Table 2. Response to Treatment in the Intention-to-Treat Population, According to Treatment Group.*

Response	Gefitinib (N=114)	Carboplatin–Paclitaxel (N=114)
	number of patients (percent)	
Complete response	5 (4.4)	0
Partial response	79 (69.3)	35 (30.7)
Complete or partial response†	84 (73.7)	35 (30.7)
Stable disease	18 (15.8)	56 (49.1)
Progressive disease	11 (9.6)	16 (14.0)
Response that could not be evaluated	1 (0.9)	7 (6.1)

* All responses differed significantly between the two groups ($P<0.001$ by Fisher's exact test).

† The percentage of patients in whom there was either a complete or a partial response was considered to be the rate of objective response.

progression-free survival from our phase 2 studies in patients with non–small-cell lung cancer and EGFR mutations. The data on overall survival first became available in 2008, when the combined analysis of Japanese phase 2 studies (Iressa — Combined Analysis of Mutation Positives [I-CAMP]) and the subgroup analyses of IPASS were reported.[7,22] We thus planned to have progression-free survival as the primary end point in the current study, because it allowed us to calculate the statistical power of the study.

Several studies have suggested that the EGFR copy number may be a better predictive biomarker for the efficacy of EGFR tyrosine kinase inhibitors than the presence of an EGFR mutation.[23] However, its predictive capacity has been reported only in placebo-controlled trials (Iressa Survival Evaluation in Lung Cancer [ISEL][24] and the BR.21 study[23]). Moreover, the subgroup analysis in IPASS showed that longer progression-free survival was significantly associated with sensitive EGFR mutations but not with a high EGFR copy number. We therefore believe that evaluation of the copy number is not necessary when an EGFR mutation test is available. In the current study, EGFR mutations were detected with the use of the PNA-LNA PCR clamp method, the usefulness of which has been validated.[15,16] With this method, EGFR mutations can be detected from small cytologic specimens, such as those from bronchial washings, pleural effusions, and sputum collection, which are frequently used for the diagnosis of advanced non–small-cell lung cancer. The results of the analyses are obtained within several days, so the treatment is usually not delayed. The PNA-LNA PCR clamp approach is readily available and is covered by health insurance in Japan.

The best timing of treatment with an EGFR tyrosine kinase inhibitor for patients with EGFR mutations remains undetermined. A recent study showed that overall survival did not differ significantly between first-line and second-line treatments with erlotinib.[25] Overall survival is considered to be influenced by the second-line or later treatment. In the current study, 95% of the patients in whom first-line carboplatin–paclitaxel failed crossed over to gefitinib therapy. Such a high crossover rate has not been reported in previous studies of EGFR tyrosine kinase inhibitors. For example, in IPASS, only 39% of patients in the first-line chemotherapy group later received an EGFR-tyrosine kinase inhibitor. Considering that in our study the median overall survival in the gefitinib group was 7 months longer than that in the chemotherapy group (30.5 months vs. 23.6 months), in which virtually all patients were given gefitinib as the second-line treatment, and that the rate of response to gefitinib was slightly worse in the second-line setting than in the first-line setting (58.5% vs. 73.7%), first-line gefitinib may be more effective than gefitinib as second-line or later therapy. This idea needs to be tested in studies with large samples or in a meta-analysis.

We believe that the prolonged progression-free survival provided by the use of first-line gefitinib is valuable for patients with advanced non–small-cell lung cancer, who have a poor prognosis. If gefitinib is administered as second-line or third-line treatment, patients may miss the opportunity to receive treatment with gefitinib because of rapidly progressive disease during or after first-line treatment. We believe that the current study, in combination with our previous study of patients with mutated-EGFR non–small-cell lung cancer and poor performance status,[26] establishes the clinical benefit of an EGFR tyrosine kinase inhibitor as first-line treatment in patients with non–small-cell lung cancer and sensitive EGFR mutations.

Predictable toxicity profiles were observed with gefitinib and with carboplatin–paclitaxel in the current study. Diarrhea and rash were seen more often in the gefitinib group, whereas hematologic and neurologic toxic effects were more common in the chemotherapy group. Gefitinib appears to

Table 3. Common Toxic Effects in the Safety Population, According to Treatment Group.*

