Dataset S2. Relationship between the Three Polymorphisms and *EGFR* Mutations

Found at doi:10.1371/journal.pmed.0040125.sd006 (55 KB DOC).

Dataset S3. Mutations Target the *CA-SSR1* Allele Having the Lower Number of Repeats

Found at doi:10.1371/journal.pmed.0040125.sd007 (48 KB DOC).

Figure S1. The Prognosis of Patients Based on the Average Length of the Shorter Allele of *CA-SSR1*

Overall survival curves for patients having a short allele of *CA-SSR1* under versus over the average length (17.5). Survival was not influenced by the minor forms of the −191 or −216 polymorphisms (data not shown). Note that none of the patients received TKI therapy.

Found at doi:10.1371/journal.pmed.0040125.sg001 (86 KB PPT).

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Author contributions. M. Nomura, H. Shigematsu, P. Estess, M. Siegelman, and A. F. Gazdar designed the study. M. Nomura, H. Shigematsu, T. Takahashi and I. I. Wistuba collected data or performed experiments for the study. M. Nomura made the primer sets for the target genes and modified the conditions of PCR reactions. M. Suzuki, H. Shigematsu, and I. I. Wistuba collected the samples for the study and their clinicopathological data. A. F. Gazdar supervised the analysis of the data. M. Nomura, L. Li, Z. Feng, H. Kato, J. D. Minna, and A. F. Gazdar analyzed the data. P. Estess interpreted early data, designed subsequent approaches, and provided expertise in experimental approaches. M. Siegelman provided technical expertise and instrumentation to perform the analysis. A. Marchetti analyzed the DNA samples for *EGFR* mutations. M. Suzuki, H. Shigematsu, A. Marchetti, M. R. Spitz, and I. I. Wistuba enrolled patients. A. Marchetti collected tissues and data from Italian patients in the study and extracted DNA samples from tissues. M. R. Spitz provided the DNA samples from normal individuals in the US. I. I. Wistuba provided the DNA samples from patients in US. J. W. Shay and J. D. Minna provided HBECs. All authors contributed to writing the paper.

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Editors' Summary

Background. Most cases of lung cancer—the leading cause of cancer deaths worldwide—are "non-small cell lung cancer" (NSCLC), which has a very low cure rate. Recently, however, "targeted" therapies have brought new hope to patients with NSCLC. Like all cancers, NSCLC occurs when cells begin to divide uncontrollably because of changes (mutations) in their genetic material. Chemotherapy drugs treat cancer by killing these rapidly dividing cells, but because some normal tissues are sensitive to these agents, it is hard to kill the cancer completely without causing serious side effects. Targeted therapies specifically attack the changes in cancer cells that allow them to divide uncontrollably, so it might be possible to kill the cancer cells selectively without damaging normal tissues. Epidermal growth factor receptor (EGFR) was one of the first molecules for which a targeted therapy was developed. In normal cells, messenger proteins bind to EGFR and activate its "tyrosine kinase," an enzyme that sticks phosphate groups on tyrosine (an amino acid) in other proteins. These proteins then tell the cell to divide. Alterations to the signaling system drive the uncontrolled growth of some cancers, including NSCLC.

Why Was This Study Done? Molecules that inhibit the tyrosine kinase activity of EGFR (for example, gefitinib) dramatically shrink some NSCLCs, particularly those in East Asian patients. Tumors driven by tyrosine kinase inhibitor–sensitive forms of EGFR often (but not always) have mutations in EGFR's tyrosine kinase. However, not all tumors with these mutations respond to TKIs, and other genetic changes—for example, amplification (multiple copies of the *EGFR* gene)—also affect tumor responsiveness to TKIs. It would be useful to know what genetic changes predict these responses, when planning treatments for NSCLC and to understand why the response to these changes varies between ethnic groups. In this study, the researchers have examined these polymorphisms—differences in DNA sequences that occur in individuals—in the *EGFR* gene in people with and without NSCLC. In addition, they have looked for associations between these polymorphisms (which are present in every cell in the body) and the *EGFR* gene mutations and allele imbalances (genes occur in pairs; amplification or loss of one copy of a pair often causes allelic imbalance) found in tumors in NSCLC.

What Did the Researchers Do and Find? The researchers measured how often three *EGFR* polymorphisms (the length of a repeat sequence called CA-SSR1, and two single-nucleotide variations, SNP1 and SNP2) in which plausibly affect how much protein is made from the *EGFR* gene occurred in normal tissue and NSCLC tissue from East Asians and individuals of European descent. They also looked for mutations in the EGFR tyrosine kinase and allelic imbalance in the tumors and then determined which genetic variations and alterations tended to occur together in people with the same ethnicity. Among many associations, the researchers found that shorter alleles of CA-SSR1 and the minor forms of the two SNPs occurred less often in East Asians than in individuals of European descent. They also confirmed that *EGFR* kinase mutations were more common in NSCLCs in East Asians than in Europeans and European descent individuals. Furthermore, mutations occurred more often in tumors with allelic imbalance, and in tumors where there was allelic imbalance and an *EGFR* mutation, the mutant allele was amplified more often than the wild-type allele.

What Do These Findings Mean? The researchers use these associations between gene variants and tumor-associated alterations to propose a model to explain the ethnic differences in mutational frequencies and responses to TKIs seen in NSCLC. They suggest that because of the polymorphisms in the *EGFR* gene commonly seen in East Asians, people from this ethnic group make less EGFR protein than people from other ethnic groups. This would explain why only a threshold level of EGFR is needed to drive cells toward malignancy. East Asians have a high frequency of amplified *EGFR* tyrosine kinase mutations in their tumors—mutation followed by amplification would be needed to activate EGFR signaling. This model, speculative help to explain some clinical findings, such as the rarity of *EGFR* mutations and of TKI sensitivity in NSCLC in East Asians. Further studies of this type in different ethnic groups and in different tumors as well as with other genes for which targeted therapies are available, should help oncologists provide personalized cancer therapies for their patients.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0040125.

- U.S. National Cancer Institute information on lung cancer and on cancer treatment for patients and professionals
- American Cancer Society information for patients about all aspects of lung cancer, including treatment with TKIs
- Wikipedia pages on lung cancer, EGFR, and gefitinib (note that Wikipedia is a free online encyclopedia that anyone can edit)

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Predictive factors associated with prolonged survival in patients with advanced non-small-cell lung cancer (NSCLC) treated with gefitinib

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This study aimed to identify predictive factors associated with prognostic benefits of gefitinib. A total of 221 Japanese patients who received gefitinib (250 mg day^{-1}) were examined retrospectively and potential predictive factors analysed. Overall response rate (ORR) was 24.4% and median survival time (MST) was 8.0 months. In a log-rank test, survival was significantly better in females, patients with adenocarcinoma, never-smokers, favourable performance status (PS) and patients with epidermal growth factor receptor (EGFR) mutation. The lower the smoking exposure (Brinkman Index (BI) = cigarettes per day × years smoked), the better the MST (BI 0: 14.5 months, BI <500: 9.5 months, BI 500 to <1000: 6.9 months, BI ⩾1000: 4.0 months). Positive-EGFR mutation status and PS 0–1 were independent predictors of favourable prognosis by multivariate analysis. Prognosis was significantly different according to EGFR mutation status (with the same smoking status), but not according to smoking status (with the same EGFR mutation status). EGFR mutation status is the most important independent predictor of survival benefit with gefitinib treatment. Although differences in prognosis were observed according to relative smoking status and smoking exposure, the results suggested that smoking is not a direct predictor of prognosis, yet is a surrogate marker of EGFR mutation status.
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Keywords: epidermal growth factor receptor (EGFR) inhibitor; EGFR mutations; gefitinib; IRESSA; non-small-cell lung cancer; smoking

Gefitinib (IRESSA) is an orally active small-molecule compound that inhibits the epidermal growth factor receptor (EGFR) tyrosine kinase (TK) by competing with adenosine triphosphate (ATP) at the ATP-binding site. In two large Phase II trials (IDEAL: IRESSA Dose Evaluation in Advanced Lung cancer 1 and 2) gefitinib-induced tumour regression and provided symptom relief in previously treated patients with non-small-cell lung cancer (NSCLC) (Fukuoka *et al*, 2003; Kris *et al*, 2003). Although a placebo-controlled Phase III study (ISEL) in previously-treated patients with NSCLC has not shown a statistically significant improvement in survival associated with gefitinib, preplanned subgroup analysis suggested survival benefits in patients of Asian origin and never-smokers (Thatcher *et al*, 2005). Patient selection criteria were not incorporated in this comparative study, which most likely contributed to the absence of a positive survival benefit in the overall population. In fact, a Phase II study in which gefitinib was used as first-line therapy for NSCLC in a subgroup of never-smokers with adenocarcinoma reported favourable outcomes, with an overall response rate (ORR) of 61% (Lee *et al*, 2005).

