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A Prospective, Molecular Epidemiology Study of *EGFR* Mutations in Asian Patients with Advanced Non–Small-Cell Lung Cancer of Adenocarcinoma Histology (PIONEER)

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Introduction: PIONEER (NCT01185314) was a prospective, multinational, epidemiological study of epidermal growth factor receptor (*EGFR*) mutations in patients from Asia with newly diagnosed advanced lung adenocarcinoma.

Methods: Eligible patients (aged ≥20 years) had untreated stage IIIB/IV adenocarcinoma. The *EGFR* mutation status (primary end point: positive, negative, or undetermined) of tumor samples (biopsy, surgical specimen, or cytology) was determined (Scorpion amplification refractory mutation system). *EGFR* mutation frequency was calculated and compared between demographic and clinical subgroups.

Results: Of 1482 patients from seven Asian regions, 43.4% of patients were female, median age was 60 years (range, 17–94), and 52.6% of patients were never-smokers. *EGFR* mutation status was evaluable in tumors from 1450 patients (97.8%) (746 [51.4%] positive; 704 [48.6%] negative). Country, sex, ethnicity, smoking status, pack-years (all $p < 0.001$), disease stage ($p = 0.009$), and histology type ($p = 0.016$) correlated significantly with *EGFR* mutation frequency. Mutation frequency was 61.1% in females, 44.0% in males; lower in patients from India (22.2%) compared with other areas (47.2%–64.2%); highest among never-smokers (60.7%); and decreased as pack-year number increased (>0–10 pack-years, 57.9%; >50 pack-years, 31.4%) (similar trend by sex). Ethnic group ($p < 0.001$) and pack-years ($p < 0.001$) had statistically significant associations with mutation frequency (multivariate analysis); sex was not significant when adjusted for smoking status.

Conclusion: PIONEER is the first prospective study to confirm high *EGFR* mutation frequency (51.4% overall) in tumors from Asian patients with adenocarcinoma. The observed high mutation frequency in demographic/clinical subgroups compared with white populations suggests that mutation testing should be considered for all patients with stage IIIB/IV adenocarcinoma, even males and regular smokers, among Asian populations.

Key Words: Epidermal growth factor receptor mutation, epidemiology, Asian, Adenocarcinoma, Non–small-cell lung cancer.

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Non–small-cell lung cancer (NSCLC) comprises approximately 80% to 85% of all lung cancers,[1] and the majority of patients present with advanced or metastatic disease.[2] Several phase III studies have demonstrated the clinical efficacy of the epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) gefitinib and erlotinib compared with chemotherapy against advanced NSCLC when used as first-line treatment for patients whose tumors harbor activating *EGFR* mutations.[3–8] Several clinical practice guidelines now recommend *EGFR* mutation testing before initiation of first-line therapy for advanced NSCLC.[9–11]

The frequency of *EGFR* mutation among Asian (Japanese) NSCLC populations is approximately 30%[12] compared with approximately 20% in white populations.[13,14] Among clinical subgroups, the frequency of mutation in Asian males and smokers is low in comparison with Asian females and never-smokers[15–17]; however, even these *low-frequency* subgroups have a higher prevalence of *EGFR* mutations compared with broad white populations.[15–17] To date, epidemiological studies of *EGFR* mutation frequency have been

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performed in white populations, but no large epidemiological studies have provided prospective *EGFR* mutation frequency data in Asian populations, other than Japanese. In addition, no large epidemiological studies have compared the frequency of *EGFR* mutations in patients of different Asian ethnicities and it is still to be confirmed whether the traditional view of a higher frequency of *EGFR* mutation in Asian patients applies to all subgroups of Asian patients. Indeed, current thinking on *EGFR* mutation testing among many clinicians is still governed largely by the idea of the clinically selected Iressa Pan-Asia Study (IPASS) population,[3] with patients of female sex, adenocarcinoma histology, never-smoking status, and Asian ethnicity considered for testing. It is important to investigate this assumption by determining the prevalence of *EGFR* mutations among different ethnic and clinical subgroups of Asian patients, the results of which will help to optimize the identification of patients likely to benefit from EGFR-TKI therapy.

A molecular epidemiology study in Asian patients with advanced NSCLC of adeno histology to assess *EGFR* mutation status (PIONEER) was an epidemiological study planned to provide prospective *EGFR* mutation data in patients from Asia with newly diagnosed adenocarcinoma NSCLC. In this study, we report the *EGFR* mutation frequency of the overall PIONEER population. The influence of demographic/clinical factors on *EGFR* mutation frequency was also investigated.