Toxic Effect	Gefitinib (N=114)					Carboplatin–Paclitaxel (N=113)					P Value for Grade ≥3
	Grade 1	Grade 2	Grade 3	Grade 4	Grade ≥3	Grade 1	Grade 2	Grade 3	Grade 4	Grade ≥3	
	no. of patients				*no. (%)*	*no. of patients*				*no. (%)*	
Diarrhea	32	6	1	0	1 (0.9)	7	0	0	0	0	<0.001
Appetite loss	7	4	6	0	6 (5.3)	39	18	7	0	7 (6.2)	<0.001
Fatigue	8	1	3	0	3 (2.6)	19	11	1	0	1 (0.9)	0.002
Rash	38	37	6	0	6 (5.3)	8	14	3	0	3 (2.7)	<0.001
Neuropathy (sensory)	0	1	0	0	0	28	27	7	0	7 (6.2)	<0.001
Arthralgia	1	2	1	0	1 (0.9)	25	21	8	0	8 (7.1)	<0.001
Pneumonitis	3	0	2	1†	3 (2.6)	0	0	0	0	0	0.02
Aminotransferase elevation	20	13	29	1	30 (26.3)	31	5	0	1	1 (0.9)	<0.001
Neutropenia	5	1	0	1	1 (0.9)	4	9	37	37	74 (65.5)	<0.001
Anemia	19	2	0	0	0	35	32	6	0	6 (5.3)	<0.001
Thrombocytopenia	8	0	0	0	0	25	3	3	1	4 (3.5)	<0.001
Any	17	44	43	4†	47 (41.2)	4	25	41	40	81 (71.7)	<0.001

* Toxic-effect grades are based on the National Cancer Institute Common Terminology Criteria (version 3.0).
† One patient counted here had a grade 5 toxic effect.

be less toxic than carboplatin–paclitaxel. The only exception was interstitial lung disease; there were three cases of severe interstitial lung disease (≥grade 3) in the gefitinib group and none in the chemotherapy group; one of the cases was fatal. The patient who died was a woman who had no history of smoking and thus had a relatively low risk of interstitial lung disease. Gefitinib sometimes causes diffuse alveolar or interstitial damage, especially during the first 3 months of treatment.[27] The estimated incidence of interstitial lung disease is low in many countries (e.g., 0.3% in United States)[28] but is relatively high (4 to 6%) in Japan.[29,30] Every patient treated with an EGFR tyrosine kinase inhibitor should be carefully monitored for this toxic effect.

In conclusion, the efficacy of first-line gefitinib was superior to that of standard chemotherapy, with acceptable toxicity, in patients with advanced non–small-cell lung cancer harboring sensitive EGFR mutations. Selection of patients on the basis of EGFR-mutation status is strongly recommended.

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APPENDIX

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Frequency of and variables associated with the EGFR mutation and its subtypes

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Mutation in the epidermal growth factor receptor (EGFR) is frequently seen in non-small cell lung cancers (NSCLCs), especially in Asian females with adenocarcinoma. The frequency of mutation and the factors associated requires to be elucidated by analyzing a large number of consecutive clinical samples. We summarized the result of the EGFR mutation analysis for 1,176 patients performed at the time of diagnosis or relapse. The PNA-LNA PCR clamp, a highly sensitive detection method for the EGFR mutation, was employed. For fresh cases a portion of samples isolated to establish the diagnosis of lung cancer was used. For cases with a relapsed disease archival tissue were tested. The variables associated with the EGFR mutation after removing the confound factors were investigated by the logistic analysis using the samples collected in our university (n = 308) where detailed information on patients were available. The frequency of the EGFR mutation and its subtypes were investigated using all samples (n = 1,176). The EGFR mutation was significantly associated with adenocarcinoma (p = 0.006) and light-smoking ($p < 0.0001$), but not gender. The deletions in exon 19 were more frequently associated with male gender while exon 21 deletions were with female gender (p = 0.0011). The overall frequency of the EGFR mutation was 31%. Our result suggests that the female predominance in the EGFR mutation rate is a reflection of a higher frequency of adenocarcinoma in females. The gender difference in the mutation subtypes may provide a clue for the mechanism of the occurrence of the EGFR mutation.

EGFR mutation is one of the most common genetic alterations in non-small cell lung cancers (NSCLCs).[1,2] It is more frequently seen in East Asians, females and non-smokers.[3] EGFR mutation is more frequently found in adenocarcinomas than in other types of cancers.[4] Since adenocarcinomas occur more frequently in females than in males, the seeming gender difference in the EGFR mutation rate may just reflect the gender difference in the rate of adenocarcinoma. The epidemiological factors associated with the EGFR mutations provide the clues to the causes that originated EGFR mutation and hence cancers. Therefore, it is important to eliminate the confounding factors and identify the factor(s) that are primarily associated with EGFR mutations. A study consisting of a large number of patients is required.

