences in the proportions of gender and *EGFR* mutations in these two year groups. To avoid that more SCC than ADC in year group 1 would cause sampling bias for the survival analysis, we have performed multivariate analyses for suitable adjustment.

Among all of the clinical variables, year group 2 (2002–2004), younger age (65 years and younger), stage I disease, and nonsmoker were associated with significant better overall survival by univariate and multivariate analyses (Table 3 and Figure 1). The median survival of year group 2 was much longer than year group 1 (88.6 months versus 21.4 months). These variables were also associated with significant better 3-year survival. For 5-year survival, only year group, age, and stage IIIA disease remained to have significant survival differences. Difference in survival between patients with SCC and ADC was not significant by univariate analysis ($p = 0.2602$) but was marginally significant by multivariate analysis ($p = 0.0543$). We also have performed the same survival analyses for SCC and ADC, respectively (supplement Table 2 and 3, http://links.lww.com/JTO/A25), and the prognosticators were quite similar to the data of overall analysis except smoking status, which was associated with shorter survival in patients with SCC ($p = 0.0018$) but not in ADC.

Because the survival difference of the two year groups was quite big, and a larger number of year group 2 patients had received adjuvant C/T or TKI treatment, we have also performed survival analysis for the year group 2 patients only. The 17 patients with TKI treatment were also excluded. The results still showed similar trends in survival difference as in the 164 patients, except that the smoking status became nonsignificant for survival (Table 4).

EGFR Mutations and Survival

When we compared the survival between patients with or without *EGFR* mutations, the median survival was much longer for patient with *EGFR* mutations (55.5 months) than wild type (34.9 months), but the difference was not statistically significant by univariate ($p = 0.3206$) or multivariate analyses ($p = 0.1173$) (Table 3). If we only compared the survival of wild-type patients with L858R mutations and exon 19 deletion only (not including double mutations), the median survival was also much longer for patient with *EGFR* mutations (54.7 months) than wild type (34.9 months). The difference was still not statistically significant by univariate analysis ($p = 0.1981$) but had borderline significance by multivariate analyses ($p = 0.0506$) (Table 3). In addition, the 3-year survival rates of patients with *EGFR* mutations were all significantly higher than wild type in both comparisons. After the 18 patients received TKI treatment were deleted, the median survival for patients with *EGFR* mutations and wild type all became shorter, but the former (52 months) remained much longer than the latter (30.1 months). When we only analyzed the *EGFR* mutations and patient survival of year group 2 and excluding the 17 patients received TKI treatment (Table 4), the trend was similar to the whole group. The median survival of patients with *EGFR* mutations should still be longer than wild type (59 months), because it has not been reached yet.

We also performed the survival analysis for *EGFR* mutations in the year group 1 patients only. The only one patient (wild type, SCC), who had received TKI treatment for tumor recurrence was excluded. In these 68 patients, none received adjuvant C/T. The median survival of the patients with *EGFR* mutations (29.6 months) remained longer than wild type (19.3 months). It was even longer, if only patients with L858R and exon 19 deletion were included for comparison (49.1 months). The Log-rank tests and PH regressions appeared nonsignificant because of hazards crossings (Supplemental Figure 1, Supplemental Digital Content 1, http://links.lww.com/JTO/A26).

We have further compared the survival differences between wild type and the three groups of patients with *EGFR* mutations. These three groups of patients all had longer median survival than wild type (62.4 months for L858R, 51.9 months for exon 19 deletion, and 56.2 months for others, respectively), but with no statistic significance by univariate analyses (Table 6). However, Kaplan-Meier estimates display large discrepancy among different *EGFR* mutation subgroups (Figure 2*A*). Only the L858R mutation

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TABLE 3. Survival Analyses of 164 Patients with NSCLC