MATERIALS AND METHODS

Study Design

PIONEER (NCT 01185314) was a prospective, multinational, epidemiological study of *EGFR* mutation status in patients from Asia with newly diagnosed advanced (stage IIIB or IV) NSCLC of adenocarcinoma histology. The primary objective of the study was to assess the overall *EGFR* mutation frequency. Secondary objectives were to investigate the correlation between *EGFR* mutation status and demographic and clinical factors; to investigate the attrition rates of *EGFR* mutation testing; and to investigate the correlation of *EGFR* mutation status between histology and cytology for patients who provided both samples.

Patients

Eligible patients were aged 20 years or older, with histological/cytological confirmed advanced (stage IIIB/IV), treatment-naïve, adenocarcinoma NSCLC. Data collected included date of the first diagnosis of NSCLC, histological type, American Joint Committee on Cancer disease stage, and number of organs with metastases. Availability of tumor samples (biopsy, surgical specimen, or cytology) was an inclusion criterion in the study.

The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation Guidelines for Good Clinical Practice, applicable regulatory requirements, and AstraZeneca's policy on bioethics, and was approved by the Ethics Committees of all study centers. All patients provided written informed consent before the initiation of data collection and sample testing.

Assessments

Detection of *EGFR* Mutations

Acquisition, preparation, and processing of tumor material were performed in line with routine clinical practice at participating hospital laboratories. Tumor *EGFR* mutation status was determined by analyzing DNA extracted from formalin-fixed, paraffin-embedded archival tumor tissue (using validated methods previously published by Fukuoka et al.[18]) or from cytology samples (including fine-needle aspirates and bronchial washings). Samples underwent central, histopathological review to ensure that they were adequate for use and where appropriate, hematoxylin and eosin-stained tissue was classified by suitably qualified pathologists according to the most recent World Health Organization classification. Samples considered suitable for downstream biomarker analysis were progressed to biomarker analysis (on the basis of quality, sample source, and tumor content [>100 tumor cells]). All samples were tested by using an amplification refractory mutation system (ARMS)-based *EGFR* mutation detection kit (Scorpion ARMS IVD2; Qiagen, Crawley, United Kingdom). The ARMS IVD2 kit is able to detect 29 mutations: three in exon 18 (G719A, G719S, and G719C; the kit was unable to distinguish between these subtypes, which are referred to as G719X hereafter), 19 deletions in exon 19, two mutations in exon 20 (S768I, T790M), three insertions in exon 20, and two mutations in exon 21 (L858R, L861Q). The *EGFR* mutation status of each patient's tumor was assessed from the individual status of all *EGFR* mutation types and recorded as one of the following: positive (mutation detected for at least one of the mutation types assayed), negative (no mutation detected in any of the mutation types assayed), or undetermined/unknown (a positive or negative result could not be determined as per laboratory assessment [assay fail, insufficient DNA, fail because of assay criteria, or no/insufficient sample]).

Statistical Analyses

The overall distribution of *EGFR* mutation status (primary end point) was summarized as the number of patients in the per-protocol (PP) population (all patients who did not significantly deviate from the study protocol) classified in each of the three mutation status categories (positive, negative, or undetermined/unknown). Percentages of patients in the positive and negative groups were calculated with corresponding 95% confidence intervals (CIs) using the Wilson score method, both overall and for demographic/clinical subgroups, including country/region, sex, ethnic group, smoking status, smoking pack-years, disease stage, and histology type. Patients with tumors of undetermined *EGFR* mutation status were not included in these calculations. The specific *EGFR* mutations detected by the ARMS kit were only summarized, with no formal statistical comparison performed.

Frequency of *EGFR* mutation was compared between demographic and clinical subgroups with the use of χ^2/Fisher's exact test, with no correction made for multiple testing. To best predict *EGFR* mutation frequency, factors with p less than 0.05 in the univariate analysis were further analyzed by multivariate logistic regression (at 1% significance level because of the large data set).

To investigate any correlation of *EGFR* mutation status between histology and cytology for patients who provided both types of samples, the probability (and 95% CIs) of agreement between sample types was calculated using the Wilson score method and Cohen's κ coefficient.

Sample size was calculated to obtain an accurate estimate of the proportion of patients with *EGFR* mutation-positive tumors. Assuming a percentage of 40%, more than 1047 samples were required to ensure a 95% CI of less than ±3% (Wilson score method). Taking into account patients with tumors of undetermined status, an overall sample size of 1270 was chosen.