The PNA-LNA PCR clamp is a highly sensitive detection method for EGFR mutation.[5,6,7] It detects mutations in the presence of 100 to 1000-fold background of the normal cells over the cancer cells, and thus enables one to detect the EGFR mutation from cytological specimens used to diagnose cancers in clinical practice.[6] Several prospective phase II studies have shown the administration of gefitinib to the EGFR mutation-positive patients to provide a survival benefit.[8,9,10–13] Even patients with a poor performance status have been shown to benefit from gefitinib if they are mutation-positive.[14] With this information, a test for the EGFR mutation for NSCLC patients has become routine in Japan. Currently more than 10,000 NSCLC patients per year are tested for EGFR mutations by the PNA-LNA PCR clamp.

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Since the development of the PNA-LNA PCR clamp in 2005, we have tested for EGFR mutations in all NSCLC patients who visited our hospital and provided informed consent. In addition, we tested for the mutation in more than 2,000 samples sent from more than 20 collaborative institutes; most of the samples were a part of a specimen isolated in order to establish the diagnosis of lung cancer. We herein summarize the results of tests and the statistical analyses for the first consecutive 1,176 patients.

Material and Methods

Clinical samples

This study was approved by the institutional review board of each institute and performed in accordance with the Declaration of Helsinki (1995, revised in Edinburgh 2000). An aliquot of the specimens which were isolated to establish the diagnosis of NSCLC, or in the case of relapsed diseases, the archival specimens from the previous treatment were subjected to the EGFR mutation test by the PNA-LNA PCR clamp after obtaining informed consent from all patients. The period of sample collection for Saitama Medical University Hospital and Saitama Medical University International Medical Center ranged from October 2004 to February 2008, while that for the collaborative institutes differed depending on the time they joined to the study.

EGFR mutation analyses

Each sample (sputum, pleural effusion, bronchial washing, needle biopsy and paraffin embedded tissue) was divided into two parts immediately after collection at each institute. When the diagnosis of cancer was pathologically established from the first part, the other part was sent to our institute to test for the EGFR mutation by the PNA-LNA PCR clamp. The investigated gefitinib sensitive mutations included G719C, G719S, G719A, L858R, L861Q and exon 19 deletions, as well as a gefitinib resistant mutation T790M. The results were reported within 1 to 3 days after the receipt of the samples so that each institute was able to utilize the results in order to select the appropriate treatment for each patient.

Statistical analysis

Any significant differences among the categorized groups were compared using either the two-sided χ^2 test or Fisher's exact test. The adjusted effects of age, sex, histology, staging and smoking history on EGFR mutation were evaluated by using a logistic regression model, and the results were described as an odd ratio with a 95% confidence interval. All analyses were performed using SPSS Statistics (SPSS version 17.0 for Windows, SPSS Inc, Chicago, IL).

Results

Samples

We were able to collect detailed information for the samples collected in our university. On the other hand, because of the privacy policy of the individual institutes, information was incomplete for the samples from other institutes. Therefore, we performed two different analyses. Firstly, we analyzed the samples collected in our university, and performed detailed analyses on the factors associated with EGFR mutations and their subtypes. Next, we put all samples together and calculated the frequencies of EGFR mutations and their subtypes.

Table 1. *EGFR* mutation identified

	No. of samples	Male	Female
Exon 18 point mutation	1		
G719S	1	0	1
Exon 19 deletions	68		
E746-A750del Type1	25	13	12
E746-A750del Type2	14	4	10
L747-A750del T751S	1	0	1
L747-S752del P753S	10	6	4
L747-E749del A750P	3	2	1
L747-T751del	10	7	3
L747-T751del insA	1	1	0
L747-S752del P753Q	1	1	0
E746-S752del insV	1	1	0
E746-A750del insKP	1	1	0
E746-T751del insLS	1	1	0
Exon 19 deletions + Exon 21 point mutation	1		
L747-T751del + L858R	1	0	1
Exon 19 deletions + Exon 20 point mutation	2		
E746-A750del Type1 + T790M	1	0	1
E746-A750del Type2 + T790M	1	1	0
Exon 21 point mutations	38		
L858R	37	14	23
L861Q	1	0	1
Exon 21 point mutation + Exon 20 point mutation	2		
L858R + T790M	2	0	2
Total	112	52	60

EGFR mutations found in 112 patients out of 308 patients who visited to Saitama Medical University or Saitama Medical University International Medical Center were summarized. Sources of the samples include bronchial washing/brushing, 167; pleural effusion, 42; sputum, 22; pleural effusion, 2; paraffin embedded tissue, 69; and surgically resected tissue, 9. A part of the data has been reported elsewhere.[6,7] E746-A750del Type1: E746-A750del (2235-2249del). E746-A750del Type 2: E746-A750del (2236-2250del).