Variables	Death/ Alive	Median Survival (mo) (95% CI)	p^a	HR[b] (95% CI) (p)	3-yr[c] (p)	5-yr[d] (p)
Year group						
1	56/13	21.4 (16.6–44.4)		1.00	0.391 (—)	0.304 (—)
2	47/48	88.6 (43.3–88.6)	0.0011	0.29 (0.15–0.55) (0.0001)[e]	0.653 (0.0023)	0.526 (0.0080)
Gender						
Female	34/20	48.5 (29.5–91.8)		1.00	0.593 (—)	0.444 (—)
Male	69/41	43.9 (28.0–67.9)	0.7218	0.67 (0.33–1.36) (0.2634)	0.518 (0.3527)	0.427 (0.8356)
Age						
<65	43/37	88.6 (50.1–125.8)		1.00	0.688 (—)	0.523 (—)
≥65	60/24	23.6 (16.6–39.6)	0.0013	3.97 (2.17–7.26) (<.0001)[e]	0.405 (0.0005)	0.345 (0.0243)
Pathology diagnosis[f]						
ADC	56/41	54.7 (38.0–121.9)		1.00	0.598 (—)	0.463 (—)
SCC	38/17	35.6 (23.5–67.9)	0.2602	0.49 (0.24–1.01) (0.0543)	0.491 (0.2195)	0.400 (0.4606)
Stage						
I	46/43	88.6 (49.1–)		1.00	0.674 (—)	0.561 (—)
II	18/6	24.4 (18.0–67.9)	0.0057	2.22 (1.01–4.87) (0.0471)	0.375 (0.0321)	0.333 (0.0859)
IIIA	39/12	23.5 (8.4–45.0)	<.0001	4.01 (2.07–7.78) (<.0001)[e]	0.392 (0.0042)	0.255 (0.0021)
Smoking status[g]						
(−)	55/41	58.0 (40.8–104.7)		1.00	0.635 (—)	0.499 (—)
(+)	38/16	22.1 (15.6–54.7)	0.0279	1.98 (1.03–3.78) (0.0399)	0.426 (0.0232)	0.352 (0.0984)
CISH[g]						
(−)	42/30	53.4 (28.7–104.7)		1.00	0.569 (—)	0.471 (—)
(+)	29/23	56.2 (31.5–)	0.8738	1.14 (0.60–2.14) (0.6948)	0.615 (0.6049)	0.481 (0.9124)
KRAS mutations[g]						
(−)	94/55	44.4 (29.5–67.4)		1.00	0.544 (—)	0.429 (—)
(+)	5/2	21.0 (11.4–88.6)	0.5053	0.88 (0.31–2.45) (0.8015)	0.429 (0.5913)	0.429 (0.9990)
EGFR mutation s[g]						
Wt	69/37	34.9 (23.2–67.9)		1.00	0.491 (—)	0.424 (—)
Mut-1	31/21	55.5 (39.6–)	0.3206	0.53 (0.24–1.17) (0.1173)	0.654 (0.0426)	0.442 (0.8291)
Wt	61/37	34.9 (23.2–67.9)		1.00	0.491 (—)	0.424 (—)
Mut-2	23/18	54.7 (39.6–)	0.1981	0.43 (0.18–1.00) (0.0506)	0.683 (0.0232)	0.488 (0.4733)
EGFR mutation[h]						
Wt	66/34	30.1 (21.0–58.0)		1.00	0.470 (—)	0.400 (—)
Mut-1	26/15	52.0 (29.5–125.8)	0.4732	0.69 (0.28–1.70) (0.4210)	0.585 (0.1952)	0.415 (0.8684)
Wt	66/34	30.1 (21.0–58.0)		1.00	0.470 (—)	0.400 (—)
Mut-2	19/12	52.0 (29.5–)	0.3650	0.53 (0.20–1.42) (0.2052)	0.613 (0.1354)	0.452 (0.5994)

