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ORIGINAL REPORT

Japanese-US Common-Arm Analysis of Paclitaxel Plus Carboplatin in Advanced Non–Small-Cell Lung Cancer: A Model for Assessing Population-Related Pharmacogenomics

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ABSTRACT

Purpose
To explore whether population-related pharmacogenomics contribute to differences in patient outcomes between clinical trials performed in Japan and the United States, given similar study designs, eligibility criteria, staging, and treatment regimens.

Methods
We prospectively designed and conducted three phase III trials (Four-Arm Cooperative Study, LC00-03, and S0003) in advanced-stage, non–small-cell lung cancer, each with a common arm of paclitaxel plus carboplatin. Genomic DNA was collected from patients in LC00-03 and S0003 who received paclitaxel (225 mg/m^2) and carboplatin (area under the concentration-time curve, 6). Genotypic variants of CYP3A4, CYP3A5, CYP2C8, NR1I2-206, ABCB1, ERCC1, and ERCC2 were analyzed by pyrosequencing or by PCR restriction fragment length polymorphism. Results were assessed by Cox model for survival and by logistic regression for response and toxicity.

Results
Clinical results were similar in the two Japanese trials, and were significantly different from the US trial, for survival, neutropenia, febrile neutropenia, and anemia. There was a significant difference between Japanese and US patients in genotypic distribution for *CYP3A4*1B* ($P = .01$), *CYP3A5*3C* ($P = .03$), *ERCC1* 118 ($P < .0001$), *ERCC2* K751Q ($P < .001$), and *CYP2C8* R139K ($P = .01$). Genotypic associations were observed between *CYP3A4*1B* for progression-free survival (hazard ratio [HR], 0.36; 95% CI, 0.14 to 0.94; $P = .04$) and ERCC2 K751Q for response (HR, 0.33; 95% CI, 0.13 to 0.83; $P = .02$). For grade 4 neutropenia, the HR for ABCB1 3425C→T was 1.84 (95% CI, 0.77 to 4.48; $P = .19$).

Conclusion
Differences in allelic distribution for genes involved in paclitaxel disposition or DNA repair were observed between Japanese and US patients. In an exploratory analysis, genotype-related associations with patient outcomes were observed for *CYP3A4*1B* and *ERCC2* K751Q. This common-arm approach facilitates the prospective study of population-related pharmacogenomics in which ethnic differences in antineoplastic drug disposition are anticipated.

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INTRODUCTION

Results may vary between different clinical trials that evaluate the same treatment regimen for many reasons, including trial design, eligibility criteria, patient characteristics, and subtle alterations in the treatment regimens themselves. An additional explanation for divergence of outcomes is host-related genetic differences associated with ethnicity, which is particularly pertinent when trials that are performed in different parts of the world are compared.

More than 10 years ago, the Southwest Oncology Group (SWOG) established a collaboration with Japanese investigators of lung cancer to provide a forum for exchange of research data, to facilitate standardization of clinical trial design and conduct, and to establish areas for joint collaboration.[1] We hypothesized that outcome differences between trials performed in Japan and the United States that evaluated similar treatment regimens in advanced-stage, non–small-cell lung cancer (NSCLC) could be explained by population-related

pharmacogenomics. To evaluate this possibility, we prospectively designed three phase III trials, (Four-Arm Cooperative Study [FACS], LC00-03, and S0003), each with similar patient eligibility criteria, staging, and treatment with a common arm of paclitaxel plus carboplatin. We have reported previously that, despite this effort at trial standardization, differences in clinical outcomes were observed in Japanese versus US patients treated on these studies.[2,3] Herein, we report the results of a clinical and pharmacogenomic analysis that involved patients from two of the three clinical trials (LC00-03 and S0003), and we report implications for additional studies by using this clinical research approach in which population-related differences in drug disposition are anticipated.

METHODS

Patients

The clinical trial methodology employed was prospective design of three separate-but-equal, randomized, phase III trials in advanced-stage NSCLC, each with its own comparator regimens but linked by a common treatment arm of paclitaxel plus carboplatin. In FACS, patients were randomly assigned to a standard treatment in Japan (irinotecan plus cisplatin) versus experimental arms of paclitaxel plus carboplatin, gemcitabine plus cisplatin, and vinorelbine plus cisplatin. LC00-03 compared paclitaxel plus carboplatin to the nonplatinum regimen of sequential vinorelbine plus gemcitabine followed by docetaxel, whereas patients on S0003 were randomly assigned to paclitaxel plus carboplatin with or without the hypoxic cytotoxin tirapazamine.

Clinical results for the three trials have been previously presented and published separately.[4-6] Common elements of eligibility criteria are summarized here. All patients had histologically or cytologically confirmed chemotherapy-naïve NSCLC with stage IV (ie, no brain metastases) or selected stage IIIB disease (ie, positive pleural or pericardial effusion or multiple ipsilateral lung nodules); measurable or assessable disease, performance status (PS) of 0 or 1; and adequate hematologic, hepatic, and renal function. All patients gave written informed consent in accordance with institutional regulations, and each protocol was approved by the respective institutional review boards; trials were conducted with adherence to the Helsinki Declaration.

Treatment Schedule, Dose Modifications, and Toxicity Assessment

Study elements of S0003, FACS and LC00-03 were designed to be as similar as possible: each study contained a common arm of paclitaxel plus carboplatin, which was repeated on a 21-day schedule. In all three studies, carboplatin was dosed at an area under the concentration-time curve (AUC) of 6.0 mg/mL/min on day 1. Paclitaxel was dosed at 225 mg/m^2 in S0003 and LC00-03 and at 200 mg/m^2 in FACS because of regulatory requirements for this study; in each study, paclitaxel was delivered as a 3-hour infusion on day 1. Premedication to prevent paclitaxel-related allergic reactions were similar. Prophylactic granulocyte colony-stimulating factor was not utilized. A complete blood count and chemistries were performed on day 1 of each cycle. Dose modifications occurred as previously described.[4] Patients were evaluated every two cycles for objective response by using RECIST (Response Evaluation Criteria in Solid Tumors) criteria[7] Toxicity grading was performed in accordance with the National Cancer Institute Common Toxicity Criteria, version 2.0, in each study.[8]

DNA Extraction and Genotyping

Specimens were not available from FACS; therefore, this analysis compares pharmacogenomic results from LC00-03 with S0003. Whole-blood specimens were collected from consenting patients at the time of enrollment on to LC00-03 and S0003. For S0003, DNA was extracted from patient plasma by using the Gentra PureGene Blood Kit (Gentra, Minneapolis, MN) and the QIAamp DNA Blood midi kit (Qiagen, Valencia, CA), and DNA was reconstituted in a buffer that contained 10 mmol/L Tris (pH 7.6) and 1 mmol/L EDTA, as previously described.[9] For LC00-03, DNA was extracted from buffy coats by using the GenElute Blood Genomic DNA Kit (Sigma-Aldrich, St Louis, MO). Selected genotypic variants related to paclitaxel disposition (ie, the ABC transporter superfamily [multidrug resistance {MDR} transporter 1 P-glycoprotein, *ABCB1* 3435C→T], the pregnane X receptor (PXR, NR1I2-206 deletion), *CYP3A4* (*CYP3A4*1B* 392A→G, 5′ untranslated region), CYP3A5 (*CYP3A5*3C* 6986A→G, splice variant), *CYP2C8* (*CYP2C8*3* 416G→A, R139K) or to platinum-related DNA repair enzymes *ERCC1* (118C→T, silent) and ERCC2 (XPD, K751Q) previously reported to be of functional consequence were analyzed by polymerase chain reaction (PCR) or pyrosequencing, as previously described.[9-13] Briefly, PCR was conducted by using Amplitaq Gold PCR master mix (ABI, Foster City, CA), 5 pmol of each primer, and 5 to 10 ng of DNA. Pharmacogenetic analysis was conducted by using the Pyrosequencing hsAPSQ96 instrument and software (Biotage, Uppsala, Sweden). The genotype was considered variant if it differed from the Reference Sequence consensus sequence for the single-nucleotide polymorphism (SNP) position (http://www.ncbi.nlm.nih.gov/RefSeq/). The *ERCC1* polymorphism was analyzed by PCR restriction fragment length polymorphism, as previously described.[14]

Statistical Methods

Comparison of clinical results among the three trials was prospectively planned and was coordinated through the SWOG statistical center. Pharmacogenomic results were assessed by Cox model for progression-free survival (PFS) and overall survival and by logistic regression for response and toxicity, adjusted for sex and histology.[15] Comparisons of patient demographics, toxicity, and efficacy parameters were made, when applicable, from the available data sets, by two-sample *t* tests, log-rank tests, and Wilcoxon rank sum tests.