RESULTS

Patients

From September 29, 2010 to July 31, 2011, 1510 patients were enrolled from 51 investigational sites in seven Asian countries/regions (China mainland, Hong Kong, India, Philippines, Taiwan, Thailand, and Vietnam) (Fig. 1). Of these patients, 1482 had no important protocol deviations, had samples available for mutation analysis, and were included in the PP population. Of note, three patients were less than 20 years old, deviating from the inclusion criteria that patients should be 20 years or older; however, this was considered a minor deviation and these patients were included in the PP population.

Overall demography/clinical characteristics for the PP population are summarized in Table 1; 43.4% (643 of 1482) were female, median age was 60 years (range, 17–94

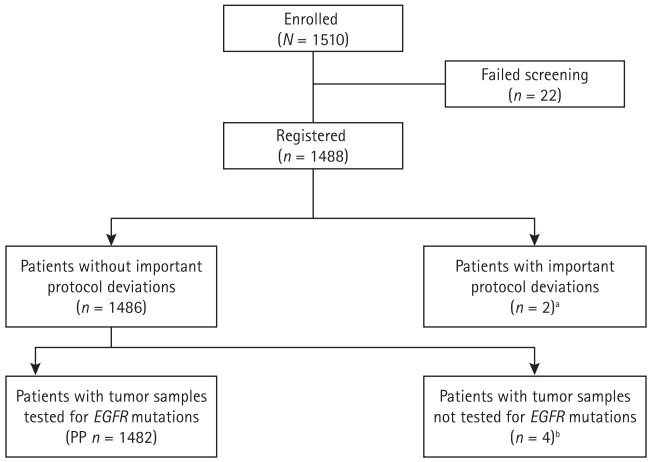

FIGURE 1. Study flow diagram. [a]Two patients had major protocol deviations: one patient did not have histologically or cytologically confirmed NSCLC, and one patient had received previous treatment for NSCLC. Three patients were <20 years old and therefore violated the study inclusion criteria. This deviation was considered to be minor and these patients were subsequently included in the PP population. [b]Four patients had tumor samples that were not tested for *EGFR* mutations: two biopsy samples were lost after pathological reading, one sample did not have a clot for preparing a tissue block, and the DNA concentration of one block was insufficient for testing. *EGFR*, epidermal growth factor receptor; NSCLC, non–small-cell lung cancer; PP, per-protocol population.

TABLE 1. Key Demographic and Clinical Characteristics (PP Population)

	PP Population (*n* = 1482)
Country/region, n (%)	
China	747 (50.4)
Hong Kong	170 (11.5)
India	81 (5.5)
Philippines	66 (4.5)
Taiwan	178 (12.0)
Thailand	119 (8.0)
Vietnam	121 (8.2)
Median age, yrs (range)	60 (17–94)
Female sex, n (%)	643 (43.4)
Ethnic group, n (%)	
Chinese	1093 (73.8)
Indian	82 (5.5)
Japanese	1 (0.1)
Kinh	121 (8.2)
Filipino	63 (4.3)
Thai	119 (8.0)
Mixed/others	3 (0.2)
Smoking history,[a] n (%)	
Never-smoker	779 (52.6)
Ex-smoker	310 (20.9)
Occasional smoker	66 (4.5)
Regular smoker	327 (22.1)
Smoking pack-years, n (%)	
0	779 (52.8)
>0–10	123 (8.3)
>10–20	138 (9.4)
>20–30	153 (10.4)
>30–40	113 (7.7)
>40–50	62 (4.2)
>50	106 (7.2)
Time from diagnosis of NSCLC, months	
<6	1398 (94.3)
6–12	35 (2.4)
>12	49 (3.3)
Stage classification	
IIIB	281 (19.0)
IV	1164 (78.5)
Other	37 (2.5)
Histology type	
Adenocarcinoma (NOS)	1406 (94.9)
Adenocarcinoma bronchoalveolar	76 (5.1)
Sample tissue type	
Primary tumor	1032 (69.6)
Metastases	412 (27.8)
Others	38 (2.6)

[a]Smoking history definitions: never-smoker (patients who had never smoked cigarettes in their lifetime); ex-smoker (patients who had previously smoked but no longer smoked); occasional smoker (patients who smoked, but not every day); and regular smoker (patients who smoked every day).

NSCLC, non–small-cell lung cancer; PP, per-protocol; NOS, not otherwise specified.

years), and 52.6% (779 of 1482) were never-smokers. Nearly three-quarters of patients (73.8% [1093 of 1482]) were of Chinese ethnicity.

