Lung adenocarcinoma patients of young age have lower *EGFR* mutation rate and poorer efficacy of EGFR tyrosine kinase inhibitors

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ABSTRACT   Patients aged ⩽50 years are rarely diagnosed with nonsmall cell lung cancer. We conducted a retrospective cohort study to understand the mutation status of *EGFR* and the efficacy of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) treatment in young Asian patients with lung adenocarcinoma.

We collected tumour specimens and malignant pleural effusions from lung adenocarcinoma patients from June 2005 to April 2014, recorded their clinical demographic data, and analysed *EGFR* mutations by reverse transcriptase PCR.

EGFR mutation data were collected from 1039 lung adenocarcinoma patients, including 161 patients aged ⩽50 years and 878 patients aged >50 years. Fewer patients aged ⩽50 years had *EGFR* mutations than older patients (p=0.043), but they showed a higher rate of uncommon *EGFR* mutations (p=0.035). A total of 524 patients with *EGFR* mutations received EGFR-TKI treatment, including 81 patients aged ⩽50 years. Younger patients had a lower response rate than older patients (p=0.038) and had the shortest progression-free survival compared with other predefined age categories (p=0.033). Multivariate analysis of overall survival revealed age ⩽50 years as a poor prognostic factor.

In conclusion, fewer Asian patients aged ⩽50 years had *EGFR* mutations, but the *EGFR* mutation types were more uncommon. Age ⩽50 years is associated with poorer efficacy of EGFR-TKI treatment.

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Introduction

Lung cancer is the leading cause of cancer deaths worldwide. Conventional platinum-based chemotherapy is the standard treatment, but the effects are limited. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have been shown to provide favourable treatment outcomes in lung cancer patients harbouring activating *EGFR* mutations. The discovery of oncogenic driver mutations in lung cancer has led to personalised and targeted treatments.

Age-related alterations at the molecular, cellular and physiological levels are associated with carcinogenesis and subsequent cancer growth [1]. Recently, younger patients of different cancer types have been shown to exhibit distinct features in terms of disease characteristics, disease biology and treatment prognosis compared with those of older patients. For example, *BRCA1/2* germline mutation or a microsatellite instability phenotype leads to the occurrence of breast cancer or colon cancer at young age. Young patients with breast cancer or colon cancer also have a more aggressive disease process [2–6].

According to the results of the Surveillance, Epidemiology and End Results (SEER) programme, >60% of patients are >60 years old at initial diagnosis of nonsmall cell lung cancer (NSCLC) in the USA; <5% of all NSCLC patients are <50 years old at diagnosis [7]. However, the incidence of lung cancer in young patients has increased gradually [8]. Furthermore, based on analysis of SEER and the California Cancer Registry, younger age as a prognostic factor predicts improved survival among lung cancer patients [9, 10].

Some oncogenic driver mutations are associated with age. Among NSCLC patients, *EML4–ALK* fusion and *ROS1* rearrangement are associated with younger age. However, the relationship between *EGFR* mutation rate and age remains controversial [11–14]. In addition, there are few studies that explore the impact of age on the efficacy of EGFR-TKI treatment [15, 16]. We therefore conducted a retrospective cohort study to understand *EGFR* mutation status and EGFR-TKI treatment efficacy for young Asian patients (defined as ⩽50 years of age) with lung adenocarcinoma in a tertiary hospital in Taiwan.

Materials and methods

Patients and tissue procurement

From June 2005 to April 2014, lung cancer tissue and malignant pleural effusion (MPE) specimens were consecutively collected in National Taiwan University Hospital (NTUH). Informed consent for molecular analyses was obtained before tissue collection. The Institutional Review Board of the NTUH Research Ethics Committee approved this study. The tumour specimens included surgically resected lung tumours, bronchoscopy biopsy/brushing specimens and pleural effusions from thoracentesis.

Histological classification of lung adenocarcinoma was performed according to the International Multidisciplinary Classification of Lung Adenocarcinoma criteria [17]. MPE was confirmed by cytology. Pulmonary adenocarcinoma was confirmed by a positive immunohistochemical stain of thyroid transcription factor (TTF)-1 for tumour biopsies or cell blocks of MPEs [17].

All enrolled patients received lung cancer staging work-up, including whole-body bone scintigraphy and computed tomography (CT) of the head, chest and abdomen [18]. Only patients who completed staging work-up were enrolled in this study. Basic demographic information, smoking status and imaging reports were recorded for each patient. Never-smokers were defined as patients who smoked <100 cigarettes in their lifetime [19]. Patients who smoked cigarettes within 1 year of diagnosis were defined as current smokers and the remaining patients were regarded as ex-smokers (quit ⩾1 year ago). The cancer stages and Eastern Cooperative Oncology Group (ECOG) performance status were also recorded [20]. The date of diagnosis, all systemic treatments, including chemotherapy and EGFR-TKIs, and response to treatment were also recorded.