RESULTS

Clinical Results Summary

Clinical results are presented for all three trials to document similarities between the two Japanese trials compared with the US S003 trial, whereas pharmacogenomic information was derived only from LC00-03 and S0003. Table 1 summarizes characteristics of patients on the paclitaxel-plus-carboplatin arms of each of the three trials. The median ages and age ranges were similar, and there were no significant differences in sex, stage, or histology. In S0003, 3% of patients self-reported Asian heritage, not additionally specified. Toxicity, efficacy, and dose delivery comparisons are listed in Table 2, which compares S0003 versus FACS/LC00-03 when applicable. Grades 3 to 4 neutropenia and febrile neutropenia were comparable

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	Trial: FACS (n = 145) No.	%	LC00-03 (n = 197) No.	%	S0003 (n = 184) No.	%	*P*
Age, years							.03*
Median	63		65		63		
Range	33-74		33-81		28-80		
Female sex	46	32	61	31	68	37	.42
Disease stage IV	117	81	162	82	161	87	.20
Nonsquamous tumor type	114	79	167	85	152	83	.17

Abbreviation: FACS, four-arm cooperative study.
*Two-sample *t* test to compare LC00-03 and S0003 data. Patient-level data not available for FACS.

Table 2. Toxicity Comparisons

Toxicity	Trial						
	FACS (n = 148)		LC00-03 (n = 197)		S0003 (n = 184)		
	No.	%	No.	%	No.	%	*P*
Neutropenia grades 3-4	130	88	137	70	70	38	< .0001
Febrile neutropenia grades 3-4	27	18	24	12	4	2	< .0001
Thrombocytopenia grades 3-4	16	11	14	7	12	6.5	.31
Anemia grades 3-4	22	15	16	8	12	7	.03
Neuropathy grades 2-4	25	17	32	16	30	16	.99

Abbreviation: FACS, four-arm cooperative study.

in FACS and LC00-03 and were significantly greater than in S0003. Anemia was more frequent in FACS compared with the two other trials (Table 2). Efficacy comparisons are summarized in Table 3. Response rates were similar between the three trials and ranged from 32% to 36%. Median PFS rates were 4.5, 6, and 4 months in FACS, LC00-03, and S0003, respectively. Median survival rates were higher in the Japanese studies at 12 and 14 months, versus 9 months in S0003, and 1-year survival was significantly higher in FACS and LC00-03 than in S0003 ($P = .0004$). Dose delivery, summarized in Table 4, was lower in FACS than in S0003 and LC00-03. Dose reductions were similar between LC00-03 and S0003. Dose reduction data were not available from FACS.

Pharmacogenomic Results

Table 5 lists allelic distributions of patients with common, heterozygous, and variant alleles in the Japanese (LC00-03) and US (S0003) trials. Fisher's exact test was used to determine whether allele distributions were different between the populations. There were significant differences between patients from Japan (LC00-03) and the United States (S0003) in genotype distribution for *CYP3A4*1B* ($P = .01$), *CYP3A5*3C* ($P = .03$), *ERCC1* 118 ($P < .0001$), *ERCC2* K751Q ($P < .001$), and *CYP2C8*3* ($P = .01$).

Across populations, genotypic correlations were observed between *CYP3A4*1B* for PFS (hazard ratio [HR], 0.36; 95% CI, 0.14 to 0.94; $P = .04$) and *ERCC2* K751Q for response (HR, 0.33; 95% CI, 0.13 to 0.83; $P = .02$). There were no other significant associations noted (Table 6). For grade 4 neutropenia, the HR for ABCB1 3425C→T was 1.84 (95% CI, 0.77 to 4.48; $P = .19$). The relationship between the ERCC2 polymorphism and patient response stems principally from US patients. All but one Japanese patient was homozygous for the common allele (A/A). Those who harbored one or more variant alleles were significantly more likely to respond to treatment compared with those who had the common genotype. The response rate for patients with variant alleles was 51% versus 19% for patients homozygous for the common allele $P = .004$). However, no differences were observed in overall survival when stratified by this locus.

In S0003 (ie, the US trial), there were seven African American patients who had specimens available for genotyping. African American patients accounted for all seven patients who were heterozygous or homozygous for the *CYP3A4*1B* allele (Table 5). Additionally, the three patients with the common allele for *CYP3A5*C* were African American.

Table 3. Efficacy Comparisons

Parameter	Trial			*P*
	FACS (n = 145)	LC00-03 (n = 197)	S0003 (n = 184)	
Response				.55
No.	47	73	61	
%	32	37	33	
PFS, months	4.5	6	4	.04*
MST, months	12	14	9	.0006*
1-year survival	51%	57%	37%	.0004

Abbreviations: FACS, four-arm cooperative study; PFS, progression-free survival; MST, median survival time.
*Log-rank test to compare LC00-03 and S0003. Patient-level data not available for FACS.

DISCUSSION

This report describes the culmination of a unique multinational and multistudy collaboration that explores the hypothesis that clinical differences in treatment outcomes between Japanese and US patients with NSCLC may be explained, in part, by pharmacogenomic factors. Potential differences in drug disposition related to ethnic variability in distribution of relevant single nucleotide polymorphisms are well recognized. To our knowledge, however, the current project represents the first attempt to prospectively incorporate study of this topic into a joint clinical trial design. To preplan such a multinational endeavor required a high level of collaboration and compromise among all participants, including, in the case of FACS, Japanese regulatory authorities. Nevertheless, this report demonstrates the overall feasibility of using a common-arm methodology to investigate this research topic, in which a single, prospectively planned, joint study cannot be conducted. Considering the limitations of the clinical and pharmacogenomic data sets generated in this effort, and considering the multiple comparisons generated, the results reported here should be viewed as exploratory only and as primarily useful for refining this common-arm model of multinational collaboration. Even so, the clinical results are remarkably consistent with those anticipated, in which expectations were for both improved efficacy and higher levels of toxicity in Japanese patients who received a similar treatment regimen. Observation of clinical differences despite reduced paclitaxel

Table 4. Treatment Delivered

Treatment Data	Trial						
	FACS (n = 145)		LC00-03 (n = 197)		S0003 (n = 184)		
	No.	%	No.	%	No.	%	*P*
Median cycles delivered	3.5		4		4		.07
Received > three cycles	35	24	118	60	100	54	< .0001
Received six cycles	16	11	58	29	68	36.5	< .0001
Dose was reduced	No data	No data	100	51	98	26	.63*

Abbreviation: FACS, four-arm cooperative study.
*Wilcoxon rank sum test to compare LC00-03 and S0003. Patient-level data not available for FACS.

dosing and drug delivery of paclitaxel plus carboplatin in the FACS Japanese study highlights the contrast.

The rationale for conducting this common-arm project specifically in collaboration with Japanese investigators was based on several factors, including the established SWOG interaction described earlier, the high quality of lung cancer investigation by Japanese cooperative groups, and prior literature that suggested that overall, Japanese patients achieve better results than their US counterparts. However, the most compelling rationale was prior pharmacogenomic literature, which suggested that relevant drug disposition differences might exist between US and Japanese populations treated with cancer chemotherapeutic agents. Well recognized here are alterations in irinotecan metabolism as a result of variability in the allelic distribution of UDP-glucuronosyltransferases, particularly *UGT1A1*28* in different ethnic groups, as Asians have a much lower frequency of variant alleles. Recently, a comparative analysis of patient-level data from phase III trials in small-cell lung cancer in Japan (J9511) and the United States (S0124) demonstrated significant differences in toxicity profiles between the two groups. In addition, a pharmacogenomic analysis of S0124 showed significant associations between genotypic variants and toxicity levels.[16,17]

Table 5. Genotype Profiles in Japanese and US Patients on LC00-03 and S0003

Polymorphism by Trial Location	No. of Patients			*P*
	Com	Het	Var	
*CYP3A4*1B*				
Japan	73	0	0	.01
United States	64	4	3	
*CYP3A5*C*				
Japan	7	16	50	.03
United States	3	7	66	
CYP2C8 (R139K)				
Japan	69	2	0	.01
United States	57	7	5	
ABCB1 (3435C→T)				
Japan	33	21	17	.11
United States	24	23	29	
NR1I2 (206 deletion)				
Japan	51	19	5	.25
United States	40	25	8	
ERCC1 (118)				
Japan	8	27	43	< .0001
United States	23	33	19	
ERCC2 (K751Q)				
Japan	73	1	0	< .001
United States	37	27	8	

NOTE. LC00-03 is the trial in Japan; S0003 is the trial in the United States. Fisher's exact test was used to determine whether allele distributions were different between the populations.
Abbreviations: Com, common allele; Het, heterozygous allele; Var, variant allele.