In 2004, mutations in the *EGFR* gene conferring increased sensitivity to gefitinib were reported (Lynch *et al*, 2004; Paez *et al*, 2004). Recently, very favourable outcomes (response rate (RR) 75%) in a Phase II study of gefitinib as first-line therapy for patients with NSCLC with *EGFR* gene mutations has been reported (Inoue *et al*, 2006).

Therefore, it is important to conduct patient selection before using gefitinib and, in particular, it is vital to identify the predictive factors that may contribute to survival. To aid future selection of patient groups for gefitinib treatment we conducted a retrospective analysis of patients who had been treated with gefitinib, assessing the relationship between clinical characteristics, the EGFR mutation status, antitumour activity and patient survival.

PATIENTS AND METHODS

Patients

A total of 221 patients who had been initiated on gefitinib monotherapy (250 mg day^{-1}) during a 3-year span from July 2002 (gefitinib was launched in Japan) to June 2005 at the Hyogo Medical Center for Adults in Japan were retrospectively examined.

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Clinical assessments

Clinical parameters studied were gender, age, smoking history (Brinkman Index (BI) = number of cigarettes per day × number of years smoked), Eastern Cooperative Oncology Group performance status (PS) and previous lines of chemotherapy.

Assessment of tumour regression was conducted according to the response evaluation criteria in solid tumours (RECIST) guideline. The National Cancer Institute Common Toxicity Criteria, version 3.0, was used to evaluate toxicity.

EGFR gene analysis

EGFR gene mutation detection was performed on samples from 106 patients: surgical specimens were obtained from 34 patients and a transbronchial lung biopsy (TBLB) was performed on 72 patients. EGFR mutation analysis was successfully performed in 91 of the 106 samples. EGFR mutation was analysed at Aichi Cancer Center Hospital in Japan. A cycleave PCR technique for codon 858 of *EGFR* gene was used on a SmartCycler system (SC-100, Cepheid, Sunnyvale, CA, USA). Deletion in exon 19 of the *EGFR* gene was detected with fragment analysis using an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA) (Yatabe *et al*, 2006). Many of the cases began treatment on gefitinib before it had been reported that EGFR mutation detection was important when treating with the drug. Moreover, many of those cases had already died before our plans to undergo EGFR mutation detection, effectively preventing us from obtaining informed consent in this regard. Accordingly, our Institutional Review Board approved our study plan, provided that samples would be processed anonymously, that samples would be analysed only for somatic mutations and not germline mutations, and that the presence of the study be publicly disclosed, strictly according to the 'Ethical Guidelines for Human Genome Research' published by The Ministry of Education, Culture, Sports, Science and Technology, The Ministry of Health, Labour and Welfare and The Ministry of Economy, Trade and Industry, Japan. (http://www.mext.go.jp/a_menu/shinkou/seimei/genome/04122801.htm).

Statistical analysis

OS analysis was conducted on all 221 and 91 patients in which EGFR mutation analysis could be successfully performed.

The differences in responders (complete response; CR + partial response; PR) by each factor (gender, PS, histology, prior chemotherapy, smoking status and mutation status) were examined with the Fisher's exact test. The difference in mutation rate among groups categorised by smoking exposure was examined with χ^2 test.

An OS curve was plotted using the Kaplan–Meier method and survival curve comparisons were conducted with the log-rank test. Univariate analysis and multivariate analysis of the impact of the factors, including gender (male vs female), smoking history (ever-smokers vs never-smokers), histology (adenocarcinoma vs others), PS (PS 0–1 vs 2–4) and EGFR mutation (positive vs negative) were conducted using the Cox regression model. All analysis was determined to be statistically significant where the *P*-value was <0.05.

Analyses were conducted using the SPSS 11.0.1.

RESULTS

Patient characteristics

The clinical characteristics of the patients are shown in Table 1. The majority of patients (89%) had adenocarcinoma histology. One hundred and thirty-one patients (59%) were ever-smokers.

EGFR mutation analysis and clinical response

TBLB or surgical samples were available from 106 patients for EGFR-mutation detection, but actual analysis was only possible for 103 patients because tumour cells were not found in three post-treatment specimens.

DNA could not be amplified in 12 cases. Analysis of the remaining 91 samples showed EGFR mutations in 28 patients (30.8%) and wild type in 63 patients (69.2%). EGFR mutation rate was high in females, never-smokers and patients with adenocarcinoma. Among ever-smokers, EGFR mutation rate was higher in patients with BI < 500 and BI 500 to 1000 than BI > 1000 (Table 2). Of the 28 EGFR mutations, 19 (67.9%) were exon 19 in-frame deletions and nine (32.1%) were exon 21-point mutations (L858R). Seven (36.8%) of the exon 19 deletions and four (44.4%) of the L858R cases were smokers. Significantly high mutation rates were observed in females and never-smokers.

In the overall population, RR was 24.4% (95% confidence interval (CI) 18.0–30.6%) (Table 1). Response rate was significantly higher among females, patients with adenocarcinoma histology, never-smokers and patients with the EGFR mutation. Disease control rate (DCR: CR + PR + stable disease; SD) was 51.1% (95% CI 44.3–57.9%) and among those with EGFR mutation, 100%.

Survival analyses

Median survival time (MST) in the overall population was 8 months, with 34.8% surviving 1 year. MST among patients showing PR was significantly longer than that in the SD cases ($P = 0.003$) and MST of the SD cases was also shown to be significantly longer than that of the PD cases ($P < 0.0001$) (Table 1).

Kaplan–Meier curves indicated significantly longer survival in patients with favourable PS ($P < 0.0001$), in patients with adenocarcinoma histology ($P < 0.0001$), in never-smokers ($P < 0.0001$), and in patients with EGFR mutations ($P < 0.0001$) (Table 1, Figure 1). L858R patients tend to survive longer than those with deletions at exon 19 ($P = 0.0539$). Multivariate analysis was conducted to identify factors contributing to survival. When all patients were analysed considering of the clinical characteristics (gender, adenocarcinoma histology, smoking status and PS), adenocarcinoma, never-smoker status and PS 0–1 were found to be prognostic factors of survival (Table 3a). However, analysis (including that on EGFR mutation status and clinical characteristics) of the patients for whom EGFR mutation results were obtained showed PS 0–1 and *EGFR* gene mutation status to be the independent prognostic factors, and the relationship between smoking status and survival did not reach statistical significance (Table 3b).

Further analysis of smoking exposure and survival indicated that the higher the exposure, the shorter the MST (Figure 2) . The presence of EGFR mutation was associated with significantly prolonged survival in both never-smokers ($P = 0.014$) and ever-smokers ($P = 0.012$). Furthermore, among EGFR mutation-positive patients, there was no statistically significant difference in median survival between never-smokers and ever-smokers ($P = 0.864$), although patient numbers were small (Figure 3).

Tolerability

Adverse events were observed in 165 out of 221 (75%) patients. Common adverse events were rash/dry skin (51%), diarrhoea (22%), liver dysfunctions (20% (2.3% were Grade 3)) and paronychia (14%). Sixteen (7%) of the patients developed interstitial lung disease (ILD) and three (1.4%) died. As three out of 14 (21%) patients with PS 3 developed ILD, patients with poorer PS were more likely to develop ILD. There were no differences in ILD incidence by gender, smoking history, age or

Table 1 Demographics and relationship between clinical variables and antitumor response/overall survival in patients treated with gefitinib

