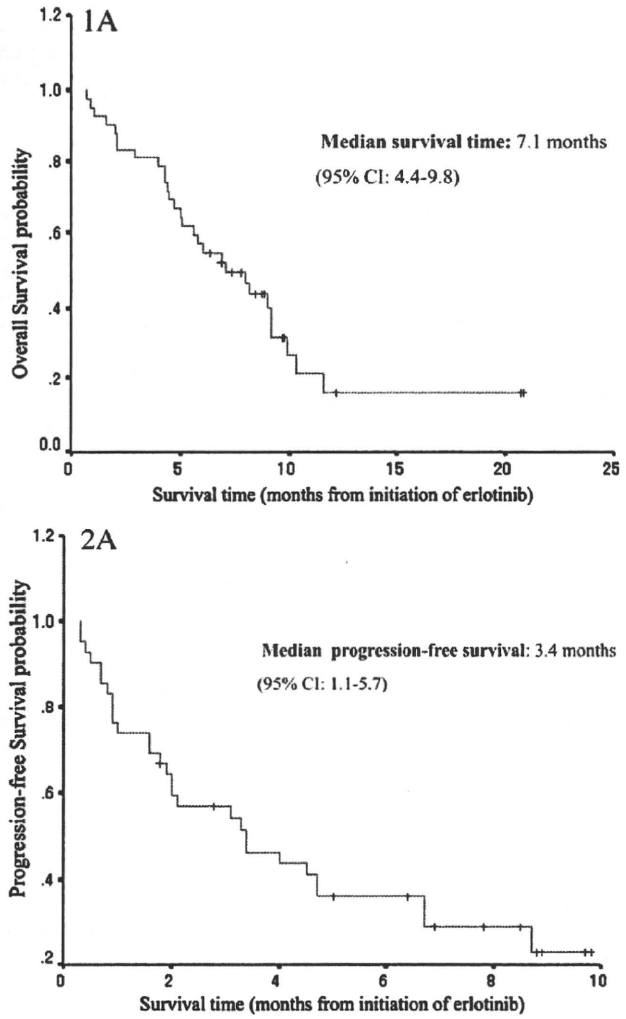


Fig. 1. Kaplan–Meier plot of survival time with erlotinib. (A) Overall survival rates and (B) progression-free survival rates of 42 patients. MST: median survival time; mPFS: median progression-free survival.

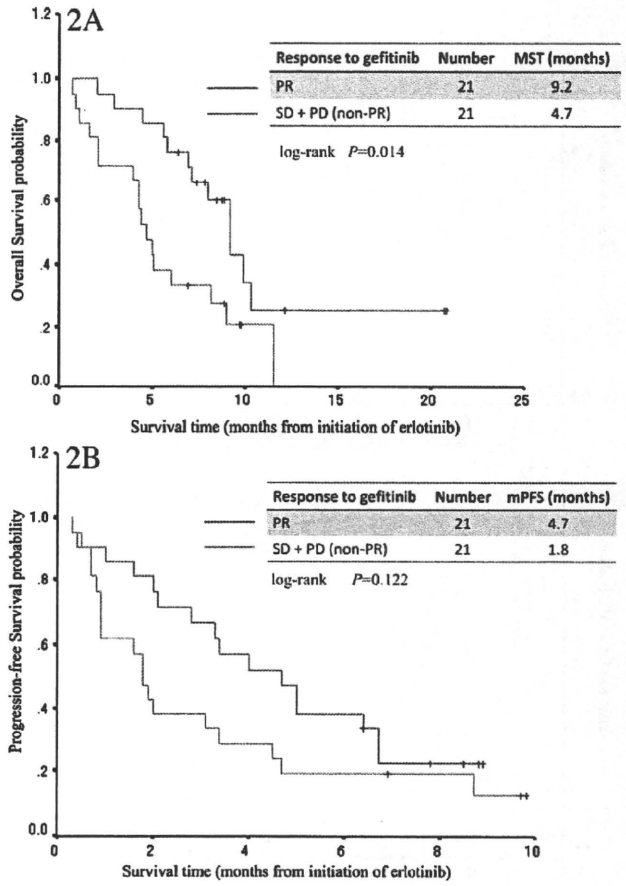


Fig. 2. Kaplan–Meier plot of survival time with erlotinib. (A) Overall survival rates and (B) progression-free survival rates stratified by response to prior gefitinib. Non-PR is defined as SD plus PD with gefitinib therapy.

was found to be the only independent prognostic factor (hazard ratio = 0.23; 95% CI: 0.08–0.64, $p = 0.005$), and time to progression with gefitinib showed borderline significance (hazard ratio = 0.34; 95% CI: 0.12–1.01, $p = 0.05$).

Kaplan–Meier curves of survival time according to response to prior gefitinib therapy are shown in Fig. 2. Patients who achieved PR while receiving gefitinib therapy showed significantly longer OS ($p = 0.014$). However, no significant difference was noted in PFS between patients with PR for gefitinib and those with non-PR (4.7 months [95% CI: 2.9–6.5 months] vs. 1.8 months [95% CI: 1.4–2.2 months]; $p = 0.122$). Time to progression with gefitinib showed a borderline significant impact on survival with erlotinib therapy. However, among patients who achieved PR with gefitinib, TTP with gefitinib therapy was strongly correlated with survival time. Kaplan–Meier curves of survival time for patients who achieved PR with gefitinib stratified according to TTP are shown in Fig. 3. Patients with TTPs of less than 12 months with gefitinib therapy were found to have significantly longer OS (10.3 months [95% CI: 7.0–13.6 months] vs. 6.4 months [95% CI: 2.6–10.2 months]; $p = 0.04$) and longer PFS (6.4 months [95% CI: 3.6–9.2 months] vs. 3.4 months [95% CI: 1.2–5.6 months]; $p = 0.19$) than patients with TTPs of 12 months or more. However, no statistically significant difference was noted between the two groups in terms of PFS ($p = 0.19$).

In addition, we found that skin rash was not predictive of survival with erlotinib therapy. All patients in the present study were affected by rash of some grade while receiving erlotinib. The degree of skin rash toxicity due to erlotinib exceeded the grade noted during gefitinib treatment in 32 patients. Seven patients required dose reduction of erlotinib due to grade 3 skin rash. Using a Cox proportional hazard model, we determined that skin rash grade had no impact on survival (hazard ratio = 0.64 [95% CI: 0.27–1.47]; $p = 0.29$).

4. Discussion

Here, we investigated survival potential in patients receiving erlotinib after failure of gefitinib, focusing on response and TTP with gefitinib. Our findings suggest that administration of erlotinib subsequent to gefitinib may exert survival benefit in former gefitinib-positive responders. Further, among those former responders, most with TTP <12 months may not yet have secondary resistance to EGFR-TKIs. Our findings suggest little chance for patients to achieve a high response with erlotinib therapy after experiencing progression with gefitinib therapy. This observation may be due to these two EGFR TKIs sharing the same mechanism of EGFR blockade or to cross resistance [5].

Our retrospective study showed that response achieved with prior administration of gefitinib was the only prognostic factor for subsequent erlotinib therapy after experiencing progression on gefitinib therapy. In particular, among patients who achieved PR with gefitinib, patients with TTPs of less than 12 months with gefitinib therapy were found to have significantly longer OS than

Please cite this article in press as: Asami K, et al. Duration of prior gefitinib treatment predicts survival potential in patients with lung adenocarcinoma receiving subsequent erlotinib. Lung Cancer (2011), doi:10.1016/j.lungcan.2010.12.014

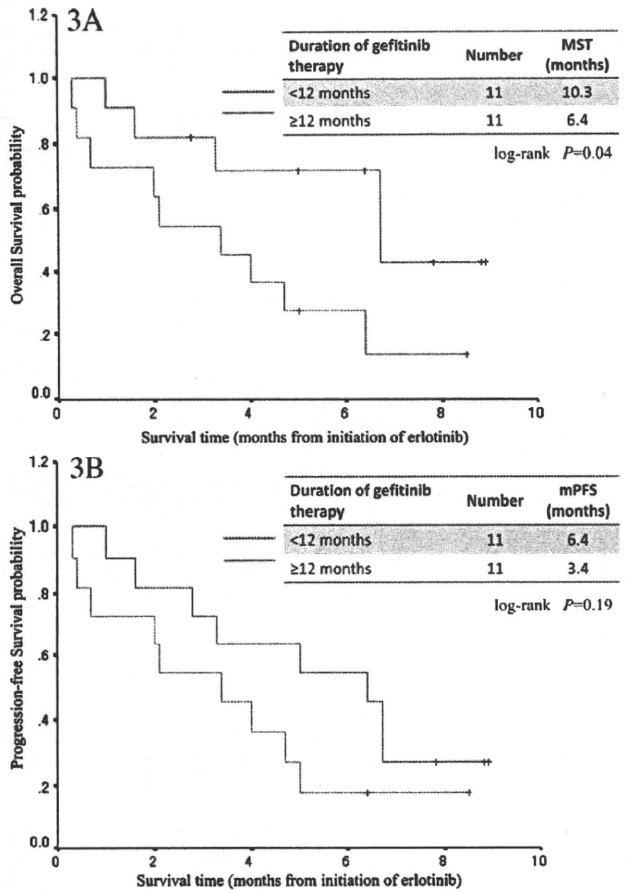


Fig. 3. Kaplan–Meier plot of survival time for patients who achieve PR with gefitinib. (A) Overall survival rates and (B) progression-free survival rates stratified by TTP with gefitinib.

patients with TTPs of 12 months or more. In addition, most of these patients showed some degree of improvement in image findings after subsequent erlotinib therapy. We noted no EGFR T790M mutations in any of three patients who underwent a second biopsy of their progressed lesions after failure with gefitinib therapy. We therefore supposed that most patients with TTP <12 months may have not yet acquired the EGFR T790M mutation. However, we only investigated the presence of a secondary EGFR T790M mutation in three patients in the present study. Validation of this hypothesis will require collection of more molecular information from patients who are no longer responsive to gefitinib in the future.

Shepherd et al. demonstrated that TTP was 2.6 months in NSCLC patients who had previously been treated with docetaxel therapy [20]. We observed that PFS was 3.4 months in patients with TTP ≥12 months who achieved PR in our study, a duration which appears improved over that demonstrated by Shepherd et al. Given these findings, we posited that, regardless of duration of gefitinib therapy, subsequent erlotinib may be able to prolong PFS compared to chemotherapy with cytotoxic agent provided the patients demonstrated a positive response with gefitinib. However, given that our results were obtained in a retrospective study with an extremely small sample population, a prospective study is warranted to clarify whether or not erlotinib administered subsequent to gefitinib can elicit greater survival benefit in gefitinib-positive responders than chemotherapy with cytotoxic agents.