The average age of menopause is 50–51 years in the USA, and 47–50 years in Korea, Lebanon, Singapore, Greece, Morocco, Mexico, Taiwan and Turkey [21]. Therefore, we defined young patients as patients aged ⩽50 years.

Treatment response evaluation of EGFR-TKIs in lung adenocarcinoma patients

The patients received single-agent EGFR-TKI daily and no concurrent chemotherapy or radiotherapy for the lung tumours was performed during EGFR-TKI therapy. To evaluate the responses to clinical treatment, the patients received chest radiographs every 2–4 weeks and chest CT scans (including the liver and adrenal glands) every 2–3 months as routine clinical practice and as needed. A unidimensional method was adopted to evaluate measurable solid tumours according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines version 1.1 [22]. Only patients who had measurable target lesions were enrolled so that treatment responses could be evaluated. The objective responses were defined as complete remission, partial response, stable disease and progressive disease [22]. Response rate was defined as the percentage of patients who achieved complete remission or partial response. Disease control rate was defined as the percentage of patients who achieved complete remission, partial response or stable disease.

Progression-free survival (PFS) was defined as the period from the date of EGFR-TKI treatment initiation to the date of the first objective or clinical sign of disease progression or death. Overall survival was defined as the period from the date of systemic treatment to the date of death.

EGFR mutation analysis
RNA was extracted from tissue specimens including lung tumours, metastatic sites and malignant effusions with a QIAamp RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Spectrophotometry was used to measure the quantity and quality of the extracted RNA. Tissue specimens were processed for EGFR mutation analysis as described previously [23, 24]. Exons 18–21 of EGFR were amplified by reverse transcriptase PCR with a OneStep RT-PCR Kit (Qiagen) as described previously [25]. Tumours harbouring EGFR exon 19 deletion (del-19) or L858R point mutations are known to show good responses to EGFR-TKIs and both were defined as classical mutations [26]. The other EGFR mutations were detected infrequently and defined as uncommon EGFR mutations.

Statistical analysis
The Chi-squared test was used for analysis of all categorical variables. The nonparametric Mann–Whitney U-test was used to compare the median ages between two groups. Survival curves were plotted using the Kaplan–Meier method and compared between groups using the log-rank test. To explore the impact of age on EGFR-TKI treatment efficacy, we used age categories in PFS and overall survival analysis: ⩽50, 51–60, 61–70, 71–80 and >80 years. Multivariate Cox models were used to estimate adjusted hazard ratios (HRs). Two-sided p-values of <0.05 were considered statistically significant. We used SPSS version 17.0 (SPSS, Chicago, IL, USA) for statistical analysis.

Results
Clinical characteristics of lung adenocarcinoma patients
From June 2005 to April 2014, we consecutively collected 2680 tissue and MPE specimens, which included 1668 lung adenocarcinoma specimens from 1066 patients. The tissue specimens included 171 surgically resected tumours, 53 bronchoscopic biopsies and 42 bronchial brushing specimens; the majority of the specimens was composed of 1402 MPEs. Of the 1066 patients, specimens from 27 patients were inadequate or insufficient for EGFR mutation analysis, yielding 1039 patients with EGFR mutation test results. Data from some patients were included in our prior studies [25, 27, 28].

Of the 1039 patients, 540 (52.0%) were female and 742 (71.4%) were never-smokers. Median (range) age was 65.4 (26.8–95.5) years. 74.1% (770 out of 1039) of the patients had stage IV disease at initial diagnosis (table 1). There were 161 (15.5%) patients aged ⩽50, including: 14 (8.7%) patients with stage I, seven (4.3%) patients with stage II, 22 (13.7%) patients with stage III and 118 (73.3%) patients with stage IV at initial diagnosis of lung cancer (table 1). Compared with patients aged >50 years, younger patients had better performance status (p<0.001). There were no significant differences in sex (p=0.908), smoking history (p=0.136) or initial cancer stages (p=0.328) between patients aged ⩽50 and >50 years (table 1).

EGFR mutation status
Of the 1039 lung adenocarcinoma patients, 673 (64.8%) patients tested positive for EGFR mutations. Females (females 71.5% versus males 57.5%; p<0.001) and never-smokers (never-smokers 71.6% versus smokers 47.8%; p<0.001) had higher EGFR mutation rates. The EGFR mutation types included 289 (27.8%) del-19, 288 (27.7%) L858R and 96 (9.2%) uncommon mutations (table 2). Among the 673 EGFR mutant patients, there were no differences in EGFR mutation types between never-smokers and ex-/current smokers (p=0.145) (supplementary table S1). There were 25 patients with exon 18 mutation of EGFR. The rates of exon 18 mutation did not reach a statistical difference between never-smokers (17 out of 531 (3.2%)) and smokers (eight out of 142 (5.6%)) (p=0.173).