[a] p value from the log-rank test.
[b] HR, which is equal to RR estimated, adjusted for other variables using Cox PH model.
[c] Three-year overall survival rate percentage.
[d] Five-year overall survival rate percentage.
[e] Significant under Holm-Bonferroni correction.
[f] Twelve cases are not included, six with adenosquamous cell carcinoma and six with other carcinomas.
[g] There are 14, 40, 8, and 6 patients with no data at the smoking status, CISH, *KRAS*, and *EGFR* mutation variables, respectively.
[h] Five patients with no data at the *EGFR* mutation variable, and 18 patients who had received TKI treatment for tumor recurrence were not included.
NSCLC, non-small cell lung cancer; CI, confidence interval; HR, hazards ratio; RR, relative risk; ADC, adenocarcinoma; SCC, squamous cell carcinoma; CISH, chromogenic in situ hybridization; *EGFR,* epidermal growth factor receptor; Wt, wild type; Mut-1, L858R group + exon 19 deletion group +others; Mut-2, L858R group + exon 19 deletion group only; TKI, tyrosine kinase inhibitor.

group had significant better overall survival than wild type by multivariate analyses ($p = 0.0374$). L858R mutation was also significantly associated with higher 3-year survival rate than wild type ($p = 0.0079$), but not in 5-year survival ($p = 0.3863$). This effect became milder, but remained significant, when the analysis was only restricted to the patients with ADC ($p = 0.0452$) (Figure 2*B*). When the 18 TKI-treated patients were excluded, the median survival becomes shorter for all mutation groups and wild type, but the trends remained the same. The L858R group still had higher 3-year survival than wild type ($p = 0.0406$).

In the case that univariate analysis gave nonsignificant result, but multivariate analysis gave significant difference, we need to explain how the confounding structure rendered these results. By further stratified analyses, we found strong stratification effects resulted from "age" and "stage" variables. The older age group ($p = 0.0211$) and stage IIIA patients ($p = 0131$) both showed significant better survival

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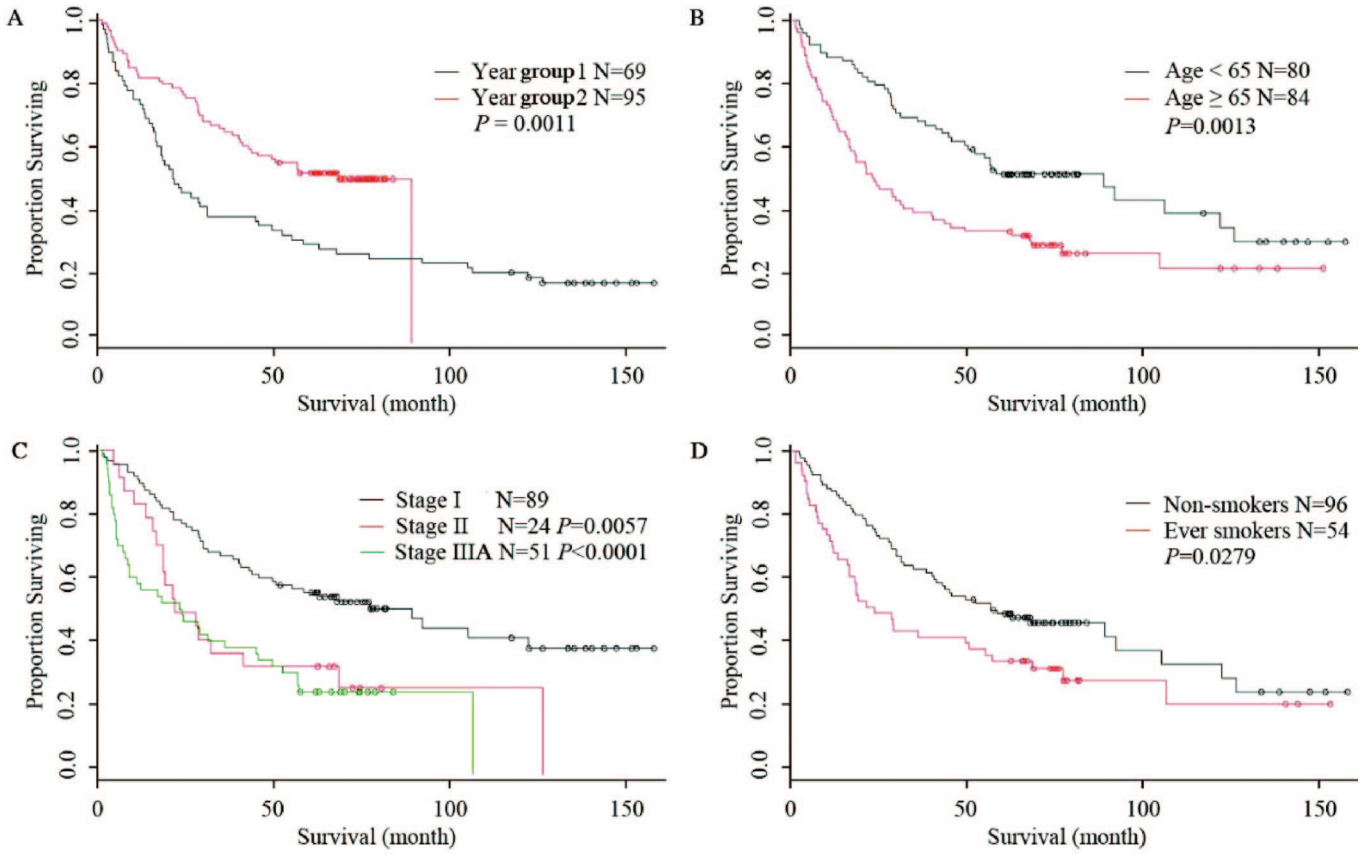