We noted here that treatment with erlotinib following gefitinib resulted in more toxic grades of skin rash in patients, findings which suggest that erlotinib may have greater biological activity than gefitinib. Several other investigators have also suggested based on their own findings that erlotinib may have higher biological activity than gefitinib. Costa et al. showed that differing efficacy between gefitinib and erlotinib was due to differences in commonly administered dosages between the two drugs [21]. Gefitinib (250 mg per day) is typically administered at one third of its maximum-tolerated dose, whereas erlotinib (150 mg per day) is administered at its maximum tolerated dose. In vitro data showed that the mean concentration of gefitinib was 0.24 μg/ml at the 300-mg daily dose and 1.1 μg/ml at 1000 mg/day. In contrast, median concentration of erlotinib at 150 mg/day was 1.26 μg/ml. These previous findings suggest that erlotinib (150 mg/day) has a higher biological dose of EGFR inhibition than gefitinib (250 mg/day).

Recent studies have demonstrated that the increased biological activity of EGFR-TKIs is associated with control of tumor clones. Yoshimasu et al. reported observing a dose-response relationship between inhibition rates and gefitinib concentration [22]. Clarke et al. reported that high-dose erlotinib was effective in controlling leptomeningeal metastases progression while receiving standard erlotinib therapy in EGFR-mutant patients [23]. These authors demonstrated that a weekly 1200-mg dose of erlotinib controlled leptomeningeal metastases in a patient who was no longer responsive to a standard daily dose of erlotinib (150 mg).

Our findings here suggest that a treatment duration of 12 months of gefitinib therapy may be the borderline period for tumor clones to attain resistance to EGFR-TKIs. However, speculation as to whether or not previously EGFR-TKI-sensitive clones gradually grow resistant to EGFR-TKIs has not been resolved. Further studies are necessary to validate our findings.

In conclusion, gefitinib responders may achieve survival benefits from erlotinib therapy after experiencing progression with gefitinib. Among patients who have been receiving gefitinib therapy for less than 12 months, tumor clones may not yet have acquired a secondary mutation. However, further studies are needed to clarify precisely how tumor clones attain such secondary resistance to EGFR-TKIs.

Conflicts of interest statement

None declared.

Acknowledgements

We are grateful to the staff of Kinki-Chuo Chest Medical Center for their helpful comments. We are especially indebted to Dr. Masahiko Ando of Kyoto University for support on statistical analyses.

References

[1] Moscatello DK, Holgado-Madruga M, et al. Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. Cancer Res 1995;55(23):5536–9.
[2] Janne PA, Engelman JA, et al. Epidermal growth factor receptor mutations in non-small-cell lung cancer: implications for treatment and tumor biology. J Clin Oncol 2005;23(14):3227–34.
[3] Baselga J, Arteaga CL. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. J Clin Oncol 2005;23(11):2445–59.
[4] Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci 2004;101:13306–11.
[5] Mitsudomi T, Kosaka T, Endoh H, et al. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. J Clin Oncol 2005;23(11):2513–20.
[6] Mok TS, Wu Y-L, et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361(September (10)):947–57.
[7] Shepherd FA, Rodrigues Pereira J, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353(2):123–32.

Please cite this article in press as: Asami K, et al. Duration of prior gefitinib treatment predicts survival potential in patients with lung adenocarcinoma receiving subsequent erlotinib. Lung Cancer (2011), doi:10.1016/j.lungcan.2010.12.014

[8] Johnson JR, Cohen M, et al. Approval summary for erlotinib for treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of at least one prior chemotherapy regimen. Clin Cancer Res 2005;11(18):6414–21.
[9] Allan S, et al. Efficacy of erlotinib in patients with advanced non-small cell lung cancer (NSCLC) relative to clinical characteristics: subset analysis from the TRUST study. In: Poster presented at ASCO. 2008.
[10] Baselga J, Rischin D, et al. Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. J Clin Oncol 2002;20(November (21)):4292–302.
[11] Hidalgo M, Siu LL, Nemunaitis J, Rizzo J, et al. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. J Clin Oncol 2001;19(July (13)):3267–79.
[12] Riely GJ, Pao W, et al. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res 2006;12(3 (Pt 1)):839–44.
[13] Choong NW, Dietrich S, Seiwert TY, Tretiakova MS. Gefitinib response of erlotinib-refractory lung cancer involving meninges-role of EGFR mutation. Nat Clin Pract Oncol 2006;3(January (1)):50–7 [quiz 1 p following 57].
[14] Mitsudomi T, Kosaka T, Endoh H, Yoshida K. Mutational analysis of the EGFR gene in lung cancer with acquired resistance to gefitinib. J Clin Oncol 2006;24(18S):7074.
[15] Pao W, Balak MN, Riely GJ, Li AR. Molecular analysis of NSCLC patients with acquired resistance to gefitinib or erlotinib. J Clin Oncol 2006;24(18S):7078.
[16] Bean J, Brennan C, Shih JY, Riely G, Viale A. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci USA 2007;104(December (52)):20932–27.
[17] Engelman JA, Zejnullahu K, Mitsudomi T. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316(May (5827)):1039–43.
[18] Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316(May (5827)):1039–43 [Epub 2007 April 26].
[19] Balak MN, Gong Y, Riely GJ, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. Clin Cancer Res 2006;12(Nov (21)):6494–501.
[20] Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 2000;18(10):2095–103.
[21] Costa DB, Schumer ST, Tenen DG, et al. Differenctial responses to erlotinib in epidermal growth factor receptor (EGFR)-mutated lung cancers with acquired resistance to gefitinib carrying the L747S or T790M secondary mutations. J Clin Oncol 2008;26(7):1182–4.
[22] Yoshimasu T, Ohta F, Oura S, et al. Histoculture drug response assay for gefitinib in non-small-cell lung cancer. Gen Thorac Cardiovasc Surg 2009;57(March (3)):138–43 [Epub 2009 March 12].
[23] Clarke JL, Pao W, Wu N, et al. High dose weekly erlotinib achieves therapeutic concentrations in CSF and is effective in leptomeningeal metastases from epidermal growth factor receptor mutant lung cancer. J Neurooncol 2010;February.

Please cite this article in press as: Asami K, et al. Duration of prior gefitinib treatment predicts survival potential in patients with lung adenocarcinoma receiving subsequent erlotinib. Lung Cancer (2011), doi:10.1016/j.lungcan.2010.12.014

Published OnlineFirst November 8, 2010; DOI:10.1158/1078-0432.CCR-10-1773

Clinical Cancer Research

Human Cancer Biology

Long Exposure of Environmental Tobacco Smoke Associated with Activating *EGFR* Mutations in Never-Smokers with Non–Small Cell Lung Cancer

Tomoya Kawaguchi[1], Masahiko Ando[2], Akihito Kubo[3], Minoru Takada[4], Shinji Atagi[1], Kyoichi Okishio[1], Kazuhiro Asami[1], Akihide Matsumura[1], Kazuyuki Tsujino[5], Sai-Hong Ignatius Ou[6], and Hidefumi Sasaki[7]

Abstract

Purpose: To examine an association between environmental tobacco smoke (ETS) and activating epidermal growth factor receptor (*EGFR*) mutations in never-smokers with non–small cell lung cancer (NSCLC).

Experimental Design: A total of 126 never-smokers with NSCLC were prospectively included in this study. Detailed ETS information was obtained through a standardized questionnaire including exposure period, place, and duration. Cumulative dose of ETS (CETS) was evaluated as a sum of the number of the exposure years at home and/or workplace. *EGFR* and *K-ras* mutations were determined using real-time PCR amplification.

Results: A total of 124 patients (98.4%) had ETS exposure with median CETS of 50 years (range: 0–118). Activating *EGFR* mutations were detected in 62.7% of the 126 patients and *K-ras* in 2 of 114 patients. The incidence of activating *EGFR* mutations was significantly higher in females than in males (67.6% vs. 26.7%; $P = 0.002$), and increased in quintile groups separated on the basis of CETS (shortest group = 44.0%, longest = 84.6%; $P = 0.0033$). In the multivariate logistic regression model, including gender, CETS, age, and family history of cancer, both gender and CETS were significantly associated with an incidence of activating *EGFR* mutations; the odds ratio for the *EGFR* mutations were 5.13 [95% confidence interval, CI = 1.47–18.0; $P = 0.0105$] for females and 1.02 (95% CI = 1.00–1.04; $P = 0.0193$) for each 1-year increment in CETS.

Conclusions: Females and increased ETS exposure are closely associated with *EGFR* mutations in never-smokers with NSCLC. *Clin Cancer Res; 17(1); 39–45. ©2010 AACR.*

Introduction

Although lung cancer is the leading cause of cancer-related death and is predominantly caused by tobacco smoke, approximately 25% of all lung cancers worldwide are not attributable to tobacco smoke (1). In fact, there were about 30% never-smokers in Japan in a large cohort study including more than 20,000 patients with non–small cell lung cancer (NSCLC; ref. 2).

Authors' Affiliations: [1]National Hospital Organization Kinki-chuo Chest Medical Center, Osaka, [2]Kyoto University, Kyoto, [3]Aichi Medical University School of Medicine, Aichi, [4]Sakai Hospital Kinki University School of Medicine, and [5]Osaka University Graduate School of Medicine, Osaka, Japan; [6]Chao Family Comprehensive Cancer Center, University of California Irvine Medical Center, Orange, California; and [7]Nagoya City University Graduate School of Medicine, Nagoya, Japan

Corresponding Author: Tomoya Kawaguchi, Department of Internal Medicine, National Hospital Organization Kinki-chuo Chest Medical Center, 1180 Nagasone-cho, Kita-ku, Sakai, Osaka 591-8555, Japan. Phone: 072-252-3021; Fax: 072-251-1372; E-mail: t-kawaguchi@kch.hosp.go.jp.

doi: 10.1158/1078-0432.CCR-10-1773

Lung cancer in never-smokers is unique in clinical characteristics and is suggested to be a distinctive disease. When considered as a separate category, lung cancer in never-smokers would constitute the seventh most common cause of cancer death worldwide (3, 4).

Recently, molecular biological studies showed that epidermal growth factor receptor (*EGFR*) mutations are detected in almost 60% of NSCLC in never-smokers (5), and the mutations happened exclusively in bronchial tree (6, 7). It has been suggested that lung cancer arises through different molecular mechanisms according to smoking status, and *EGFR* mutations are associated with never-smokers and *K-ras* mutations in ever-smokers; both mutations are almost exclusively found in adenocarcinomas (3). A high frequency of *EGFR* mutations in NSCLC in never-smokers leads to the conclusion that these mutations are one of the important biomarkers to clarify the disease.

