Abstract. To investigate the association between tumor response to thoracic radiotherapy and epidermal growth factor receptor (EGFR) mutation status in patients with lung adenocarcinoma, we collected 48 patients treated between January 2010 and December 2013. Of the 18 patients with EGFR mutation, 15 (83.3%) had a single mutation, and three (16.7%) had double mutation. Different EGFR mutation subtypes exhibited different responses to radiotherapy. The identified double EGFR mutations were associated with reduction of residual tumor burden (RTB) after radiotherapy. In univariate analysis, EGFR mutations in exon 18, 20, and 21 and double EGFR mutation were significant factors predicting RTB. In multivariate analysis, exon 20 mutation was the only significant factor. Patients with EGFR mutation seemed to have longer overall survival (OS) compared to the group with wild-type EGFR (31.1 vs. 26.6 months, p=0.49). The median and mean OS in patients with double EGFR mutation vs. wild-type EGFR were 20.1 vs. 16.9 months and 28.9 vs. 26.6 months, respectively. Further studies with larger sample size are warranted to clarify the association of EGFR mutation status with the lung tumor response after radiotherapy.

Lung cancer has been the most frequent cancer in the world for several decades and is the major cause of cancer-related deaths worldwide (1). Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer cases, and approximately 40% of NSCLCs are adenocarcinomas. Lung radiotherapy is the standard treatment for patients with unresectable, locally advanced NSCLC and is an effective approach to relieve the symptoms in patients with end-stage NSCLC.

In East Asians, epidermal growth factor receptor (EGFR) mutations were detected in 51.4% of all lung adenocarcinomas (2). The mutation frequency was 61.1% in females and 44.0% in males and was highest among the never-smokers (60.7%). The occurrence of EGFR mutation is a strong predictor in patients with advanced adenocarcinoma who are treated with the EGFR-tyrosine kinase inhibitor (TKI). However, EGFR-mutated adenocarcinomas are heterogeneous in their response to EGFR-TKI (3). Patients with mutation in exons 18, 19, or 21 are sensitive to EGFR-TKI therapy, whereas mutations in exon 20 are associated with EGFR-TKI resistance. Furthermore, the patients harboring EGFR mutations display a trend toward a better response to chemotherapy (4, 5). The EGFR mutation status may influence the way in which a lung cancer behaves and how well the tumors might respond to a specific treatment. Indeed, a previous study revealed a higher intracranial treatment response rate to radiotherapy in patients with EGFR-mutant NSCLC with brain metastasis (6). Another study demonstrated a better overall response rate and fewer locoregional relapses in EGFR-mutant patients (7).

Therefore, the aim of this study was to investigate the association between tumor response after thoracic radiotherapy and EGFR mutation status in patients with lung adenocarcinoma. In addition, we explored the influence of the EGFR mutation subtypes on radiosensitivity and overall survival (OS).
Materials and Methods

**Patients and EGFR mutation status determination.** Between January 2010 and December 2013, 95 patients with lung adenocarcinoma from three university hospitals (namely Shuang Ho Hospital of Taipei Medical University, Taipei Municipal Wanfang Hospital, and Taipei Medical University Hospital) were referred to receive lung tumor radiotherapy. Under the Institutional Review Board-approved protocol (TMU-JIRB No.: 201411055), we reviewed the medical records of all these patients. The lung adenocarcinoma diagnosis was based on histological examination and immunohistochemical staining of biopsied tumor specimens. Patients without complete course of radiotherapy, *EGFR* mutation testing, or documentation of follow-up imaging at 4 months after completion of radiotherapy were excluded from the study and consequently 48 patients were enrolled in the analysis. The *EGFR* mutational status of all patients was analyzed using the same tumor specimen, fixed by formalin and embedded in paraffin, as that obtained at the time of initial diagnosis. Positive *EGFR* mutations were validated by using the QIAamp DNA Mini Kit (QIAGEN, Germantown, MD, USA) or the Cobas *EGFR* Mutation Test (Roche Molecular Diagnostics, Pleasanton, CA, USA).

**Radiotherapy technique and prescribed dose.** All patients received radiotherapy for a single lung tumor to control disease progression or relieve tumor-related symptoms. Computed tomography (CT)-based simulation and three-dimensional conformal radiotherapy planning were used. For the reason that both conventional (daily dose 1.8-2 Gy) and hypofractionation (daily dose 3-10 Gy) schemes were adopted, the radiation dose prescribed to the tumor was converted into biologically effective dose using α/β=10 (BED10) according to the linear quadratic modeling method (8).