The genes evaluated in this study were selected on the basis of their potential to influence paclitaxel disposition or DNA damage repair. Paclitaxel is principally eliminated through multiple hydroxylation reactions mediated by cytochrome isoforms *CYP2C8, CYP3A4,* and *CYP3A5.*[18,19] The *CYP2C8*3* variant (R139K), which is associated with decreased metabolism of paclitaxel, occurs at a frequency of 9% to 15% in white patients but is rare in African and Asian populations.[20-23] In this study, the allele frequency in the US population was 12%, which was significantly different from the less-than-1% frequency in the Japanese cohort (P = .01). *CYP2C8* genotypic variability at R139K was not significantly associated with patient outcome. CYP3A isozymes account for 45% to 60% of paclitaxel metabolism.[24] In white patients, the *CYP3A5* allele is commonly nonfunctional as a result of a transition in intron 3 that produces a truncated splice variant.[25] Our findings are consistent with that of Hustert et al,[25] who reported frequencies of functional *CYP3A5* as 5% in white patients, 29% in Japanese patients, and 73% in African American patients. Of patients enrolled onto the S0003 trial conducted in the US, three of three with the functional allele (indicated as common in Table 5) were African Americans, as were three of the seven heterozygous patients. Although trends were observed, *CYP3A5*3C* genotypic variability was not significantly associated with patient outcome (overall survival *P* = .07; PFS *P* = .09), perhaps related to the small sample size. Similarly, the *CYP3A4*1B* allele was observed in seven of seven African American patients but was absent in white and Japanese patients. In vitro studies suggest that the *CYP3A4*1B* variant has enhanced activity over common allele.[26] An association was observed between occurrence of the *CYP3A4*1B* and PFS (*P* = .04); however, this association should be interpreted in the context that only African American patients harbored this allele. Thus, it remains unclear whether this potential relationship with outcome is associative or causative. The PXR (*NR1I2-206* deletion) is a master regulator of genes involved in xenobiotic detoxification and influences transcription of *CYP3A4, CYP3A5, CYP2C8,* and *MDR-1 (ABCB1).*[27-29] Paclitaxel can activate PXR, which enhances drug clearance through increased activity of MDR1.[30] No significant differences by genotype were observed for PXR or ABCB1, although there was a trend toward

Table 6. Cox Model to Compare Outcomes by Polymorphism

Outcome by Polymorphism	Analyses			
	Comparison	HR	95% CI	*P*
ABCB1 3425				
Overall survival	Com *v* Het/Var (CC *v* CT/TT)	1.09	0.71 to 1.67	.69
PFS		1.04	0.70 to 1.56	.82
Response		0.97	0.39 to 2.38	1.00
Neutropenia		0.54	0.22 to 1.30	.19
CYP2C8 R139K				
Overall survival	Com *v* Het/Var (GG *v* GA/AA)	1.09	0.61 to 1.96	.76
PFS		1.12	0.63 to 2.00	.69
Response		1.92	0.46 to 11.11	.51
Neutropenia		1.30	0.35 to 5.00	.87
*CYP3A4***1B*				
Overall survival	Com *v* Het/Var (AA *v* AG/GG)	0.74	0.32 to 1.72	.48
PFS		0.36	0.14 to 0.94	.04
Response		0.63	0.10 to 4.76	.84
Neutropenia		0.44	0.04 to 2.94	.58
*CYP3A5***3C*				
Overall survival	Com/Het *v* Var (AA/AG *v* GG)	1.64	0.95 to 2.86	.07
PFS		1.56	0.93 to 2.63	.09
Response		1.61	0.53 to 4.76	.47
Neutropenia		1.30	0.44 to 3.85	.78
ERCC1 (118)				
Overall survival	TT *v* TC/CC	1.20	0.74 to 1.96	.45
PFS		1.11	0.69 to 1.82	.65
Response		1.45	0.48 to 4.17	.61
Neutropenia		0.57	0.20 to 1.61	.35
ERCC2 K751Q				
Overall survival	Com *v* Het/Var (AA *v* AC/CC)	0.97	0.63 to 1.49	.89
PFS		0.85	0.55 to 1.30	.45
Response		0.33	0.13 to 0.83	.02
Neutropenia		0.75	0.30 to 1.85	.63
nr1I2-206 del				
Overall survival	Com *v* Het/Var 206 deletion	0.82	0.53 to 1.25	.35
PFS		0.93	0.63 to 1.39	.75
Response		0.82	0.34 to 2.00	.77
Neutropenia		0.88	0.37 to 2.08	.90

Abbreviations: HR, hazard ratio; PFS, progression-free survival; Com, common allele; Het, heterozygous allele; Var, variant allele.

neutropenia ($P = .19$) for patients who harbored the ABCB1 3435 common allele.

The *ERCC2* gene, also known as xeroderma pigmentosum complementation group D, encodes a DNA helicase which complexes with TFIIH, a transcription factor essential for replication and nucleotide excision repair.[31] Several nonsynonymous SNPs have been described in this gene, including an Asp→Asn (G→A) at codon 312 in exon 10 and a Lys→Gln (A→C) at codon 751 in exon 23 and are likely in linkage disequilibrium with each other.[32,33] The functional consequences of these SNPs are still in contention, and the majority of studies indicate that variants in these alleles result in reduced DNA repair capacity.[34-41] Additionally, most studies indicate that *ERCC2* variants confer an increased risk of lung cancer.[32,34,35,42-48] In this study, 51% of patients (ie, 37 of 72 patients) from the US were homozygous wild type for the common (A) allele. These patients were significantly less likely to respond to treatment compared with US patients who had one or more variant alleles (A/C or C/C). However, no differences in overall survival were observed on the basis of *ERCC2* K751Q allele frequencies. In addition, this allele cannot account for the improved survival experienced by Japanese patients, as they uniformly harbored the common A/A genotype (and only one patient harbored A/C). The *ERCC1* 118 C→T SNP does not result in an amino acid substitution, although studies have nevertheless identified associations with patient outcome in various tumor types.[49] It has been suggested that this variant may modulate *ERCC1* mRNA and protein expression and/or may be in linkage disequilibrium with other functional SNPs.[14,50,51] However, three reports in NSCLC found no associations between the *ERCC1* 118 and patient outcome.[52-54] Here, we found a highly significant divergence in allele frequency between Japanese and US patients ($P < .0001$); however, no impact on patient outcome was observed.

In summary, the results of cancer clinical trials to test the same regimen may differ for a variety of reasons, including differences related to ethnicity. FACS, LC00-03, and S0003 were prospectively designed to facilitate a comparison of patient outcomes and pharmacogenomic results, in a setting where joint clinical trials sponsored by the US National Cancer Institute were not possible. Our

results suggest that global clinical trials (ie, those conducted internationally) should be carefully designed and conducted to account for potential genetic differences in the patient populations studied. This common-arm approach provides a model for the prospective study of population-related pharmacogenomics in which ethnic differences in antineoplastic drug disposition are anticipated.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Usefulness of cumulative smoking dose for identifying the *EGFR* mutation and patients with non-small-cell lung cancer for gefitinib treatment

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We examined the diagnostic accuracy of the cumulative smoking dose for identifying the epidermal growth factor receptor (*EGFR*) exon 19 deletion and L858R mutation among Japanese patients with non-small-cell lung cancer (NSCLC). *EGFR* mutations in exon 19 and exon 21 were determined in 1001 NSCLC patients. A receiver–operating characteristic (ROC) curve methodology was applied to estimate the diagnostic accuracy. *EGFR* mutations were detected in 314 patients (31.4%). A cumulative smoking dose of less than 13 pack-years (PY) was the optimal cut-off point for predicting a positive *EGFR* mutation status, producing a balance between the sensitivity (73.5%) and the specificity (77%). The area under the ROC curve was 0.77, indicating that the smoking dose had a moderate diagnostic accuracy. The median survival time or the median progression-free survival time of patients who had smoked less than 13 pack-years (PY) were 18.6 and 6.3 months, respectively, while those of patients with equal to or more than 13 PY were 9.6 and 2.4 months, respectively. The overall survival (OS) and progression-free survival (PFS) rates were significantly different between the two groups (OS: hazard ratio [HR] = 0.64, 95% confidence interval [CI] = 0.51–0.80, $P = 0.0001$) (PFS: HR = 0.58, 95% CI = 0.47–0.71, $P < 0.0001$). Our study indicated that the smoking dose predicted *EGFR* mutations with a moderate diagnostic accuracy. Thus, patients who have smoked less than 13 PY might be candidates for gefitinib treatment when *EGFR* mutation status cannot be determined. (*Cancer Sci* 2009; 100: 1931–1934)

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is highly expressed in cancer cells.[1] Mutations in *EGFR* have been reported in non-small-cell lung cancers (NSCLC).[2–4] *EGFR* mutations are frequently located in exons 18 to 21 of the EGFR tyrosine kinase domain and play an oncogenic role, especially in adenocarcinoma.[5] Gefitinib and erlotinib are reversible EGFR tyrosine inhibitors (EGFR-TKI) and are used for the treatment of NSCLC patients.[6] Previous studies have focused on identifying factors that are useful indicators for selecting candidates for EGFR-TKI treatment. An adenocarcinoma histology, a never-smoking status, and the female sex have been shown to be associated with sensitivity to gefitinib. Since 2004, *EGFR* mutations, especially exon 19 deletions and the L858R mutation, have been demonstrated to be associated with sensitivity to EGFR-TKIs and are considered to predict a favorable clinical outcome for NSCLC patients treated with EGFR-TKIs.[7,8] Based on accumulating data, an examination of the *EGFR* mutation status prior to the start of EGFR-TKI treatment is now encouraged. However, there are some situations when a mutation analysis is not feasible, such as, for instance, when the available clinical samples are inappropriate for determining the *EGFR* genotype. Previous studies have shown that smoking status, adenocarcinoma histology, and East Asian ethnicity were significantly related to *EGFR* mutations.[4] In addition, we and others have reported that the presence of *EGFR* mutations is inversely correlated with the cumulative smoking dose, suggesting that the degree of smoking predicts the prevalence of *EGFR* mutations.[9–11] Pham *et al.* reported that a smoking history could be a predictor of *EGFR* mutation based on a study using a receiver–operating characteristic (ROC) curve methodology, suggesting that the total smoking dose could assist clinicians in assessing the likelihood of *EGFR* mutations.[11] Because the frequency of *EGFR* mutation differs according to ethnicity, the relationship between *EGFR* mutation and smoking dose is an issue of interest in East Asian patients, including Japanese patients, in countries where gefitinib and erlotinib have been approved for the treatment of NSCLC.