Detailed analysis

We had 311 patients who visited to our university and were tested for the EGFR mutation, and 308 of them provided informative results. The source of the samples included all types of cytological and tissue specimens that are used for the diagnosis of the cancer. A total of 112 (36.4%) had EGFR mutations (Table 1). We identified the gefitinib resistant

Table 2. Association of each variable with the *EGFR* mutation

	Negative	Positive	χ^2 value	*P*-value
Sex				
Male	155	52	33.015	<0.0001
Female	41	60		
Histology				
Adenocarcinoma or Adeno-squamous cell carcinoma	138	104	20.201	<0.0001
Squamous cell carcinoma and others	58	8		
Smoking history*				
Less than 20 pack-year or never	37	59	41.398	<0.0001
More than 20 pack-year	150	43		
Age				
Younger than 65 years	70	54	4.125	0.0423
Older than 65 years	126	58		
Clinical stage				
Stage I-II	27	12	0.612	0.7364
Stage III-IV	146	86		
Post-operative	23	14		

*Nineteen patients who had unknown smoking history were excluded.

Table 3. Logistic regression analysis for the association with EGFR mutation ($N = 289$)

		Odds ratio (95% confidence interval)	*P*-value
Age	less than 64 yrs/ over 65 yrs	1.57 (0.90–2.70)	0.12
Gender	Male/female	0.69 (0.35–1.38)	0.30
Histology	Adenocarcinoma and adenosquamous carcinoma/squamous cell carcinoma and others	3.18 (1.39–7.23)	0.006
Smoking history	Packs a year < 20/20 ≤ packs a year	3.84 (1.92–7.65)	<0.0001
Stage	Stages I and II/Stages III and IV	0.84 (0.37–1.90)	0.67

Nineteen patients who had unknown smoking history were excluded.

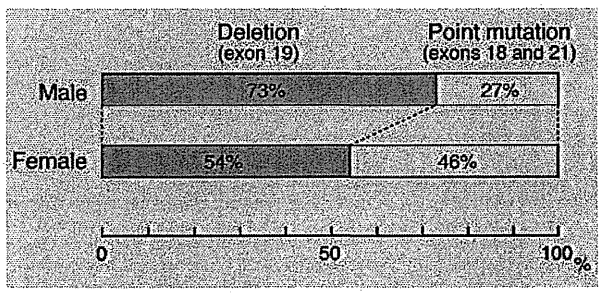

Figure 1. The type of EGFR mutations according to gender. The exon 19 deletions are more frequently found in males than in females (Fisher's exact test, $p < 0.047$).

Table 4. Logistic regression analysis for the association with exon 19 deletion (N=102)

		Odds ratio (95% confidence interval)	*P*-value
Age	less than 64 yrs/ over 65 yrs	2.16 (0.87–5.36)	0.10
Gender	Male/female	5.10 (1.92–13.54)	0.0011
Histology	Adenocarcinoma and adenosquamous cell carcinoma/squamous cell carcinoma and others	2.26 (0.43–11.8)	0.34
Smoking history	pcyear < 20/20 ≤ pcyear	0.64 (0.25–1.64)	0.35
Stage	Stage III to IV/ Stage I to II	2.94 (0.67–12.8)	0.15

Ten patients who had unknown smoking history were excluded.

mutation T790M in 4 samples, for all of which the gefitinib sensitive mutations co-existed. All patients from whom these samples were isolated had been treated by gefitinib. Patients who were not previously treated with gefitinib did not have the T790M mutation.

We investigated the association of several variables with the EGFR mutation (Table 2). Two-sided χ^2 tests revealed gender (female), histology (adenocarcinoma and adenosquamous cell carcinoma) and smoking history (less than 20 packs/year) to be significantly associated with the presence of the EGFR mutation.

The variables studied here may be associated with each other, and they may act as confounding factors in the analyses of other variables. For example, the smoking rate is several-fold different between males and females in Japan and thus the variables affected by smoking may show a seeming gender difference. To eliminate the effect of such confounding variables, we performed a logistic regression analysis (Table 3). The analysis revealed histology and smoking history to be significantly associated with EGFR mutation, while gender, which has often been used as a criterion for selecting a patient group populated with EGFR mutations in clinical medicine, was not significantly associated.

In a subgroup analysis where only mutation-positive patients were studied, we noticed that exon 19 deletions were more frequently found in males, and the difference was significant according to Fisher's exact test (Figure 1). We again performed a logistic regression analysis and confirmed that exon 19 deletions were significantly associated with a male gender after removing the influence of other variables (Table 4). We concluded that not the frequency of the EGFR mutation but the subtype of it shows a gender difference.