EGFR Mutation Analyses

Sample Flow Attrition Rates

Of 1486 patients with no important protocol deviations, tumor samples from four were not tested: two biopsy samples were lost after pathological reading, one sample did not have a clot for preparing a tissue block, and the DNA concentration of one block was insufficient for testing. Of the remaining 1482 samples tested, 169 (11.4%) were cytology samples. In the overall PP population, *EGFR* mutation analysis was successful in samples from 1450 of 1482 patients (97.8%; 95% CI, 97.0%–98.5%); 32 of 1482 (2.2%; 95% CI, 1.5%–3.0%) were undetermined (unknown), of which eight were cytology samples. Among the 1450 evaluable samples, 746 (51.4%; 95% CI, 48.9–54.0) were *EGFR* mutation positive and 704 (48.6%; 95% CI, 46.0–51.1) were *EGFR* mutation negative.

EGFR Mutation Test Time

Of patients with a known time interval between physicians requesting and obtaining a test result (n = 1475), the mean (SD) time interval for reporting the test was 17.6 (13.3) days (median, 15.0; range, 1–148 days); for the majority of patients (1168; 79.2%), the time interval was less than 21 days.

Associations between *EGFR* Mutation Frequency and Demographic/Clinical Factors

Tumor *EGFR* mutation frequency for patients in demographic/clinical subgroups is presented in Table 2. Factors with a statistically significant association with *EGFR* mutation status (χ^2 or Fisher's exact test) were country, sex, ethnicity, smoking status, smoking pack-years (all $p < 0.001$), disease stage ($p = 0.009$), and histology type ($p = 0.016$), and are briefly summarized below. Caution is advised when interpreting results of these univariate analyses because individual demographic/clinical factors may be influenced by others and therefore may not represent a true effect of that variable:

Country/Region—EGFR mutation frequency was highest in patients from Vietnam (64.2% [77 of 120]) and lowest in patients from India (22.2% [16 of 72]) (other countries 47.2% [76 of 161] to 62.1% [108 of 174]).

Sex—EGFR mutation frequency was significantly higher in females (61.1% [384 of 628]) than males (44.0% [362 of 822]).

Ethnic Group—EGFR mutation frequency was highest for those of Kinh (Vietnamese) ethnicity (64.2% [77 of 120]) and lowest for those of Indian ethnicity (21.9% [16 of 73]).

Smoking Status—EGFR mutation frequency was highest among never-smokers (60.7% [462 of 761]) compared with ex-smokers (43.2% [130 of 301]), occasional smokers (51.6% [33 of 64]), or regular smokers (37.3% [121 of 324]).

Smoking Pack-Years—EGFR mutation frequency was highest among never-smokers (60.7%) and decreased as pack-year number increased (>0–10 pack-years: 57.9%; >50 pack-years: 31.4%). A similar trend was observed by sex: males (0–10 pack-years: 55.9% [161 of 288], >10–30 pack-years: 46.6% [123 of 264], and >30 pack-years: 28.2% [74 of 262]); females (0–10 pack-years: 62.5% [371 of 594], >10–30 pack-years: 37.5% [9 of 24], and >30 pack-years: 40.0% [4 of 10]).

Disease Stage—EGFR mutation frequency was significantly higher among patients with stage IV disease (53.5% [612 of 1144]) compared with IIIB (43.2% [117 of 271]) or other stage (48.6% [17 of 35]).

Histology Type—EGFR mutation frequency was significantly higher among patients with adenocarcinoma not otherwise specified histology (52.2% [718 of 1376]) compared with adenocarcinoma bronchoalveolar histology (37.8% [28 of 74]).

Factors not significantly correlating with *EGFR* mutation frequency were age ($p = 0.565$), time from diagnosis ($p = 0.612$), existence of malignant pleural effusion ($p = 0.265$), primary tumor stage (tumor staging T1-TX; $p = 0.454$), regional lymph node involvement ($p = 0.075$), and tumor grade ($p = 0.369$).

Multivariate logistic regression (with 1% significance level) identified ethnicity ($p < 0.001$) and smoking pack-years ($p < 0.001$) to be independent predictive factors for *EGFR* mutation status (Table 3). When stratified by smoking status or pack-years, sex was no longer found to be significant (Fig. 2).

Individual mutation types, including multiple mutations, are summarized in Table 4. Of the 1450 evaluable samples, 671 (46.3%) harbored activating (sensitizing) mutations alone, 42 (2.9%) had resistance mutations alone, and 33 (2.3%) had a combination of activating and resistance mutations. The most common mutations detected were deletion in exon 19 (deletion alone 22.1% [321 of 1450]; alone and in combination with others 24.3% [352 of 1450]) and L858R point mutation in exon 21 (L858R alone 20.9% [303 of 1450]; alone and in combination with others 22.9% [332 of 1450]). Tumors from 21 patients (1.4%) harbored T790M resistance mutations, of which five (0.3%) had T790M alone.