Although several possible explanations have been proposed, the cause of lung cancer in never-smokers still remains unclear. Explanations include environmental tobacco smoke (ETS) exposure (8), radon exposure (9), occupational exposure (10), oncogenic virus (11), genetic change (12), and estrogen hormone (13, 14). A Japan

Published OnlineFirst November 8, 2010; DOI:10.1158/1078-0432.CCR-10-1773

Translational Relevance

Although there has been a growing interest in lung cancer in never-smokers and significant body of work, the cause still remains unclear. Several possible explanations have been proposed including exposure of environmental tobacco smoke (ETS). Molecular biological studies have suggested that epidermal growth factor receptor (*EGFR*) mutations seem to play a critical role in the carcinogenesis of lung cancer in never-smokers, and the mutations are exclusively observed in bronchial tree. We hypothesized that development of the mutations are associated with ETS, and we conducted prospective study using a detailed questionnaire to evaluate the association. Here we showed that female gender and longer exposure to ETS are closely associated with *EGFR* mutations in never-smokers with non–small cell lung cancer (NSCLC).

Public Health Center-based prospective study showed that second-hand smoke exposure is clearly related to the development of lung adenocarcinoma in never-smokers in Japan (8). The study identified a statistically significant dose-response relationship between the quantity and intensity of husbands' smoking and their wives' incidence of lung cancer.

Given this background, we hypothesized that development of *EGFR* mutations are associated with ETS exposure, and we conducted prospective study using a detailed questionnaire to evaluate the association.

Patients and Methods

Data collection

We consecutively enrolled prospective patients with newly diagnosed, histologically or cytologically proven NSCLC between March 2008 and January 2010 in National Kinki-chuo Chest Medical Center. Patients who were never-smokers (<100 cigarettes in the lifetime) and whose tumor tissue samples were available for analysis were eligible for the study. ETS was defined as regular exposure to tobacco smoke produced by an active smoker within a confined space for at least 1 year.

Detailed ETS information was obtained through a standard questionnaire that was carefully supported by interview by trained personnel. The questionnaire included items for years of ETS exposure from parents and other relatives as a child, years of ETS exposure from spouse/partner and/or children at home, and years of ETS exposure from co-workers at workplace. We also included family history of cancer in first-degree relatives (parents, siblings, and offspring) and menopausal status, if the patients are female. This study was approved by the institutional review board of the National Hospital Organization Kinki-chuo Chest Medical Center. All patients gave their written informed consent before enrollment.

EGFR and *K-ras* mutation analysis

A genetic analysis was done to detect *EGFR* mutations from exons 18 to 21 and codon 12, and 13 mutations in exon 1 of *K-ras* gene. The nucleotide sequence of the kinase domain of the *EGFR* gene was determined using PCR-INVADER assay of the individual exons (15), and that of *K-ras* was done using real-time PCR amplification and genotyping (16).

Statistical analysis

The cumulative dose of ETS (CETS) exposure was assessed in total smoker-years. This assessment was constructed to add exposure years of ETS from the 3 different parts in the questionnaire: 1) from parents and/or other relatives in his/her childhood; 2) from spouse/partner and/or children at home; and 3) from co-workers at a workplace. In each part, the same year was counted only once to avoid overlap of exposure periods from the different sources (e.g., in part 2, exposure from a spouse at age 30–50 years and his/her child at age 25–35 years would present a total ETS as 25, and if total ETS was 20 from part 1 and 3 respectively, the CETS of the patient would be $20 + 25 + 20 = 65$).

Assuming the proportion of patients with activating *EGFR* mutations to be 60% among never-smokers with NSCLC, we planned to recruit at least 100 patients to detect a 10-year difference in CETS between *EGFR* mutation positive and negative populations at a significance level of 0.05 with a 90% statistical power.

All patients were divided into quintile groups by CETS. Significant differences in the variables were tested using the Pearson's, chi-square, Fisher's exact, and Mantel extension tests, wherever appropriate.

The odds ratios for the risk of activating *EGFR* mutations were calculated in a multivariate logistic regression model, including gender, ETS exposure, age, and family history of lung cancer in all the patients and those with adenocarnoma, respectively. To evaluate a dose–response relationship closely, CETS was treated as a continuous variable or quintile in the model. In the quintile model, the odds ratios for the second, third, fourth, and longest quintiles relative to the shortest were calculated. To test for a linear trend across the quintiles, we coded each quartile as 0, 1, 2, 3, or 4, and then included it in the model as a single variable. All the statistical analyses were done with SAS version 9.2 (SAS Institute).

Results

Clinical characteristics

A total of 126 patients were enrolled, and characteristics are summarized in Table 1. Among those, 124 patients (98.4%) had the ETS exposure, and the median CETS was 50 years (range: 0–118). Most patients were female (88.1%) with an adenocarcinoma histologic type (96.8%). The median age was 65 years (range: 29–88 years) in all the patients; 67 years (29–88 years) in females and 54 years (36–75 years) in males. About 23% of the patients

Published OnlineFirst November 8, 2010; DOI:10.1158/1078-0432.CCR-10-1773

Table 1. Characteristics and activating *EGFR* mutations

	CETS			*EGFR* mutations			Site of activating mutations		
	Number (CETS <50/total)	Frequency, %	*P*	Number (mutation +/total)	Frequency, %	*P*	Number		*P*
							Exon 19	Exon 21	
Gender									
Male	10/15	33.3	0.213	4/15	26.7	0.002	2	2	0.855
Female	55/111	49.5		75/111	67.6		34	41	
Histology									
Adenocarcinoma	63/122	51.6	0.512	79/122	64.8	0.031	36	43	1.000
SQ	1/3	33.3		0/3	0		0	0	
LCNEC	1/1	100		0/1	0		0	0	
Family history of cancer									
Yes	10/22	45.5	0.527	15/22	68.2	0.558	5	10	0.290
No	55/104	52.9		64/104	61.5		31	33	
Age at diagnosis, y									
30–39	3/3	100	0.029	0/3	0	0.005	0	0	0.016
40–49	9/11	81.8		5/11	45.5		4	1	
50–59	14/25	56.0		14/25	56.0		6	8	
60–69	10/29	34.5		20/29	69.0		14	6	
70–79	24/44	54.5		27/44	37.0		9	18	
80 or older	5/14	35.7		13/14	92.9		3	10	
Menopausal age, y									
Premenopausal	3/5	60.0	0.343	3/5	60.0	0.23	2	1	0.825
50 or younger	23/39	59.0		22/39	56.4		10	12	
51 or older	27/60	45.0		44/60	73.3		20	24	
Unknown	2/7	28.6		6/7	85.7		2	4	

Abbreviations: SQ, squamous cell carcinoma; LCNEC, large cell neuroendocrine carcinoma.

were more than 75 years in this population, and age was significantly associated with cumulative ETS, as expected.

EGFR and *K-ras* mutations

Genetic analysis was successfully done for EGFR status on all 126 patients from paraffin-embedded samples. Activating mutations were detected in 79 of 126 patients (62.7%), consisting of 36 in-frame deletions in exon 19, 43 L858R in exon 21, and the other 4 mutations were detected in 2 G719C in exon 18, 1 in-frame deletion in exon 18, and 1 T790M in exon 20. Forty-two samples were positive for *EGFR* mutations in the 66 surgical specimens, 33 in the 51 biopsy samples from bronchoscopy, and 8 in the 9 cytology samples from pleural effusion. There was no significant difference in EGFR detection rates among sample sources ($P = 0.707$). The remaining samples after EGFR analysis were 114, enough for K-ras analysis. Mutations were detected in 2 of 114 patients, and both were point mutations in codon 12, one of which was a G to C transversion and the other a G to A transition, respectively. No mutation was detected in codon 13. When the patients were categorized into 2 groups on the basis of EGFR status, incidence of the mutations was significantly higher in females and according to age ($P = 0.002$, $P = 0.005$).

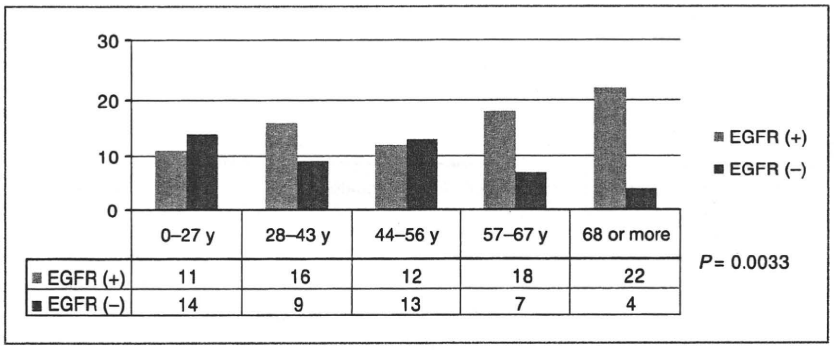
When the female patients were divided into 2 groups on the basis of menopausal age, there was no association between them. There was no significant association between family history of cancer and *EGFR* mutations. When separated by exon 19 and exon 21 mutations in the 79 cases, the elderly tended to have exon 21 *EGFR* mutations.

ETS exposure and activating *EGFR* mutations

We examined the association between cumulative ETS and activating *EGFR* mutations in the quintile groups separated by cumulative ETS. As presented in Figure 1, the incidence of *EGFR* mutations increased (shortest group = 44.0%; second group = 64.0%; third group = 48.0%; fourth group = 72.0%; longest group = 84.6%; $P = 0.0033$).

Next, we examined the association between the type of ETS and *EGFR* mutations (Table 2). The odds ratios for the *EGFR* mutations were analyzed for each 1-year increment in the total ETS. In Japan, age of 20 years is traditionally defined as reaching adulthood; also, home and workplace are major, separate places to live. We divided the patients into 2 groups on the basis of CETS in period by age of 20 years (childhood or adulthood) and place (household or

Published OnlineFirst November 8, 2010; DOI:10.1158/1078-0432.CCR-10-1773



	0–27 y	28–43 y	44–56 y	57–67 y	68 or more
EGFR (+)	11	16	12	18	22
EGFR (–)	14	9	13	7	4

Figure 1. Association between cumulative ETS and activating *EGFR* mutations. The horizontal line is exposure period of ETS, and the vertical line is number of the patients. The incidence of *EGFR* mutations increased significantly with the exposure period of ETS.

workplace). There was a significant association between adulthood exposure ($P = 0.0136$) and household exposure ($P = 0.0049$), and *EGFR* mutations.

Impact of female gender and ETS on *EGFR* mutations

In the multivariate logistic regression model, including gender, cumulative ETS, age, and family history of lung cancer, both gender and cumulative ETS were significantly associated with the incidence of *EGFR* mutations in all cases (Tables 3 and 5) and in adenocarcinoma (Tables 4 and 6). When cumulative ETS was evaluated as continued variable in all cases (Table 3), the odds ratios for the *EGFR* mutations were 5.13 (95% CI = 1.47–18.0; $P = 0.0105$) for female gender, 1.02 (95% CI = 1.00–1.04; $P = 0.0193$) for each 1-year increment in CETS, 1.05 (95% CI = 0.48–2.33; $P = 0.9014$) for age, and 1.32 (95% CI = 0.46–3.83; $P = 0.6040$) for family history of cancer. In the quintile models (Tables 5 and 6), gender and CETS were also significantly associated with *EGFR* mutations. Moreover, the exposure duration of ETS of more than 68 years was the only significant effect on the mutations (Odds ratio = 5.44, 95% CI = 1.40–21.1; $P = 0.0144$).