FIGURE 1. Kaplan-Meier curves for overall survival of 164 patients with non-small cell lung cancer for *A*, Year group 1 versus year group 2; *B*, Younger age (65 years and younger) versus older age (65 years or older); *C*, Stages I, II, and IIIA; and *D*, non-smoker versus ever-smoker. These four variables all were associated with significant survival differences.

for L858R group than wild type (Supplemental Figure 2, Supplemental Digital Content 2, http://links.lww.com/JTO/A27). This specific effect was lower, when the 12 TKI-treated patients (six had L858R and six were wild type) were not included, but the trends were still all the same. However, the difference became statistically nonsignificant mainly due to small case numbers (Supplemental Figure 3, Supplemental Digital Content 3, http://links.lww.com/JTO/A28).

Survival Analyses for *KRAS* Mutations and *EGFR* Gene Copy Number

Patients with *KRAS* mutations had a shorter median survival (21 months) than wild-type patients (44.4 months). However, the difference was not significant statistically. If only year group 2 patients were analyzed, this trend no longer existed (Table 4).

For *EGFR* gene copy number analyses, CISH (+) patients (≧5 copies per nucleus) also had a longer survival (56.2 months) than CISH (−) patients (53.4 months). But the difference was quite small and was not significant statistically. If only year group 2 patients were analyzed, this trend also no longer existed (Table 4).

DISCUSSION

In this study, we have demonstrated that patients with *EGFR* mutations had significant higher 3-year survival rates, and much longer median survival (55.5 months) than wild type (34.9 months). If we performed the survival analysis only in the year group 1 patients with no adjuvant C/T and no TKI treatment, the median survival of the patients with *EGFR* mutations remained much longer than wild type (Table 5, and Supplement Figure 1). Our study is the first one to demonstrate the differences of median survival between patients with or without EGFR mutations in patients with surgically resectable NSCLC. All previous reports had only overall survival data due to limitation of follow-up time.[14,20–22,25]