Characteristic	No. of patients (%)	PR (n)	RR (%)	(95% CI)	P-value[a]	MST (months)	(95% CI)	P-value
All	221	54	24.4	(18.9–30.6)		8	(6.66–9.34)	
Gender								
Male	142 (64)	20	14.1	(8.8–20.9)	<0.001	6.8	(5.04–8.56)	0.036
Female	79 (36)	34	43	(31.9–54.7)		13.3	(8.84–17.76)	
Age								
65<	100	20	20	(12.7–29.2)	0.208	9	(6.41–11.59)	0.2852
<65	121	34	28.1	(20.3–37.0)		7.3	(5.88–8.72)	
ECOG PS								
0–1	160 (72)	44	27.5	(20.7–35.1)	0.114	11.1	(8.30–13.90)	<0.001
2–4	61 (28)	10	16.4	(8.2–28.1)		2.1	(1.26–2.94)	
Histology								
Adenocarcinoma	196 (89)	52	26.5	(20.5–33.3)	0.048	9.3	(7.66–10.94)	0.137
Others	25 (9)	2	8	(1.0–26.0)		3.6	(2.13–5.07)	
Prior chemotherapy								
Yes	188 (85)	45	24.6	(18.5–43.3)	1	8.1	(6.67–9.53)	1
No	33 (15)	9	25.7	(12.5–43.3)		8.4	(5.72–11.08)	
Smoking history (n = 220)								
No	89 (40)	37	41.6	(31.2–52.5)	<0.001	14.5	(10.87–18.13)	<0.001
Yes	131 (15)	17	13	(7.7–20.0)		6.5	(4.36–8.64)	
BI 1 <500	25 (11)	5	20	(6.8–40.7)		9.5	(6.41–12.59)	
BI 500 to <1000	59 (27)	9	15.3	(7.2–27.0)		6.9	(5.64–8.16)	
BI≥1000	45 (21)	2	4.4	(0.5–15.1)		4	(3.00–5.00)	
EGFR gene status (n = 91)								
Wild type	63 (69)	7	11.1	(4.6–21.6)	<0.001	7.4	(4.84–9.96)	<0.001
Mutation positive	28 (31)	20	71.4	(51.3–86.8)		24.9	(14.27–35.53)	
Exon 19 deletion	19 (21)	15	78.9	(54.4–93.9)		16.1	(6.22–25.98)	
Exon 21 (L858R)	9 (10)	5	55.6	(21.2–86.3)		>34.5	—	
Tumor response (n = 191)								
PR	54	—	—			26.2	(15.76–36.64)	0.003
SD	59	—	—			11.9	(7.47–16.33)	<0.0001
PD	78	—	—			5.6	(3.20–8.00)	

Abbreviations: BI = Brinkman Index; BI = defined as number of cigarettes per day × number of years smoking; CI = confidence interval; EGFR = epidermal growth factor receptor; ECOG PS = Eastern Cooperative Oncology Group performance status; MST = median survival time; PD = progressive disease; PR = partial response; RR = response rate; SD = stable disease. [a]Fisher's exact test.

Table 2 Mutation rate by patient background

Population	N	Mutation (%)	95%CI	P-value[a]
All samples	91	28 (30.8)		
Male	59	12 (20.3)	11.0–32.8	0.005
Female	32	16 (50.0)	31.9–68.1	
Never-smoker	38	17 (44.7)	28.6–61.7	0.014
Ever-smoker	53	11 (20.8)	10.8–34.1	
Adeno	81	27 (33.3)	23.2–44.7	0.166
Non-adeno	10	1 (10.0)	0.3–44.5	
Brinkman Index				
0	38	17 (44.7)	28.6–61.7	0.055[b]
1≤500	9	2 (22.2)	2.8–60.0	
500≤1000	25	7 (28.0)	12.1–49.4	
1000<	18	2 (11.1)	1.4–34.7	

[a]Fisher's exact test (two-sided). [b]χ^2-test (likelihood ratio).

histology. ILD was experienced by four out of 63 patients with wild type and two out of 28 patients with EGFR mutation (both with an exon 19 deletion).

DISCUSSION

The data from this retrospective study suggest that in a practical setting

(1) A favourable PS, adenocarcinoma histology, never-smoking and presence of an EGFR mutation are predictive of increased antitumour activity with gefitinib,
(2) Although PR cases showed longer median survival than SD cases, SD cases also displayed significantly longer median survival than PD cases,
(3) Although, in terms of clinical characteristics, PS 0–1, adenocarcinoma histology and never-smoking status are predictive factors of survival with gefitinib in the overall population, PS 0–1 and EGFR mutation status were identified as independent predictive factors in patients in which EGFR mutation status has been detected,
(4) The relationships between smoking/EGFR mutation status and survival suggest that the latter is more related to prognosis. Conceivably, smoking has a strong confounding relationship with EGFR mutation status and smoking exposure can result in a different prognosis.

IDEAL 1 reported favourable antitumour activity in females and patients with adenocarcinoma histology (Fukuoka *et al*, 2003).

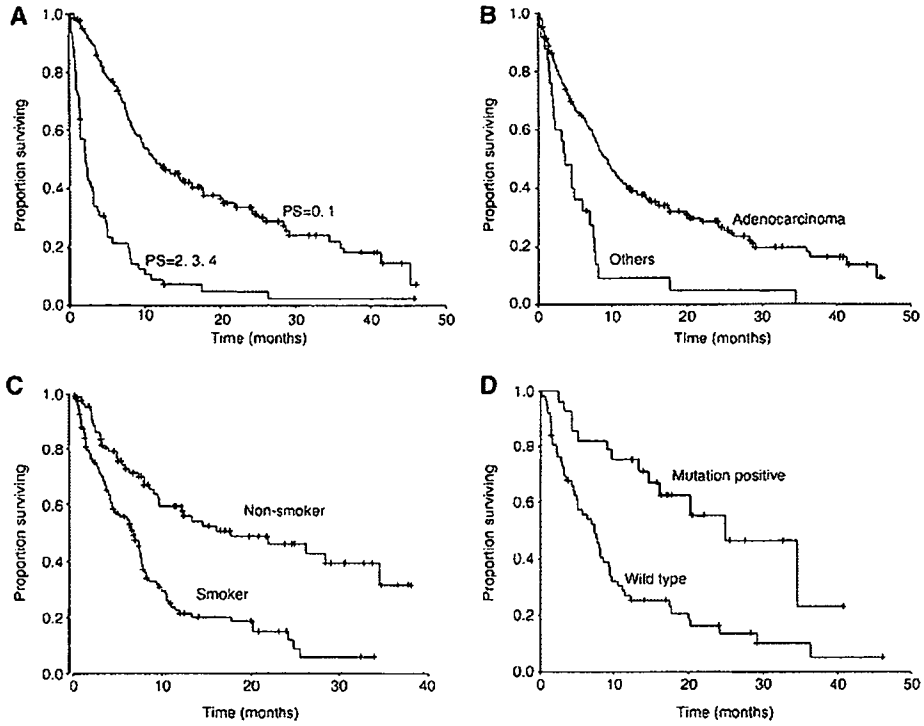


Figure 1 Kaplan–Meier plots of survival for patients receiving gefitinib treatment classified according to (**A**) PS, (**B**) histology, (**C**) smoking status and (**D**) *EGFR* gene mutation status.

Table 3a COX Proportional Hazard Model for Survival Analysis in Overall Population ($N = 221$)

Variable	HR	95%CI	*P*-value
Never-smoker/Ever-smoker	0.413	0.294–0.582	<0.001
Adeno/Non-adeno	0.416	0.265–0.654	<0.001
PS 0, 1/2–4	0.205	0.145–0.291	<0.001

Stepwise method (include <0.05, exclude >0.2). Tested variables: gender, smoking, histology, PS, excluded variable; gender.

Table 3b COX proportional hazard model for survival analysis in patients in which EGFR mutation status has been detected ($n = 91$)

Variable	HR	95%CI	*P*-value
Adeno/Non-adeno	0.581	0.288–1.171	0.129
Never-smoker /ever-smoker	0.607	0.351–1.048	0.073
Mutation negative/positive	2.543	1.345–4.808	0.004
PS 0, 1/2–4	0.166	0.091–0.303	<0.001

Tested variables: gender, smoking, histology, PS, mutation excluded variable; gender.

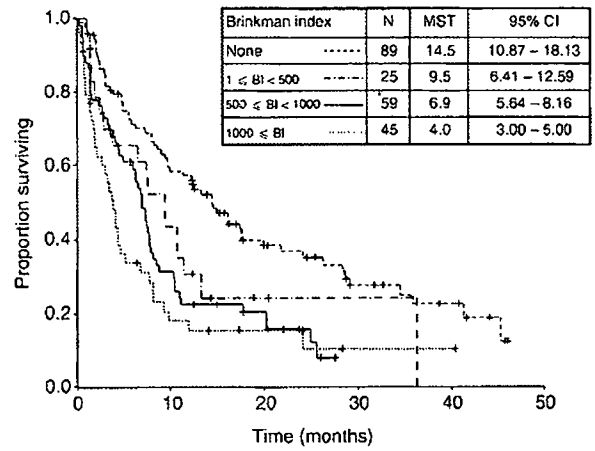
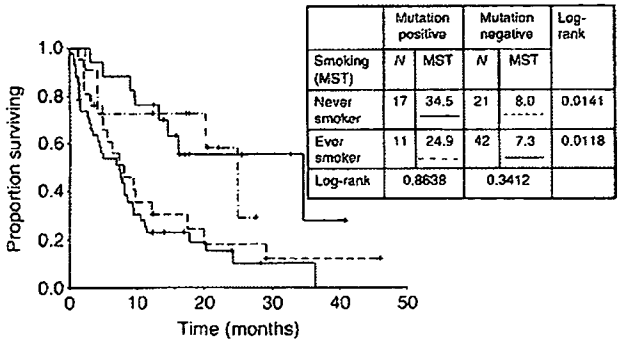


Figure 2 Survival stratified by smoking exposure (classified by BI).