**Review of charts.** The medical record of each patient was reviewed thoroughly and the potential confounding variables affecting tumor response to radiotherapy and patient’s survival were recorded for analysis. These variables relate to immunohistochemical status of tumor tissue [thyroid transcription factor 1 (TTF1), cytokeratin 7 (CK7), cytokeratin 20 (CK20), and *EGFR* mutations], tumor serum markers [carcinoembryonic antigen (CEA), cancer antigen 19.9 (CA19.9), and cancer antigen 125 (CA125)], and tumor irradiated dose (BED10). The other clinical factors selected for analysis were gender, age, cigarette smoking status, clinical staging according to the seventh edition of the cancer staging system of the American Joint Committee on Cancer (AJCC) (9)], combined systemic treatment modalities, and the sum of the longest tumor diameter (SLD) obtained by pre-radiotherapy CT scan. In accordance with the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1 (10), we measured the longest diameters of tumors, from which the SLD was calculated, by CT scans using 5 mm slice thickness for the 48 included patients.

**Evaluation of tumor response and patient survival.** The tumor response for each patient was assessed as the residual tumor burden (RTB), which was calculated based on the ratio of SLD on CT scans obtained within the preceding 2 months before beginning of radiotherapy to that obtained within the 4 months following completion of radiotherapy. The secondary endpoint was the duration of survival after the initiation of radiotherapy.

**Statistical analysis.** The statistical evaluation was performed using IBM SPSS statistics software version 22 (IBM Corp., Armonk, NY, USA). We analyzed the differences of the selected variables between patients with lung adenocarcinoma with *EGFR* mutations and those with wild-type *EGFR* using the Pearson chi-square test or Fisher exact test for binary data and Student’s *t*-test for cardinal data. To identify the factors affecting RTB after radiotherapy, univariate and multivariate analyses were performed using the linear regression model. The OS was estimated by the Kaplan–Meier method and the log-rank test was used to compare survival according to the *EGFR* mutational status. The Cox proportional hazards model was applied to investigate the association between OS and the tested factors. *p*-Value less than 0.05 was considered statistically significant.

Results

**Patient characteristics.** The patients’ baseline characteristics are shown in Table I. The study population consisted of 32 men and 16 women. The median ages were both 65 years (range=29-93 years) in patients with mutated or wild-type *EGFR*. Female patients had a higher proportion harboring *EGFR* mutation, which did not reach statistical significance (38.9% vs. 30%; *p*=0.75), and the patients with mutated *EGFR* had a statistically lower ratio of cigarette smoking habit. Most patients had locoregionally advanced or metastatic disease (the clinical AJCC stage was I in three patients, II in one, III in 19, and IV in 25). Regarding the treatment modality, 37 patients received combined chemotherapy and radiotherapy, six patients received radiotherapy with EGFR-TKIs, and five received radiotherapy alone. The total radiation dose with conventional regimen ranged from 44 to 70 Gy (mean=59.9±5.2 Gy), while that with hypofractionation regimen ranged from 40 to 51 Gy (mean=47.2±3.7 Gy). The BED10 value ranged from 52.8 to 100 Gy and was a mean value of 72.9±7.5 Gy. No significant difference in age, clinical stage, and radiotherapy BED10 values were found. A higher proportion of patients with mutated *EGFR* had received concurrent EGFR-TKIs, while patients with wild-type *EGFR* tended to receive concurrent chemotherapy. Among the 18 patients with *EGFR* mutations, 15 (83.3%) harbored a single *EGFR* mutation, and three (16.7%) harbored double *EGFR* mutations. The proportions of single *EGFR* mutation cases according to each mutation status were as follow. Exon 19 deletion mutation was observed in seven patients, exon 20 S768I mutation in one, exon 21 L858R in 27, and exon 21 L858R plus exon 20 S768I mutations in one. Of the three patients with double *EGFR* mutation, two had exon 18 G719X plus exon 20 S768I mutations, and one had exon 18 G708L plus exon 21 L858R mutations.

**Tumor response.** The tumor response assessed by the RECIST criteria and the RTB values are summarized in Table II. RTB value was calculated as the SLD ratio acquired on the CT scans before beginning and after the completion
of radiotherapy. The mean RTB in all 48 patients was 55.9±29.0%. There was no obvious difference in RTB between the mutated EGFR group and the wild-type EGFR group (56.7% vs. 55.4%). Notably, the mean RTB in the group with double EGFR mutation was lower than that of the group with single EGFR mutation (19.9% vs. 64.0%) and wild-type EGFR group (55.4%). Objective tumor response was observed in 34 out of the 48 patients (70.8%). Two patients had complete response and stable disease was observed in 14 patients (29.2%). There was no disease progression observed after radiotherapy.

