only), 22, 29, 36, 43 and 50 and during the post-treatment observation period or at the time of withdrawal from the study. Cetuximab serum concentration data were generated using a validated sandwich enzyme-linked immunosorbent assay (ELISA) carried out by MDS PS Pharma Services Switzerland AG (Fehraltorf, Switzerland) essentially as described [11].

Results

Patients and demographics

EGFR expression was detected immunohistochemically in the tumor tissue of 43 of 47 screened patients (91%). Of these 43 patients, 30 fulfilled all the inclusion criteria and were enrolled in the study; all received at least one dose of the study drug. Summarized for all patients in Table 1, the demographic characteristics of the individual treatment groups were generally similar. There were no major differences between the dose levels with regard to medical history other than cancer. Twenty-nine patients were suffering from adenocarcinoma of the colon or rectum and the remaining patient had adenocarcinoma of the lung. The majority of patients had metastatic disease at study entry, and 8 (27%), 10 (33%) and 9 (30%) patients had 1, 2 and 3 organs involved, respectively. Most commonly involved organs were the lung in 22, liver in 20 and the lymph nodes in 15 patients. All patients had received previous chemotherapy or hormonal therapy; 29 had undergone surgery, 6 had received radiotherapy, and three had received other treatments. Similar percentages of patients received concomitant medication across dose levels, except for a higher incidence of the use of antihypertensive medications at dose levels 4 and 5.

Table 1 Patients characteristics

Characteristic	$N = 30$
Gender, *N* (%)	
Male	15 (50.0)
Female	15 (50.0)
Age (years)	
Median (min–max)	54 (36–73)
ECOG PS, *N* (%)	
0	20 (67.7)
1	9 (30.0)
2	1 (3.3)
Diagnosis, *N* (%)	
Colorectal cancer	29 (96.7)
NSCLC	1 (3.3)
Prior therapy, *N* (%)	
Chemotherapy	30 (100)
5-Fluorouracil	27
S-1	7
UFT	7
Irinotecan	28
Oxaliplatin	1
Radiotherapy	6 (20)

ECOG PS Eastern Cooperative Oncology Group performance status, *NSCLC* non-small cell lung cancer

Dose-limiting toxicity assessment

The safety population comprised all 30 patients, each of whom had received at least one dose of the study medication. Four patients did not complete the DLT evaluation period after withdrawing from treatment as a consequence of PD after one (one patient) or three infusions (three patients). DLT analyses were therefore performed on 26 evaluable patients (5 patients each at dose levels 2–5 and 6 patients at dose level 1). The median duration of treatment was 14.0 weeks and the median cumulative cetuximab dose was 2,450 mg/m^2. No DLT was reported during the evaluation period and consequently, the MTD was not reached even at the highest dose level. Eighteen patients continued treatment with cetuximab after completion of the preset weekly repeated treatment schedule (on day 50).

Adverse events

AEs and cetuximab-related AEs were reported in 30 (100%) and 29 (97%) patients, respectively. The most common AEs according to system organ class (SOC) distribution were skin and subcutaneous tissue disorders and investigations (both reported for 28/30, 93% of patients) followed by gastrointestinal disorders and general disorders and administration site conditions (both 26/28 patients, 87%).

The most common cetuximab-related AEs observed (summarized in Table 2) were acneiform dermatitis (83%), rash and skin reaction (both 47%), dry skin (40%), pruritus (33%), paronychia (37%), pyrexia (57%), diarrhea (33%) and fatigue and stomatitis (both 30%). Hypersensitivity reaction (HSR) was reported in only one patient at dose level 1. This patient experienced HSR twice: a grade 1 HSR on the day of the first cetuximab infusion and a grade 2 HSR on the second day after administration at week 6. Both reactions resolved. Pyrexia and headache appeared to more common at the higher dose levels and were mainly reported in a close temporal relationship with cetuximab infusion, suggesting that they may have been infusion-related events. Grade 3 or 4 AEs were reported in nine patients after DLT evaluation and in two cases, were considered to be