Several subsequent retrospective studies have reported that female gender, adenocarcinoma histology, bronchioloalveolar subtype, never-smokers and patients with favourable PS are predictive factors of response (Miller *et al*, 2004; Kim *et al*, 2005; Lim *et al*, 2005; Ando *et al*, 2006). EGFR mutation has been reported as a predictor of efficacy of gefitinib and erlotinib (Lynch *et al*, 2004; Pao *et al*, 2004). There have been several reports of clinical factors associated with EGFR mutations, and per the univariate analysis, mutation frequency is high in patients of East Asian ethnicity, females, never-smokers and adenocarcinomas (Kosaka *et al*, 2004; Paez *et al*, 2004; Shigematsu *et al*, 2005; Tokumo *et al*, 2005). Moreover, multivariate analysis has shown that adenocarcinoma histology and never-smoker status are independent factors associated with EGFR mutation (Kosaka *et al*, 2004; Tokumo *et al*, 2005). Reports to date have shown that approximately 90% of EGFR mutations are centred around the L858R point mutation in exon 21 and deletions centred around codons 746–750 in exon 19 (Kosaka *et al*, 2004; Shigematsu *et al*, 2005; Sonobe *et al*, 2005; Tokumo *et al*, 2005). As association between these two types of EGFR mutations and the antitumour activity and prolonged survival with gefitinib has been reported (Han *et al*, 2005; Mitsudomi *et al*, 2005), we conducted analysis on these two types of mutations only. Our results were compatible with those from



	Mutation positive		Mutation negative		Log-rank
Smoking (MST)	*N*	MST	*N*	MST	
Never smoker	17	34.5	21	8.0	0.0141
Ever smoker	11	24.9	42	7.3	0.0118
Log-rank	0.8638		0.3412		

Figure 3 Survival stratified by smoking status and *EGFR* gene mutation status.

prospective Phase II studies conducted in patients with EGFR mutation (Inoue *et al*, 2006; Okamoto *et al*, 2006). Epidermal growth factor receptor mutation therefore appears to be a more specific criterion for gefitinib use than patient selection according to clinical characteristics.

The relatively high incidence of ILD (3.5–5.8%) in patients treated with gefitinib has been reported in Japan. It also revealed that male gender, ever-smokers, poor PS and the coincidence of interstitial pneumonia were predictive factors for the development of ILD (Yoshida, 2005; Ando *et al*, 2006). Although these predictive factors contrast with those for the presence of an EGFR mutation, two of the 28 patients with an EGFR mutation developed ILD in our study.

In our examination of prognostic factors, we analysed the relationship particularly between smoking status (ever-/never-smoker, smoking exposure) and two types of EGFR mutations, as well as the relationship between smoking status and EGFR mutation. Our findings indicated that patients with EGFR mutation had significantly longer MST in both ever- and never-smokers, and there was no significant difference in MST between ever- and never-smokers with the same mutation status. This led to the conclusion that the essential factor associated with survival is EGFR mutation status. Though better MST has been reported in L858R cases in a comparison of survival between exon 19 deletion and L858R missense (Shigematsu *et al*, 2005), recent reports have shown better survival in patients with exon 19 deletion (Jackman *et al*, 2006; Riely *et al*, 2006). Incidentally, we found MST to be better in L858R cases. As reported by Jackman *et al* (2006), RR was more favourable among the exon 19 deletion cases. Although this was conceivably due to factors including the ILD being experienced in two cases with exon 19 deletion and the impact of post-gefitinib treatment, the relatively small sample did not allow for any clarification in this respect.

Our data also show that the larger the smoking exposure, the shorter the survival.

There have been several reports of an inverse correlation between smoking exposure and EGFR mutation rate (Han *et al*, 2005; Pham *et al*, 2006; Sugio *et al*, 2006; Tam *et al*, 2006; Toyooka *et al*, 2006). In line with these studies, our data show that smoking status, unlike EGFR mutation status, is not an independent prognostic factor. Considered in combination with past reports on smoking exposure and EGFR mutation rates, the inverse correlation between smoking exposure and MST shown by our data might conceivably reflect that mutation rates differ according to smoking exposure. They also indicate that smoking status is a very powerful surrogate marker of EGFR mutation status, which is a prognostic factor for prolonged survival with gefitinib treatment. Our multivariate analysis in terms of clinical characteristics indicates that smoking status is a significant predictor. However, the multivariate analysis adding EGFR mutation status eliminates the significant difference with regard to smoking, demonstrating that EGFR mutation status and PS 0–1 are independent prognostic factors. This also suggests that ECOG PS and EGFR mutation status are factors that can be used to predict the intrinsic effect of gefitinib on patients as well as their prognosis, supporting the claim that smoking could be a surrogate marker of EGFR mutation status for prediction of survival benefit. Although RR in never-smokers and cases with EGFR mutation on erlotinib, which is also an EGFR-TKI, has been significantly favourable, there has only been marginal significant interaction between survival and smoking status (Clark *et al*, 2006). However, no significant differences have been reported in regard to EGFR mutation status, and detection of the EGFR mutation is considered unnecessary in treatment using erlotinib (Tsao *et al*, 2005; Clark *et al*, 2006). Our results showing that EGFR mutation and smoking status can function as predictors of survival benefit differ from reports on erlotinib. However, they concur with reports to date on gefitinib, presumably suggesting the necessity to select patients before using gefitinib. Further clinical studies are warranted to examine the survival benefits of gefitinib according to EGFR mutation status, that is, to make the EGFR mutation status an inclusion criterion. Considerations should be made in clinical practise to analyse actively EGFR mutations status where possible. However, it is often very difficult to obtain histological specimens of advanced and recurrent lung cancer for which gefitinib is indicated. In fact, in this study we were only able to obtain analytical results for EGFR mutation status for 91 out of 221 (42%) patients. Another problem is EGFR mutation analysis takes time, about 1–3 weeks, necessitating a wait-time before treatment. Therefore, when a certain clinical environment does not allow for, or complicates the detection of EGFR mutations, smoking exposure/smoking status could be a quick and inexpensive reference as a surrogate marker of EGFR mutation status. In future, it will be necessary to evaluate the survival benefits of gefitinib via a Phase III study in patients with these better predictive factors.

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ORIGINAL ARTICLE

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Preliminary experience with a modified premedication protocol that included intravenous diphenhydramine and calcium bromide for the prophylaxis of paclitaxel-related hypersensitivity reactions

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Abstract
Background. Paclitaxel often causes severe hypersensitivity reactions (HSRs) rapidly after infusion, even in patients given prophylactic therapy. The purpose of this study was to analyze the incidence of paclitaxel-related HSRs in patients with non-small cell lung cancer (NSCLC) retrospectively, and to assess the feasibility of a modified premedication protocol.
Methods. One hundred and seven patients who were pretreated with either a conventional premedication regimen (two doses of dexamethasone) or a short premedication regimen (single dose of dexamethasone with oral diphenhydramine and intravenous ranitidine), prior to paclitaxel infusion were retrospectively analyzed. A modified premedication regimen, consisting of 12.5 ml of Rescalmin (intravenous diphenhydramine 50 mg and calcium bromide 437.5 mg), intravenous ranitidine 100 mg, and intravenous dexamethasone 20 mg, was given 30 min prior to paclitaxel, with oral dexamethasone 8 mg given on the night before the paclitaxel. Patients received paclitaxel intravenously at 175 mg/m^2 over 3 h, followed by carboplatin, AUC 5, over 1 h on day 1 every 3 weeks.
Results. In the conventional premedication group, 21 patients had HSRs (32.3%); in 1 of these patients the HSR was considered to be severe (1.5%). In the short premedication group, 19 patients had HSRs (45.2%); in 6 of these patients the HSRs were considered to be severe (14.3%). The incidence of severe HSRs was significantly higher in the short premedication group than in the conventional premedication group ($P = 0.027$). In the modified premedication protocol study, HSR events were recorded in 14 patients (63.6%); 14 showed flushing, 2 had skin rash, and 1 had tachycardia. No severe HSRs were seen.
Conclusions. The incidence of HSRs in the short premedication group tended to be higher than that in the conventional premedication group. The modified premedication protocol was found to be feasible for preventing paclitaxel-related HSR, but case accumulation is needed.