In the present study, we determined the diagnostic accuracy of the cumulative smoking dose for identifying the *EGFR* mutation status of Japanese NSCLC patients. We also showed the survival of patients according to the smoking dose, as determined using an ROC curve methodology.

Materials and Methods

Patients' characteristics. We collected the data of 1001 NSCLC patients undergoing surgical procedures between 2000 to 2008 that contained clinical records and *EGFR* mutation status from Aichi Cancer Center Hospital (410 patients), Nagoya, Japan, and Okayama University Hospital (591 patients), Okayama, Japan. Total patients consisted of 365 (36.5%) females and 636 (63.5%) males, 375 (37.5%) never-smokers and 626 (62.5%) ever-smokers (median, 43.7 pack-years [PY]; range, 0.15–324 PY), 779 (77.8%) adenocarcinomas and 222 (22.2%) non-adenocarcinomas. Smoking categories were defined as follows: never-smokers were those with lifetime exposure of 100 cigarettes or less, ever-smokers were those with lifetime exposure of more than 100 cigarettes. Patients' characteristics are shown in Table 1. We previously reported the survival of 408 gefitinib-treated patients with clinical records and *EGFR* mutation status collected from four institutions.[12] Briefly, 408 NSCLC patients consisting of

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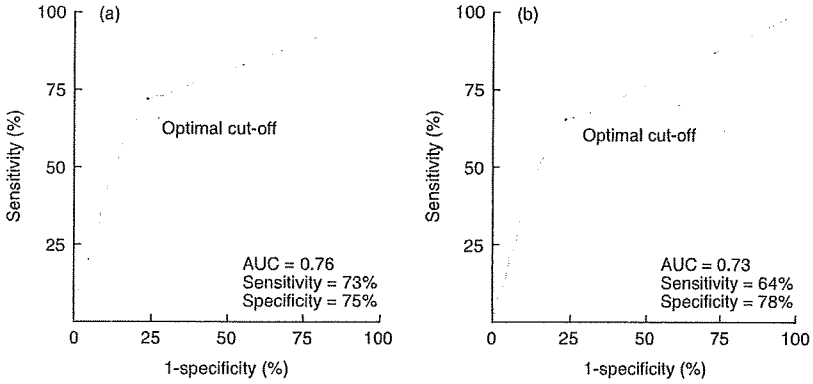

Fig. 1. Receiver–operating characteristic (ROC) curve of the association between epidermal growth factor receptor (*EGFR*) mutation and cumulative smoking dose. The optimal cut-off for the test is the point closest to the upper-left corner of the graph, which corresponds to a mutation in *EGFR*. AUC, area under the ROC curve (a) ROC for total 1001 cases. (b) ROC for 779 adenocarcinoma cases.

Table 1. Patients' characteristics and relevance of *EGFR* mutation

Variables	Number	*EGFR* mutation (%)	*P*-values
Sex			
Female	365	198 (54.2)	<0.0001
Male	636	116 (18.2)	
Smoking history			
Never	375	223 (59.5)	<0.0001
Ever	626	91 (14.5)	
Histology			
Ad	779	308 (39.5)	<0.0001
Non-Ad	222	6 (2.7)	

Ad, adenocarcinoma; *EGFR*, epidermal growth factor receptor.

Table 2. Cumulative smoking dose and *EGFR* mutation

Smoking dose (pack-years [PY])	Number	*EGFR* mutation (%)	*P*-values*
0 (Never-smokers)	375	223 (59.5)	
0 < PY ≤ 10	29	12 (41.2)	0.070
10 < PY ≤ 20	42	12 (28.6)	0.0002
20 < PY ≤ 40	181	28 (15.5)	<0.0001
40 < PY ≤ 60	187	20 (10.7)	<0.0001
60 < PY	187	19 (10.1)	<0.0001

*The difference was examined between pack-year categories and never-smokers. *EGFR*, epidermal growth factor receptor.

362 adenocarcinomas and 46 non-adenocarcinomas were obtained form from the National Cancer Center Hospital (207 patients), Aichi Cancer Center Hospital (103 patients), and Okayama University Hospital with NHO Yamaguchi–Ube Medical Center (98 patients). All patients had advanced or recurrent NSCLC and initiated gefitinib treatment (250 mg/day) between November 2000 and August 2006 in each institution.[12] We used this cohort to investigate the clinical outcome of patients treated with gefitinib.

Tumor response was assessed based on World Health Organization criteria (National Cancer Center Hospital and Okayama University Hospital with Sanyo National Hospital)[8,13,14] and image analysis and serum carcinoembryonic antigen level as reported (Aichi Cancer Center Hospital).[7] The overall survival (OS) and progression-free survival (PFS) were calculated from the start of gefitinib treatment until the date of death or the last follow-up for OS and until confirmed disease progression or death for PFS. The Kaplan–Meier method was applied to estimate OS and PFS. This study was permitted by the Institutional Review Board at each institution and informed consents were obtained from each patient.

Detection of *EGFR* mutations in primary tumors. The DNA-based analysis using direct-sequencing or PCR-based length polymorphisms (exon 19) or RFLP (exon 21) assays were performed to detect EGFR mutation in samples from Okayama University.[15] The RNA-based analysis using one-step reverse transcription–polymerase chain reaction for *EGFR* mutation detection was carried out at Aichi Cancer Center.[9]

Receiver–operating characteristic (ROC) curve analysis for prediction of the *EGFR* mutation. We used ROC analysis to determine the cut-off point for the smoking dose at which optimal sensitivity and specificity were achieved, maximizing accuracy. The best cut-off point for balancing the sensitivity and specificity of a test was assumed to be the point on the curve closest to the (0, 1) point.[16] The diagnostic accuracy of smoking dose for predicting the incidence of *EGFR* mutations was summarized as the area under the curve (AUC). The AUC greater than 0.9 has high accuracy, while 0.7–0.9 indicates moderate accuracy; 0.5–0.7, low accuracy; and 0.5 a toss-up.[17]

Statistical analyses. The differences of significance among categorized groups were compared using the χ^2-test. Differences in OS and PFS among groups were assessed by the log-rank test. Statistical data were analyzed with StatView 5.0 for Windows (SAS Institute, Cary, NC, USA). All statistical tests were two-sided and $P < 0.05$ were defined as being statistically significant.

Results

Frequency of *EGFR* mutation and clinicopathological factors. *EGFR* mutations were present in 314 of the 1001 patients and were comprised of 164 mutations in exon 19 and 153 mutations in exon 21. Three patients had mutations in both exons 19 and 21. The relationships between the *EGFR* mutation status and clinicopathological factors are shown in Table 1. *EGFR* mutations were significantly more frequent among females ($P < 0.0001$), patients with an adenocarcinoma histology ($P < 0.0001$), and never-smokers ($P < 0.0001$). Regarding smoking status, *EGFR* mutations were identified in 223 (59.5%) never-smokers and 91 (14.5%) ever-smokers ($P < 0.0001$). According to the cumulative smoking dose, *EGFR* mutation was present in 12 (41.2%) patients with a PY of >0 and 10, 12 (28.6%) patients with a PY >10 and 20, 28 (15.5%) patients with a PY >20 and 40, 20 (10.7%) patients with a PY >40 and 60, and 19 (10.1%) patients with a PY >60 (Table 2). Overall, the incidence of *EGFR* mutations decreased as the number of PY increased.