Table 2 Relevant common any grade and grade 3/4 cetuximab-related adverse events

Adverse event	Number of patients with any grade (grade 3/4)					Any grade total (%)	Grade 3/4 total (%)
	Dose level						
	1	2	3	4	5		
	Dose[a] (mg/m²)						
	100/100 $N = 6$	250/250 $N = 6$	400/250 $N = 6$	500/250 $N = 6$	400/250 $N = 6$	$N = 30$	
Any adverse event	5 (1)	6	6	6 (1)	6	96.7	6.7
Acneiform dermatitis	5	6	4	5 (1)	5	83.3	3.3
Rash	3	2	5	2 (1)	2	46.7	3.3
Skin reaction	3	3	3	2	3	46.7	
Dry skin	1	2	1	4	4	40.0	
Pruritus	2	1	3	3 (1)	1	33.3	3.3
Paronychia	3			4	4	36.7	
Pyrexia		2	4	5	6	56.7	
Diarrhea	2 (1)	1	2	2	3	33.3	3.3
Fatigue	1	1	4	2	1	30.0	
Stomatitis	3	1	1	4		30.0	
Anorexia			2	4	2	26.7	
Nausea	1		2	3	1	23.3	
Vomiting		1	3	2	1	23.3	

[a] Dose: initial dose/weekly dose

cetuximab-related (grade 3 diarrhea, one patient at dose level 1; grade 3 acneiform dermatitis, pruritus and rash, one patient at dose level 4).

Although cetuximab-related AEs did not lead to discontinuation of cetuximab in any patient, the primary reason for discontinuation in two patients was an aggravation of disease symptoms. The weekly dose for one patient (dose level 4) was reduced from 250 to 200 mg/m² at the 38th week of administration due to grade 3 skin toxicity in accordance with the study protocol. There were no other dose reductions. One patient died within 30 days of the end of study treatment from an unrelated respiratory failure due to progressive lung metastases.

Pharmacokinetics

A full PK profile suitable for PK analysis following initial cetuximab infusion was available from all patients. Individual PK parameters after non-compartmental and compartmental analysis were in good agreement. In general, interpatient variability in the cetuximab concentration values was not large. Cetuximab serum concentration time profiles are displayed in Fig. 2. Mean trough concentrations for dose level 5 were constant from week 4 (day 22) onwards (Fig. 3).

PK parameters, based on non-compartment analysis and data obtained at 2 weeks later (day 15) in dose level 1–4 and at a week later (day 8) in dose level 5, are shown in Table 3. Dose-proportional increases in mean C_{max} (range 49.0–396.7 μg/mL) were observed across the dose range of 100–500 mg/m². Moderate deviations from dose proportional increases were observed for $AUC_{0-\infty}$ (range 3,469–3,4817 μg/mL h), especially at the low doses. However, in general, maximum serum concentrations following infusion and the exposure to cetuximab as measured by $AUC_{0-\infty}$ are predictable for each dose used. Mean CL values decreased with dose at the lower dose levels. At doses of ≥400 mg/m², CL values appeared to level off. Mean terminal half-life ($t_{1/2}$) values increased from 54 to 111 h over the 100–500 mg/m² dose range. At the dose of 400 mg/m² (equivalent to the standard regimen), the mean $t_{1/2}$ values were 101 (dose level 3) and 106 h (dose level 5). Values for the volume of distribution at steady state (V_{ss}) were independent of dose and consistent with distribution of cetuximab in the theoretical vascular space.

Pre- and post-dose samples for the determination of HACA levels were available for 21 patients. The analytical results suggested that there had been no induction of such antibodies in these patients.

Efficacy

Six patients were excluded from the efficacy analysis, three because follow-up evaluation was not available (all

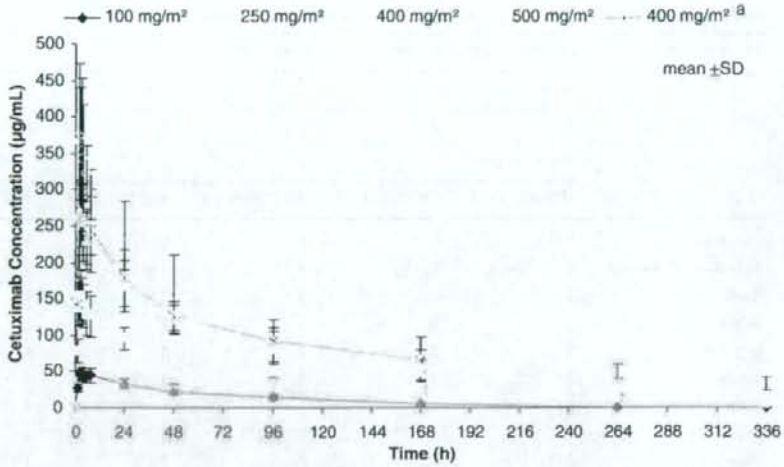

Fig. 2 Cetuximab serum concentration time profile

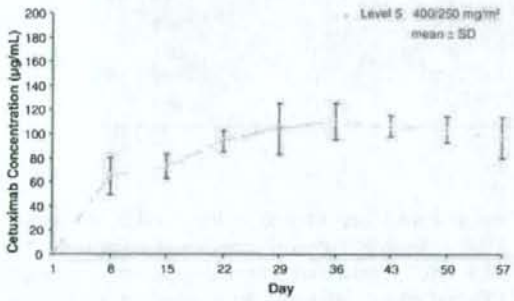

Fig. 3 Trough serum cetuximab concentrations (linear scale) after the 400 mg/m² initial dose and following weekly administrations of 250 mg/m² (dose level 5)