Compared with the patients aged >50 years, EGFR mutations were less frequent in younger patients (57.8% versus 66.1%; p=0.043). Interestingly, younger patients had a higher rate of uncommon EGFR mutations than older patients (13.7% versus 8.4%; p=0.035) (table 2).

Clinical characteristics of EGFR-TKI-treated patients with lung adenocarcinoma harbouring EGFR mutations
As patients harbouring tumours with the de novo T790M mutation are associated with primary resistance to EGFR-TKI treatment [29, 30], we excluded five patients with L858R+de novo T790M from this study, all >50 years old.

There were 524 EGFR mutant patients who had measurable target lesions and had taken EGFR-TKIs, including 366 who took gefitinib, 137 who took erlotinib and 21 who took afatinib. The demographic

TABLE 1 Clinical characteristics of the lung adenocarcinoma patients

	Total patients	Age ≤50 years	Age >50 years	p-value
Subjects	1039	161 (15.5)	878 (84.5)	
Age years	65.4 [26.8–95.5]	43.8 [26.8–49.9]	68.2 [50.1–95.5]	
Sex				0.908
Female	540	83 (51.6)	457 (52.1)	
Male	499	78 (48.4)	421 (47.9)	
Smoking history#				0.136
Never-smoker	742	118 (73.3)	624 (71.2)	
Ex-smoker	130	13 (8.1)	117 (13.4)	
Current smoker	165	30 (18.6)	135 (15.4)	
ECOG PS				<0.001
0–1	887	154 (95.7)	733 (83.5)	
2–4	152	7 (4.3)	145 (16.5)	
Stage at initial diagnosis				0.328
I	118	14 (8.7)	104 (11.8)	
II	45	7 (4.3)	38 (4.3)	
III	106	22 (13.7)	84 (9.6)	
IV	770	118 (73.3)	652 (74.3)	

Data are presented as n, n (%) or median (range), unless otherwise stated. ECOG PS: Eastern Cooperative Oncology Group performance status. #: data missing from two patients.

characteristics of these patients are listed in table 3. Among them, 308 were female (58.8%) and 419 were never-smokers (80.0%). Median (range) age was 64.7 (29.5–92.1) years. The *EGFR* mutation status included 233 del-19, 228 L858R and 63 uncommon mutations (table 4). EGFR-TKIs were taken as first-line treatment by 339 patients (64.7%), second-line treatment by 117 patients (22.3%) and subsequent-line (third-line or greater) treatment by 67 patients (13.0%).

81 patients (15.5%) were aged ≤50 years (table 3). Younger patients showed better performance status (p=0.002), more current smokers (p=0.010) and more uncommon *EGFR* mutations (22.2% *versus* 10.2%; p=0.009) than patients aged >50 years. In addition, there were 21 patients who received afatinib treatment. Afatinib was prescribed more frequently to younger patients (aged ≤50 years) than to older patients (aged >50 years) (8.6% *versus* 3.2%). Their *EGFR* mutation types included six del-19, nine L858R and six uncommon mutations. Of the six patients with uncommon *EGFR* mutations, five had a partial response to EGFR-TKI treatment and one had progressive disease.

Treatment response rate and PFS for EGFR-TKIs among *EGFR* mutant patients

Of the 524 *EGFR* mutant patients who received EGFR-TKI treatment, the response rate was 79.0% and the PFS was 8.9 (95% CI 8.21–9.59) months. Patients aged ≤50 years had a lower disease response rate (70.4% *versus* 80.6%; p=0.038) and disease control rate (75.3% *versus* 84.8%; p=0.035) than older patients (supplementary table S2).

In addition, PFS for EGFR-TKIs analysed by predefined age categories revealed that the shortest median PFS occurred among patients aged ≤50 years (7.3 months), followed by those aged 51–60 years (8.7 months), >80 years (8.8 months), 61–70 years (9.6 months) and 71–80 years (10.1 months) (p=0.033) (figure 1).

TABLE 2 Difference in *EGFR* mutations among lung adenocarcinoma patients

	Wild-type	Del-19	L858R	Uncommon	Total
Age years					
≤50	68 (42.2)	37 (23.0)	34 (21.1)	22 (13.7)	161
>50	298 (33.9)	252 (28.7)	254 (28.9)	74 (8.4)	878
Total	366 (35.2)	289 (27.8)	288 (27.7)	96 (9.2)	1039

Data are presented as n (%) or n. p=0.043 for lung adenocarcinoma patients aged ≤50 *versus* >50 years for positive and negative *EGFR* mutations. p=0.035 for lung adenocarcinoma patients aged ≤50 *versus* >50 years with tumours harbouring uncommon *EGFR* mutations.