Diagnostic accuracy of smoking dose in identifying *EGFR* mutation status. The smoking dose, described in terms of PY, predicted the prevalence of *EGFR* mutations among all the patients and among only the adenocarcinoma patients (areas under the ROC curves were 0.77 and 0.73, respectively) (Fig. 1a). According to the ROC curve for all the patients, a 12.8 PY dose level was the best cut-off of a positive *EGFR* mutation status, with a 73.5%

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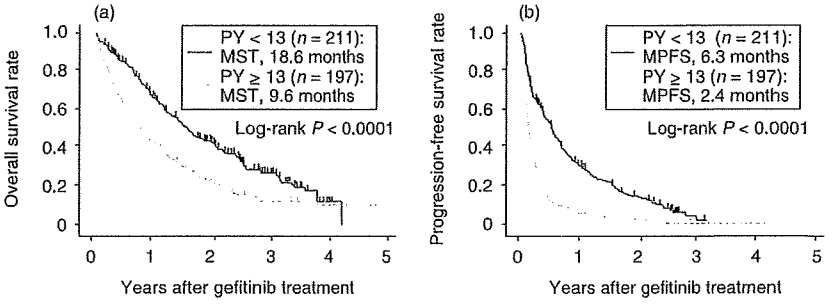

Fig. 2. Kaplan–Meier plot of survival times stratified by the cumulative smoking dose (13 pack-years [PY]). (a) Overall survival of patients treated with gefitinib. (b) Progression-free survival of patients treated gefitinib. MPFS, median progression-free survival time; MST, median survival time. *P*-values were calculated using the log-rank test.

Table 3. Cumulative smoking dose and clinical outcomes

Smoking dose (pack-years [PY])	Number	Response rate (%)	*P*-values*	MST (months)	*P*-values*	MPFS (months)	*P*-values*
0 (Never-smokers)	178	94 (52.8%)		18.3		6.3	
0 < PY < 13	33	15 (45.5%)	0.78	18.7	0.71	5.1	0.61
13 ≤ PY ≤ 40	92	26 (28.3%)	0.019	9.6	0.0024	2.6	0.0015
40 < PY	105	21 (20.0%)	0.0004	9.5	0.0015	2.2	< 0.0001

*The difference was examined between pack-year categories and never-smokers.
MPFS, median progression-free survival time; MST, median survival time.

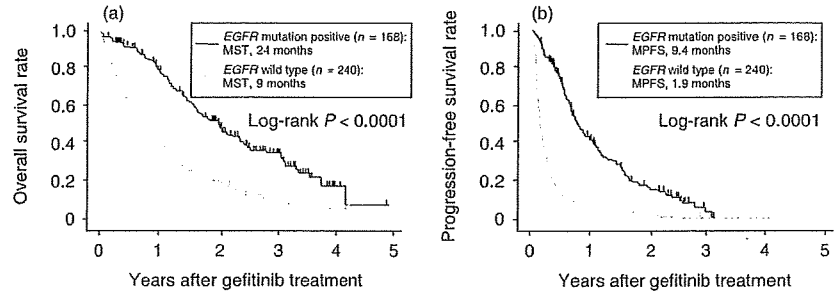

Fig. 3. Kaplan–Meier plot of survival times stratified by epidermal growth factor receptor (*EGFR*) mutation status. (a) Overall survival of patients treated with gefitinib. (b) Progression-free survival of patients treated gefitinib. MPFS, median progression-free survival time; MST, median survival time; Mut, *EGFR* mutation; Wt, *EGFR* wild-type. *P*-values were calculated using the log-rank test.

sensitivity and a 77% specificity for prediction. For convenience, we choose a dose level of 13 PY as the best cut-off value for a positive *EGFR* mutation status. Among only the adenocarcinoma patients, the ROC curve indicated that a cut-off of 11.3 PY was the best predictor for a positive *EGFR* mutation status (67.3% sensitivity and 77.6% specificity) (Fig. 1b). The ROC curves for specific mutation types, i.e. in exon 19 or exon 21, were also similar (data not shown). Though the frequency of *EGFR* mutation was higher among patients with adenocarcinomas than among all the patients with NSCLCs, the optimal cut-off value was similar and the sensitivity and the specificity were not superior to those obtained for all the NSCLC patients.

Response and survival of patients treated with gefitinib stratified according to smoking dose. Analyses for tumor response and survival were performed in 408 patients who were treated with gefitinib.[12] A total of 211 patients had smoked less than 13 PY and 197 patients had smoked 13 PY or more. A total of 109 (51.7%) patients with less than 13 PY smoking history showed tumor response and 47 (23.9%) patients with equal or more than 13 PY showed response ($P = 0.0001$). The median survival time (MST) or the median progression-free survival time (MPFS) of patients who smoked less than 13 PY were 18.6 and 6.3 months, respectively, while those of patients with equal to or more than 13 PY were 9.6 and 2.4 months, respectively. Significant differences in OS and PFS were observed between the two groups (OS: hazard ratio [HR] = 0.64, 95% CI = 0.51–0.80, $P = 0.0001$) (PFS: HR = 0.58, 95% CI = 0.47–0.71, $P < 0.0001$) (Fig. 2a,b). Furthermore, tumor response rate, MST, and MPFS according to smoking dose are shown in Table 3. When the patients were stratified according to *EGFR* mutation status, significant differences in OS and PFS were observed between the patients with *EGFR* mutations and the wild-type patients, as expected (OS: HR = 0.43, 95% CI = 0.33–0.54, $P < 0.0001$) (PFS: HR = 0.30, 95% CI = 0.24–0.37, $P < 0.0001$) (Fig. 3a,b).

Discussion

Previous studies have indicated that an *EGFR* mutation, but not smoking status or sex, was a predictor of a better clinical outcome among patients treated with gefitinib.[12] However, understanding the ability or limitation of clinical factors as a predictor of patients' prognosis when treated with EGFR-TKI would be useful if the *EGFR* mutation status were not available. In this study, we determined the utility of the cumulative smoking dose for identifying the *EGFR* mutation status in a Japanese cohort. Our results showed that a cumulative smoking dose of less than 13 total PY yielded the highest discriminative ability with a 73.5% sensitivity and 77% specificity for predicting the presence of *EGFR* mutations. The AUC was 0.77, indicating that the cumulative smoking dose had a moderate diagnostic accuracy for predicting *EGFR* mutation status.[18]

For the survival analysis, we used a previously reported cohort that had demonstrated the impact of *EGFR* mutation.[12] Both the OS and PFS were significantly longer among patients who had smoked less than 13 PY, compared with patients who had a smoking history of 13 PY or more. Although the OS and PFS were longer in patients with a positive *EGFR* mutation status than in patients with a smoking history of less than 13 PY, our results indicated that the cumulative smoking dose was a predictor of survival among patients treated with gefitinib when

EGFR mutation status was unknown. It should be noted that never- or light-smoking status itself could be a favorable prognostic factor of NSCLC. On this point, Hotta *et al.* reported that the effect of smoking dose on survival was more significant in patients with gefitinib treatment than those without treatment, indicating that smoking status was a predictive factor among patients for gefitinib treatment, rather than a prognostic factor.[19] In addition, the tumor response rate was also better in patients with never- or light-smoking history than in patients with heavy smoking history. Taken together, patient selection based on smoking dose may be useful when *EGFR* mutation status is not available and might be a determiner for EGFR-TKI treatment.

Using an ROC curve methodology, Pham *et al.* reported that a smoking history of less than 15 PY had an 82% sensitivity and a 70% specificity for predicting the presence of *EGFR* mutations, with an AUC of 0.78.[11] While there are some differences, their ROC results are similar to our data. The fact that the ROC curve and the optimal cut-off were very similar between American and Japanese patients is interesting, since the frequency of *EGFR* mutation differs between these two groups. Matsuo *et al.* suggested that smoking itself might not cause *EGFR* mutation.[20] The reason why *EGFR* mutation seemed to be low in smokers is that lung cancers in smokers have more chance of having other molecular alterations such as *K-ras* mutation, *LKB1* alteration, or DNA methylation.[4,21,22] The effect of smoking on lung cancer in patients without an *EGFR* mutation might be similar between American and Japanese patients, although further investigation of this possibility is necessary. While Pham *et al.* reported that smoke-free years were an effective predictor of *EGFR* mutation status, this type of data was not available in our cohort.

In conclusion, cumulative smoking dose predicted *EGFR* mutation status with a moderate diagnostic accuracy. NSCLC patients who have smoked less than 13 PY might be candidates for gefitinib treatment.

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Disclosure Statement

Authors have no conflict of interest to disclose.