Discussion

We demonstrated the occurrence of activating *EGFR* mutations were significantly associated with female gender and long exposure of ETS in NSCLC in never-smokers in this prospective study. A large randomized clinical study, IRESSA Pan-Asian Study (IPASS; ref. 17), demonstrated that females and elderly had more *EGFR* mutations. The study included about 1,200 Asian never/light, former smokers with NSCLC and more than 90% of the patients were never-smokers. The rate was 63.0% for females versus 49.0% for males, and 68.5% for more than 65 years old versus 56.7% for less than 65 years old. Elderly patients have a chance for longer exposure to ETS and females have a higher chance of being exposed to ETS from a spouse, and this study accords well to our results.

EGFR mutations were detected in 65.9% in all the patients, and the 2 activating mutations of exon 19 deletions and L858R point mutations accounted for about 95.2% of all mutations included. Also, in this population, there were 2 patients who had *K-ras* mutations, and they were all EGFR wild type. These results are consistent with the previous published data (17, 18).

It has been reported that the development of activating *EGFR* mutations is inversely proportional to pack-years of smoking (19, 20). That carcinogen from cigarettes is similar in toxic effect for active and passive smoking and our results that longer exposure of ETS are significantly associated with *EGFR* mutations may be contradictory. However, we also demonstrated that the duration of exposure beyond a certain amount only affected the mutations. According

Table 2. Type of passive smoking and activating *EGFR* mutations

	Odds ratio[a]	95% CI	*P*
Exposure period			
Childhood (19 y or younger)	1.01	0.96–1.05	0.8032
Adulthood (20 y or older)	1.02	1.01–1.04	0.0136
Exposure place			
Household	1.03	1.01–1.05	0.0049
Workplace	0.99	0.97–1.02	0.7793

[a]Odds ratio for 1-year increment in CETS.

Table 3. Multivariate analysis on activating *EGFR* mutations

	Odds ratio	95% CI	*P*
CETS (continuous)	1.02	1.00–1.04	0.0193
Gender (female/male)	5.13	1.47–18.0	0.0105
Age at diagnosis (65 years/<65 years)	1.05	0.48–2.33	0.9014
Family history of cancer (yes/no)	1.32	0.46–3.83	0.6040

NOTE: Risk of activating *EGFR* mutations (all cases).

Published OnlineFirst November 8, 2010; DOI:10.1158/1078-0432.CCR-10-1773

Table 4. Multivariate analysis on activating *EGFR* mutations

	Odds ratio	95% CI	*P*
CETS (continuous)	1.02	1.00–1.04	0.0280
Gender (female/male)	5.63	1.60–19.8	0.0070
Age at diagnosis (65 years/<65 years)	1.13	0.50–2.55	0.7757
Family history of cancer (yes/no)	1.18	0.40–3.44	0.7659

NOTE: Risk of activating *EGFR* mutations (adenocarcinoma).

to the study of diagnostic accuracy for the mutations, the optimal cutoff for predicting a positive was less than about 15 pack-years in cumulative dose (19, 20). We speculate a mechanism that low dose of tobacco carcinogens and long exposure induce activating *EGFR* mutations regardless of smoking status (Fig. 2). Another important aspect is that mainstream smoke and sidestream smoke from tobacco combustion are different, and sidestream smoke is produced at a lower burning temperature, and the quantities of its chemical constituents in both vapor and particulate phases differ from those of mainstream smoke (21). Mutational spectrum may possibly be different depending on the intensity and duration of tobacco exposure. In addition, formation of carcinogens indoors by surface-mediated reactions of nicotine with nitrous acid was recently reported to lead to potential third hand smoke hazards (22). Development of *EGFR* mutations may be associated with this third chemical that may affect healthy subjects in a time-lag manner.

Table 5. Multivariate analysis on activating *EGFR* mutations

	Odds ratio	95% CI	*P*
CETS (quintile group)			Trend $P = 0.0131$
1st (shortest)	Reference		
2nd	2.35	0.72–7.71	0.1579
3rd	1.15	0.36–3.65	0.8084
4th	3.33	0.99–11.3	0.0528
5th (longest)	5.44	1.40–21.1	0.0144
Gender (female/male)	4.67	1.31–16.7	0.0177
Age at diagnosis (65 years/<65 years)	1.07	0.47–2.40	0.8779
Family history of cancer (yes/no)	1.35	0.45–4.07	0.5925

NOTE: Risk of activating *EGFR* mutations (all cases).

Table 6. Multivariate analysis on activating *EGFR* mutations

	Odds ratio	95% CI	*P*
CETS (quintile group)			Trend $P = 0.0201$
1st (shortest)	Reference		
2nd	2.20	0.66–7.33	0.1986
3rd	1.47	0.43–4.97	0.5363
4th	3.07	0.90–10.5	0.0742
5th (longest)	4.93	1.25–19.4	0.0224
Gender (female/male)	5.09	1.36–15.5	0.0122
Age at diagnosis (65 years/<65 years)	1.14	0.50–2.62	0.7593
Family history of cancer (yes/no)	1.19	0.40–3.60	0.7538

NOTE: Risk of activating *EGFR* mutations (adenocarcinoma).

According to our study and other epidemiologic studies (8), ETS play some part in the development of NSCLC in never-smokers. Based on reduced consumption of tobacco in the United States and Japan, the incidence of the disease will decrease as well. However, it is still uncertain that whether the actual number of never-smokers with NSCLC will increase or decrease (23). Defining molecular biomarkers for tumors in never-smokers remains a work in progress. Etinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-AKT) gene dislocation has been discovered apart from *EGFR* mutations (24) that

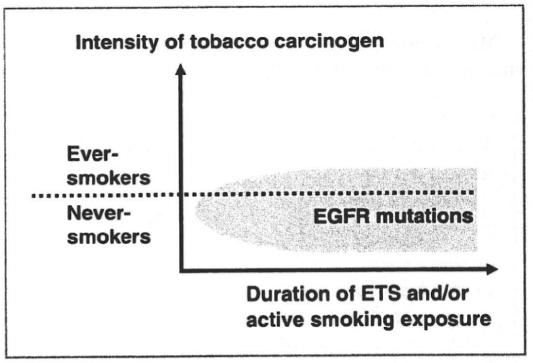


Figure 2. A model for relationship between activating *EGFR* mutations and amount of tobacco carcinogen. The horizontal line is duration of passive and/or active smoking exposure, and the vertical line is intensity of tobacco carcinogen. Low-dose tobacco carcinogens and long exposure induce activating *EGFR* mutations regardless of route of exposure (active or passive).

Published OnlineFirst November 8, 2010; DOI:10.1158/1078-0432.CCR-10-1773

is also specific for never-smokers, and some of the tumors were reported to relate to *K-ras* transition and *HER2* mutations (25). The influence of ETS was modest, and more comprehensive approaches are required.

Recently, a study on relationship between ETS and activating *EGFR* mutations was reported from Korea (26). The approach was similar to ours, but the results were inconsistent. Gender was not associated with the mutations, which was more frequently observed numerically in males in the study. Also, long ETS exposure was inversely associated with *EGFR* mutations, which is opposite to our study. One possible explanation for the difference is duration of the exposure. Frequency of the patients exposed to never-ETS was 24.6% in Korea and 1.6% in ours, and the median total exposure was 30 years in Korea whereas it was 50 years in ours. As mentioned above, the duration of the exposure beyond a certain amount of time was important for mutation development. In fact, *EGFR* mutation rate in never-smokers was 44.1% in that study that is lower compared with about 60% from Japanese studies (5, 20, 27), and the IPASS (17). Another possible explanation is intensity or concentration of ETS that may be different between the 2 studies. Although intensity of ETS may have a different impact on the development of the mutations, it is still difficult to grade and evaluate it. In fact, our questionnaire included ETS intensity, whereas most of the patients declined to fill them for inaccuracy of their memory, particularly as a child or at workplace. Because of few reply of ETS intensity in the questionnaire, we did not include them for analysis in this study, and further study may be required.

The main limitation of our study is lack of validation for the questionnaire because of the potential for recall bias by the patients. However, because there has been no biomarker to evaluate ETS to date, a detailed questionnaire is essential for study of ETS. Also, support by interview by trained personnel is important to obtain reproducible and accurate information. Moreover, standardized questionnaire will be required for comparison among ethnicity in future global study. Another weak point in this study may be the relatively small number of patients, although sample size was considered statistically in advance of the study. However, multivariate analysis did demonstrate that our data were significant, and the large clinical trial IPASS supports and enhances it.

In conclusion, *EGFR* mutations are significantly associated with female gender and long exposure of ETS in never-smokers with NSCLC. *EGFR* mutations are most prevalent and specific mutations in never-smokers. However, defining molecular biomarkers for tumors in never-smokers is still in progress, and a large molecular epidemiologic study will be required to clarify the disease comprehensively.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This study was supported in part by a grant-in-aid for Cancer Research from the Ministry of Health and Welfare, Japan.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 5, 2010; revised September 24, 2010; accepted October 21, 2010; published OnlineFirst November 8, 2010.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2. Kawaguchi T, Takada M, Kubo A, et al. Performance status and smoking status are independent favorable prognostic factors for survival in non-small cell lung cancer: a comprehensive analysis of 26,957 patients with NSCLC. J Thorac Oncol 2010;5:620–30.
3. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers–a different disease. Nat Rev Cancer 2007;7:778–90.
4. Rudin CM, Avila-Tang E, Samet JM. Lung cancer in never smokers: a call to action. Clin Cancer Res 2009;15:5622–5.
5. Toyooka S, Matsuo K, Shigematsu H, et al. The impact of sex and smoking status on the mutational spectrum of epidermal growth factor receptor gene in non small cell lung cancer. Clin Cancer Res 2007;13:5763–8.
6. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.
7. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339–46.
8. Kurahashi N, Inoue M, Liu Y, et al. Passive smoking and lung cancer in Japanese non-smoking women: a prospective study. Int J Cancer 2008;122:653–7.
9. Krewski D, Lubin JH, Zielinski JM, et al. A combined analysis of North American case-control studies of residential radon and lung cancer. J Toxicol Environ Health A 2006;69:533–97.
10. Reid A, Heyworth J, de Klerk N, Musk AW. The mortality of women exposed environmentally and domestically to blue asbestos at Wittenoom, Western Australia. Occup Environ Med 2008;65:743–9.
11. Cheng YW, Wu MF, Wang J, et al. Human papillomavirus 16/18 E6 oncoprotein is expressed in lung cancer and related with p53 inactivation. Cancer Res 2007;67:10686–93.
12. Li Y, Sheu CC, Ye Y, et al. Genetic variants and risk of lung cancer in never smokers: a genome-wide association study. Lancet Oncol 2010;11:321–30.
13. Raso MG, Behrens C, Herynk MH, et al. Immunohistochemical expression of estrogen and progesterone receptors identifies a subset of NSCLCs and correlates with EGFR mutation. Clin Cancer Res 2009;15:5359–68.
14. Nose N, Sugio K, Oyama T, et al. Association between estrogen receptor-beta expression and epidermal growth factor receptor mutation in the postoperative prognosis of adenocarcinoma of the lung. J Clin Oncol 2009;27:411–7.
15. Thyagarajan B, Anderson KE, Kong F, Selk FR, Lynch CF, Gross MD. New approaches for genotyping paraffin wax embedded breast tissue from patients with cancer: the Iowa women's health study. J Clin Pathol 2005;58:955–61.
16. Sasaki H, Okuda K, Kawano O, et al. Nras and Kras mutation in Japanese lung cancer patients: genotyping analysis using LightCycler. Oncol Rep 2007;18:623–8.
17. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57.