TABLE 3 Clinical characteristics of *EGFR* mutant patients treated with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs)

	Total patients	Age ⩽50 years	Age >50 years	p-value
Subjects	524	81 (15.5)	443 (84.5)	
Age years	64.7 (29.5–92.1)	43.9 (29.5–49.6)	68.1 (50.1–92.1)	
Sex				0.405
Female	308	51 (63.0)	257 (58.0)	
Male	216	30 (37.0)	186 (42.0)	
Smoking history				0.010
Never-smoker	419	62 (76.5)	357 (80.6)	
Ex-smoker	52	4 (4.9)	48 (10.8)	
Current smoker	53	15 (18.5)	38 (8.6)	
ECOG PS				0.002[#]
0–1	449	78 (96.3)	371 (83.7)	
2–4	75	3 (3.7)	72 (16.3)	
***EGFR* mutation**				0.009
Del-19	233	33 (40.7)	200 (45.1)	
L858R	228	30 (37.0)	198 (44.7)	
Uncommon	63	18 (22.2)	45 (10.2)	
EGFR-TKI				0.051
Gefitinib	366	51 (63.0)	315 (71.1)	
Erlotinib	137	23 (28.4)	114 (25.7)	
Afatinib	21	7 (8.6)	14 (3.2)	
TKI sequence				0.140
First-line	339	49 (60.5)	290 (65.5)	
Second-line	117	16 (19.8)	101 (22.8)	
Third-line or greater	68	16 (19.8)	52 (11.7)	

Data are presented as n, n (%) or median (range), unless otherwise stated. ECOG PS: Eastern Cooperative Oncology Group performance status. [#]: Fisher's exact test.

We stratified the patients into del-19, L858R and uncommon mutations to investigate the impact of age on the effect of EGFR-TKIs for different *EGFR* mutation types. Among patients with del-19, there were no differences in PFS for EGFR-TKIs between young and old patients (8.3 *versus* 9.9 months; p=0.279) (figure 2a). Among patients with L858R, younger patients had shorter PFS on EGFR-TKI than those aged >50 years (7.3 *versus* 9.6 months; p=0.003) (figure 2b). For those with uncommon *EGFR* mutations, there was no significant difference between the two age groups (3.0 *versus* 5.2 months; p=0.834) (figure 2c).

We then stratified the patients according to sex to investigate the impact of sex on the effect of EGFR-TKIs. Among female patients, younger patients had shorter PFS for EGFR-TKIs than those aged >50 years (7.4 *versus* 9.9 months; p=0.027) (supplementary figure S1). Among male patients, the difference (⩽50 *versus* >50 years) also showed a similar trend, although not statistically significant (6.9 *versus* 8.8 months; p=0.055) (supplementary figure S2).

As prior reports showed that smoking may impact the treatment efficacy of EGFR-TKIs [31, 32], we analysed the association between the clinical response of patients on EGFR-TKIs and their smoking history. Current smokers (34 out of 53 (64.2%)) had the lowest response rate, followed by never-smokers (336 out of 419 (80.2%)) and ex-smokers (44 out of 52 (84.6%)) (p=0.015). Current smokers (6.9 months) also had the shortest PFS for EGFR-TKIs compared with never-smokers (9.1 months) and ex-smokers (9.4 months) (p=0.015) (supplementary figure S3). We also stratified the patients according to smoking history and then investigated the impact of age on the effect of EGFR-TKIs. Among never-smokers, younger patients had shorter PFS on EGFR-TKI than those aged >50 years (7.7 *versus* 9.7 months; p=0.047) (supplementary figure S4a). However, there were no significant differences between age groups among ex-smokers (p=0.115) and current smokers (p=0.148) (supplementary figure S4b and c).

Multivariate analysis was performed using the Cox regression model for potential predictive factors of PFS, including predefined age categories, sex, smoking history, performance status and *EGFR* mutation types (table 5). The result showed that the youngest age category (age ⩽50 years) had a significant shorter PFS compared with all other age categories except for the group aged 51–60 years. In addition, ECOG performance status 2–4 (HR 1.86, 95% CI 1.38–2.50; p<0.001) was significantly associated with shorter