colorectal cancer), and three because the disease at baseline was not measurable (two colorectal cancer and one lung adenocarcinoma). Twenty-four patients were therefore evaluable for efficacy. Two patients treated at dose level 1 showed partial response, giving an overall response rate of 8.3% in the efficacy-evaluable population [95% confidence interval (CI): 1.0, 27.0]. Furthermore, 12 patients achieved stable disease (3, 3, 1, 3 and 2 patients at dose levels 1–5, respectively) to give an overall disease control rate of 58.3% (95% CI: 36.6, 77.9).

Discussion

Cetuximab has been shown to be effective and generally well tolerated in mixed but mainly Caucasian patient groups and the PK profile of this agent administered as a single dose of 20–500 mg/m^2 has been extensively characterized in a variety of separate studies in such populations [15]. Two recent studies have examined cetuximab single-dose PK in US patients with solid tumors using a similar

Table 3 Mean (standard deviation) pharmacokinetic parameters at day 15 (non-compartment analysis)

	Dose level (dose mg/m^2)				
	1 (100) $N = 6$	2 (250) $N = 6$	3 (400) $N = 6$	4 (500) $N = 6$	5 (400)[a] $N = 6$
C_{max} (µg/mL)	49.0 (8.5)	157.0 (31.9)	287.2 (37.9)	396.7 (83.6)	297.8 (30.5)
% CV	17	20	13	21	10
$AUC_{0-\infty}$ (µg/mL × h)	3,469 (583)	12,132 (2.300)	25,823 (6,525)	34,817 (11,498)	29,213 (6,431)
% CV	17	19	25	33	22
$t_{1/2}$ (h)	54 (17)	74 (12)	101 (31)	111 (19)	106 (24)
CL (L/h)	0.046 (0.007)	0.035 (0.009)	0.026 (0.009)	0.026 (0.013)	0.022 (0.005)
V_{ss} (L)	3.46 (0.59)	3.98 (0.78)	3.34 (0.48)	3.51 (0.56)	3.1 (0.5)

CV coefficient of variation, C_{max} maximum concentration, $AUC_{0-\infty}$ area under the concentration-time curve, $t_{1/2}$ terminal half-life, *CL* total body clearance, V_{ss} volume of distribution at steady state

[a] Dose level 5: pharmacokinetic parameters are based on concentration data measured up to timepoint 168 h (day 8) following the initial 400 mg/m^2 dose

dose-escalation protocol to that employed in the current study [11, 12]. Patients in both of these studies received either: 50, 100, 250, 400 or 500 mg/m^2 initial infusions, followed after a 3-week interval by weekly infusions of 250 mg/m^2. The similarity in schedules and type of patient included in these analyses allows a comparative evaluation of cetuximab PK and safety in the non-Japanese and Japanese patient groups. Mean C_{max} values were comparable for initial cetuximab dose levels of 100 and 250 mg/m^2 in the two populations. However, at the higher doses of 400 and 500 mg/m^2, C_{max} values were higher in the Japanese (287 and 397 μg/mL) compared with the non-Japanese populations 205/229 and 243/246 μg/mL, respectively). Likewise, mean $AUC_{0-\infty}$ values were comparable at the lower doses but higher in the Japanese compared with the non-Japanese patient groups at the 500 mg/m^2 dose level (34,817 vs. 30,870 and 24,740 μg/mL h).

However, the results of the current study confirm that the PK profile in Japanese patients is broadly similar to that obtained for non-Japanese patient groups. In particular, linear relationships for both mean C_{max} and $AUC_{0-\infty}$ with dose that were previously noted in the non-Japanese populations were also observed in the Japanese population, indicating that the exposure to cetuximab is predictable across the dose-range. Dose-dependent relationships were observed in the current study for $t_{1/2}$ and CL at lower doses, with the apparent leveling of CL seen at the higher doses mirroring the earlier studies in non-Japanese patients and supportive of receptor saturation at these doses. In addition, V_{ss} was independent of dose and consistent with a distribution of cetuximab in the theoretical vascular space, which is similarly consistent with the data from non-Japanese populations. The cetuximab mean trough concentrations following repeated weekly doses of 250 mg/m^2 (dose level 5) in the Japanese population were constant from fourth week (day 29) onwards and were in good agreement with previously reported pharmacologically active trough concentration values following the standard dosing regimen (equal to dose level 5) of cetuximab [16].