Correlation of *EGFR* Mutation Status between Histology and Cytology for Patients Who Provided Both Samples

In total, 23 patients (1.5% of PP population) provided both histology and cytology samples for mutation analysis. The *EGFR* mutation status (and specific mutations found) of matched histology and cytology samples were concordant for 21 patients (positive n = 13, negative n = 8; 91.3% concordance [Wilson score 95% CI, 73.2%–97.6%; κ coefficient 0.817]). Matched samples from two patients were discordant (one patient's samples were positive for histology and negative for cytology, with the other patient having a reverse of this result).

DISCUSSION

In PIONEER, the first epidemiological study of *EGFR* mutation frequency across Asian countries/regions, approximately half of the unselected patients with adenocarcinoma NSCLC had tumors that harbored *EGFR* mutations. Frequency of tumor mutation was significantly lower in Indian patients compared with other countries, and at 22.2%, was

TABLE 2. *EGFR* Mutation Frequency for Demographic and Clinical Characteristic Subgroups (PP Population)

		EGFR Mutation-Positive		*EGFR* Mutation-Negative		
	N	n (%)	95% CI	n (%)	95% CI	*p*
Country/region						<0.001
China	741	372 (50.2)	46.6–53.8	369 (49.8)	46.2–53.4	
Hong Kong	161	76 (47.2)	39.6–54.9	85 (52.8)	45.1–60.4	
India	72	16 (22.2)	14.2–33.1	56 (77.8)	66.9–85.8	
Philippines	65	34 (52.3)	40.4–64.0	31 (47.7)	36.0–59.6	
Taiwan	174	108 (62.1)	54.7–68.9	66 (37.9)	31.1–45.3	
Thailand	117	63 (53.8)	44.8–62.6	54 (46.2)	37.4–55.2	
Vietnam	120	77 (64.2)	55.3–72.2	43 (35.8)	27.8–44.7	
Sex						<0.001
Female	628	384 (61.1)	57.3–64.9	244 (38.9)	35.1–42.7	
Male	822	362 (44.0)	40.7–47.5	460 (56.0)	52.5–59.3	
Ethnic group						<0.001
Chinese	1074	556 (51.8)	48.8–54.7	518 (48.2)	45.3–51.2	
Indian	73	16 (21.9)	14.0–32.7	57 (78.1)	67.3–86.0	
Japanese	1	0 (0.0)	0.0–79.3	1 (100.0)	20.7–100.0	
Kinh	120	77 (64.2)	55.3–72.2	43 (35.8)	27.8–44.7	
Filipino	62	31 (50.0)	37.9–62.1	31 (50.0)	37.9–62.1	
Thai	117	63 (53.8)	44.8–62.6	54 (46.2)	37.4–55.2	
Mixed/others	3	3 (100.0)	43.9–100.0	0 (0.0)	0.0–56.1	
Smoking history[a]						<0.001
Never-smoker	761	462 (60.7)	57.2–64.1	299 (39.3)	35.9–42.8	
Ex-smoker	301	130 (43.2)	37.7–48.8	171 (56.8)	51.2–62.3	
Occasional smoker	64	33 (51.6)	39.6–63.4	31 (48.4)	36.6–60.4	
Regular smoker	324	121 (37.3)	32.3–42.7	203 (62.7)	57.3–67.7	
Smoking pack-years						<0.001
0	761	462 (60.7)	57.2–64.1	299 (39.3)	35.9–42.8	
>0–10	121	70 (57.9)	48.9–66.3	51 (42.1)	33.7–51.1	
>10–20	135	62 (45.9)	37.7–54.3	73 (54.1)	45.7–62.3	
>20–30	153	70 (45.8)	38.1–53.7	83 (54.2)	46.3–61.9	
>30–40	110	29 (26.4)	19.0–35.3	81 (73.6)	64.7–81.0	
>40–50	60	17 (28.3)	18.5–40.8	43 (71.7)	59.2–81.5	
>50	102	32 (31.4)	23.2–40.9	70 (68.6)	59.1–76.8	
Stage classification						0.016
IIIB	271	117 (43.2)	37.4–49.1	154 (56.8)	50.9–62.6	
IV	1144	612 (53.5)	50.6–56.4	532 (46.5)	43.6–49.4	
Other	35	17 (48.6)	33.0–64.4	18 (51.4)	35.6–67.0	
Histology type						0.009
Adenocarcinoma (NOS)	1376	718 (52.2)	49.5–54.8	658 (47.8)	45.2–50.5	
Adenocarcinoma bronchoalveolar	74	28 (37.8)	27.6–49.2	46 (62.2)	50.8–72.4	

p Values are from χ^2 or Fischer's exact test, refer to overall comparisons across all subgroups, and are not corrected for multiple testing. All patients had stage IIIB/IV disease; however, some provided tumor samples at an earlier stage of their disease, and were therefore classified as *other*. Caution is advised when interpreting the results of these univariate analyses as the results of an individual demographic and clinical factor may be influenced by the others and may therefore not represent a true effect of that variable.