TABLE 4 Uncommon *EGFR* mutations				
EGFR mutation	Age ⩽50 years	Age >50 years	Total	EGFR-TKI response
G719A	2	1	3	2 PR, 1 PD
G719D	0	1	1	1 PD
G719S	1	0	1	1 PR
L747P	0	2	2	1 PR, 1 PD
A763_Y764 ins FQEA	2	0	2	1 PR, 1 PD
A767–V769 dup ASV	0	4	4	4 PD
S768–D770 dup SVD	1	3	4	1 SD, 3 PD
V769_D770 ins GSV	1	0	1	1 PD
D770_N771 ins G	1	0	1	1 PD
Del D770 ins GY	0	1	1	1 PD
Del N771 ins KH	0	1	1	1 PD
P772–773H dup PH	0	1	1	1 PD
P772_H773 ins YNP+H773Y	1	0	1	1 PD
K806E	0	1	1	1 PR
R831H	1	0	1	1 PR
L861P	0	1	1	1 PR
L861Q	0	5	5	2 PR, 2 SD, 1 PD
L861R	0	1	1	1 PR
G719A+S720F	0	1	1	1 PR
G719A+S768I	0	1	1	1 PD
G719A+V769M	1	0	1	1 PD
G719C+S768I	0	1	1	1 PR
G719D+L861Q	0	1	1	1 PD
G719S+Q701L+I706T	0	1	1	1 PR
G719S+E709K	0	1	1	1 PR
G719S+L747S	0	1	1	1 PR
G719S+L861Q	1	0	1	1 PR
P772_H773insYNP+H773Y	1	0	1	1 PD
Del-19+K754T	0	1	1	1 PR
Del-19+V769M	0	1	1	1 PD
L858R+E709A	1	0	1	1 PR
L858R+E709G	1	3	4	3 PR, 1 PD
L858R+K754R+E762K	0	1	1	1 PR
L858R+S768I	1	1	2	2 PR
L858R+R776H	0	2	2	2 PR
L858R+V834L	1	3	4	2 PR, 2 PD
L858R+A859S	0	1	1	1 PR
L858R+K860I	1	0	1	1 PR
L858R+A871E	1	0	1	1 PR
L861Q+E746G	0	1	1	1 PR
L861Q+R776H	0	1	1	1 PD
L861R+R831C	0	1	1	1 PR

Data are presented as n. EGFR: epidermal growth factor receptor; TKI: tyrosine kinase inhibitor; PR: partial response; SD: stable disease; PD: progressive disease.
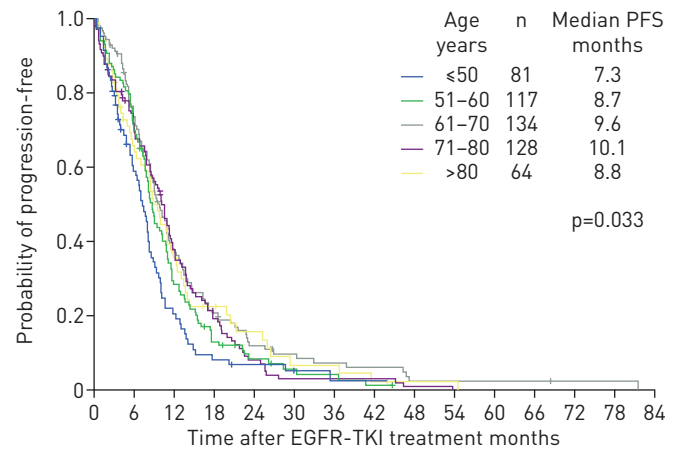
PFS. Classical *EGFR* mutations, del-19 (HR 0.63, 95% CI 0.46–0.86; p=0.003) and L858R (HR 0.72, 95% CI 0.53–0.99; p=0.041), were associated with longer PFS.

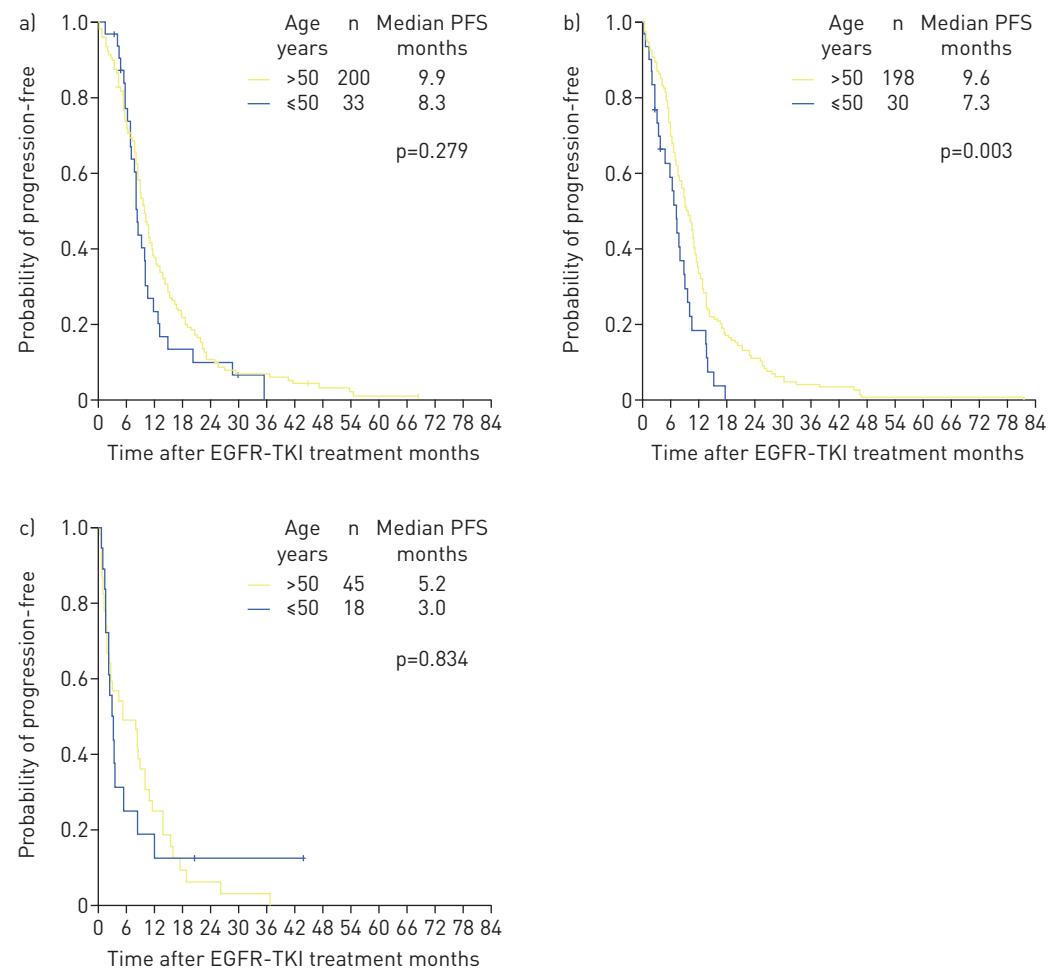### Overall survival and prognostic factors of EGFR *mutant patients treated with EGFR-TKIs*