Published OnlineFirst November 8, 2010; DOI:10.1158/1078-0432.CCR-10-1773

18. Subramanian J, Govindan R. Molecular genetics of lung cancer in people who have never smoked. Lancet Oncol 2008;9:676–82.
19. Pham D, Kris MG, Riely GJ, et al. Use of cigarette-smoking history to estimate the likelihood of mutations in epidermal growth factor receptor gene exons 19 and 21 in lung adenocarcinomas. J Clin Oncol 2006;24:1700–4.
20. Jida M, Toyooka S, Mitsudomi T, et al. Usefulness of cumulative smoking dose for identifying the EGFR mutation and patients with non-small-cell lung cancer for gefitinib treatment. Cancer Sci 2009;100:1931–4.
21. Besaratinia A, Pfeifer GP. Second-hand smoke and human lung cancer. Lancet Oncol 2008;9:657–66.
22. Sleiman M, Gundel LA, Pankow JF, Jacob P 3rd, Singer BC, Destaillats H. Formation of carcinogens indoors by surface-mediated reactions of nicotine with nitrous acid, leading to potential thirdhand smoke hazards. Proc Natl Acad Sci U S A 2010;107:6576–81.
23. Thun MJ, Henley SJ, Burns D, Jemal A, Shanks TG, Calle EE. Lung cancer death rates in lifelong nonsmokers. J Natl Cancer Inst 2006;98:691–9.
24. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561–6.
25. Sasaki T, Rodig SJ, Chirieac LR, Janne PA. The biology and treatment of EML4-ALK non-small cell lung cancer. Eur J Cancer 2010;46:1773–80.
26. Lee YJ, Cho BC, Jee SH, et al. Impact of environmental tobacco smoke on the incidence of mutations in epidermal growth factor receptor gene in never-smoker patients with non-small-cell lung cancer. J Clin Oncol 2009;28:487–92.
27. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919–23.

Cancer Chemother Pharmacol
DOI 10.1007/s00280-010-1506-7

ORIGINAL ARTICLE

Phase I study of LY2181308, an antisense oligonucleotide against survivin, in patients with advanced solid tumors

M. Tanioka · H. Nokihara · N. Yamamoto · Y. Yamada · K. Yamada · Y. Goto · T. Fujimoto · R. Sekiguchi · K. Uenaka · S. Callies · T. Tamura

Received: 15 July 2010 / Accepted: 26 October 2010

Abstract
Purpose LY2181308 is an antisense oligonucleotide that complementarily binds to survivin mRNA and inhibits its expression in tumor tissue. This phase I dose escalation study evaluated the tolerability, pharmacokinetics, and anticancer activity of LY2181308 in Japanese.
Methods Patients with solid tumors refractory to standard therapy received LY2181308 (400, 600, or 750 mg) as a 3-h intravenous infusion for 3 consecutive days and thereafter once a week.
Results LY2181308 was administered to 14 patients, aged 44–73 (median 60) years. Flu-like syndrome, prolonged prothrombin time-international normalized ratio (PT-INR), thrombocytopenia, and fatigue were common reversible grade 1/2 toxicities. The dose-limiting toxicity was reversible grade 3 elevation of ALT/AST/γ-GTP in 1 patient treated at the 750-mg dose. Pharmacokinetic analysis showed a long terminal half-life of 21 days and an extensive tissue distribution of LY2181308. In 12 evaluable patients, one patient had stable disease, while the remaining 11 patients had progressive disease.
Conclusions LY2181308 monotherapy is well tolerated up to 750 mg with a manageable toxicity, the pharmacokinetic profile warrants further evaluation of LY2181308 in combination with cytotoxic agents or radiotherapy.

Keywords Antisense oligonucleotide · Pharmacokinetics · Phase I · Survivin

Introduction

Survivin, a member of the inhibitor of apoptosis family of proteins (IAP), regulates apoptosis and promotes cell proliferation [1]. Survivin is highly expressed during fetal development and rarely detectable in normal adult tissues [1]. However, overexpression of survivin has been reported in the vast majority of solid tumors and leukemias [2, 3].

LY2181308 is a novel second-generation antisense oligonucleotide (ASO) designed to complementarily bind to human survivin mRNA, inhibit the gene/protein expression, and consequently restore the normal apoptotic pathway in cancer cells. The antitumor activity of LY2181308 is correlated with inhibition of survivin [4, 5]. Furthermore, LY2181308 potently inhibited the expression of survivin mRNA and protein in human tumor cell lines [6]. These results justified its evaluation in clinical studies. Recently, a first-in-human dose study established the tolerability of LY2181308 at 750 mg [7]. ASOs are known to accumulate in the liver, where they can cause off-target hepatic toxicities [8].

In Japanese patients, genetic variations that influence metabolism and safety of anticancer drugs, such as UDP-glucuronosyltransferase (UGT) 1A1 and multidrug resistance protein (MRP) 1, have been identified [9]. Also, there are genetic variations that can predispose Japanese patients

M. Tanioka · H. Nokihara · N. Yamamoto · Y. Yamada · K. Yamada · Y. Goto · T. Tamura (✉)
Division of Internal Medicine, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: ttamura@ncc.go.jp

T. Fujimoto · R. Sekiguchi · K. Uenaka
Eli Lilly Japan K.K., Sannomiya Plaza Bldg., 7-1-5, Isogamidori, Chuo-ku, Kobe 651-0086, Japan

S. Callies
Eli Lilly and Company, Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK

Published online: 16 November 2010

to an altered response to inflammation as highlighted by polymorphisms of the TNF gene [10]. Because ASO administration is associated with complement activation leading to subsequent inflammatory reactions, including TNF release [11], Japanese patients may be more susceptible to ASO administration and its associated off-target inflammation. Hence, the objective of this study is to determine the tolerability, pharmacokinetics, and anticancer activity of LY2181308 in Japanese patients with advanced solid tumors.

Materials and methods

Patient eligibility

Patients with malignant solid tumors were eligible after failing standard therapies or if there was no approved treatment available. Eligibility criteria included: age 20–75 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1; adequate bone marrow, hepatic, renal, and coagulative function (absolute neutrophil count $\geq$1,500/μl, platelet count $\geq$100,000/μl, hemoglobin level 9.0 g/dl, total bilirubin $\leq$ upper limit of normal range [ULN], aspartate aminotransferase [AST] or alanine aminotransferase [ALT] $\leq$ 2.5 times ULN, estimated creatinine clearance $\geq$ 50 ml/min, normal prothrombin time-international normalized ratio [PT-INR, 0.8–1.2], and normal activated partial thromboplastin time [APTT, 23-40 s]); discontinuation of prior anticancer therapy 28 days before enrollment to this study or conclusion of palliative radiotherapy 14 days prior to starting on study; written informed consent. Exclusion criteria included the following: diagnosed pregnancy or ongoing lactation; symptomatic brain metastasis; active hepatitis B, C, or human immunodeficiency virus (HIV); and concomitant use of any anticoagulant drugs. The protocol was approved by the Institutional Review Board. The study was consistent with Good Clinical Practice and all applicable laws and regulations in Japan.

Drug administration and dose escalation procedure

LY2181308 (Eli Lilly and Company, IN, USA) was administered via intravenous infusion over 3 h on day 1–3 of the initial cycle (cycle 1 [day 1–7]) as a loading dose and then once a week as maintenance dose (cycle 2 [day 8–28] and onwards [28 days/cycle]). The content of each vial containing 100 mg in 4 ml of buffer was added to 500 ml of saline. The starting dose was 400 mg, with subsequent dose escalations to 600 and 750 mg in a classical 3 + 3 design. Dose-limiting toxicities (DLTs) were evaluated at each dose level (400:600:750 mg = 3:3:6 patients). The maximum tolerated dose (MTD) was defined as the highest dose level at which no or one patient experienced a DLT.

DLT was defined as any event that met at least one of the following criteria: (1) febrile neutropenia or grade 4 neutropenia persisting more than 4 days; (2) grade 4 decreased hemoglobin or thrombocytopenia, or thrombocytopenia requiring blood transfusion; (3) grade 3 APTT prolongation observed at the end of the study drug administration and persisting $\geq$48 h after administration; (4) non-hematological toxicities of grade 3 or higher (nausea, vomiting, anorexia, fatigue, constipation, diarrhea, and abnormal electrolytes were not considered DLTs if controllable with appropriate treatment); (5) discontinuation/postponement of study drug administration due to a toxicity other than those mentioned above and the total amount of administered study drug <80% of the total amount scheduled for the first 28 days; and (6) other toxicities that were judged as DLTs by the investigator.

Treatment assessment

Tolerability was evaluated in all patients for toxicities, clinical laboratory tests, coagulants, and vital signs. Toxicities were graded according to the Common Terminology Criteria for Adverse Events version 3.0 [12].

Tumor responses were assessed in patients who had measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) guideline version 1.0 [13].

Pharmacokinetics

Pharmacokinetic evaluation was performed in all patients during cycles 1 and 2. The concentration of LY2181308 in plasma was measured by enzyme-linked immunosorbent assay (ELISA) using antidigoxigenin-AP (Roche) to capture LY2181308. The minimum quantifiable concentration was 1.875 ng/ml in plasma. Non-compartmental pharmacokinetic parameters were calculated. Population pharmacokinetic analysis was also conducted in a model-dependent manner using a NON-linear Mixed Effect Model (NONMEM) program version VI.

Results

Patient characteristics

Fourteen patients in this study had the typical characteristics in a phase I study (Table 1). The most common histology was lung cancer (six patients), followed by pancreatic cancer (four patients). Approximately, half of the patients had more than four prior chemotherapies. A total of 31

Table 1 Patient characteristics

Variable	400 mg ($n = 4$)	600 mg ($n = 4$)	750 mg ($n = 6$)	Total ($n = 14$)
Sex				
Male	3	4	4	11
Female	1	0	2	3
Age, years				
Median	58.5	63.5	59.0	60.0
Range	44–73	60–69	49–66	44–73
Primary tumor type				
Lung	1	1	4	6
Pancreas	2	2	0	4
Intrahepatic cholangiocarcinoma	0	1	0	1
Uterus	1	0	0	1
Esophagus	0	0	1	1
Breast	0	0	1	1
ECOG PS				
0	1	1	6	8
1	3	3	0	6
Prior therapy				
Cancer-related surgery	3	1	4	8
Radiotherapy	0	1	5	6
Chemotherapy	4	4	6	14
≥4 prior chemotherapy regimens	1	2	3	6

ECOG PS eastern cooperative oncology group performance status

cycles were administered; the median number of cycles administered per patient was 2 (range 1–4). The maximum treatment period was 93 days.