[a]Smoking history definitions: never-smoker (patients who had never smoked cigarettes in their lifetime); ex-smoker (patients who had previously smoked but no longer smoked); occasional smoker (patients who smoked, but not every day); and regular smoker (patients who smoked every day).

CI, confidence interval; PP, per-protocol; NOS, not otherwise specified; *EGFR*, epidermal growth factor receptor.

more comparable with the frequency for a broad white population (approximately 20%)[13] than to the East Asian countries/regions in the study (approximately 47–64%), or the approximately 30% and approximately 36% reported in Japanese and Korean patients, respectively.[12,19] Interestingly, a higher tumor mutation frequency of 44% was reported in a similarly sized (n = 75) adenocarcinoma population of Indian patients in a study by Sahoo et al.,[20] using Scorpion ARMS polymerase

TABLE 3. Multivariate Logistic Regression Analysis for *EGFR* Mutation Status (PP Population)

Variable	Contrast	Regression Coefficient Estimate	SE	Odds Ratio Estimate (95% CI)	*p*
Intercept		0.431	0.075		
Ethnic group[a]	Indian vs. Chinese	−1.437	0.303	0.24 (0.13–0.43)	<0.001
	Thai vs. Chinese	0.135	0.202	1.14 (0.77–1.70)	
	Kinh vs. Chinese	0.717	0.212	2.05 (1.35–3.10)	
	Filipino vs. Chinese	0.070	0.274	1.07 (0.63–1.83)	
Pack-years	>10–30 vs. 0–10	−0.683	0.140	0.51 (0.38–0.66)	<0.001
	>30 vs. 0–10	−1.343	0.154	0.26 (0.19–0.35)	

Total number of patients in the regression analysis was 1438.
[a]Japanese (n = 1) and mixed/others (n = 3) were excluded from ethnic groups because of the very small patient numbers.
CI, confidence interval; *EGFR*, epidermal growth factor receptor; PP, per-protocol; SE, standard error.

chain reaction. However, only 90 of 220 tumor samples could be histologically subclassified, which may have resulted in potential bias. In contrast, the large data set and consistent use of mutation test methodology in PIONEER permitted comprehensive and reliable subgroup analysis. In PIONEER, a patient's ethnicity and smoking status/pack-years were independent predictive factors for tumor *EGFR* mutation status. Of note, there was no association between sex and tumor *EGFR* mutation status when results were stratified by smoking status.