In relation to safety, cetuximab was generally well tolerated at all dose levels in Japanese patients and the MTD was not reached at the highest dose-level tested (500 mg/m^2 initial infusion followed by 250 mg/m^2 weekly). No specific toxicities were identified in Japanese patients compared with mainly Caucasian groups, and the incidence of cetuximab-related grade 3/4 AEs was low (2/30 patients) and as expected. The most common AEs at any grade according to SOC distribution were skin and subcutaneous tissue disorders, which were reported for 93% of patients. Acneiform dermatitis, which was noted in 83% of patients, was the most commonly occurring cetuximab-related AE. Although skin reactions are a class effect of EGFR-targeted agents, the level of incidence of this mainly mild adverse drug reaction in this study is in the upper range of what has been commonly reported for mixed but mainly Caucasian populations. A considerable number of studies in a range of cancer types including mCRC have noted a correlation between the incidence and severity of acne-like rash or skin reactions and efficacy [1, 3, 10–13]. The high level of skin toxicity noted in Japanese patients may therefore be a promising indicator for cetuximab efficacy in this population, a hypothesis that should be addressed in future clinical studies. The disease control rate of 58% achieved for cetuximab monotherapy in Japanese patients is encouraging in this context. On balance, the safety profile for all dose regimens in the current study was essentially consistent with the safety profile of cetuximab as described in the previous comparable studies in non-Japanese patient populations [11, 12].

In conclusion, the current study has demonstrated that cetuximab PK and safety profiles are similar between Japanese and non-Japanese patient populations. Given this assessment, it would appear that the standard dose of an initial 2-h infusion of 400 mg/m^2 followed thereafter by weekly 1-h infusions of 250 mg/m^2 is feasible for future clinical studies in Japanese patients.

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A Phase II Trial of Gefitinib Monotherapy in Chemotherapy-Naïve Patients of 75 Years or Older with Advanced Non-small Cell Lung Cancer

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Background: Gefitinib has shown modest activity in patients with recurrent non-small cell lung cancer (NSCLC) after platinum-based chemotherapy. However, the activity of gefitinib as first-line chemotherapy remains unclear, especially unknown in elderly patients. A multicenter phase II trial was conducted to evaluate the efficacy and tolerability of gefitinib for elderly patients with chemotherapy-naïve NSCLC.

Methods: Elderly chemotherapy-naïve patients with advanced NSCLC, ECOG PS of 0–2, and adequate organ functions received 250 mg/day of gefitinib. The primary objective of this study was to determine the objective response rate (RR). Secondary endpoints were tolerability, disease-related symptom using lung cancer subscale (LCS) in FACT-L, progression free survival (PFS) and overall survival (OS). We investigated mutation status of the epidermal growth factor receptor (EGFR) gene in cases with available tumor samples.

Results: Fifty patients were enrolled, of whom 49 were eligible. Median age (range) was 80 (75–90) years. Thirty-two patients were female (65%) and 40 patients had adenocarcinoma (82%). The objective RR was 25% (CI 95%, 13–39). Median survival time was 10 months (CI 95%, 7–20) and 1-year survival rate was 50%. The most frequent adverse events were skin disorders (76%). Fifteen patients (30%) experienced toxicities ≥grade 3. There were four patients with possible interstitial lung disease including two treatment-related deaths. Symptom improvement rate using LCS was 49% at 4 weeks of gefitinib therapy. Tumor samples from 17 patients were analyzed for *EGFR* mutation status. *EGFR* mutations were detected in tumor tissues from 7 patients, of which 5 had partial responses (71%).

Conclusions: Gefitinib monotherapy is effective and relatively well tolerated in chemotherapy-naïve elderly patients with advanced NSCLC. Gefitinib has potential as a first-line therapeutic option in elderly patients with advanced NSCLC.

Key Words: Gefitinib, Non-small cell lung cancer, Elderly.