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Gender Difference in Treatment Outcomes in Patients with Stage III Non-small Cell Lung Cancer Receiving Concurrent Chemoradiotherapy

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Objective: To identify any gender differences in the outcomes of concurrent platinum-based chemotherapy and thoracic radiotherapy for unresectable stage III non-small cell lung cancer (NSCLC).
Methods: A comparative retrospective review of the clinical characteristics and treatment outcomes between female and male NSCLC patients receiving chemoradiotherapy.
Results: Of a total of 204 patients, 44 (22%) were females and 160 (78%) were males. There was no difference in age, body weight loss, performance status or disease stage between the sexes, whereas never-smokers and adenocarcinoma were more common in female patients (55% vs. 3%, $P < 0.001$, and 73% vs. 55%, $P = 0.034$, respectively). Full cycles of chemotherapy and radiotherapy at a total dose of 60 Gy were administered to ~70% and >80% of the patients, respectively, of both sexes. Grade 3–4 neutropenia was observed in 64% of the female patients and 63% of the male patients. Severe esophagitis was encountered in <10% of the patients, irrespective of the sex. The response rate was higher in the female than in the male patients (93% vs. 79%, $P = 0.028$), but the median progression-free survival did not differ between the sexes. The median survival time in the female and male patients was 22.3 and 24.3 months, respectively ($P = 0.64$).
Conclusions: This study failed to show any gender differences in the survival or toxicity among patients treated by concurrent chemoradiotherapy. These results contrast with the better survival in female patients undergoing surgery for localized disease or chemotherapy for metastatic disease.

Key words: gender – female – non-small cell lung cancer – chemotherapy – radiotherapy

INTRODUCTION

Lung cancer in women differs from that in men with respect to its incidence, association with smoking, and histological distribution (1). Several epidemiological studies have shown that female smokers have a 1.5- to 3-fold higher risk of developing lung cancer than male smokers, suggesting that women may have an increased susceptibility to the carcinogens in tobacco. Never-smokers with lung cancer are more likely to be female than male, and in East Asian countries, as high as 70% of the women diagnosed with lung cancer have never smoked in their lives. Women are more likely to develop adenocarcinoma than squamous cell carcinoma, the latter being more common in men. This difference cannot be explained fully by differences in the smoking patterns, and potentially suggests basic differences in the etiology of lung cancer between the sexes (1).

Prospective cohort studies and a large population-based study have consistently shown that female gender is a favorable prognostic factor in patients with non-small cell lung cancer (NSCLC). These studies, however, included patients

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with all stages of cancer, and the therapies administered are not specified (2–4). The existence of a gender difference in survival remains controversial among patients with locally advanced NSCLC receiving radiation-based treatment. Some studies have shown better survival in females than in males (5–7), whereas others have shown no difference in survival between the sexes (8,9). Many patients in these studies, however, received radiotherapy alone, which is no longer the standard treatment for locally advanced disease. Furthermore, all but one of these studies included patients with stage I–II disease who were considered unsuitable for surgical treatment because of poor general condition. One study that addressed gender differences in unresectable stage III NSCLC patients treated by chemoradiotherapy showed a median survival time in women of 19.7 months and in men of 21.7 months ($P = 0.26$) (10). The objectives of this study were to compare the outcomes of concurrent chemoradiotherapy between female and male patients with stage III NSCLC.

PATIENTS AND METHODS

Study Population

Patients with unresectable stage III NSCLC who underwent concurrent platinum-based chemotherapy and thoracic radiotherapy at the National Cancer Center Hospital between 1994 and 2005 were eligible for this study. A total of 204 patients were identified. Patients treated by sequential chemotherapy and thoracic radiotherapy were excluded from this study, because we consider that the standard of care for unresectable stage III NSCLC without effusion is concurrent chemoradiotherapy, and sequential treatment is only given to patients in poor general condition or those with tumors too large for radiotherapy initially, which are expected to shrink sufficiently for radiotherapy after chemotherapy. All patients underwent a systematic pre-treatment evaluation and standardized staging procedures, which included physical examination, chest X-rays, computed tomographic (CT) scans of the chest and abdomen, CT or magnetic resonance imaging of the brain, and bone scintigraphy. Chemotherapy consisted of cisplatin combined with either vinorelbine ($n = 125$), vindesine with or without mitomycin ($n = 46$), or other drugs ($n = 6$) repeated every 4 weeks, carboplatin and docetaxel ($n = 10$) administered weekly, and nedaplatin and paclitaxel administered every 4 weeks ($n = 17$).

A retrospective review of the medical charts of the patients was conducted to determine the gender, age, smoking history, body weight loss, performance status, clinical stage, histology, success of treatment delivery, incidence/severity of hematological toxicity and esophagitis, tumor responses, and survival parameters. The histological classification of the tumor was based on the criteria of the World Health Organization (11). Toxicity was graded according to the Common Terminology Criteria for Adverse Events v3.0. Objective tumor responses were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (12).

Statistical Methods

The demographic, clinical and histopathologic characteristics were compared between the genders. The χ^2 and Mann–Whitney tests were used to evaluate the differences in the categorical and continuous variables, respectively. Overall survival was measured from the start of chemotherapy to death from any cause. For progression-free survival (PFS), both the first evidence of disease progression and death from any cause were counted as an event. A patient who did not develop any event at the last follow-up was censored at that time. Survival curves were calculated according to the Kaplan–Meier method. Cox's proportional hazard models were used to adjust for potential confounding factors such as tumor stage and performance status (13). The significance of P value was set to be <0.05. All of the above-mentioned analyses were performed using the Dr. SPSS II 11.0 for Windows software package (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Patient Demographics

Of the 204 patients, 44 (22%) were females and 160 (78%) were males (Table 1). There were no differences in age, body weight loss or performance status between the sexes, whereas never-smokers were more common among female patients (55% vs. 3%, $P < 0.001$). Adenocarcinoma accounted for the main histological type in both sexes, but was more common in female patients (73% vs. 55%, $P = 0.034$). No difference in the distribution of the clinical stage was noted between the sexes.

Treatment Delivery

The delivery of chemoradiotherapy was good in both sexes. Three to four cycles of chemotherapy were administered in 68% of the female patients and 69% of the male patients. A total radiation dose of 60 Gy was given to 89% of the female patients and 86% of the male patients.

Toxicities

Grade 3–4 neutropenia was observed in 64% of the female patients and 63% of the male patients (Table 2). The frequency of febrile neutropenia was also the same between the sexes. Severe esophagitis was encountered in <10% of the patients, irrespective of the sex.

Treatment after Recurrence

The use of epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) was evaluated in

43 of the 44 female patients and 153 of the 160 male patients. Gefitinib was given to 7 female and 25 male patients, and erlotinib to 1 female and 1 male patient. Thus, in all, EGFR-TKIs were given to 8 (18.2%) female and 26 (16.3%) male patients.

Table 1. Patient characteristics

Characteristics	Female ($n = 44$)		Male ($n = 160$)		P value
	N	%	N	%	
Age					
Median (range)	57 (29–74)		58 (35–78)		0.28
Smoking history					
Never	24	55	5	3	<0.001
Former	5	11	77	48	
Current	15	34	78	49	
Body weight loss					
≤4.9%	36	82	126	79	0.66
≥5.0%	8	18	34	21	
Performance status					
0	12	27	51	32	0.62
1	32	73	107	67	
2	0		2	1	
Histology					
Adenocarcinoma	32	73	88	55	0.034
Non-adenocarcinoma	12	27	72	45	
Stage					
IIIA	17	39	69	43	0.53
IIIB	27	61	91	57	
Period					
1994–99	17	39	47	29	0.24
2000–05	27	61	113	71	

Table 2. Grade 3–4 toxicity

Toxicity	Grade	Female ($n = 44$)		Male ($n = 160$)		P value
		N	%	N	%	
Leukopenia	3	23	52	79	49	0.44
	4	9	21	33	21	
Neutropenia	3	13	30	49	31	0.19
	4	15	34	51	32	
Thrombocytopenia	3	1	2	5	3	0.97
	4	0		1	1	
Febrile neutropenia	3	9	21	37	23	0.59
	4	1	2	1	1	
Esophagitis	3	2	5	14	9	0.79

Response and Survival

There were 3 patients showing complete response (CR), 38 showing partial response (PR) and 2 showing stable disease (SD) among the 43 female patients evaluable for response, and 10 patients showing CR, 116 showing PR, 24 showing SD and 7 showing progressive disease among the 157 male patients evaluable for response. The response rate was higher in the female than in the male patients (93% vs. 79%, $P = 0.028$). Disease progression was noted in 36 of the 44 (82%) female patients and 131 of the 160 (82%) male patients. The median PFS did not differ significantly between the sexes: 9.2 months in the females and 9.7 months in the males ($P = 0.67$, Fig. 1). The median survival time in the female and male patients was 22.3 and 24.3 months, respectively ($P = 0.64$, Fig. 2). Survival analyses in subgroups showed the

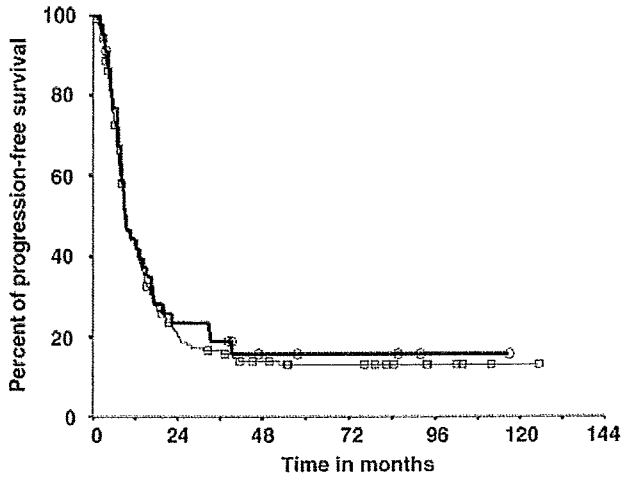

Figure 1. Progression-free survival by sex. Thick line, females; thin line, males.