Generally, female sex, adenocarcinoma histology, never-smoking status, and Asian ethnicity are considered the most important factors associated with *EGFR* mutation and response to EGFR-TKIs.[21] In PIONEER, univariate analyses showed that country, sex (subsequently negated when stratified by smoking status), ethnic group, smoking status, pack-years, disease stage, and adenocarcinoma histology type all had a statistically significant association with *EGFR* mutation status (note that our study was ongoing before the publication of the new adenocarcinoma classification system).[22] The highest frequency of *EGFR* mutation was among female never-smokers, in agreement with previous studies.[17,23–25] However, tumor *EGFR* mutations can be found in patients with clinical characteristics other than female sex, adenocarcinoma histology, never-smoking status, or Asian ethnicity. Indeed, the frequency of *EGFR* mutation in tumors from Asian males in PIONEER was 44% (ARMS), in sharp contrast to the 8.2% reported in a comparable European male population (DNA sequencing).[14] Similarly, frequency of *EGFR* mutation in Asian heavy-smokers was approximately 30% in PIONEER, much higher than the 5.8% observed in European heavy-smokers.[14] Thus, physicians should not discount these other populations from *EGFR* mutation testing on the basis of clinical characteristics. In a large, retrospective U.S. study of 2142 patients with stage I to IV NSCLC, *EGFR* mutations in tumors from former/current smokers represented 40% of all mutations detected (direct sequencing; 201 of 503; 95% CI, 36%–44%), and those from men represented 31% (157 of 503; 95% CI, 27%–35%)[13]; if only female never-smokers had been tested, 57% of mutation-positive tumors would have remained undetected. In PIONEER, more than 50% of patients with *EGFR* mutation-positive tumors came from subpopulations other than female never-smokers. These data highlight that *EGFR* mutation testing is warranted even in males and smokers, particularly in Asian populations, and emphasize that *EGFR* mutation status can only be confirmed by performing *EGFR* mutation testing. EGFR-TKI efficacy in non-Asian patients with *EGFR* mutation-positive NSCLC was demonstrated in the phase III European Tarceva versus Chemotherapy (EURTAC) study.[7] This study of 173 European white patients with *EGFR* mutation-positive tumors (DNA sequencing) reported significantly longer progression-free survival (PFS) with first-line erlotinib (n = 86; 9.7 months) compared with chemotherapy (n = 87; 5.2 months) (hazard ratio [HR], 0.37; 95% CI, 0.25–0.54; $p < 0.0001$), with benefit in both female (n = 126; PFS HR, 0.35; 95% CI, 0.22–0.55) and male subgroups (n = 47; PFS HR, 0.38; 95% CI, 0.17–0.84). These data further strengthen the rationale for routine assessment of tumor *EGFR* mutations in all patients (where possible) before initiation of NSCLC therapy. Indeed, recent molecular testing guidelines copublished by three societies recommended the use of *EGFR* mutation testing to guide patient selection for therapy with an *EGFR* inhibitor, in all patients with advanced-stage adenocarcinoma, regardless of sex, race, smoking history, or other clinical risk factors, and to prioritize *EGFR* testing over other molecular predictive tests.[26] In PIONEER, the success rate (known positive or negative result) of *EGFR* mutation analysis was very high at 97.8%, and only 2.2% of patients had tumor samples for which mutation status could not be determined. This high success rate is very encouraging as it indicates that even though the acquisition, preparation, and processing of tumor material varied between test centers/participating laboratories because of differences in routine clinical practice, the quality of most samples was such that mutation testing was successful and a definite result obtained. The high success rate also indicates that the in vitro diagnostic mutation ARMS kit used throughout the study was suitable for a range of samples where the collection method was not standardized, highlighting its suitability for adoption/routine use at local test centers. Indeed, previous studies have found ARMS to be successful at detecting *EGFR* mutations in a variety of sample types, including those of cytological origin, with a reported increase in detection of *EGFR* mutations with ARMS when compared with direct sequencing.[27–29] Although the success rate of mutation testing in PIONEER was very high, the median time interval between requesting and reporting a result was 15.3 days; not ideal from a clinical perspective. However, *EGFR*