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The incidence of lung cancer, the major cause of cancer-related mortality worldwide, increases with age.[1] More than 30% of lung cancer patients are diagnosed at an age of ≥75 years,[2] and this proportion is likely to increase over the coming years. Approximately 80 to 85% of lung cancer subtypes are of non-small cell histology. In patients with advanced non-small cell lung cancer (NSCLC) chemotherapy improves survival, disease-related symptoms, and quality of life (QoL) compared with best supportive care[3] and combination chemotherapy involving newer agents is now considered the standard first-line treatment for most patients with advanced NSCLC.[4,5] However, combination chemotherapy causes increased hematologic and neuropsychiatric toxicity in older patients,[6,7] and >90% of elderly patients experience a grade ≥3 toxicity when treated with a platinum-based doublet.[8] Therefore, single-agent chemotherapy is considered as a standard treatment for elderly patients with advanced NSCLC.[9,10] An effective, less toxic therapy might help extend potentially beneficial treatment to a greater proportion of older patients who would otherwise be considered unsuitable for chemotherapy. Recently, molecular-targeted agents have been introduced for the treatment of NSCLC. Gefitinib, an orally active epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), is a leading agent in the field of EGFR-targeted

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therapy.[11] In two international, randomized phase II trials (Iressa Dose Evaluation in Advanced Lung cancer [IDEAL]-1 and IDEAL-2) in patients with advanced or metastatic NSCLC after platinum-based chemotherapy, treatment with gefitinib 250 mg monotherapy resulted in response rates (RRs) of 12 to 18%, good tolerability and symptom improvement.[12,13] The subset analyses in both trials revealed no significant difference in terms of the RR and adverse events (AEs) reported with gefitinib between younger and older patients. Despite the high incidence of NSCLC and its high mortality rate in elderly patients,[1] the likelihood of receiving systemic chemotherapy seems to decrease with increasing age—in particular, the age of ≥75 years—due to the progressive decline in organ function, and age-related comorbidities. Therefore, we conducted a phase II study of gefitinib monotherapy in chemotherapy-naive patients ≥75 years of age with advanced NSCLC.

PATIENTS AND METHODS

Patient Population

Patients with histologically or cytologically confirmed NSCLC that was inoperable as a result of substantial comorbidity, impairment of respiratory function or anatomic contraindication were eligible. Patients were to be aged 75 years or older, and to have an Eastern Co-operative Oncology Group Performance Status 0 to 2, measurable disease, Pao_2 ≥60 mmHg and adequate organ function. Exclusion criteria were: prior chemotherapy or thoracic radiotherapy; interstitial pneumonia or pulmonary fibrosis; as determined by chest computed tomography (CT); paralytic ileus or vomiting; symptomatic brain metastases; active infection; active concomitant malignancy; pregnancy or breast-feeding; and severe allergy to study drugs. All patients gave written informed consent before enrolment. This protocol was approved by the Institutional Review Boards of the participating centers.

Treatment Plan

Baseline assessment included a medical history and physical examination, standard laboratory studies, ECG, CT of the chest and abdomen, head CT or magnetic resonance imaging, and disease-related symptoms using lung cancer subscale (LCS) in The Functional Assessment of Cancer Therapy–Lung.[14] Patients were treated with gefitinib 250 mg daily until disease progression, severe or intolerable toxicity, or withdrawal of consent.

Patients were evaluated for objective response every month using Response Evaluation Criteria in Solid Tumors guidelines.[15] The objective response was evaluated centrally by an independent review board. All AEs reported during gefitinib treatment were recorded and severity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 2.0.[16] Treatment interruptions up to 14 days were allowed for toxicities ≥grade 3, and patients were restarted if toxicities recovered to ≤grade 2 within 14 days.

Disease-Related Symptom Analysis

Disease-related symptoms were assessed by LCS before therapy and at 1, 2, and 4 weeks. Symptom improvement was defined as a 2-point or greater increase in score on the summed LCS.[17]

Mutation Analysis of the EGFR Gene

Tumor specimens (paraffin embedded) were collected from previous diagnostic or surgical procedures. DNA was extracted from tumor tissues derived either by macrodissection or by laser-capture microdissection performed to enrich tumor cells, using the QIAamp Micro kits (QIAGEN KK, Tokyo, Japan). The amplification-refractory mutation system (ARMS) with designed "scorpion" primers were applied for the allele-specific detection of *EGFR* mutations.[18] Only the following mutations described in previous studies were classified as mutations in the present study: G719X in exon 18, deletion of E746 to A750 or of neighboring residues in exon 19, and L858R and L861Q in exon 21. All mutations were confirmed by analysis of at least two independent amplification products.