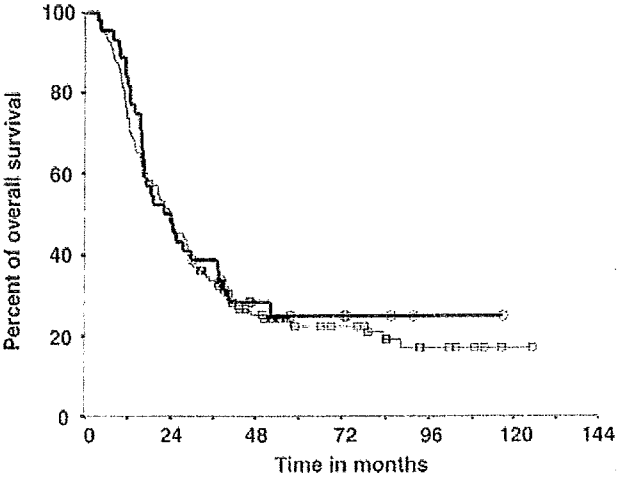

Figure 2. Overall survival by sex. Thick line, females; thin line, males.

Table 3. Factors associated with overall survival

Variables	Hazard ratio (95% confidence interval)	
	Univariate analyses	Multivariate analyses
Age	1.01 (0.99–1.03)	—
Sex		
Female	1	1
Male	1.10 (0.74–1.62)	1.16 (0.71–1.90)
Smoking habit		
No	1	1
Yes	1.00 (0.63–1.59)	0.75 (0.41–1.36)
Body weight loss		
≤4.9%	1	—
≥5.0%	1.19 (0.81–1.75)	—
Performance status		
0	1	1
1–2	1.59 (1.11–2.28)	1.44 (0.97–2.15)
Histology		
Adenocarcinoma	1	1
Non-adenocarcinoma	0.76 (0.53–1.10)	0.74 (0.51–1.08)
Stage		
IIIA	1	1
IIIB	0.96 (0.70–1.32)	0.79 (0.56–1.11)
Period		
1994–99	1	1
2000–05	0.62 (0.45–0.86)	0.65 (0.45–0.92)

absence of any gender differences either among patients with adenocarcinoma or among those with non-adenocarcinoma. Similarly, no gender differences were observed either among smokers or among never-smokers. Univariate Cox's proportional hazard analyses showed that the performance status and treatment period were significantly associated with the survival (Table 3). After adjustment for the smoking history and histological type, the gender had no impact on the overall survival (Table 3).

DISCUSSION

Although prospective cohort studies and a population-based study have reported better survival in women than in men with NSCLC, these results may be biased by potential confounding factors, because these studies included highly heterogeneous patients in terms of the stage, therapy, co-morbidities and other prognostic factors (2–4). Thus, whether there is any significant difference in survival between male and female patients receiving radiation-based treatment remained controversial, and this study failed to show any significant gender difference in the survival in NSCLC patients receiving concurrent chemoradiotherapy.

Several previous studies have suggested a better prognosis in female than in male NSCLC patients treated by surgery (2,14–18), whereas our results were inconsistent with this suggestion. This may be attributable to the difference in the distribution of the disease stage (pathological stages I, II and III) between these studies and our study, including pathological stages I, II and III. The magnitude of the gender difference in survival has been suggested to vary with the disease stage. Some studies have shown a diminishing gender difference as the disease stage advanced from stages I to III, with disappearance of the gender difference among patients with stage III disease (14,15), whereas others have shown relatively constant gender difference through all the disease stages (2,16,17). A study on the gender difference in the survival in surgically resected NSCLC patients showed a better overall survival in women than men, but no significant difference in the cancer-specific survival between the two sexes (18). These results suggest that the gender difference in survival in NSCLC patients undergoing curative surgery, especially patients with early-stage disease, can be explained by the mortality related to diseases other than lung cancer.

Among local or locally advanced NSCLC patients receiving radiotherapy-based treatment, the gender difference in survival has been controversial (5–9), but potential confounding factors in these studies prevent an accurate interpretation of the results. In these studies, as high as 30% of the patients had medically inoperable stage I–II disease and 3–22% of the patients had a performance status of 2. In addition, 36–100% of patients were treated by thoracic radiation alone, whereas the others also received some form of chemotherapy as part of the treatment. Neither the current study nor another previous study showed any gender difference in the survival (10). The patients in both of these studies were limited to stage III NSCLC patients with a performance status of 0–1 who were treated by concurrent chemoradiotherapy.

Several studies have been conducted on the gender differences in survival among patients with stage IIIB–IV disease treated by systemic chemotherapy (19–24). Of these, many showed a better survival in female patients than in male patients (19–22), but the causes of this gender difference in survival remain unknown. Our previous study also showed a better survival in female patients, which was explained partly by the large number of female patients (56% vs. 44%) receiving gefitinib, and the 4-fold longer duration of gefitinib treatment (144 vs. 35 days) in these patients (25). In contrast, only 18% of the female patients and 16% of the male patients received EGFR-TKIs in this study. Thus, treatment with EGFR-TKIs had little influence on the patient survival in this study.

Clear difference in the frequency of adenocarcinoma and smoking history between female and male patients has been reported repeatedly, and this study also showed that adenocarcinoma and never-smokers were more common among the female patients. Thus, it would be reasonable to think that differences in the tumor cell characteristics between the

female and male patients may be responsible for the difference in survival between the two sexes. However, survival analyses conducted separately in subgroups among patients with adenocarcinoma and those with non-adenocarcinoma, or among smokers and non-smokers have failed to reveal any gender differences in the survival among any subgroups. In addition, a multivariate analysis showed no difference in survival between the sexes after adjustment for the tumor histology and smoking history.

The threshold for drug toxicity may also differ between women and men. In general, chemotherapy-related toxicity is reported to be slightly more severe in women, and to the best of our knowledge, there are no reports on the gender difference in radiation-related toxicity. This study showed no difference in the severity of esophagitis or hematological toxicity between the two sexes. We did not examine pulmonary toxicity in this study, because our previous large retrospective study showed no difference in the incidence or grade of pulmonary toxicity between the sexes (26).

Among several limitations of this study, the most important is the small sample size that made it difficult to draw definitive conclusions. Indeed, small difference in survival between the sexes, if any, could not be detected in this small number of patients. It is difficult, however, to expand the study population without an increase in its heterogeneity. A population-based study with >20 000 patients, for example, included patients with all stages of lung cancer, and the therapies administered were not specified. Furthermore, the quality of data on diagnosis and treatment was not uniform (4). Thus, the results of that study may be biased, despite of the huge number of patients. We cannot overlook this problem especially when analyzing stage III NSCLC patients treated with radiation-based treatment, because the quality control of radiotherapy has not been fully developed in Japan, and therefore, indication, methods and outcomes of thoracic radiotherapy may vary among hospitals.

In conclusion, this study failed to reveal any significant differences in the treatment outcomes, including survival and treatment toxicity, between female and male patients with stage III NSCLC receiving concurrent chemoradiotherapy. These results are in sharp contrast to the reported better survival in female patients with localized disease treated by surgery or those with metastatic disease treated by systemic chemotherapy.

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Conflict of interest statement

None declared.

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Gefitinib or Carboplatin–Paclitaxel in Pulmonary Adenocarcinoma

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ABSTRACT

BACKGROUND

Previous, uncontrolled studies have suggested that first-line treatment with gefitinib would be efficacious in selected patients with non–small-cell lung cancer.

METHODS

In this phase 3, open-label study, we randomly assigned previously untreated patients in East Asia who had advanced pulmonary adenocarcinoma and who were nonsmokers or former light smokers to receive gefitinib (250 mg per day) (609 patients) or carboplatin (at a dose calculated to produce an area under the curve of 5 or 6 mg per milliliter per minute) plus paclitaxel (200 mg per square meter of body-surface area) (608 patients). The primary end point was progression-free survival.