Safety and tolerability

DLT was evaluated in 12 of the 14 patients. At the 750-mg dose, one patient with small cell lung cancer and a PS of 0 experienced DLTs of grade 3 elevation including AST, ALT, and γ-GTP on day 8. These events were asymptomatic and resolved without medication. Another patient at the 600-mg dose experienced a grade 3 total bilirubin elevation, which was not considered a DLT but rather a result of tumor progression. Hence, the MTD was established at the 750-mg dose level.

In addition to these toxicities, we observed flu-like symptoms (fever, chills, and hyperhidrosis), prolonged PT-INR, thrombocytopenia, and fatigue as grades 1/2 (Table 2). Almost all flu-like symptoms were observed during the loading dose of day 1–3. They were manageable with oral antipyretic such as acetaminophen. Prolonged PT-INR and thrombocytopenia were also grade 1/2, and there was no medical treatment required such as blood transfusions. Grade 1 fatigue occurred in eight patients. Grade 3 lymphocytopenia and anemia were only observed in one patient treated at the 400-mg dose; they were not considered drug related. Concentrations of complement fragments Bb and C3a were elevated on day 3 and returned to baseline after day 8 (Fig. 1).

Anticancer activity

Twelve of the fourteen patients met RECIST guideline for antitumor response assessment. At the 600-mg dose, 1 patient with intrahepatic cholangiocarcinoma had stable disease, but the remaining 11 patients had progressive disease.

Pharmacokinetics

Pharmacokinetic analysis was performed in all 14 patients (Fig. 2). The interindividual coefficient of variation (CV%) of AUC for LY2181308 was moderate. Pharmacokinetic parameters on day 3 were similar to those on day 1, suggesting no accumulation of study drug in plasma. The multiphasic disposition pharmacokinetic profile of LY2181308 was adequately described by a 4-compartment model with elimination from the peripheral compartment. The mean terminal $t_{1/2}$, distribution clearance (CL), elimination CL, and V_{ss} estimated by the model were 21 days, 2.0 l/h,

Table 2 Drug-related toxicities at selected dose levels

	400 mg ($n = 4$)		600 mg ($n = 4$)		750 mg ($n = 6$)		All ($n = 14$)			
Grade	1–2	≥3	1–2	≥3	1–2	≥3	1	2	≥3	All
Hematological toxicities										
Decreased platelet count	3	0	3	0	4	0	7	3	0	10
Decreased lymphocyte count	0	1	1	0	2	0	2	1	1	4
Decreased Hb	0	1	2	0	0	0	1	1	1	3
Decreased WBC	0	0	1	0	2	0	3	0	0	3
Non-hematological toxicities										
Increased PT-INR	3	0	3	0	5	0	11	0	0	11
Hyperhidrosis	2	0	3	0	6	0	11	0	0	11
Pyrexia	2	0	3	0	6	0	8	3	0	11
Fatigue	3	0	1	0	4	0	8	0	0	8
Increased CRP	2	0	4	0	2	0	8	0	0	8
Prolonged prothrombin time	1	0	1	0	5	0	7	0	0	7
Increased β-2 microglobulin urine	1	0	4	0	2	0	7	0	0	7
Anorexia	2	0	0	0	3	0	4	1	0	5
Chills	1	0	1	0	3	0	5	0	0	5
Diarrhea	2	0	1	0	2	0	5	0	0	5
Increased ALT	1	0	1	0	2	1[a]	3	1	1	5
Decreased blood albumin	2	0	0	0	2	0	4	0	0	4
Headache	1	0	0	0	3	0	4	0	0	4
Injection site pain	2	0	1	0	1	0	4	0	0	4
Nausea	0	0	1	0	3	0	3	1	0	4
Prolonged APTT	1	0	0	0	3	0	4	0	0	4
Increased AST	1	0	1	0	1	1[a]	3	0	1	4
Flushing	1	0	1	0	1	0	3	0	0	3
Hypothermia	1	0	0	0	2	0	0	3	0	3
Increased ALP	0	0	0	0	3	0	2	1	0	3
Increased blood triglycerides	0	0	0	0	3	0	2	1	0	3
Increased γ-GTP	0	0	0	0	1	2[a]	1	0	2	3

ALP alkaline phosphatase, *ALT* alanine aminotransferase, *APTT* activated partial thromboplastin time, *AST* aspartate aminotransferase, *CRP* c-reactive protein, *γ-GTP* gamma-glutamyl transpeptidase, *Hb* hemoglobin, *PT-INR* prothrombin time-international normalized ratio

[a] Grade 3 elevations in AST/ALT/γ-GTP in one patient were judged as DLTs

28.1 l/h, and 2.05×10^5 l, respectively. Model analysis showed that 84.5% of plasma LY2181308 was distributed to tissue within 8 h after the initiation of administration.

Discussion

Survivin is attracting considerable interest as a potential target for cancer therapy because it is upregulated in most malignancies and may play a role in blocking apoptosis in cancer cells [14]. LY2181308 is the first ASO to successfully inhibit survivin. Overall, LY2181308 was generally well tolerated in patients with advanced solid tumors. MTD was reached at 750 mg, and the 750-mg dose was recommended for further investigations. This study revealed a potential mild hepatotoxicity at the 750-mg dose in contrast to the first-in-human dose study in which this event was only observed at doses of 900 and 1,000 mg [7]. Other clinically important toxicities included flu-like symptoms, elevated PT-INR, thrombocytopenia, and fatigue. The frequency of flu-like symptoms, such as fever, was higher (79%) than that of previous study (32%) [7]. Despite these two main differences, LY2181308 had similar tolerability profile in Japanese as in Caucasian or other races [7]. However, the low-grade toxicity, the reversibility profile, and the medical treatment with antipyretics suggest that these differences between two studies may be marginal or insignificant.

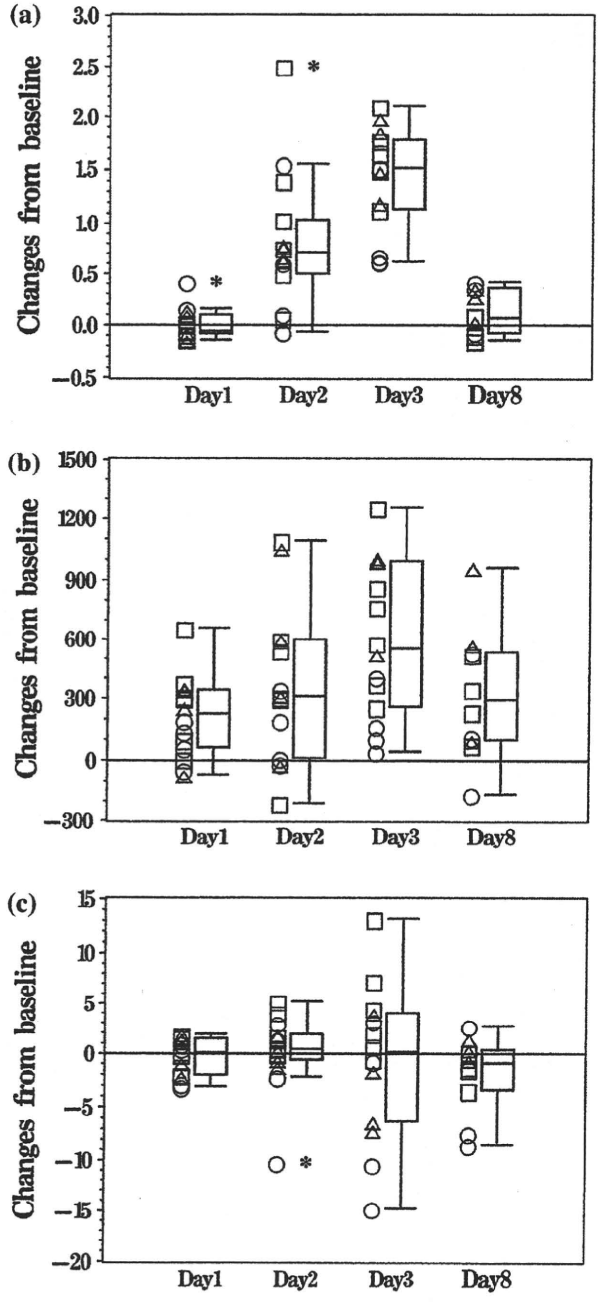


Fig. 1 Change in plasma concentration of complement fragments. Change in plasma concentration of complement fragments from baseline (before administration) to 15 min after the completion of infusion (**a**, Bb; **b** C3a; **c**, C5a). Symbols = *circles* 400 mg/time; *triangles* 600 mg/time; *squares* 750 mg/time. Normal range = Bb, 0.35–0.85 μg/ml; C3a, 305–1,239 ng/ml; C5a, 13.5–58.7 ng/ml

One reason for the induction of flu-like symptoms would be the off-target activation of complement after infusion of ASOs. Increase in levels of complement fragments C3a and Bb on day 3 was comparable to those found in preclinical studies using monkeys [15] and past phase I studies of other ASOs [16]. These complement elevations were acceptable since there was no signs of abnormal immune responses including anaphylaxis. Also, increases in C-reactive protein were equally distributed across all three dose groups, thus suggesting that the complement activation was similar in all patients.

Thrombocytopenia and decreased hemoglobin were observed as grade 1/2. In just one case, grade 3 was observed, but hematologic toxicities were generally mild. The ASO effect on reducing platelet counts may have two potential mechanisms: one is related to the well-known off-target effect of ASOs [8] and the other to the inhibition of survivin. For instance, survivin-depleted mice have reduced hemoglobin, white blood cell, and platelet counts as a result of loss in hematopoietic progenitor cells [17]. Lastly, it should be noted that despite the high accumulation of ASOs in the kidney, no elevation or signs and symptoms related to kidney dysfunction were observed.

The pharmacokinetic profile had a long half-life of 21 days. Consistent with the long terminal $t_{1/2}$, the CL to tissue and elimination CL were low to moderate, and the V_{ss} was large. It is thought that the long terminal $t_{1/2}$ represents plasma-tissue concentration during the tissue elimination phase of LY2181308 once the distribution equilibrium between plasma and tissues has been reached [8]. These results were comparable to the pharmacokinetic results from LY218308 studies in mice and monkeys, showing that rapid tissue distribution (within 24 h) cleared approximately 90% of the drug from plasma. The linearity of LY2182308 was not evaluated in this study due to the small number of patients.