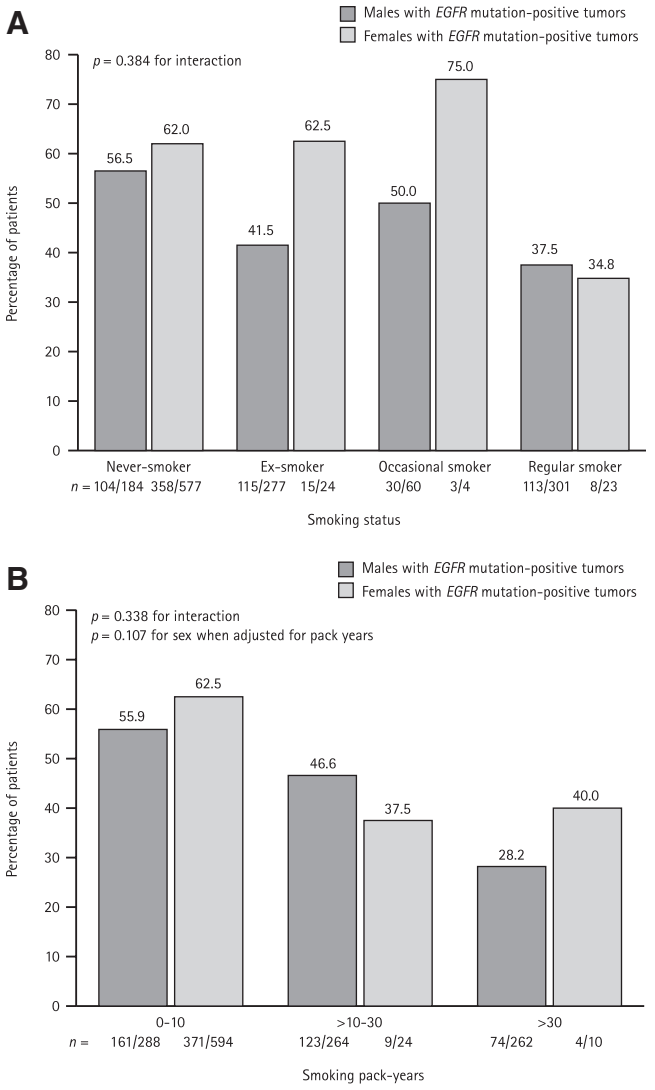

FIGURE 2. Combined effect of sex and (*A*) smoking status and (*B*) pack-years on frequency of *EGFR* mutation (PP population). *A*, *p* values for logistic regression model. Smoking history definitions: never-smoker (patients who had never smoked cigarettes in their lifetime); ex-smoker (patients who had previously smoked but no longer smoked); occasional smoker (patients who smoked, but not every day); regular smoker (patients who smoked every day). *B*, *p* values for logistic regression model. *EGFR*, epidermal growth factor receptor; PP, per-protocol.

mutation testing was not performed routinely in some participating countries, and performing tests solely for the experimental purposes of our study will have exacerbated this time interval. Commercial test centers routinely performing tests are currently reporting turnaround times of 8 to 10 days[30]; however, even this may not be satisfactory, given that patients need access to the most appropriate treatment as quickly as possible. Research into more rapid mutation identification, such as allele-specific testing, may help to reduce this waiting period and provide more rapid access to appropriate personalized therapies. Mutation test results were concordant for the majority of patients in PIONEER who provided matched histology and cytology samples, indicating that cytology samples could be considered for mutation testing if tumor biopsy samples are not available. However, the small number of samples (n = 23) limits the interpretation of these findings, although mutation test methodology studies are providing more conclusive evidence which corroborates these data.[27]

TABLE 4. Summary of Individual *EGFR* Mutation Types (Including Multiple Mutations)

	n	%
Patients with an evaluable *EGFR* mutation test	1450	100.0
Sensitizing mutations alone	671	46.3
G719X	15	1.0
G719X, deletion	4	0.3
G719X, L861Q	2	0.1
Deletion	321	22.1
Deletion, L858R	11	0.8
L858R	303	20.9
L858R, L861Q	2	0.1
L861Q	13	0.9
Resistance mutations	42	2.9
T790M	5	0.3
S768I	14	1.0
S768I, exon 20 other (insertion)	4	0.3
Exon 20 other (insertion)	19	1.3
Combination of sensitizing and resistance mutations	33	2.3
G719X, deletion, T790M, S768I, L858R, L861Q, exon 20 other (insertion)	2	0.1
G719X, S768I	3	0.2
G719X, exon 20 other (insertion)	1	0.1
Deletion, T790M	7	0.5
Deletion, T790M, L858R	1	0.1
Deletion, S768I, L858R	1	0.1
Deletion, S768I, exon 20 other (insertion)	1	0.1
Deletion, exon 20 other (insertion)	4	0.3
T790M, L858R	6	0.4
S768I, L858R	3	0.2
S768I, L861Q, exon 20 other (insertion)	1	0.1
L858R, exon 20 other (insertion)	3	0.2
Patients with a negative *EGFR* mutation test	704	48.6

EGFR, epidermal growth factor receptor.

There is currently a need to generate data for a pan-Asian guideline for the management of NSCLC.[31] Such a guideline has been difficult to establish owing to differences in ethnicity and medical care across Asian countries/regions, in addition to longer drug approval times compared with the European Union and United States.[31–34] Lack of standardization in testing methodology has also reduced the feasibility of large-scale testing.[32] However, the consensus from a recent meeting to discuss *EGFR* mutation testing in East Asia recommended testing all recently diagnosed patients with nonsquamous NSCLC (as is current practice in some centers and community practices), and patients with squamous NSCLC with clinical features associated with higher prevalence of

EGFR mutations.[32] Tissue acquisition and pre-test sample evaluation were also considered important steps to increase sensitivity/specificity, and thus help standardize mutation test methodology. The substantial body of data generated by PIONEER therefore has valuable clinical implications for the treatment of advanced NSCLC across Asia, and indicates that large-scale testing across countries is feasible, can be standardized, and can result in a high analysis success rate. Further multinational studies are required to help establish guidelines and realize these recommendations.

In summary, the observed frequency of tumor *EGFR* mutation in demographic and clinical subgroups of Asian patients in PIONEER suggests that *EGFR* mutation testing should be considered for all patients with stage IIIB/IV adenocarcinoma NSCLC in an Asian population. Such an approach should help ensure the optimal identification and treatment of patients whose tumors harbor *EGFR* mutations.

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