Univariate analysis of prognostic factors was conducted for the 524 *EGFR* mutant patients who had taken EGFR-TKIs as shown in table 6. Stratified by predefined age categories, the shortest median overall survival occurred among patients aged >80 years (14.3 months), followed by those aged 71–80 years (19.0 months), ⩽50 years (20.7 months), 61–70 years (26.3 months) and 61–70 years (31.5 months) (p<0.001) (figure 3). Current smokers (15.4 months) had the shortest overall survival followed by never-smokers (25.0 months) and ex-smokers (30.3 months) (p=0.002).

Multivariate analysis was performed using the Cox regression model for potential prognostic factors of overall survival, including predefined age categories, sex, smoking, performance status, *EGFR* mutation types, EGFR-TKI sequence, platinum use and pemetrexed use (table 6). The result showed that the

FIGURE 1 Kaplan–Meier curves of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) progression-free survival (PFS) for *EGFR* mutant lung adenocarcinoma patients stratified into predefined age categories: ⩽50, 51–60, 61–70, 71–80 and >80 years. The difference was statistically significant (p=0.033, log-rank test).

FIGURE 2 Kaplan–Meier curves of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) progression-free survival (PFS) were constructed based on different *EGFR* mutation types. PFS curves of lung adenocarcinoma patients aged >50 and ⩽50 years were plotted separately for patients with a) del-19, b) L858R and c) uncommon *EGFR* mutations.

TABLE 5 Multivariate analysis of predictive factors for progression-free survival (PFS) in *EGFR* mutant patients treated with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs)

Factors	Patients n	Median PFS months	Univariate analysis p-value	Multivariate analysis	
				Hazard ratio (95% CI)	p-value
Age years			0.033		
⩽50	81	7.3		1	
51–60	117	8.7		0.80 (0.59–1.08)	0.144
61–70	134	9.6		0.66 (0.49–0.90)	0.009
71–80	128	10.1		0.69 (0.50–0.93)	0.016
>80	64	8.8		0.54 (0.37–0.80)	0.002
Sex			0.247		
Female	308	9.1		1	
Male	216	8.7		1.09 (0.88–1.36)	0.428
Smoking history			0.015		
Never-smoker	419	9.1		1	
Ex-smoker	52	9.4		0.94 (0.66–1.32)	0.705
Current smoker	53	6.9		1.40 (1.00–1.96)	0.052
ECOG PS			0.001		
0–1	449	9.4		1	
2–4	75	6.0		1.86 (1.38–2.50)	<0.001
***EGFR* mutation**			0.002		
Uncommon	63	3.6		1	
Del-19	233	9.7		0.63 (0.46–0.86)	0.003
L858R	228	9.1		0.72 (0.53–0.99)	0.041
TKI sequence			0.487		
First-line	339	9.0		1	
Second-line	117	8.5		0.89 (0.70–1.13)	0.336
Third-line or greater	68	9.0		0.94 (0.71–1.24)	0.643

ECOG PS: Eastern Cooperative Oncology Group performance status.

youngest age category (age ⩽50 years) had a significantly shorter overall survival compared with the age groups of 51–60 and 61–70 years. In addition, ECOG performance status 2–4 (HR 2.84, 95% CI 2.13–3.78; p<0.001) was significantly associated with shorter overall survival. Pemetrexed use was a favourable prognostic factor (HR 0.57, 95% CI 0.45–0.72; p<0.001).