Key words Paclitaxel · Premedication · Hypersensitivity reactions · Prophylaxis · Diphenhydramine

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Introduction

Paclitaxel is a highly active drug used for the treatment of lung, ovarian, breast, head and neck, bladder, and other epithelial cancers. In early phase I trials, a high frequency of severe hypersensitivity reactions (HSRs) was observed when paclitaxel was administered.[1–3] HSRs usually occur just after the start of paclitaxel administration. The reaction likely occurs due to the release of histamine and other vasoactive compounds from mast cells in response to the polyoxyethylated castor oil vehicle (Cremophor El, Sigma Chemical Co., St. Louis, MO).[4] Severe HSRs, characterized by chest pain, dyspnea, bronchospasm, urticaria, and/or hypotension were initially reported in 10.6% of patients who were not premedicated prior to paclitaxel infusion.[4] After the initial report of HSR events to the National Cancer Institute, it was recommended that all patients receiving paclitaxel be given conventional premedication, which contained two doses of oral dexamethasone, intravenous diphenhydramine, and intravenous cimetidine or ranitidine.[5] Consequently, the incidence of severe HSRs has decreased to 1%–3%.[6–8] It has become common practice to pretreat patients with various regimens prior to paclitaxel administration.

Currently, the standard recommended prophylactic therapy regimen is a single dose of intravenous dexamethasone, intravenous diphenhydramine, and intravenous cimetidine or ranitidine,[8–11] which is called a short premedication regimen. However, it has been reported that short premedication may not be the optimum prophylactic therapy for paclitaxel-related HSRs.[12,13] In Japan, oral diphenhydramine has usually been used as a prophylactic H1 antagonist, because pure intravenous diphenhydramine has not been not available. We hypothesized that the blood concentration of diphenhydramine when the oral form is used may be more influenced by the patient's condition (for example, by the presence of gastrointestinal disease, or advanced age) than when the intravenous form is used.

In this study, we retrospectively analyzed the incidence of paclitaxel-related HSRs in patients with non-small cell lung cancer (NSCLC). Also, we carried out prophylactic treatment with a modified premedication protocol, using Rescalmin (diphenhydramine with calcium bromide; Nissin, Yamagata, Japan) – a product which is usually used intravenously for allergic rhinitis – to assess its feasibility for preventing paclitaxel-related HSRs.

Patients and methods

Definition of paclitaxel-related hypersensitivity reactions

Reactions were scored as "severe" if, during paclitaxel infusion, the patient experienced one or more of the following grade 3–4 toxicities: angioderma, chest and/or back pain, dyspnea and/or wheezing, hypotension requiring vasopressor agent support, or cardiac arrest. If patients with a grade 2 HSR, such as chest or back pain, strongly desired to stop the infusion, we classified the HSR as severe in such patients. Reactions were scored as mild if one of the following grade 1–2 toxicities was recorded: flushing, mild hypotension, skin rash, or palpitation.

Retrospective historical cohort analysis of paclitaxel-related hypersensitivity reactions

We retrospectively analyzed the incidence of paclitaxel-related HSRs in patients with NSCLC. A pharmacy database at Osaka Prefectural Medical Center for Respiratory and Allergic Diseases identified all patients who had received paclitaxel with either conventional or short premedication from April 1999 to March 2002 (Table 1). All the patients had received both H1 and H2 antagonists (50 mg oral diphenhydramine and 100 mg intravenous ranitidine) 30 min prior to the paclitaxel infusion. In addition, the conventional premedication group had received two 20 mg doses of intravenous dexamethasone, at 12 and 6 h prior to the paclitaxel. The short premedication group had received a single 20-mg dose of intravenous dexamethasone 30 min prior to the paclitaxel. Paclitaxel was administered at a dose of 175–200 mg/m^2 by infusion over 3 h.

Treatment evaluations consisted of a complete medical history and physical examination, which included a blood cell count, urinalysis, ECG, chest X-ray, bone scan, and computed tomography. HSRs were graded according to the National Cancer Institute common toxicity criteria (NCI-CTC version 2.0; January 30, 1998) for adverse reactions to chemotherapy. Statistical significance was calculated with the Yate's corrected χ^2 statistic. A difference with a P value of less than 0.05 was considered to be significant. Statistical analysis software (StatMate III, ATMS, Tokyo, Japan) was used for the analysis.

Table 1. Premedication details and characteristics in patients receiving paclitaxel

	Total	Conventional	Short
Total no. of patients	107	65	42
Sex			
Male	77	48	29
Female	30	17	13
Median age, years (range)	61 (32–75)	61 (32–75)	62.5 (34–74)
Performance status			
0–1	83	50	33
2–3	24	15	9
Histological type			
Adenocarcinoma	84	52	32
Squamous cell carcinoma	19	10	9
Large cell carcinoma	4	3	1
Prior chemotherapy			
Yes	25	13	12
No	82	52	30
Allergic history			
Yes	5	2	3
No	102	63	39

Modified premedication protocol for prophylaxis of paclitaxel-related hypersensitivity reactions

We conducted a prospective trial to assess the feasibility of using a modified premedication protocol for the prophylaxis of paclitaxel-related HSRs. To be eligible, patients had to have histologically or pathologically documented NSCLC. Measurable disease was not necessary. Patients were required to have, at study entry, an Eastern Cooperative Oncology Group (ECOG) performance score of 0 to 2, and were required to have an absolute neutrophil count of 2000/μl or more, a platelet count of 100 000/μl or more, a WBC count of 3500/μl or more, and a hemoglobin level of 9.5 g/dl or more. The total bilirubin level was required to be less than 1.5 times the upper normal limit. The serum creatinine level was required to be less than the upper normal limit. Patients were required to have recovered from toxicities of prior chemotherapy, and may not have had either radiation therapy or investigational drug therapy within 4 weeks of initiating paclitaxel and carboplatin. This protocol was reviewed and approved by the institutional review board, and all patients gave written informed consent before participation.

All patients received 12.5 ml of Rescalmin (50 mg intravenous diphenhydramine with 437.5 mg calcium bromide; Nissin), 100 mg intravenous ranitidine, and 20 mg intravenous dexamethasone, 30 min prior to the paclitaxel infusion,

after having oral dexamethasone 8 mg the night before the paclitaxel. Paclitaxel was administered intravenously at 175 mg/m^2 over 3 h, followed by carboplatin, AUC 5, over 1 h on day 1 every 3 weeks. The calculated dose of paclitaxel was diluted in 500 ml of 5% dextrose in water. Polyolefin containers and polyethylene-lined tubing were used for drug administration because of concern that the vehicle in which paclitaxel was prepared, Cremophor EL, might leach plasticizer from polyvinylchloride-containing intravenous sets.

During the infusion, patients' vital signs (heart rate, respiratory rate, and blood pressure) were determined every 15 min for the first hour, and every 30 min for the next 2 h. Continuous cardiac monitoring was required until 6 h after the completion of the paclitaxel infusion.

Treatment cycles were repeated every 3 weeks, provided toxic effects were not prohibitive and there was no evidence of tumor progression. Doses were to be reduced in the event of treatment-related febrile neutropenia, grade 4 neutropenia, or grade 3 nonhematological toxicity. Paclitaxel was discontinued if there was more than grade 2 neurologic toxicity, cardiac arrhythmias, heart block, or a significant HSR. Minor reactions were to be managed by stopping the infusion, if judged medically necessary, and by administering symptomatic medications such as additional antihistamines, corticosteroids, or bronchodilators.

Results

Retrospective historical cohort analysis

One hundred and seven patients were identified in the database. Up to November 2000, 65 patients had received the conventional prophylactic regimen, and from December 2000, 42 patients had received the short prophylactic regimen. Table 2 shows the incidence of HSRs in the two prophylactic regimens. In the conventional premedication group, 21 patients had HSRs (32.3%); in 1 of these patients, the HSR was considered to be severe (1.5%). In the short premedication group, 19 patients had HSRs (45.2%); in 6 of these patients, the HSRs were considered to be severe (14.3%). In this historical cohort analysis, the overall incidence of HSRs in the short premedication group was not significantly different from that in the conventional premedication group (χ^2; $P = 0.177$), but the incidence of severe HSRs was found to be significantly higher in the short premedication group (χ^2; $P = 0.027$). Table 3 shows a summary of the severe HSRs in the 7 patients. In the 6 patients in the short premedication group, the hypersensitivity events occurred soon after the paclitaxel was initiated in the second course, and the reactions included chest or back pain, and dyspnea with or without bronchospasm. In the 1 patient in the conventional premedication group, grade 3 dyspnea with bronchospasm occurred during the first course. Paclitaxel infusion was discontinued immediately in all the patients with severe HSRs, and they received corticosteroid treatment. None of the patients were in a critical state, and there were no treatment-related deaths.