Finally, 12 patients met the RECIST guideline for tumor response assessment. Only one patient had stable disease, while the others had progressive disease. While these data do not suggest any single-agent activity of LY2181308, it should be stressed that this was also not expected. LY2181308 is expected to have activity in conjunction with apoptosis-inducing agents, such as chemotherapy or radiation, and render previously apoptosis-resistant cells sensitive to pro-apoptotic treatments. For instance, paclitaxel or docetaxel resistance has been associated with increased survivin expression [18–20]. Besides, radiotherapy treatment is enhanced after blocking survivin expression in colorectal cancer models [21]. Based on these observations and the favorable toxicity profile of LY2181308, three phase 2 studies are being/have been conducted in conjunction with cytotoxic drugs: (1) combination with cytarabine and idarubicin for acute myeloid leukemia (completed), (2) combination with docetaxel and prednisone for prostate cancer (on-going), and (3) combination with docetaxel for non-small cell lung cancer (ongoing).

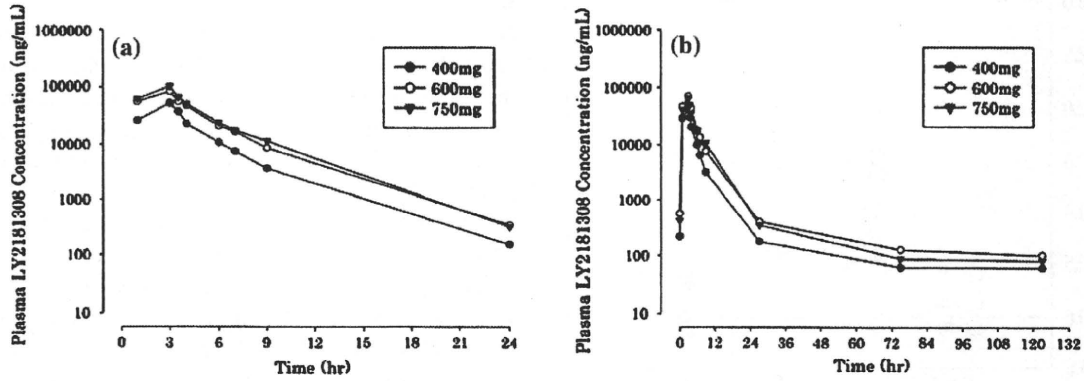


Fig. 2 Mean plasma concentration–time profiles of LY2181308. Mean plasma concentration–time profiles of LY2181308 on day 1 (**a**) and day 3–7 (**b**). C_{max} and AUC at 750 mg were comparable between Japanese and non-Japanese patients [21]

Acknowledgments The study was sponsored and supported by Eli Lilly Japan K.K. We thank Dr. Michael Lahn, Dr. Hisashi Taniai and Dr. Sotaro Enatsu for their review of the manuscript, and Toshinori Mishina for editorial assistance.

Conflict of interest Results from this study were presented in part at the 21st (2009) Molecular Targets and Cancer Therapeutics Conference (EORTC-NCI-AACR Symposium; Boston, 15–19 November) [22]. T. Tamura serves as a consultant to Eli Lilly Japan K.K. T. Fujimoto, R. Sekiguchi and K. Uenaka are full-time employees of Eli Lilly Japan K.K. S. Callies is a full-time employee of Eli Lilly and Company. M. Tanioka, H. Nokihara, N. Yamamoto, Y. Yamada, K. Yamada and Y. Goto have no conflicts of interest to disclose.

References

1. Ambrosini G, Adida C, Altieri DC (1997) A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 3:917–921. doi:10.1038/nm0897-917
2. Altieri DC (2003) Validating survivin as a cancer therapeutic target. Nat Rev Cancer 3:46–54. doi:10.1038/nrc968
3. Shen YC, Hu FC, Jeng YM, Chang YT, Lin ZZ, Chang MC, Hsu C, Cheng AL (2009) Nuclear overexpression of mitotic regulatory proteins in biliary tract cancer: correlation with clinicopathologic features and patient survival. Cancer Epidemiol Biomarkers Prev 18(2):417–423. doi:10.1158/1055-9965.EPI-08-0691
4. Carter BZ, Wang RY, Schober WD, Milella M, Chism D, Andreeff M (2003) Targeting survivin expression induces cell proliferation defect and subsequent cell death involving mitochondrial pathway in myeloid leukemic cells. Cell Cycle 2:488–493
5. Ansell SM, Arendt BK, Grote DM, Jelinek DF, Novak AJ, Wellik LE, Remstein ED, Bennett CF, Fielding A (2004) Inhibition of survivin expression suppresses the growth of aggressive non-Hodgkin's lymphoma. Leukemia 18:616–623. doi:10.1038/sj.leu.2403281
6. Li F, Ackermann EJ, Bennett CF, Rothermel AL, Plescia J, Tognin S, Villa A, Marchisio PC, Altieri DC (1999) Pleiotropic cell-division defects and apoptosis induced by interference with survivin function. Nat Cell Biol 1:461–466
7. Talbot DC, Davies J, Callies S, Andre V, Lahn M, Ang J, De Bono JS, Ranson M (2008) First human dose study evaluating safety and pharmacokinetics of LY2181308, an antisense oligonucleotide designed to inhibit survivin. 44th ASCO Annual Meeting
8. Geary RS, Yu RZ, Watanabe T, Henry SP, Hardee GE, Chappell A, Matson J, Sasmor H, Cummins L, Levin AA (2003) Pharmacokinetics of a tumor necrosis factor-alpha phosphorothioate 2′-O-(2-methoxyethyl) modified antisense oligonucleotide: comparison across species. Drug Metab Dispos 31:1419–1428. doi:10.1124/dmd.31.11.1419
9. Robert J, Le Morvan V, Smith D, Pourquier P, Bonnet J (2005) Predicting drug response and toxicity based on gene polymorphisms. Crit Rev Oncol Hematol 54:171–196. doi:10.1016/j.critrevonc.2005.01.005
10. Tokushige K, Takakura M, Tsuchiya-Matsushita N, Taniai M, Hashimoto E, Shiratori K (2007) Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. J Hepatol 46(6):1104–1110. doi:10.1016/j.jhep.2007.01.028
11. Jason TL, Koropatnick J, Berg RW (2004) Toxicology of antisense therapeutics. Toxicol Appl Pharmacol 201(1):66–83. doi:10.1016/j.taap.2004.04.017
12. Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events, version 3.0, DCTD, NCI, NIH, DHH. Publish date: December 12, 2003. Available: http://www.ctep.cancer.gov. Accessed 01 May 2010
13. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92:205–216. doi:10.1093/jnci/92.3.205
14. Ryan BM, O'Donovan N, Duffy MJ (2009) Survivin: a new target for anti-cancer therapy. Cancer Treat Rev 35:553–562. doi:10.1016/j.ctrv.2009.05.003
15. Henry SP, Giclas PC, Leeds J, Pangburn M, Auletta C, Levin AA, Kornbrust DJ (1997) Activation of the alternative pathway of complement by a phosphorothioate oligonucleotide: potential mechanism of action. J Pharmacol Exp Ther 281:810–816
16. Goel S, Desai K, Bulgaru A, Fields A, Goldberg G, Agrawal S, Martin R, Grindel M, Mani S (2003) A safety study of a mixed-backbone oligonucleotide (GEM231) targeting the type I regulatory subunit alpha of protein kinase A using a continuous infusion schedule in patients with refractory solid tumors. Clin Cancer Res 9:4069–4076
17. Leung CG, Xu Y, Mularski B, Liu H, Gurbuxani S, Crispino JD (2007) Requirements for survivin in terminal differentiation of erythroid cells and maintenance of hematopoietic stem and progenitor cells. J Exp Med 204:1603–1611. doi:10.1084/jem.20062395
18. Rahman KM, Banerjee S, Ali S, Ahmad A, Wang Z, Kong D, Sakr WA (2009) 3, 3′-Diindolylmethane enhances taxotere-induced apoptosis in hormone-refractory prostate cancer cells through survivin down-regulation. Cancer Res 69:4468–4475. doi:10.1158/0008-5472.CAN-08-4423

19. Xing H, Weng D, Chen G, Tao W, Zhu T, Yang X, Meng L, Wang S, Lu Y, Ma D (2008) Activation of fibronectin/PI-3 K/Akt2 leads to chemoresistance to docetaxel by regulating survivin protein expression in ovarian and breast cancer cells. Cancer Lett 261:108–119. doi:10.1016/j.canlet.2007.11.022
20. Zaffaroni N, Pennati M, Colella G, Perego P, Supino R, Gatti L, Pilotti S, Zunino F, Daidone MG (2002) Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer. Cell Mol Life Sci 59:1406–1412. doi:10.1007/s00018-002-8518-3
21. Rödel F, Frey B, Leitmann W, Capalbo G, Weiss C, Rödel C (2008) Survivin antisense oligonucleotides effectively radiosensitize colorectal cancer cells in both tissue culture and murine xenograft models. Int J Radiat Oncol Biol Phys 71:247–255. doi:10.1016/j.ijrobp.2008.02.011
22. Nokihara H, Yamamoto N, Yamada Y, Yamada K, Goto Y, Tanioka M, Nambu Y, Uenaka K, Sekiguchi R, Tamura T (2009) A phase 1 study of an antisense oligonucleotide against survivin (LY2181308) in Japanese patients with solid tumors. Mol Cancer Ther 8:B48. doi:10.1158/1535-7163.TARG-09-B48

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2010;40(5)404–411
doi:10.1093/jjco/hyp192
Advance Access Publication 4 February 2010

Phase I and Pharmacokinetic Study of ABI-007, Albumin-bound Paclitaxel, Administered Every 3 Weeks in Japanese Patients with Solid Tumors

Kazuhiko Yamada[1], Noboru Yamamoto[1], Yasuhide Yamada[1], Toru Mukohara[2,3], Hironobu Minami[2,3] and Tomohide Tamura[1]*

[1]Department of Internal Medicine, National Cancer Center Hospital, Tokyo and [2]Department of Oncology/Hematology, National Cancer Center Hospital East, Kashiwa, Japan

[3]Present address: Medical Oncology, Department of Medicine, Kobe University Hospital, Kobe, Japan.

*For reprints and all correspondence: Tomohide Tamura, Department of Internal Medicine, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: ttamura@ncc.go.jp

Received August 25, 2009; accepted December 20, 2009

Objective: ABI-007 is a novel Cremophor® EL-free nanoparticle albumin-bound paclitaxel. This Phase I study was designed to evaluate tolerability and determine recommended dose for Japanese patients when ABI-007 was administered in every-3-week schedule. Pharmacokinetics of paclitaxel was also assessed.

Methods: Patients with advanced solid tumors refractory to standard therapy received a 30 min intravenous infusion of ABI-007 every 3 weeks without pre-medications at 200, 260 or 300 mg/m^2, respectively. Tolerability and recommended dose were determined by the standard '3 + 3' rule.