When we further divided the mutations into three groups, the median survivals (62.4 months for L858R, 51.9 months for exon 19 deletion, and 56.2 months for others, respectively) were all longer than wild type. Interestingly, the median survival and 3-year survival rate of the L858R group were all better than exon 19 deletion group (Table 6). This result was similar to the report of Shigematsu et al.,[20] which also found that L858R had the best overall survival and the exon 19 deletion had the worst, even shorter than the wild type (the difference was nonsignificant statistically). After the patients treated by TKI were excluded, the median survival and 3-year survival rate of L858R mutation group remained to be the best. This survival data was quite different from the studies on patients with advanced-stage NSCLC treated by TKIs, which usually showed higher TKI response rate or a

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TABLE 4. Survival Analyses of 73 Patients with NSCLC without TKI Treatment in Year Group 2 (2002–2004)

Variables	Death/ Alive	Median Survival (mo) (95% CI)	p^a	HR[b] (95% CI) (p)	3-yr[c] (p)	5-yr[d] (p)
Gender						
Female	15/13	48.5 (29.5–)			0.571 (—)	0.464 (—)
Male	22/23	67.9 (39.6–)	0.6216	1.01 (0.43–2.39) (0.9783)	0.644 (0.5429)	0.532 (0.5792)
Age						
<65	12/21	— (56.2–)			0.758 (—)	0.634 (—)
≥65	25/15	37.0 (23.2–)	0.0140	4.31 (1.83–10.16) (0.0008)[e]	0.500 (0.0255)	0.400 (0.0500)
Pathology diagnosis						
ADC	24/25	— (31.5–)			0.633 (—)	0.509 (—)
SCC	10/9	67.9 (34.3–)	0.9901	0.40 (0.13–1.23) (0.1092)	0.632 (0.9939)	0.526 (0.8991)
others	3/2	20.7 (3.4–)	0.3319	0.85 (0.20–3.58) (0.8288)	0.400 (0.4111)	0.400 (0.6700)
Stage						
I	12/23	— (50.1–)			0.800 (—)	0.657 (—)
II	9/5	36.1 (20.7–)	0.0282	4.36 (1.53–12.41) (0.0058)[e]	0.500 (0.0935)	0.429 (0.1988)
IIIA	16/8	28.2 (8.0–)	0.0028	6.72 (2.42–18.62) (0.0003)[e]	0.417 (0.0108)	0.333 (0.0303)
Smoking status[f]						
(−)	23/25	— (39.6–)			0.646 (—)	0.519 (—)
(+)	14/11	— (35.6–)	0.5028	1.86 (0.76–4.56) (0.1746)	0.560 (0.4901)	0.480 (0.7551)
CISH						
0	19/22	— (35.6–)			0.634 (—)	0.561 (—)
1	18/13	43.3 (28.5–)	0.3183	0.73 (0.23–2.34) (0.5942)	0.581 (0.6514)	0.419 (0.2479)
KRAS						
0	35/34	67.9 (39.6–)			0.623 (—)	0.506 (—)
1	2/2	— (4.3–)	0.7750	0.85 (0.16–4.51) (0.8522)	0.500 (0.6655)	0.500 (0.9815)
EGFR mutation						
Wt	25/23	59.0 (29.5–)		1.00	0.583 (—)	0.500 (—)
Mut-1	12/13	— (31.5–)	0.6211	0.69 (0.23–2.09) (0.5153)	0.680 (0.4021)	0.520 (0.8704)
Wt	25/23	59.0 (29.5–)		1.00	0.583 (—)	0.500 (—)
Mut-2	8/10	— (31.5–)	0.5415	0.63 (0.20–1.91) (0.4097)	0.667 (0.5146)	0.556 (0.6776)

[a] p value from the log-rank test.
[b] HR, which is equal to RR estimated, adjusted for other variables using Cox PH model.
[c] Three-year overall survival rate percentage.
[d] Five-year overall survival rate percentage.
[e] Significant under Holm-Bonferroni correction.
[f] There are four patients with no data at the smoking status.

NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; CI, confidence interval; HR, hazards ratio; RR, relative risk; ADC, adenocarcinoma; SCC, squamous cell carcinoma; CISH, chromogenic in situ hybridization; *EGFR,* epidermal growth factor receptor; Wt, wild type; Mut-1, L858R group + exon 19 deletion group +others; Mut-2, L858R group + exon 19 deletion group only.

longer survival for patients with exon 19 deletion than L858R.[29,33,34] The underlying mechanism will need further investigations.

In this study, the patients of year group 2 showed significant better overall survival than year group 1 by univariate and multivariate analyses. This improvement in survival could involve multiple factors, including improved socioeconomic status and better supportive care, etc. Among them, the improvement of systemic C/T, such as third generation chemotherapeutic agents (gemcitabine, paclitaxel, docetaxel, and vinorelbine) for NSCLC might have played some roles.[35,36] These new chemotherapeutic agents became reimbursed by the National Health Insurance and were more widely used in Taiwan after 1999. Therefore, most of the year group 1 patients, especially stage II and stage IIIA, would have fewer chances to be treated by these new reagents than year group 2. This could also explain why there were 10 patients in year group 2 received adjuvant C/T but none in year group 1.

It is puzzling to find that patients with NSCLC with *EGFR* mutation had better survival than wild type, because EGFR-over expressed malignant tumors arising from other major organs, such as breast, colon, and head and neck cancers were usually associated with poor prognosis and decreased survival.[37–39] The underlying mechanism of this survival difference is not clear at this point. It is possible that *EGFR* mutants have provided full spectrum of growth advantage because of their constitutive activation status. Therefore, those *EGFR* mutation-harboring cancer cells were not required to obtain many other mutations that either promote survival or prevent cell death during their developmental process. Because *EGFR* KD mutations rarely occur in other types of epithelial cancers, the presence of *EGFR* KD mutations might be less favorable for the progression of these

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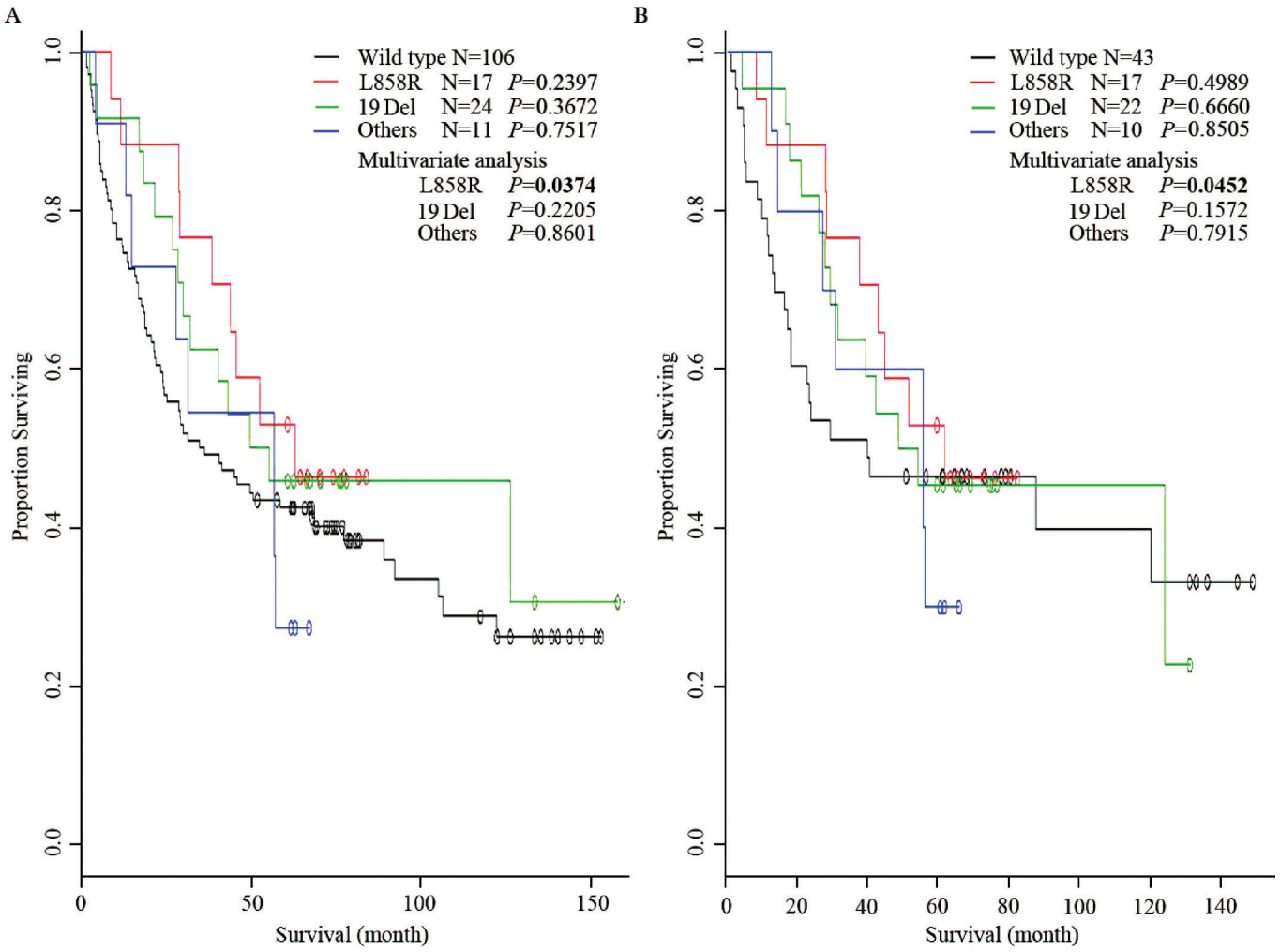