Discussion

Lung adenocarcinoma patients aged ⩽50 years had a shorter PFS and a lower disease control rate for EGFR-TKIs than those aged >50 years. Age ⩽50 years also predicts a poor overall survival. In addition, lung adenocarcinoma patients aged ⩽50 years had different *EGFR* mutations compared with older patients. Younger lung adenocarcinoma patients had tumours with a lower *EGFR* mutation rate but more uncommon mutation types. Age ⩽50 years and uncommon *EGFR* mutations were both independent predictive factors of EGFR-TKI response and were associated with a shorter PFS.

Prior studies reported that older patients have higher response rates to EGFR-TKI and longer overall survival [16, 33]. Hsu *et al.* [34] also noted that lung adenocarcinoma patients aged ⩽45 years have a shorter PFS (6.0 months) and poorer treatment response to EGFR-TKIs compared with the general population. This finding is compatible with the present study. The disease control rate for EGFR-TKIs was lower in patients aged ⩽50 years than in those >50 years. We showed that age ⩽50 years was an unfavourable prognostic factor for PFS on EGFR-TKI use compared against all other older age cohorts as well as for overall survival compared with patients aged 51–70 years according to the multivariate analysis.
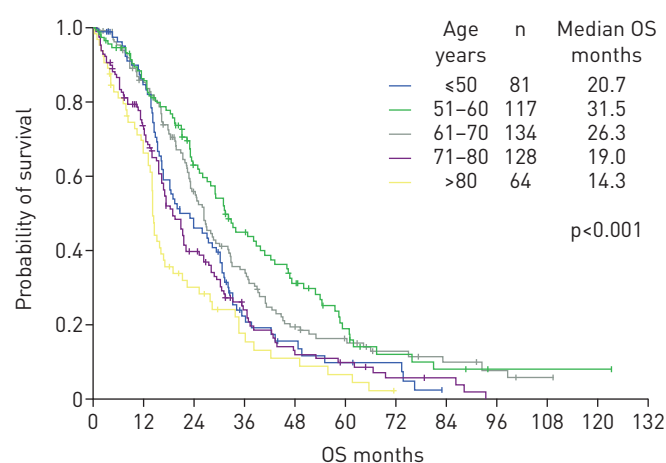
However, WHEATLEY-PRICE *et al.* [15] did not find age as a significant variable of response rate, PFS and overall survival for erlotinib treatment according to retrospective analysis of the BR.21 trial, which enrolled unselected patients with the majority having *EGFR* wild-type. The controversial conclusion of the previous studies might stem from the use of different study designs and the small sample size. Variations in the definitions of young age between different studies may also contribute to heterogeneous results. In addition, the present study took *EGFR* mutation status into consideration for all enrolled patients. *EGFR* mutation is the most important factor in determining the effect of EGFR-TKIs. Our study enrolled a larger

TABLE 6 Multivariate analysis of prognostic factors for overall survival in *EGFR* mutant patients treated with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs)

Factors	Patients n	Median overall survival months	Univariate analysis p-value	Multivariate analysis	
				Hazard ratio (95% CI)	p-value
Age years			<0.001		
⩽50	81	20.7		1	
51–60	117	31.5		0.66 (0.47–0.92)	0.015
61–70	134	26.3		0.69 (0.50–0.95)	0.025
71–80	128	19.0		0.82 (0.59–1.15)	0.246
>80	64	14.3		0.73 (0.49–1.11)	0.142
Sex			0.079		
Female	308	25.3		1	
Male	216	22.4		1.25 (0.99–1.57)	0.065
Smoking history			0.002		
Never-smoker	419	25.0		1	
Ex-smoker	52	30.3		0.96 (0.67–1.37)	0.801
Current smoker	53	15.4		1.66 (1.17–2.36)	0.005
ECOG PS			<0.001		
0–1	449	27.0		1	
2–4	75	11.0		2.84 (2.13–3.78)	<0.001
***EGFR* mutation**			0.060		
Uncommon	63	19.0		1	
Del-19	233	27.9		0.81 (0.59–1.11)	0.193
L858R	228	23.1		0.93 (0.68–1.27)	0.634
TKI sequence			<0.001		
First-line	340	19.5		1	
Second-line	117	28.0		0.70 (0.54–0.91)	0.007
Third-line or greater	67	34.7		0.58 (0.43–0.78)	<0.001
Platinum use			<0.001		
No	224	15.4		1	
Yes	300	30.3		0.85 (0.65–1.11)	0.232
Pemetrexed use			<0.001		
No	252	15.2		1	
Yes	272	32.2		0.57 (0.45–0.72)	<0.001

ECOG PS: Eastern Cooperative Oncology Group performance status.

FIGURE 3 Kaplan–Meier survival curves of overall survival (OS) for *EGFR* mutant lung adenocarcinoma patients who received epidermal growth factor receptor tyrosine kinase inhibitor treatment stratified into predefined age categories: ⩽50, 51–60, 61–70, 71–80 and >80 years. The difference was statistically significant (p<0.001, log-rank test).

series of patients with complete *EGFR* mutation reports, and the impact of age on PFS and overall survival was also determined based on predefined age categories for EGFR-TKI-treated patients.