Statistical Analysis

This study was a multicenter, single-arm, noncomparative phase II clinical trial. The primary end point was objective RR. Secondary endpoints were tolerability, disease-related symptoms using LCS in Functional Assessment of Cancer Therapy–Lung, progression free survival (PFS) and overall survival (OS). With the target activity level of 30% and the lowest RR of interest set at 15%, 48 patients were required with $\alpha = 0.048$ and $\beta = 0.18$ assuming binomial distribution of RR. Allowing for a loss, a total of 50 patients were planned to enroll. All eligible patients were included in the analysis of response. The 95% confidence interval (CI) of the RR was calculated by the exact method, assuming a binomial distribution of data. PFS was defined as the time from registration until objective tumor progression or death. Patients whose disease had not progressed at the time of discontinuation of the study treatment continued to be assessed until progression was documented. If a patient died without documentation of disease progression, the patient was considered to have had tumor progression at the time of death, unless there was sufficient documented evidence to conclude otherwise. PFS and OS were estimated using the Kaplan-Meier method.[19] To examine the association of tumor response to gefinitib and OS with clinical factors including *EGFR* mutations, two-sided Fisher's exact test and Log-rank test, respectively, were used. To examine the time tendency of disease related symptoms, Generalized Estimation Equation model was used.[20] The model includes the repeated measured LCS as a continuous dependent variable and time in week as a continuous explanatory variable. A two-sided $p < 0.05$ was considered statistically significant.

RESULTS

Patients and Treatment Administration

Fifty patients were enrolled between April 2004 and May 2006 at 22 centers across Kyushu in Japan. Of these, 49 patients were deemed eligible (one stage IB patient with no indications for surgery or curative radiotherapy due to severe emphysema). One patient was ineligible because of stage IIIA with an indication for curative radiotherapy. Patient characteristics are listed in Table 1. Median age (range) was

TABLE 1. Patient Characteristics

Characteristics	No. of Patients	%
Patients enrolled	50	
Patients eligible	49	
Age		
Median	80	
Range	75–90	
Sex		
Male	17	35
Female	32	65
Performance status		
0	13	27
1	24	49
2	12	24
Stage at enrolment		
IB[a]	1	2
IIIB	8	16
IV	40	82
Histology		
Adenocarcinoma	40	82
Squamous cell carcinoma	6	12
Large cell carcinoma	0	0
Other	3	6
Smoking status		
Never	30	61
Former	16	33
Current	3	6

[a] No indications for surgery or curative radiotherapy due to severe emphysema.

80 (75–90) years. Thirty-two patients were female (65%) and 12 patients (24%) had a performance status (PS) of 2. The most common histologic NSCLC subtype was adenocarcinoma (82%). Most patients (82%) had stage IV disease or recurrence after surgical resection and 30 patients were never smokers (61%). Of the 49 patients, 46 patients discontinued gefitinib treatment by reason of progression of disease (n = 31), treatment-related AEs (n = 13), patient request (n = 1), and another disease (n = 1). The median treatment period was 2.8 months (range: 0.1–16 months). Three patients are still receiving treatment with gefitinib.

Adverse Events

Table 2 lists the AEs by grade. The most frequent AEs were skin disorders (76%) including rash, dry skin, pruritus, acne, and nail changes. In general, toxicities were mild (grade 1–2) and easily managed. Fifteen patients (30%) experienced toxicities ≥grade 3. Thirteen were withdrawn from the study because of treatment-related AEs–four patients with interstitial lung disease (ILD), four with rash, one with asthenia, one with diarrhea, one with mucositis, one with hypoxemia, and one with hepatic toxicity. There were four patients with possible ILD including two treatment-related deaths.

Response

There were 12 partial responses (PRs) among the 49 patients, yielding an overall RR of 25% (95% CI, 13–39%). Seventeen patients (35%) achieved stable diseases (SDs) and

TABLE 2. Common Adverse Events (n = 50)

	All		Grade 3–5[a]	
Toxicity	No.	%	No.	%
Dermal[b]	38	76	10	20
Anaemia	33	66	3	6
Anorexia	25	50	6	12
Fatigue	21	42	5	10
Hepatic	20	40	11	22
Diarrhoea	14	28	2	4
Nausea	11	22	1	2
Neutropenia	11	22	0	0
Renal	11	22	0	0
Mucositis	7	14	1	2
ILD	4	8	3	6
Vomiting	3	6	0	0

[a] Two patients experienced grade 5 events related to gefitinib-induced ILD.
[b] Dermal toxicities including rash, dry skin, pruritus, acne and nail changes.
ILD, interstitial lung disease.

the overall disease control rate (PR + SD) was 59% (95% CI, 44–73%). Four patients were removed from the study before being evaluated for response; two patients had ILD, one had a stroke, and one had deteriorated PS. (Table 3).