Modified premedication regimen experience

Patients

From January 2004 to May 2004, 22 patients were enrolled in this study (Table 4). The patients were predominantly male (20 of 22 patients), and the median age was 65 years (range, 38 to 74 years). Nineteen (86.4%) of the 22 patients had an ECOG performance status of 0 or 1, 8 (36.4%) had metastatic lesions (stage IV), 17 (77.3%) had adenocarcinoma, and 7 (31.9%) had had prior chemotherapy.

Adverse events

Toxicity data were available for all 22 patients who had received at least one dose of paclitaxel. Overall, the therapy was generally well tolerated and manageable. The patients' nonhematological toxicities are listed in Table 5. In this study, HSRs were recorded in 14 patients (63.6%); 14 showed flushing (grade 1), 2 had skin rash (1 of grade 1 and 1 of grade 2), and 1 had tachycardia (grade 1). No severe

Table 2. Comparison of incidence of hypersensitivity reactions (HSRs) with the two prophylactic regimens

	Conventional	Short	*P* value
Overall HSR			0.177
(+)	21 (32.3%)	19 (45.2%)	
(–)	44 (67.7%)	23 (54.8%)	
Severe HSR			0.027
(+)	1 (1.5%)	6 (14.3%)	
(–)	64 (98.5%)	36 (85.7%)	

Table 3. Summary of severe hypersensitivity reactions

Patient no.	Age (years)	Sex	Premedication	Symptoms	Onset[a]	Course	NCI-CTC
1	71	M	Short	Back pain	Soon	2	2
2	70	F	Short	Angioderma, dyspnea without bronchospasm	5 min	2	3
3	64	F	Short	Chest pain, back pain, flushing	Soon	2	2
4	52	F	Short	Chest pain, back pain, flushing	Soon	2	2
5	71	M	Short	Dyspnea with bronchospasm	10 min	2	3
6	34	M	Short	Dyspnea with bronchospasm	Soon	2	3
7	69	M	Conventional	Dyspnea with bronchospasm	10 min	1	3

[a] In relation to paclitaxel infusion

Table 4. Characteristics of patients with modified premedication protocol ($n = 22$)

Total no. of patients	22
Sex	
Male	20
Female	2
Median age, years (range)	65 (38–74)
Performance status	
0–1	19
2	3
Stage	
IIIA	8
IIIB	6
IV	8
Histological type	
Adenocarcinoma	17
Squamous cell carcinoma	2
Large cell carcinoma	3
Chemotherapy	
First-line	15
Second-line	6
Third-line	1

Table 5. Nonhematological toxicity observed in patients with modified premedication protocol ($n = 22$)

	Grade (no. of patients)							
	1	(%)	2	(%)	3	(%)	4	(%)
Nausea	11	(50)	1	(4)	1	(4)	–	
Vomiting	1	(4)	0	(0)	1	(4)	0	(0)
Appetite loss	12	(54)	1	(4)	2	(9)	0	(0)
Neuropathy	8	(36)	3	(13)	0	(0)	0	(0)
Myalgia	5	(22)	6	(27)	0	(0)	0	(0)
Arthralgia	6	(27)	8	(36)	0	(0)	0	(0)
Diarrhea	1	(4)	0	(0)	1	(4)	0	(0)
Alopecia	2	(9)	9	(40)	–	–		
Fatigue	14	(63)	2	(9)	1	(4)	0	(0)
Somnolence	11	(50)	0	(0)	0	(0)	0	(0)
Hypersensitivity reactions								
Flushing	14	(63)	0	(0)	0	(0)	0	(0)
Tachycardia[a]	1	(4)	0	(0)	0	(0)	0	(0)
Skin rash[a]	1	(4)	1	(4)	0	(0)	0	(0)

Toxicity assessed according to NCI-CTC version 2
[a]Three patients also showed flushing

HSRs were seen. All adverse events resolved naturally without corticosteroid administration. Other nonhematological toxicities, some of which were grade 3, were mainly digestive toxicities, such as appetite loss, vomiting, constipation, and diarrhea. In addition, 11 (50%) of the 22 patients had mild somnolence, which symptom disappeared immediately after the end of treatment.

Drug delivery

Overall, the median cumulative paclitaxel exposure of the 22 patients was 665 mg/m^2 (range, 175–1050 mg/m^2). The average number of cycles delivered was 3.5 (range, 1–6). The dose was reduced in 18% of the patients because of hematological toxicity.

Discussion

Although paclitaxel-based chemotherapy is widely used for patients with NSCLC,[14] severe HSRs are reported more frequently with paclitaxel treatment than with other cytotoxic chemotherapeutic drugs. If severe HSRs occur, the paclitaxel treatment is discontinued. This is a disadvantage for patients, so prophylactic treatment has been used.[4–7] Dexamethasone is a long-acting glucocorticoid with a biologic half-time of approximately 48 h.[15] Currently, a short premedication regimen including single-dose intravenous dexamethasone has been recommended.[8–11] A comparative prospective study has reported that the incidence of paclitaxel-related HSRs was not significantly different between conventional and short premedication regimens.[14] However, Kwon et al.[12] retrospectively showed that a single-dose intravenous corticosteroid prophylactic regimen was associated with a significantly higher rate of HSRs than the two-dose oral corticosteroid regimen. Moreover, Kloover et al.[13] have reported that short premedication may not be a suitable prophylactic therapy for paclitaxel-related HSR because of a fatal outcome.

We retrospectively analyzed, in a historical cohort, the incidence of paclitaxel-related HSRs in patients who had received oral diphenhydramine, plus a single dose or two doses of intravenous dexamethasone. We found that six of the patients with a short premedication regimen had severe HSRs, which events occurred as soon as paclitaxel was initiated in the second course. These events included chest or back pain, hypoxia, dyspnea, and bronchospasm, and the incidence of severe HSRs was significantly higher than that in the conventional premedication group. Since obtaining the results of the historical analysis, we immediately stopped using the short premedication regimen. The incidence of severe HSRs in the patients in the short premedication group was quite high compared with that in past reports. Possibly, our definition of severe HSR may have differed from that used previously. However, grade 2 chest or back pain should be considered as severe, and paclitaxel infusion should be stopped for safety, because such symptoms have a possibility to lead to serious toxicity, such as cardiac arrest.[13]

In Japan, oral diphenhydramine had usually been used as a prophylactic H1 antagonist, because pure intravenous diphenhydramine was not available. With the oral product, the blood concentration is thought to be more influenced by the patient's general condition (for example, by the presence of gastrointestinal disease, or advanced age) than when the intravenous form is used:. Bearing in mind its pharmacological properties, oral diphenhydramine plus single-dose intravenous dexamethasone is unlikely to result in an adequate level of immunosuppression during the infusion of paclitaxel. This may explain the results of our historical analysis. As a result of these concerns, we employed a modified premedication protocol, using Rescalmin (Nissin) intravenously instead of oral diphenhydramine, with a dose of oral dexamethasone being administered the night before the paclitaxel infusion.

Paclitaxel treatment using the modified premedication protocol was performed smoothly and good compliance was obtained. There were no severe HSRs, and no treatment was discontinued because of toxic allergic reactions. This treatment regimen seems to be effective for the prophylaxis of paclitaxel-related HSRs, although the number of patients in our study was small. This treatment regimen has several advantages, as follows. First, it ensures that an intravenous H1 antagonist is administered prior to paclitaxel, in contrast to the administration of the oral product. Second, because the dose of oral dexamethasone given the night before the paclitaxel infusion is lower than the conventional dose, patients' compliance is better. Third, mild somnolence seems to be a favorable effect of receiving chemotherapy in anxious patients. Finally, this treatment regimen might be useful not only for paclitaxel but also for other chemotherapeutic drugs such as docetaxel, oxaliplatin, and cetuximab. In fact, in a patient with docetaxel-related HSR, re-administration of docetaxel succeeded with the modified premedication protocol. Our modified protocol might also be useful with oxaliplatin, a platinum salt which is particularly effective in treating colorectal cancer, but with which, as a result of its increasing clinical use, a rising incidence of HSRs has been observed.[16] HSRs have also been observed with cetuximab, a monoclonal antibody that is an inhibitor of epidermal growth factor receptor.[17]

In conclusion, in our historical cohort analysis, the incidence of HSRs in the short premedication group tended to be higher than that in the conventional premedication group. Our modified premedication protocol was found to be feasible for preventing paclitaxel-related HSRs, but case accumulation is needed.