Factors affecting tumor response. The primary endpoint was the RTB after radiotherapy. The results of univariate and multivariate analyses of the RTB prognostic factors are listed in Table III. Using univariate analysis, we found that the EGFR exon 18, 20, and 21 mutations, as well as the double EGFR mutation, represented significant factors, with β coefficient values of −38.40, −48.67, 23.32 and −35.45, respectively. In multivariate analysis, the only variable that was significantly associated with the tumor response was EGFR exon 20 mutation obtained by stepwise variable selection with β coefficient value of −48.671 [95% confidence interval (CI)=−80.81 to −16.54, p=0.004].

Overall survival. Among the 48 patients analyzed, 22 (45.8%) died before the end of the median follow-up period of 20 months (range=1 to 54 months). The estimated overall survival rate at 1, 2, and 4 years from the start of radiotherapy was 65.7%, 45.1%, and 40.6%, respectively. In univariate analysis, only the pre-radiotherapy SLD was identified as a significant prognostic factor (hazard ratio=1.01, 95% CI=1.00−1.01, p=0.04) (Table IV). The RTB after radiotherapy, radiation BED_{10}, clinical stage, and EGFR mutation status did not show a significant influence on OS. There were no relevant factors associated with OS in multivariate analysis. The patients with mutated EGFR appeared to have a longer mean OS (Figure 1) than those with wild-type EGFR (31.1 vs. 26.6 months, p=0.49). The estimated 2-year OS rates after radiotherapy among patient with mutated EGFR and those the wild-type EGFR were 50.9% and 41.3%, respectively. Although the tumor response was significantly better and the RTB was the least in the group with double EGFR mutation (Table II), this difference did not translate into a significant OS benefit (adjusted hazard ratio=1.81, p=0.61). The estimated median OS and mean OS in the patients with double EGFR mutant vs. those with wild-type EGFR were 20.1 (95% CI=11.6 to 28.6) vs. 16.9 (95% CI=6.2 to 27.6) months and 28.9 (95% CI=10.4 to 47.4) vs. 26.6 (95% CI=18.4 to 37.8) months, respectively.
Discussion

This study demonstrated that there was no significant difference in lung tumor response to radiotherapy between patients with mutated EGFR and those with wild-type EGFR. However, we noted that EGFR mutation status may influence the tumor response, particularly in the presence of mutation at exon 18, 20, 21, or double mutation. The lung tumors showed a greater radiotherapy response in patients harboring the EGFR exon 18, 20, or double mutation. In contrast, the patients harboring exon 21 mutation showed a poorer lung tumor response to radiotherapy.

We found that the local tumor responsiveness was similar between mutated and wild-type EGFR groups. Yagishita et al. analyzed the EGFR mutational status in patients with stage III NSCLC who were treated by chemoradiotherapy (CRT) (11). They reported that the patients with EGFR mutations showed a longer local tumor control (adjusted hazard ratio=0.49, p=0.043). However, the patients with EGFR mutations showed a similar objective response rate as compared to the patients expressing wild-type EGFR (79% vs. 76%). On the other hand, Akamatsu et al. reported a significantly higher overall tumor response rate in the mutated EGFR group as compared to the wild-type EGFR group (76.9% vs. 41.9%, p=0.02) (7). Furthermore, Hsiao et al. reported a higher intracranial tumor response rate after radiotherapy in patients with metastatic brain disease harboring EGFR mutations (88% vs. 59%, p=0.005) (6). In this study, we noted that radiotherapy efficacy differed in different subtypes of patients with EGFR mutations.

In the literature, the incidence of double EGFR mutations ranged from 2% to 18% among the patients with mutated EGFR (12-15). We found that the frequency of double EGFR mutation was 16.7% (3/18). These patients included two cases with the G719X plus S768I mutations and one case with the G708L plus L858R mutations. It is well known that ionizing radiation-induced cell death is primarily due to double-strand DNA breaks. Cells have some mechanisms to repair DNA damage, and EGFR may play a role in these mechanisms. Dittmann et al. reported that import of EGFR into the nucleus triggered by ionizing radiation was associated with activation of DNA-dependent kinase (DNA-PK) (16). An increase in the nuclear kinase activity of DNA-PK is essential for repairing DNA strand breaks. In vitro studies have revealed that EGFR-mutant NSCLC cells exhibited significant delays in DNA repair because of a lack of nuclear EGFR accumulation, which contributed to their radiosensitivity (17-19). In addition, clinical researchers have reported that patients with locally advanced lung adenocarcinomas with EGFR mutations presented a longer locoregional control after definitive CRT (7,11). In this study, we found that the mean tumor RTB was not significantly different between patients with and without EGFR mutation (56.7% vs. 55.4%). However, the double EGFR mutants clearly had the least mean RTB as compared to the wild-type EGFR group (19.9% vs. 55.4%). Our study demonstrated an obvious association between double EGFR mutation and radiosensitivity during thoracic irradiation.