The definitive mechanism for the impact of age on the efficacy of EGFR-TKIs is unknown. The present study showed that *EGFR* mutant patients aged ⩽50 years have more uncommon *EGFR* mutations. The uncommon *EGFR* mutation rate of the whole study population was 9.5%, which was comparable to ∼10% reported in previous studies [35, 36]. Compared with patients with L858R or del-19, patients with uncommon *EGFR* mutations had a lower response rate and shorter PFS [28, 37]. This may indirectly confirm the poorer EGFR-TKI treatment outcome among young patients [38]. In addition, most of the patients enrolled in this study received first-generation EGFR-TKIs. The second-generation EGFR-TKI afatinib has been shown to be active in patients with some uncommon *EGFR* mutations [39]. Larger cohort studies are necessary to clarify the complex mechanism.

Interestingly, the present study showed the most significant difference of PFS in patients with the L858R mutation after stratifying by *EGFR* mutation types. The different subtypes of *EGFR* mutations may cause different clinical characteristics and treatment prognosis for EGFR-TKIs [25]. More studies are needed to investigate the complex interactions between EGFR-TKI efficacy and the different *EGFR* mutation types.

The impact of age on oncogenic gene alteration has been explored for different cancers. The present study showed a lower *EGFR* mutation rate in patients aged ⩽50 years, which was compatible with prior studies. *EGFR* mutations accumulate with age in lung cancer patients [11, 12]. The possible mechanism that results in carcinogenesis proceeds through the accumulation of genetic changes. Cancer cells accumulate such genetic alterations over time [40]. However, some studies did not reach the same conclusion [13, 14]. Recently, SACHER *et al.* [14] showed that the frequency of targetable genomic alteration was highest among younger patients (<40 years) and steadily decreased with age. However, younger patients had more *EGFR* mutations than older patients [14]. Larger studies are necessary to address the issue in the future.

For Asian patients, the PIONEER study detected an *EGFR* mutation rate of 51.4% in lung adenocarcinoma samples (biopsy, surgical specimen or cytology) using the Scorpion amplification refractory mutation system [36]. The study reported that the *EGFR* mutation rate of lung adenocarcinoma patients in Taiwan was higher, up to 62.1% [36]. The present study showed that the *EGFR* mutation rate was 64.8%, which was also higher than the average *EGFR* mutation rate, but comparable to the Taiwanese data of the PIONEER study. The discrepancy is likely due to differences in specimen collection for *EGFR* mutation analysis. Our prior study reported that patients with lung adenocarcinoma MPEs had a higher *EGFR* mutation rate than surgically resected specimens [25, 27]. In addition, we collected TTF-1-positive tissue samples that are known to have higher *EGFR* mutation rates than TTF-1-negative tissue samples [41]. Furthermore, different *EGFR* mutation detection methods may also contribute to the difference.

Prior reports showed that smoking may lower the effects of EGFR-TKI treatment *in vitro* [42] and in clinical observation studies [31, 32]. The current study also showed that smoking had an impact on EGFR-TKI treatment response. More patients may be necessary to elucidate the inconspicuous effect and the possible mechanism.

The study has some limitations. First, although the current study enrolled a large series of *EGFR* mutant patients, the enrolled patients were all Asians, known to have a higher *EGFR* mutation rate. The result should be confirmed in other areas of the world, especially where lung adenocarcinoma has a low *EGFR* mutation incidence. Second, we did not examine all reported gene alterations that caused primary resistance to EGFR-TKIs, such as *de novo MET* amplification [43], *KRAS* mutations [44] or loss of *PTEN* [45]. MPEs are not suitable for fluorescence *in situ* hybridisation assays to detect *MET* amplification and the availability of cancer tissue samples in this cohort was limited. In addition, *EGFR* and *KRAS* mutations are mutually exclusive [46, 47]. In the current study, we chose to focus on *EGFR* mutant patients to dissect the impact of age on EGFR-TKI efficacy. Third, we confirmed the diagnosis of lung adenocarcinoma based on TTF-1 positivity. As not all lung adenocarcinomas express TTF-1, this may represent a selection bias. Fourth, we did not compare the smoking of patients of different ages expressed in pack-years because some detailed smoking information was missing from the retrospective records. We just adjusted the smoking factor in treatment efficacy by never-, ex- and current smokers. Fifth, our analyses did not differentiate between the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society lung adenocarcinoma subtypes for small biopsies/cytology and resection specimens, different lines of EGFR-TKI usage as well as the different EGFR-TKIs used, and a potential time period bias which may have influenced therapeutic strategies and thereby outcome over the long study period has not been further explored.

In conclusion, younger patients had less *EGFR* mutations but more uncommon *EGFR* mutations. Aged ⩽50 years is associated with poorer efficacy of EGFR-TKI treatment.

Acknowledgements

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