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Review

Problems with Registration-Directed Clinical Trials for Lung Cancer in Japan

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Sekine, I., Nokihara, H., Yamamoto, N., Kunitoh, H., Ohe, Y., Saijo, N. and Tamura, T. *Problems with Registration-Directed Clinical Trials for Lung Cancer in Japan.* Tohoku J. Exp. Med., 2007, **213** (1), 17-23 —— New anticancer agents against lung cancer are needed because efficacy of chemotherapy is limited. The long time required, low quality, and considerable costs of registration-directed clinical trials in Japan ("Chiken") have been pointed out. The quality of 24 phase I and 41 phase II trials of an anticancer drug for lung cancer were analyzed according to the approval year of the drug. The human resources and infrastructure to support oncology clinical practice and clinical trials were compared between Japan and the USA. A maximum tolerated dose was not defined in any of seven phase I trials before 1989, and was determined in two of six trials between 1989 and 1996 and in seven of 10 trials thereafter. Before 1989, 29 (20%) of 142 patients registered in two trials were ineligible, and the number of ineligible patients was not reported in the five trials. Sample size calculations were not performed in any of seven phase II trials before 1989 and were performed in only four of 10 trials between 1989 and 1996 and in all 23 trials conducted thereafter. The shortage of human resources, including medical oncologists, oncology nurse practitioners and clinical research coordinators, is serious and acute. The infrastructure to support clinical trials also remains insufficient in Japan. In conclusion, registration-directed clinical trials of anticancer agents have advanced significantly during last three decades but remain unsatisfactory. The development of infrastructure and human resources is an urgent task to ensure high-quality clinical trials without unnecessary delays. ——— clinical trials; medical oncologists; nurse practitioners; lung cancer; anticancer agents

Lung cancer is one of the most common malignancies and the leading cause of cancer-related deaths in many countries. In the year 2000, the annual number of deaths from lung cancer was estimated to be 1.1 million worldwide, and global lung caner incidence is increasing at a rate of 0.5% per year (Schottenfeld and Searle 2005). About 80% of patients with lung cancer have already developed distant metastases or pleural effusion, either by the time of the initial

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diagnosis or by the time recurrence is detected after surgery for local disease. These patients can be treated with systemic chemotherapy, but the efficacy of currently available anticancer agents is limited to the extent that patients with advanced disease rarely live long. Therefore, new chemotherapeutic agents continue to be developed against lung cancer (Sekine and Saijo 2000).

The Japanese Pharmaceutical Affairs Law (PAL) was enacted in 1948, and was first amended in 1960 to provide for regulations to ensure the maintenance of the quality, efficacy, and safety of drugs and medical devices, and to promote research and development of these medical and pharmaceutical products. Good Clinical Practice (GCP) was enforced by the Bureau Notification of the Ministry of Health and Welfare of Japan ("Kyokuchou-Tsuuchi") in 1989 (the former GCP). In 1996, the PAL and its related laws were amended to strengthen GCP (the new GCP), Good Laboratory Practice, Good Post-Marketing Surveillance Practice, and standard compliance reviews, conforming to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. In contrast to the laws prevailing in the US and EU, marketing approval for anticancer agents in Japan has been granted based on reports of the anti-tumor effects of the new agents in phase II trials (Fujiwara and Kobayashi 2002).

Under this Japanese drug approval system regulated by the PAL, 23 anticancer drugs have been approved for use against lung cancer during the last five decades (Fig. 1). Of these, 9 drugs are original to Japan, some of which are routinely used all over the world. Several problems, however, have been pointed out in registration-directed clinical trials in Japan ("Chiken"), including the long time required, low quality, and considerable cost (The Ministry of Health, Labour and Welfare of Japan 2002; The Ministry of Education, Science and Culture and the Ministry of Health, Labour and Welfare 2003). As a result, Japanese cancer patients must wait for a long time

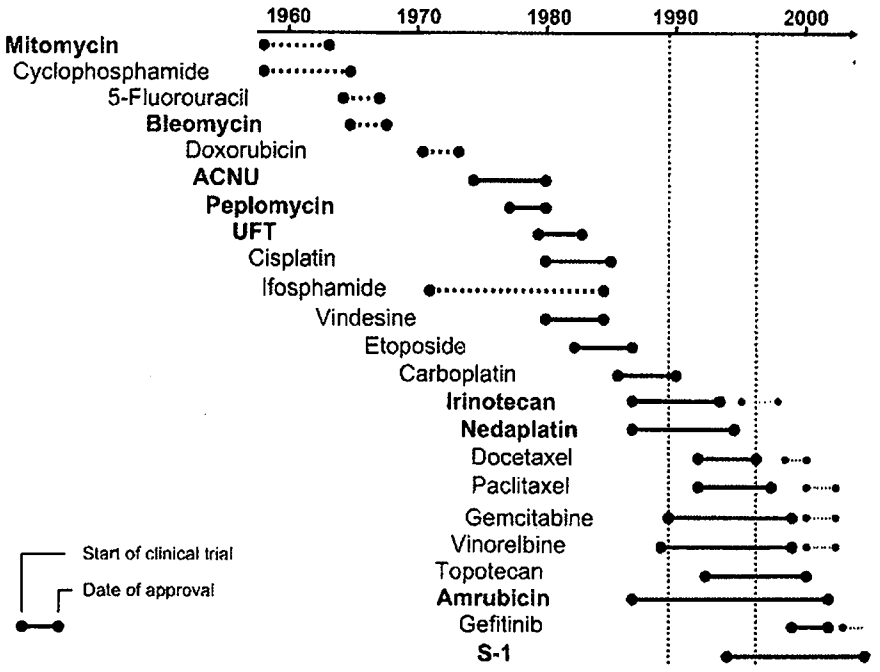

Fig. 1. Anticancer drugs approved for lung cancer in Japan.
Bold: original to Japan. Dotted line: case series studies, solid thick line: investigational new drug phase I-II trials for approval, and dotted thin line: post-marketing sponsored phase III trials. Vertical dotted lines indicate the year when the former and new GCP were issued.

until they receive new anticancer drugs which have been approved long before in other countries (The Ministry of Health, Labour and Welfare of Japan 2005). We discuss the aspects and issues of registration-directed trials in Japan by reviewing such trials for the 23 anticancer drugs.

Review of registration-directed clinical trials for the 23 anticancer drugs

A total of 65 phase I and II trials of an anticancer drug for approval were reviewed in terms of definition of eligibility criteria, maximum tolerated dose (MTD), sample size, response criteria, and extramural review for tumor responses. The MTD is the dose associated with seriouis but reversible toxicities in a sizeable proportion of patients and the one that offers the best chance for a favorable therapeutic ratio (Piantadosi 1997). The number of patients accrued in a trial, percentage of ineligible patients, number of participant hospitals in a trial, and the study period defined as the months between the first and last patient accrual were also analyzed. They were obtained from a published paper for 53 trials, from a meeting abstract and in-company resource for one trial, and from in-company resource alone for the remaining 11 trials. The clinical developmental period of an anticancer drug was defined as years between the start month of the first phase I trial and the month of the approval for lung cancer. These parameters are compared according to the approval year of the drug. We categorized three periods of approval: 1) before 1989, 2) between 1989 and 1996, and 3) between 1997 and 2004, because the former GCP was enforced in 1989, and the new GCP in 1997 (Fujiwara et al. 2002).

Of the 23 anticancer drugs, six drugs whose clinical development started before 1974 were approved on the basis of the clinical experience of the use of the drug without clinical trials (Fig. 1). A total of 24 phase I trials were identified (Table 1). The MTD was not defined in the protocol of any trials before 1989, but was defined in 33% of trials between 1989 and 1996, and in 70% of trials after 1996. Instead of the MTD, maximum acceptable dose, defined as the dose associated with grade 2 or severer toxicity in two thirds or more patients, was used in a trial after 1996. About twice more patients were registered in a trial before 1989 than thereafter, but 20% of the registered patients before 1989 were ineligible. The study period of a phase I trial got longer as the number of participant hospitals decreased, from 7 months and 11 hospitals before 1989 to 13 months and 4 hospitals after 1996, respectively.