Previous studies have reported that in-frame deletion mutations in exon 19 and exon 21 L858R mutation accounted for approximately 90% of all EGFR mutations (20-24). Yamamoto et al. published a comprehensive review of the literature (25). They found the distribution of EGFR mutations was as follows: 48% in exon 19, 43% in exon 21, 4% in exon 20, and 3% in exon 18. EGFR mutations have been identified as predictors of response to EGFR-TKIs in NSCLC. However, EGFR-mutated NSCLCs are heterogeneous in their response to EGFR-TKIs. Cheng et al. published a literature review on the association between the EGFR mutation type and response to EGFR-TKIs (3). Exon 19 deletions, L858R mutation in
exon 21, G719X mutation in exon 18, and L861Q and L861R mutations in exon 21 were identified as biomarkers that could predict the probable benefit of EGFR-TKIs therapy for adenocarcinoma. Conversely, insertion mutations, T790M mutation in exon 20, and D761Y mutation in exon 19 were associated with resistance to the EGFR-TKIs. In this study, we found that NSCLC with mutated EGFR appeared to be heterogeneous in response to thoracic irradiation. Better radiosensitivity was observed in the patients with an exon 18 or exon 20 mutation and poorer radiosensitivity was observed in the patients with an exon 21 mutation. Using multivariate analysis, we found that only exon 20 mutation was significantly associated with the lung tumor radiotherapy response ($\beta$ coefficient $-48.67$, 95% CI $-80.81$ to $-16.54$, $p=0.004$). The type of exon 20 mutation identified was S768I in all cases ($n=3$), and two of them had this mutation combined with an exon 18 mutation (G719X). Beau-Faller et al. analyzed rare EGFR exon 18 and exon 20 mutations in NSCLC (26). They found that the EGFR mutations associated with the highest EGFR-TKI sensitivity were double mutations. We also found a similar result, with patients win the rare double EGFR mutations showing a better radiosensitivity as compared to those with single EGFR mutations.

Akamatsu et al. retrospectively analyzed the OS in patients with stage III NSCLC who received concurrent CRT. They reported a median OS of 30.7 months in patients expressing wild-type EGFR and of 57.9 months in the patients harboring EGFR mutations, although the difference was not statistically significant (7). Yagishita et al. also reported longer median OS in patients with EGFR mutations in a retrospective analysis. They identified OS of 33.3 months in the patients expressing wild-type EGFR and of 46.9 months in the patients harboring EGFR mutations ($p=0.285$) (11). Our survival analysis demonstrated that the OS was not significantly different between patients, regardless of their EGFR mutation status ($p=0.49$). However, we noted a similar trend for a longer mean OS in the group with mutated EGFR as compared to the wild-type EGFR group (31.2 vs. 26.6 months, respectively). In addition, the mean OS in the patients with double EGFR mutant was longer than in those with single EGFR mutation (28.9 vs. 24.8 months, respectively).
The present study had several limitations. Our population was of a small sample size. Indeed, only three patients with double EGFR mutation were identified in our analysis. In addition, there was selection bias in our population. NSCLC patients with EGFR mutations may have been excluded because of the lack of post-radiotherapy follow-up CT imaging evaluation. This may affect the conclusion of the outcome comparisons after thoracic irradiation among the patients with different EGFR mutation status.

In conclusion, there was no significant difference in local tumor response between patients with wild-type EGFR and patients with EGFR with mutations other than wild-type. EGFR exon 18, exon 20 and exon 21 mutations together with double mutation may represent factors that predict the lung tumor response after radiotherapy. The patients with mutated EGFR seemed to have a longer mean or median OS than those with wild-type EGFR.

Future studies with larger sample sizes may help clarify the association between the subtypes of EGFR mutation status and lung tumor response after radiotherapy in patients with lung adenocarcinoma.

**Conflicts of Interest**

The Authors declare they have no conflict of interest in regard to this study.

**References**

3. Cheng L, Alexander RE, MacLennan GT, Cummings OW, Montironi R, Lopez-Beltran A, Cramer HM, Davidson DD and