FIGURE 2. Kaplan-Meier curves for overall survival of wild type versus three groups of EGFR mutations: L858R, exon 19 deletion, and others, respectively. The *p* values of multivariate analyses were shown. *A*, A total of 158 patients with non-small cell lung cancer. *B*, Ninety-two patients with adenocarcinoma. Only L858R group was significantly better than wild type by multivariate analysis.

TABLE 5. Survival Analyses of *EGFR* Mutations in 68 Patients with NSCLC in Year Group 1 (1996–1998)[a]

Variables	Death/ Alive	Median Survival (mo) (95% CI)	p^b	HR[c] (95% CI) (*p*)	3-yr[d] (*p*)	5-yr[e] (*p*)
EGFR mutation						
Wt	41/11	19.3 (15.2–30.8)		1.00	0.365 (—)	0.308 (—)
Mut-1	14/2	29.6 (17.6–54.7)	0.8563	5.93 (0.47–75.4) (0.1702)	0.438 (0.5886)	0.250 (0.6641)
Wt	41/11	19.3 (15.2–30.8)		1.00	0.365 (—)	0.308 (—)
Mut-2	11/2	49.1 (21.0–62.4)	0.6163	0.04 (0.01–8.9) (0.2439)	0.539 (0.2161)	0.308 (0.9999)

[a] One patient who had received TKI treatment was excluded.
[b] *p* value from the log-rank test.
[c] HR, which is equal to RR estimated, adjusted for other variables using Cox PH model.
[d] Three-year overall survival rate percentage.
[e] Five-year overall survival rate percentage.
EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; CI, confidence interval; HR, hazards ratio; RR, relative risk; Wt, wild type; Mut-1, L858R group + exon 19 deletion group +others; Mut-2, L858R group + exon 19 deletion group only; TKI, tyrosine kinase inhibitor.

epithelial cancers in tissues other than lung. The above hypothesis could explain the higher susceptibility to anticancer treatment, such as C/T or R/T, for patients with advanced-staged NSCLC with *EGFR* mutations.[17,18,40]

For other genetic markers, *KRAS* mutations have been associated with shorter survival irrespective of therapeutic regimens by various studies from Asia or western countries.[41,42] In this study, patients with *KRAS* mutations also had