RESULTS

The 12-month rates of progression-free survival were 24.9% with gefitinib and 6.7% with carboplatin–paclitaxel. The study met its primary objective of showing the noninferiority of gefitinib and also showed its superiority, as compared with carboplatin–paclitaxel, with respect to progression-free survival in the intention-to-treat population (hazard ratio for progression or death, 0.74; 95% confidence interval [CI], 0.65 to 0.85; P<0.001). In the subgroup of 261 patients who were positive for the epidermal growth factor receptor gene (*EGFR*) mutation, progression-free survival was significantly longer among those who received gefitinib than among those who received carboplatin–paclitaxel (hazard ratio for progression or death, 0.48; 95% CI, 0.36 to 0.64; P<0.001), whereas in the subgroup of 176 patients who were negative for the mutation, progression-free survival was significantly longer among those who received carboplatin–paclitaxel (hazard ratio for progression or death with gefitinib, 2.85; 95% CI, 2.05 to 3.98; P<0.001). The most common adverse events were rash or acne (in 66.2% of patients) and diarrhea (46.6%) in the gefitinib group and neurotoxic effects (69.9%), neutropenia (67.1%), and alopecia (58.4%) in the carboplatin–paclitaxel group.

CONCLUSIONS

Gefitinib is superior to carboplatin–paclitaxel as an initial treatment for pulmonary adenocarcinoma among nonsmokers or former light smokers in East Asia. The presence in the tumor of a mutation of the *EGFR* gene is a strong predictor of a better outcome with gefitinib. (ClinicalTrials.gov number, NCT00322452.)

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Inhibitors of the epidermal growth factor receptor (EGFR) tyrosine kinase have clinical efficacy, as compared with the best supportive care[1] or standard chemotherapy,[2] when given as second-line or third-line therapy for advanced non–small-cell lung cancer. Treatment with EGFR tyrosine kinase inhibitors is most effective in women, patients who have never smoked, patients with pulmonary adenocarcinomas, and patients of Asian origin. In these populations, such treatment is associated with favorable rates of objective responses, progression-free survival, and overall survival.[1,3,4] These populations also have a relatively high incidence of somatic mutations in the region of the *EGFR* gene that encodes the tyrosine kinase domain.[5,6] Studies have shown that in patients with pulmonary adenocarcinoma who had a base-pair deletion at exon 19 (del746_A750) or a point mutation at exon 21 (L858R), the tumors were highly responsive to EGFR tyrosine kinase inhibitors,[7-9] and subsequent studies of first-line therapy with these agents showed objective response rates of 54.8 to 81.6% and progression-free survival of 9.7 to 13.3 months among patients with these mutations.[10-12]

On the basis of these and other studies,[1,4,13-16] we hypothesized that in a selected population, first-line therapy with an oral EGFR tyrosine kinase inhibitor would be at least as effective as chemotherapy with carboplatin–paclitaxel. In this study, we compared the efficacy, safety, and adverse-event profile of gefitinib with those of carboplatin–paclitaxel when these drugs were used as first-line treatment in nonsmokers or former light smokers in East Asia who had adenocarcinoma of the lung. We also examined the role of an *EGFR* mutation as a predicator of the efficacy of gefitinib or carboplatin–paclitaxel.

METHODS

STUDY DESIGN AND PATIENTS

The First Line Iressa versus Carboplatin/Paclitaxel in Asia (Iressa Pan-Asia Study [IPASS]) study was a phase 3, multicenter, randomized, open-label, parallel-group study comparing gefitinib (Iressa, AstraZeneca) with carboplatin (Paraplatin, Bristol-Myers Squibb) plus paclitaxel (Taxol, Bristol-Myers Squibb) as first-line treatment in clinically selected patients in East Asia who had advanced non–small-cell lung cancer. The primary end point was progression-free survival. Secondary end points included overall survival (an early analysis, since follow-up is ongoing), the objective response rate, quality of life, reduction in symptoms, safety, and the adverse-event profile. Evaluations of efficacy according to the baseline biomarker status of EGFR were planned exploratory objectives.

Patients were eligible for inclusion in the study if they were 18 years of age or older, had histologically or cytologically confirmed stage IIIB or IV non–small-cell lung cancer with histologic features of adenocarcinoma (including bronchoalveolar carcinoma), were nonsmokers (defined as patients who had smoked <100 cigarettes in their lifetime) or former light smokers (those who had stopped smoking at least 15 years previously and had a total of ≤10 pack-years of smoking), and had had no previous chemotherapy or biologic or immunologic therapy. Other eligibility criteria are described in the Supplementary Appendix, available with the full text of this article at NEJM.org.

The principal investigators and the members of the steering committee (see the Appendix at the end of this article) designed the study in collaboration with the sponsor (AstraZeneca) and supervised the conduct of the trial. The sponsor collected and analyzed the data. The lead academic author had unrestricted access to the data and vouches for the validity and completeness of the results of the trial (see the Supplementary Appendix for further details). All patients provided written informed consent; separate consent was provided for the assessment of EGFR biomarkers. An independent ethics committee at each participating institution approved the study protocol. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization Guidelines for Good Clinical Practice, applicable regulatory requirements, and AstraZeneca's policy on bioethics. One planned interim analysis was performed by an independent statistician and reviewed by an independent data and safety monitoring committee (see the Supplementary Appendix).

STUDY TREATMENT

Patients were randomly assigned, in a 1:1 ratio, to receive gefitinib (250 mg per day, administered orally) or paclitaxel (200 mg per square meter of body-surface area, administered intravenously over a 3-hour period on the first day of the cycle) fol-

lowed immediately by carboplatin (at a dose calculated to produce an area under the concentration–time curve of 5.0 or 6.0 mg per milliliter per minute, administered intravenously over a period of 15 to 60 minutes) in cycles of once every 3 weeks for up to 6 cycles. Randomization was performed with the use of dynamic balancing[17] with respect to performance status, as assessed by the World Health Organization (WHO) performance scale measuring activity (0 or 1, or 2 on a scale of 0 to 4, with lower numbers indicating a higher degree of activity); smoking status (nonsmoker or former light smoker); sex; and center. Treatment continued until progression of the disease, development of unacceptable toxic effects, a request by the patient or physician to discontinue treatment, serious noncompliance with the protocol, or completion of six chemotherapy cycles. Among patients assigned to gefitinib therapy, those whose tumor progressed were offered the opportunity to switch to treatment with carboplatin–paclitaxel; however, if the patient declined or was not a good candidate for that treatment, he or she could receive another approved therapy of the physician's choice. Among patients who were receiving carboplatin–paclitaxel, further therapy after progression of the disease was at the physician's discretion.

ASSESSMENTS

Progression-free survival was assessed from the date of randomization to the earliest sign of disease progression, as determined by means of the Response Evaluation Criteria in Solid Tumors (RECIST),[18] or death from any cause. Overall survival was assessed from the date of randomization until death from any cause. Tumor response was assessed every 6 weeks until disease progression. Quality of life was assessed with the use of the Functional Assessment of Cancer Therapy–Lung (FACT–L) questionnaire (in which scores range from 0 to 136, with higher scores indicating better quality of life) and the Trial Outcome Index (TOI, which is the sum of the physical well-being, functional well-being, and lung-cancer subscale [LCS] scores of FACT-L; scores range from 0 to 84, with higher scores indicating better quality of life), and symptoms were assessed with the use of the LCS score (scores range from 0 to 28, with higher scores indicating fewer symptoms). The FACT-L questionnaire[19] was administered at randomization and at week 1, once every 3 weeks until day 127, once every 6 weeks from day 128 until disease progression, and when the study drug was discontinued. Clinically relevant improvement was predefined as an improvement of six points or more in FACT-L and TOI scores or an improvement of two points or more in LCS scores, with the higher scores maintained for at least 21 days.[20] Safety and tolerability were assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Tumor samples from patients who consented to have biomarkers assessed were analyzed at two central laboratories to determine biomarker status, with *EGFR* mutation status the first priority. Patients were considered to be positive for the *EGFR* mutation if 1 of 29 *EGFR* mutations was detected with the use of the amplification refractory mutation system (ARMS) and the DxS EGFR29 mutation-detection kit.[21,22]

STATISTICAL ANALYSIS

The primary end point (progression-free survival) was analyzed with the use of a Cox proportional-hazards model in the intention-to-treat population (all randomly assigned patients) to assess the noninferiority of gefitinib as compared with carboplatin–paclitaxel, with the WHO performance status (0 or 1, or 2), smoking status (nonsmoker or former light smoker), and sex as covariates. For noninferiority to be demonstrated, the 95% confidence interval for the hazard ratio had to lie entirely below the predefined noninferiority limit of 1.2. We estimated that with a total of 944 progression events, the study would have 80% power to demonstrate noninferiority if the treatments were truly equal, with a two-sided 5% probability of an erroneous demonstration of noninferiority. If the 95% confidence interval for the hazard ratio was also below 1, the P value would be less than 0.05 and superiority could be concluded from the same analysis without statistical penalty (closed test procedure).[23] Supportive secondary analyses are described in the Supplementary Appendix. Planned subgroup analyses were performed to compare progression-free survival between treatments in groups defined according to WHO performance status (0 or 1, or 2), smoking status (nonsmoker or former light smoker), sex, age at randomization (<65 years or ≥65 years), disease stage at screening (stage IIIB or IV), and presence or absence of biomarkers. Tests to determine in-