Results: No dose-limiting toxicity was observed, despite the dose escalation. In another cohort, 260 mg/m^2 was re-evaluated and resulted in no dose-limiting toxicity. Grade 3 or 4 neutropenia was reported for the majority of patients ($n = 8$) but no incidence of febrile neutropenia. Non-hematological toxicities were generally mild except for Grade 3 sensory neuropathy ($n = 3$). Pharmacokinetic study demonstrated the area under the curve of paclitaxel increased with increasing the dosage, and comparable pharmacokinetic parameters to the western population. Partial response was observed in three non-small cell lung cancer patients. Two of whom had received docetaxel-containing chemotherapy prior to the study.

Conclusions: ABI-007 administered in every-3-week schedule was well tolerated up to 300 mg/m^2, and recommended dose was determined at 260 mg/m^2 in consideration of efficacy, toxicities and similarity of pharmacokinetic profile in western studies. Additional studies of single-agent ABI-007 as well as platinum-based combinations, particularly in patients with non-small cell lung cancer, are warranted.

Key words: nanoparticle albumin-bound paclitaxel – ABI-007 – Phase I – pharmacokinetic – Japanese

INTRODUCTION

ABI-007 (Abraxane®; Abraxis Bioscience, Los Angels, CA, USA) is a novel Cremophor® EL (polyoxyethylated castor oil)-free albumin-bound nanoparticle formulation of paclitaxel. This formulation allows for a higher paclitaxel concentration in the suspension, serving to reduce the administration volume and time. No pre-medication to prevent the Cremophor® EL-induced hypersensitivity reaction is needed. In addition, non-polyvinyl chloride infusion system and in-line filtration are not necessarily applied given no leaching of plasticizers (1,2).

In the Phase I study of every-3-week (Q3W) schedule conducted in the USA, the dose of ABI-007 was escalated from 135 to 375 mg/m^2, and maximum tolerated dose (MTD) and recommended dose (RD) were established at 300 mg/m^2. It was exceedingly higher than that of solvent-based paclitaxel (Taxol®; Bristol–Myers Squibb, Princeton, NJ, USA), 175 mg/m^2 (1). Dose-limiting toxicities (DLTs) were keratitis, blurred vision, sensory neuropathy, stomatitis and neutropenia. Maximum concentration (C_{max}) and the area under the curve from time zero to infinity (AUC_{inf}) of paclitaxel increased linearly over the ABI-007 dose range of 135–300 mg/m^2 administered over 30 min. Volume of distribution of ABI-007 is characterized by the larger distribution than solvent-based paclitaxel, indicating extensive extravascular distribution of the drug (3). C_{max} and AUC_{inf} values for individual patients correlated well with toxicities.

In the Phase III pivotal study of 454 patients with metastatic breast cancer, Q3W schedule of ABI-007 260 mg/m^2 produced the superior outcome to the same schedule of solvent-based paclitaxel, 175 mg/m^2: significantly higher response rate and prolonged time to progression [33% vs. 19% ($P < 0.001$) and 23.0 vs. 16.9 weeks ($P = 0.006$), respectively] and significantly lower incidence of Grade 4 neutropenia, despite a 49% higher paclitaxel dose [9% vs. 22% ($P < 0.001$)] (4). The dosage and schedule used in this Phase III study lead to the approved labeling worldwide.

According to the clinical utility and study data reported overseas, ABI-007 seems to be an effective treatment. This Phase I study aimed to evaluate tolerability, DLT and RD in Japanese patients with solid tumors when administered in Q3W schedule. Efficacy, toxicity and pharmacokinetics (PK) were also evaluated as secondary objectives, followed by the evaluation on ethnic difference in PK.

PATIENTS AND METHODS

Patient Eligibility

Patients aged 20–74 years with histologically or cytologically diagnosed malignant solid tumors refractory to standard therapies or for which there was no effective treatment were eligible. They had to have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2, and a life expectancy of ≥60 days. Eligibility criteria also included adequate renal, liver and bone marrow function, defined as serum creatinine (Cr) ≤1.5 mg/dl, serum total bilirubin (TB) ≤1.5 mg/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) <100 IU/l, respectively, serum albumin ≥3.0 g/dl, white blood cell count ≤12 000/mm^3, absolute neutrophil count ≥2000/mm^3, platelets ≥100 000/mm^3 and hemoglobin ≥9.0 g/dl. Patients with prior exposure to taxanes were eligible for the study. Key exclusion criteria included the following: (i) surgery within 4 weeks; (ii) chemotherapy within 3 weeks; (iii) radiotherapy within 3 weeks; (iv) history of radiation to more than 30% of hematopoietic marrow; (v) pre-existing sensory neuropathy ≥Grade 2; (vi) pleural effusion and ascites that required drainage; (vii) brain metastasis showing symptoms or requiring treatment; (viii) hepatitis B or C virus or human immunodeficiency virus infection; (ix) chronic steroid treatment; (x) pregnancy, lactation, suspicion of being pregnant; (xi) serious pre-existing medical conditions such as uncontrolled infections, pulmonary fibrosis, diabetes, severe heart disease and psychogenic disorders.

This study was approved by the Institutional Review Board at the National Cancer Center and conducted according to Japanese Good Clinical Practice guidelines. All patients provided written informed consent prior to study entry.

Study Design and Treatment

This Phase I, open label, dose-finding study was conducted at National Cancer Center and National Cancer Center East.

ABI-007 was supplied by TAIHO Pharmaceutical Co., Ltd, Tokyo Japan. Each vial contained 100 mg of paclitaxel and ~900 mg of frozen-dried Albumin Human (United States Pharmacopeia). The prescribed dose of ABI-007 was prepared in 5 mg (paclitaxel)/ml of physiological saline as a suspension. The drug was administered via 30 min i.v. without pre-medication and in-line filtration.

Evaluated dose levels were 200, 260 or 300 mg/m^2, as shown in Table 1, repeated every 3 weeks. The rationale for selected dose range was the following: the upper level, 300 mg/m^2—MTD determined in a US Phase I study; the middle level, 260 mg/m^2—the approved dose in the US/EU, and the lower level, 200 mg/m^2—one dose level below MTD examined in the foregoing Phase I study. The dose range also factored in PK: linear PK of ABI-007 over the dose range 80–300 mg/m^2 and the same level and activity of CYP2C8 and CYP3A4 between Japanese and Caucasians (5). Dose escalation was capped at 300 mg/m^2. In the event that MTD exceeded the cap, further steps in investigation would be discussed among study sponsor, principal investigator and medical experts.

The dose escalation followed the standard '3 + 3' rule. Three patients were evaluated at the first dose level, and in the absence of DLTs, three additional patients were entered at the next dose level. If one of the three patients encountered a DLT, another cohort was to be added at the same dose level. The MTD was defined as the dose level at which two out of three to six patients experienced DLT. The RD

Table 1. Dose levels

Level	Dose (mg/m^2)	No. of patients entered	No. of courses
1	200	3	9
2	260	6	23
3	300	3	14

was defined as the dose level that is one level below MTD, and consequently, a total of six patients were to be treated at RD to further evaluate the safety profile.

DLTs were pre-defined as any of the following drug-related toxicities that had occurred during the first course: (i) Grade 4 thrombocytopenia; (ii) Grade 3 thrombocytopenia requiring platelet transfusion; (iii) Grade 4 neutropenia over 4 days; (iv) Grade 3 or 4 febrile neutropenia; and (v) Grade 3 or 4 non-hematologic toxicity. Dose was reduced by one level when DLT occurred in the first course, and reduction was allowed when the toxicities corresponding to DLT or Grade 2 neuropathy occurred in the second course or later.

Patient Evaluation

Pre-treatment evaluation included a complete history and physical examination, a complete blood count with differential, serum chemistry profile, urinalysis including pregnancy test, chest X-ray and electrocardiogram. Serum chemistry profile included electrolytes, Cr, urea nitrogen, TB, AST, ALT, lactic dehydrogenase, alkaline phosphatase, total protein, albumin and C-reactive protein. Baseline imaging studies and serum tumor marker levels were obtained at the discretion of treating physician. Toxicity assessment, physical examination and all blood tests except serum tumor markers were repeated on a weekly basis.

Toxicities were graded according to Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. Patients were considered evaluable for toxicity if they received at least one dose of the study drug. Objective response to therapy was assessed every 4–6 weeks according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0 (6).

Blood Sampling and PK Analysis

Whole blood samples of 7 ml each were collected in 6 ml of heparinized tube and 1 ml of K3-EDTA tube to determine the PK of ABI-007 at time points: 0, 0.25, 0.5 (end of infusion), 0.75, 1, 1.5, 2, 4, 10, 24, 48 and 72 h. Heparinized samples were immediately centrifuged at 1000 *g* for 15 min in 4°C and resultant plasma was stored in aliquot, whereas K3-EDTA samples were softly mixed in normal temperature. These samples were stored at less than or equal to −20°C until analyzed. The sample was analyzed for paclitaxel using liquid chromatography/tandem mass spectrometry in Alta Analytical Laboratory (El Dorado Hills, CA, USA). The limit of quantification for paclitaxel in plasma and whole blood was 1.00 and 5.00 ng/ml, respectively, and the range of reliable response in these samples was 1.00–500 and 5.00–5000 ng/ml, respectively.

PK parameters were determined from each patient's whole blood/plasma paclitaxel concentration profile. They were evaluated by non-compartmental analysis using the WinNonlin software package (Ver4.1, Pharsight Corp., CA, USA). The C_{max} of paclitaxel was obtained directly from experimental data. The elimination constant (λz) was obtained by log-linear regression analysis of the terminal phase of the whole blood/plasma concentration vs. time profile. The elimination half-life ($t_{1/2}$) was determined by taking the ratio of natural log of 2 and λz. The AUC_{inf} was estimated by summing the areas from time zero to the last measured concentration–time point (AUC_{0-t}), calculated using the linear-logarithmic trapezoidal method, and the extrapolated area. The dose–area relationship (i.e. total ABI-007 dose divided by AUC_{inf}) was used to determine total body clearance (CL). The volume of distribution (Vz) was determined by taking the ratio between CL and λz.

Table 2. Patient characteristics

Characteristics	No. of patients
Total no. of patients	12
Male/female	10/2
Age (years)	
Median	61
Range	45–69
ECOG performance status	
0	3
1	9
Tumor type	
NSCLC	6
Parotid gland	1
Ovary	1
Bladder	1
Pharyngeal and esophageal	1
Colon	1
Thymoma	1
Prior treatment	
Surgery	9
Radiotherapy	3
Chemotherapy	12
No. of prior chemotherapy	
1	1
2	4
≥3	7
Prior taxane therapy	
Yes	
Solvent-based paclitaxel	1
Docetaxel	5
Solvent-based paclitaxel and docetaxel	2
No	4

ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small cell lung cancer.