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TABLE 6. Survival Analyses of Three *EGFR* Mutation Groups in 158 Patients with NSCLC

Variables	Death/ Alive	Median Survival (mo) (95% CI)	p^a	HR[b] (95% CI) (p)	3-yr[c] (p)	5-yr[d] (p)
EGFR mutations						
Wt	69/37	34.9 (23.2–67.9)		1.00	0.491 (—)	0.424 (—)
L858R	9/8	62.4 (38.0–)	0.2937	0.32 (0.11–0.94) (0.0374)	0.765 (0.0079)	0.529 (0.3863)
19 Del	14/10	51.9 (29.5–)	0.3672	0.56 (0.22–1.41) (0.2205)	0.625 (0.1957)	0.458 (0.7570)
others	8/3	56.2 (14.2–)	0.7517	0.91 (0.32–2.62) (0.8601)	0.546 (0.7163)	0.273 (0.3831)
EGFR mutation[e]						
Wt	66/34	30.1 (21.0–58.0)		1.00	0.470 (—)	0.400 (—)
L858R	6/5	62.4 (28.3–)	0.3676	0.37 (0.10–1.40) (0.1423)	0.727 (0.0406)	0.546 (0.3012)
19 Del	13/7	44.4 (26.2–)	0.6027	0.67 (0.24–1.84) (0.4349)	0.550 (0.4913)	0.400 (0.9933)
others	7/3	43.5 (14.2–)	0.8985	1.49 (0.46–4.84) (0.5050)	0.500 (0.8528)	0.300 (0.5637)

[a] p value from the log-rank test.
[b] HR, which is equal to RR estimated, adjusted for other variables using Cox PH model.
[c] Three-year overall survival rate percentage.
[d] Five-year overall survival rate percentage.
[e] Five patients with no data at the *EGFR* mutation variable, and 18 patients who had received TKI treatment for tumor recurrence were not included.
EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; CI, confidence interval; HR, hazards ratio; Wt, wild type; RR, relative risk; TKI, tyrosine kinase inhibitor.

shorter median survival (21 months) than those with wild type (44.4 months), but the difference could not reach statistical significance. It is most likely due to the very low *KRAS* mutation rate (7 of 164, 4.3%) in our patients with NSCLC in this study and in our previous report.[27]

For the *EGFR* gene copy numbers and survival, CISH study was performed. CISH examination was the same as fluorescence in situ hybridization (FISH) except the detection method. The former used chromogen and the latter used fluorescence. Good correlations between CISH and FISH examination have been reported by multiple research centers.[43–45] This study demonstrated that CISH (+) patients (*EGFR* polysomy) had a longer survival (56.2 months) than CISH (−) patients (53.4 months). But the difference was much smaller than in *EGFR* mutations and was not significant statistically. It is similar to our previous study for *EGFR* gene copy numbers and TKI response.[26] We also demonstrated that tumors with *EGFR* polysomy were strongly associated with *EGFR* mutations, but the correlation with TKI response had only borderline significance ($p = 0.0665$). Only *EGFR* mutations were significantly associated with TKI response ($p < 0.0001$). The above findings suggested that the association of EGFR polysomy with TKI responses or longer median survival might be all driven by the overlap of high EGFR gene copy number with a positive EGFR mutation status. A recent published clinical trial (IPASS) for only Asian patients with NSCLC also made similar conclusions about *EGFR* polysomy (by FISH study) and TKI responsiveness.[18] But this association might not be the same in patients with NSCLC of western countries, because several reports have demonstrated that patients with NSCLC with *EGFR* polysomy (FISH positive) were significantly associated with TKI response in western countries.[46,47]

The better survival associated with *EGFR* mutations shown in this study would pose new insight to the mechanism of cancer progression. For such a tumor biology, in the future clinical trials for adjuvant therapy in patients with NSCLC, a stratification by *EGFR* mutation may be necessary to avoid an uneven distribution of the *EGFR* mutation that may confound the final survival data.

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