PFS and Survival

All 49 eligible patients were assessed for PFS and survival. At the time of analysis, there were 31 deaths, 17 patients confirmed alive, and one patient lost to follow-up. Median survival time was 10 months (95% CI, 7–20; Figure 1*A*). Median PFS was 4 months (95% CI, 3–8; Figure 1*B*). The 1- and 2-year survival rates were 50% and 23%, respectively.

Disease-Related Symptoms

With respect to disease-related symptoms, the mean LCS scores were 21, 22, 22, and 23 points before the therapy and at 1, 2, and 4 weeks, respectively. The time tendency of change in LCS scores was significant (p = 0.017) (Table 4). There was no significant association between response and symptom improvement (Table 5).

TABLE 3. Best Overall Objective Response (n = 49)

Type of Response	No. of Patients	% of Patients	
Complete response	0	0	
Partial response	12	25	
Stable disease	17	35	
Progressive disease	16	33	
Not evaluable[a]	4	8	
Overall response (CI 95%)	12	25	(13–39)

[a] Four patients were removed from study before being evaluated for response; two patients had interstitial lung disease (ILD), one had stroke and one had deteriorated performance status (PS).
CI 95%, 95% confidence interval.

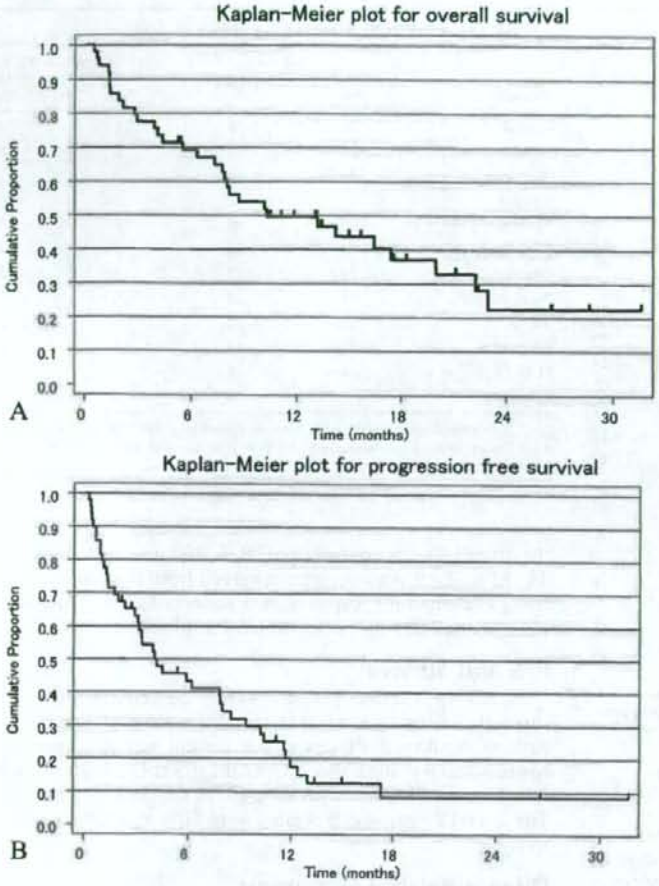

FIGURE 1. *A*, overall survival (OS) and (*B*) progression-free survival (PFS) of all eligible patients ($n = 49$). Median OS was 10 months. Median PFS was 4 months.

Mutation Analysis of the EGFR Gene

Tumor samples from 17 patients were analyzed for *EGFR* mutations by Scorpion-ARMS. *EGFR* mutations, consisting of in-frame deletions in exon 19 ($n = 2$) and point mutations in exon 21 ($n = 5$) were detected in 7 tumor tissues (41%). All patients harboring *EGFR* mutations achieved either PRs or SDs. Five patients with *EGFR* mutations had PRs and the RR was 71%. Of the 10 patients without *EGFR* mutations (59%), 2 patients had PRs, 3 had SDs and 5 had progressive diseases. The presence of *EGFR* mutations was associated with prolonged survival in this trial (Table 5).

TABLE 4. Disease-Related Symptoms Evaluated by Lung Cancer Subscale

		Before Therapy $n = 42$	After 1 wk $n = 41$	After 2 wk $n = 39$	After 4 wk $n = 35$
Mean LCS score		21	22	22	23
Change of LCS Score from Baseline			**No. of Patients (%)**		
≥+2	Improved		15 (37)	14 (36)	17 (49)
≤−2	Worsened		5 (12)	10 (26)	8 (23)
Otherwise	No change		21 (51)	15 (39)	10 (29)

The time trend in repeated measured LCS was statistically significant ($p = 0.017$) according to Generalized Estimation Equation model analysis.
LCS, lung cancer subscale.