In this review, 41 phase II trials for approval were analyzed (Table 2). Calculation of the sample size was not made in any trials before 1989, was seen in 40% of trials between 1989 and 1996, and in all trials thereafter. Response criteria were

TABLE 1. Investigational new drug phase I trials for approval.

	Before 1989	1989-1996	1997 or thereafter
Total number of trials	7	6	11
Defined, number (%) of trials			
Eligibility criteria	4 (57)	6 (100)	11 (100)
Maximum tolerated dose*	0 (0)	2 (33)	7 (70) ‡
Results of trials, median (range)			
Number of patients**	61 (32-170)	24 (18-54)	29 (9-43)
% of ineligible patients	20 (20-21) †	8 (0-33)	6 (0-22)
Number of hospitals	11 (1-21)	9 (1-18)	4 (1-17)
Study period in months	7 (5-30)	10 (5-11)	13 (8-24)

*Statistically significant difference obtained ($p = 0.014$ by the chi-square test); **Statistically significant difference obtained ($p < 0.01$ by the Kruskal Wallis test); †Data were available in 2 trials only; ‡Data were available in 10 trials only.

TABLE 2. Investigational new drug phase II trials for approval.

	Before 1989	1989-1996	1997 or thereafter
Total number of trials	7	11	23
Defined, number (%) of trials			
Eligibility criteria	4 (57)	11 (100)	23 (100)
Sample size calculation*	0 (0)	4 (40) [‡]	23 (100)
Response criteria	6 (86)	11 (100)	23 (100)
Extramural review	3 (43)	9 (82)	23 (100)
Results of trials, median (range)			
Number of patients	71 (10-127)	68 (18-153)	61 (11-102)
% of ineligible patients	18 (0-29) [†]	3 (0-22)	3 (0-12)
Number of hospitals	27 (3-103)	17 (1-30)	20 (5-46)
Study period in months	18 (12-36)	12 (6-34)	26 (4-48) [§]

*Statistically significant difference obtained ($p < 0.01$ by the chi-square test); [†]Data were available in 5 trials only; [‡]Data were available in 10 trials only; [§]Data were available in 22 trials only.

defined in almost all studies, but an extramural review was conducted only after 1989. The median number of registered patients in a trial was constant through the three periods, but the percentage of ineligible patients was high in trials conducted before 1989. The number of patients in a trial, and the number of hospitals in a trial were similar regardless of the year. The median study period in recent trials was 26 months.

The clinical development period was evaluated in the 23 drugs. Cisplatin was approved for germ cell tumors in 1983 and additionally approved for non-small cell lung cancer (NSCLC) in 1986. S-1 was firstly approved for gastric cancer in 1999, and additionally approved for NSCLC in 2004. The other drugs were approved for lung cancer for the first time. The median (range) clinical development period was 5.2 (3.2-14.5) years before 1989, 6.0 (4.8-9.1) years between 1989 and 1996, and 9.0 (3.9-15.4) years in 1997 or thereafter.

Development and recent problems of phase I and phase II trials in Japan

The concept of the "clinical trial" was not widely followed in Japan until 1974, when a phase I trial of nimustine hydrochloride (ACNU) was launched as one part of the United States-Japan Cooperation Cancer Research Program on the basis of the agreement between the National Cancer Institute and Japan Society for the Promotion of Science (Sugano 1982; Niitani 1999). Phase I trials before 1989 required the accrual of many patients, because 1) the maximum tolerated dose was not defined, 2) many patients were treated at unnecessary dose levels because the modified Fibonacci dose escalation schedule was not applied, and 3) the percentage of ineligible patients was high. Some of these issues were improved in 1997 or thereafter, but the maximum tolerated dose is still not defined in as many as 40% of trials. Recently, oncology phase I trials came to be conducted among fewer hospitals than before, as more participants were recruited in each hospital. This facilitated communication among phase I investigators, which is important to complete phase I trials safely.

Phase II trials play the central role in anticancer agent approval in Japan, because the approval can be granted based on the response rate in these trials. The quality of protocols for phase II trials suggested by eligibility criteria, sample size calculation, response criteria, and extramural review has been improved significantly. The study period of phase II trials, however, was and is still too long, as long as 4 years in recent trials. To increase participant hospitals, however, is not necessarily a desirable solution,

because a certain number of patients per hospital are needed to maintain the quality of trials by training doctors in the application of a new drug. Thus, enhancing patient recruitment in each hospital participating in the trial is the most important consideration.

A high standard of oncology clinical practice as the basis for clinical trials

Since a high standard of clinical practice is the basis for all clinical trials, the infrastructure for oncological clinical practice should be promptly advanced. The shortage of human resources including medical oncologists and oncology nurse practitioners in Japan is serious and acute. In the United States, medical oncology was established as a separate discipline by the American Board of Internal Medicine in 1971, and approximately 8,000 certified internists as of 2003 have been further certified by the Board in the subspecialty of medical oncology (Holland et al. 2003). In contrast, medical oncology has not been established as an academic unit or a regular university course in many medical schools in Japan. The Japanese Society of Medical Oncology was launched as an association in 1993, and framed the system of cancer medical specialists in 2003. A total of 1,479 doctors were certified as a tentative medical oncology supervisor between 2003 and 2005, and 47 doctors as a medical oncology specialist in 2005 (Table 3) (Japanese Society of Medical Oncology 2005).

To deal with complex cancer care, oncology nurse practitioners in the United States have become an integral part of the multidisciplinary team in the care of patients. As of 2002, more than 19,000 oncology nurse practitioners have been certified by the Oncology Nursing Society in the United States (Rieger 2003). In contrast, the number of oncology nurse practitioners registered in the Japanese Nursing Association was only 44 as of 2005 (Table 3) (Japanese Nursing Association 2005). Introduction of oncology nurse practitioners in clinical practice should lessen the burden on oncologists significantly and help them to have the incentive to take part in registration-directed clinical trials.

The infrastructure and human resources to support clinical trials

The infrastructure to support in-house clinical trials remains insufficient and even lacking in almost all institutes in Japan, while it has been advanced systematically in the United States. In the 1960s, General Clinical Research Centers were founded with the support of National Institutes of Health in 80 universities and academic institutions to provide the primary resources and optimal environment necessary for investigators to conduct clinical research. They include experienced nursing, laboratory, computer system, and biostatistical staff (Robertson and Tung 2001; General Clinical Research Centers 2005). To carry out a multicenter trial, a central data center

TABLE 3. Medical oncology professionals in Japan and the USA.

Professionals	*n* of medical oncology professionals	
	Japan	USA
Medical oncologists	47 [1]	8,000 [2]
Oncology nurse practitioners	44 [3]	19,000 < [4]
Clinical research coordinators	335 [5]	10,723 [6]

[1] Certified by the Japanese Society of Medical Oncology in 2005.
[2] Certified by the American Board of Internal Medicine as of 2003.
[3] Certified by the Japanese Nursing Association as of 2005.
[4] Certified by the Oncology Nursing Society as of 2002.
[5] Certified by the Japanese Society of Clinical Pharmacology and Therapeutics as of 2005.
[6] Certified by the Association of Clinical Research Professionals as of 2005.

is needed to deal with the increased administrative difficulties and quality assurance problems associated with this type of trial (Pollock 1994). The quality control and quality assurance system of the Japan Clinical Oncology Group has been significantly developed during the last two decades (Japan Clinical Oncology Group 2005). Using Internet resources may facilitate developing national and regional networks for clinical trials by reducing the burden associated with the extensive research time and considerable cost of all these processes (Paul et al. 2005).

The new GCP demands more of the clinical researchers in time, resources and money to enhance the science, credibility, and ethics of clinical trials for approval (Sweatman 2003). The clinical research coordinator (CRC) plays a key role in the clinical trial process by supporting investigators. The CRCs are involved in every aspect of registration-directed clinical trials, including protocol development, checking eligibility criteria, informed consent, organizing study schedules, checking clinical tests, filling in case report forms, and providing support for monitoring and auditing the trials (Rico-Villademoros et al. 2004; Sakamoto 2004). Association of Clinical Research Professionals in the USA has offered the CRC certification since 1992, and there are 10,723 CRCs to date (Association of Clinical Research Professionals 2006). The Japanese Society of Clinical Pharmacology and Therapeutics launched the certified CRC system in 2003, and there were 335 certified CRCs as of 2005 (Table 3) (The Japanese Society of Clinical Pharmacology and Therapeutics 2005).

In conclusion, clinical trials of anticancer agents for approval have been developing significantly, but still remain at an unsatisfactory level. Development of the infrastructure and human resources for clinical trials is an urgent task to complete good quality clinical trials for approval without delay.

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