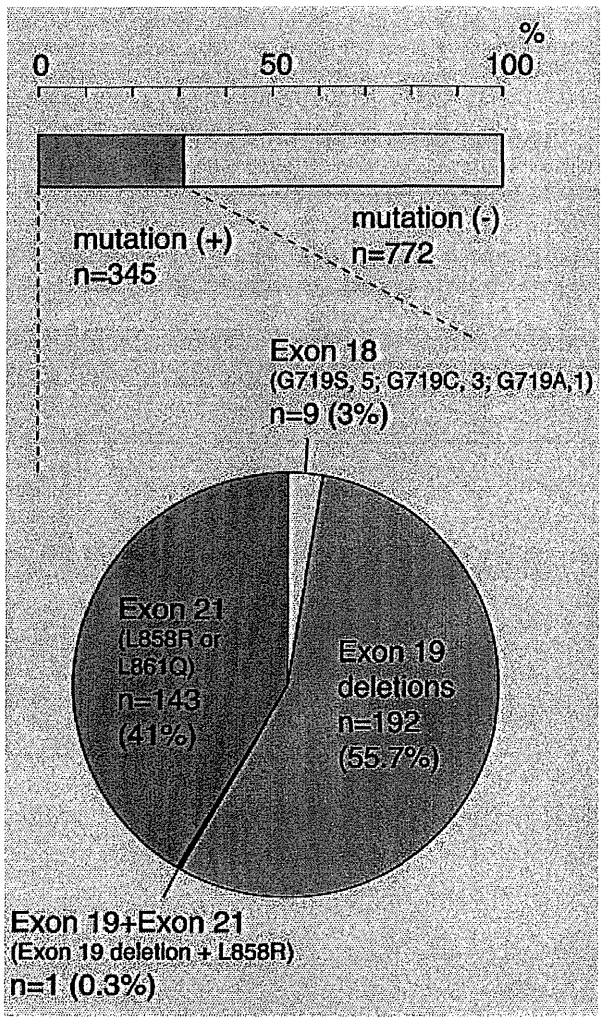

Figure 2. The rate of EGFR mutations. A total of 1176 samples were analyzed, of which 1120 samples provided informative results. Of the 56 uninformative samples, 52 were paraffin-embedded tissues. The number of paraffin-embedded tissue specimens examined was 344, meaning that about 1/8 of them were not suitable for the mutation analysis.

Frequency of the EGFR mutation

The results of the EGFR mutation analysis where samples from our university and those from other institutes were combined are shown in Figure 2. Out of 1176 samples, 1120 samples provided informative results. The EGFR mutation rate was 31%, which was very similar to the rate obtained in the detailed analysis described above.

Discussion

Our result shows the rate of EGFR mutations to be 43% in adenocarcinoma and 12% in the other types of NSCLCs. The overall frequency of EGFR mutations was around 30% in both the samples studied in detail and in all the samples combined. We are in the field of internal medicine, as are our collaborators, and therefore advanced cancers are preferably referred to us. Our samples may better represent those from more advanced stages of NSCLCs. Instead, the frequency was found to be consistent with what determined using surgical samples[3,15,16] which may better represent earlier stages of NSCLCs. Our results suggest that the rate of the EGFR mutation is similar irrespective of the stages of NSCLCs.

The results showing EGFR mutation to be associated with adenocarcinoma but not gender are considered to have clinical implications. Under a setting where an EGFR mutation test is not readily available, the targets for gefitinib therapy have been determined based on the patient characteristics and one of the criteria frequently employed has been adenocarcinomas which occurred in females. According to our results, there is no reason to select the patients by gender.

In contrast to the rate of the EGFR mutations, the mutation subtypes showed a gender difference. Although the exact mechanism by which each subtype occurs has not yet been elucidated, chromosomal recombination that involves DNA double strand breaks and repairs is likely to be involved in exon 19 deletions. It is well known that the meiotic recombination rate shows a clear gender difference.[17] Instead, to our knowledge, there have been no reports showing a gender difference in the rate of somatic, chromosomal deletion mutations. The EGFR mutation may therefore be an interesting model to pursue the gender difference of cancers from the viewpoint of the DNA repair mechanisms.

In the current study, we reported the result of 1176 samples and found a close association between adenocarcinoma and EGFR mutations as well as the gender difference in the mutation subtypes. We also provided the frequencies of EGFR mutations in the samples that are considered to better represent the later stages of NSCLCs. Since tests for EGFR mutations are now widely performed, studies consisting of a large number of samples are now becoming realistic. Such studies will provide further valuable information on the genesis of EGFR mutations.

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