Second-Line Chemotherapy

Thirteen patients received second-line chemotherapy (gefitinib rechallenge, $n = 5$; docetaxel monotherapy, $n = 4$; gemcitabine plus tegafur-uracil, $n = 2$; gemcitabine plus vinorelbine, $n = 1$; or gemcitabine monotherapy, $n = 1$). Altogether, two patients partially responded to second-line gemcitabine plus tegafur-uracil, or gemcitabine monotherapy, with no responses among patients receiving other regimens. Overall RR to second-line chemotherapy was 15%.

DISCUSSION

Given the increasing number of elderly individuals with advanced NSCLC, it is important for clinicians to be ready to manage these challenging patients in the coming decades. Prospective randomized trials in elderly (70 years or older)

TABLE 5. Association of Tumor Response to Gefitinib and Median Overall Survival with Clinicopathological Factors

Characteristics (no. of patients)	ORR		MS	
	%	P_1	mo	P_2
Gender				
Male (17)	0	<0.01	8	0.12
Female (32)	38		17	
Histology				
Adeno ca. (40)	28	0.42	13	0.06
Nonadeno ca. (9)	11		4	
Smoking				
Never (30)	37	0.02	17	0.10
Current/Former (19)	5		8	
LCS score				
Improved/No change (27)[a]	33	0.39	17	0.43
Worsened (8)[a]	13		6	
EGFR mutation				
Positive (7)	71	0.06	>27[b]	0.01
Negative (10)	20		7	

[a] This is at 4 wk of therapy.
[b] MS has not yet been reached.
ORR, objective response rate; MS, median survival; P_1, two-sided P for difference in ORR; P_2, two-sided P for difference in overall survival; LCS, lung cancer subscale.

advanced NSCLC patients are now available. The Elderly Lung Cancer Vinorelbine Italian Study Group reported significantly superior survival and QoL with single-agent vinorelbine over best supportive care.[9] The conclusive results were reported in other Multicenter Italian Lung Cancer in the Elderly Study, which enrolled more than 700 patients and reported no significant survival difference between single-agent vinorelbine, single-agent gemcitabine, or a regimen with both agents combined.[10] These studies together provide evidence that single-agent chemotherapy is considered as a standard treatment for advanced NSCLC elderly patients.

The present phase II study was designed to evaluate the efficacy and tolerability of single agent treatment with gefitinib in previously untreated NSCLC patients ≥75 years of age. The observed RR of 25% (95% CI, 13–39%), median PFS of 4 months, median survival of 10 months and 1-year survival rate of 50% are promising in elderly advanced NSCLC. One potential limitation in interpreting efficacy data from this trial is a possible selection bias on the part of treating physicians. Although this study planned to recruit unselected patients, a higher percentage of women (65%), never smokers (61%), and patients with adenocarcinoma (82%) were enrolled. This was a result of the growing evidence that these clinical characteristics are more often associated with benefit from EGFR-TKIs.[12,13,21] A randomized phase II study (median age <70) comparing combination chemotherapy with another EGFR-TKI, erlotinib for chemotherapy-naive advanced NSCLC patients with a PS of 2 (who accounted for 24% of our study enrollment) showed a superior response rate, PFS and OS in combination chemotherapy relative to erlotinib. However, subgroup analyses revealed a longer PFS in erlotinib relative to combination chemotherapy for women, never smokers, and patients with adenocarcinoma.[22] Indeed, our subgroup analyses demonstrated that all responders in the present study were women, and never smokers had a higher response rate than smokers (37 versus 5%). Imbalance of responders by gender may be attributed to the fact that most women enrolled in this study were never smokers. On the other hand, histologic subtype as another predicting factor did not show any significant differences in response rate, possibly due to the small sample size.

The recent discovery of somatic mutations in the tyrosine kinase domain of EGFR and of the association of such mutations with a high response rate to EGFR-TKIs has had a profound impact on the treatment of advanced NSCLC.[23–25] In the present study, we used Scorpion-ARMS which is more sensitive than direct sequencing for detection of the known *EGFR* mutations that reflect responsiveness to EGFR-TKIs.[18] Our analyses demonstrated that *EGFR* mutations were detected in 7 of 17 patients (41%), and those 5 patients achieved PRs and 2 patients had SDs. It is noteworthy that the presence of *EGFR* mutations was associated with longer survival (median >27 months), although it remains unclear whether *EGFR* mutations are predictive of EGFR-TKIs treatment benefit or merely prognostic of prolonged survival. Disease-related symptom improvement may be more important factors than tumor response and survival in the treatment of elderly patients with advanced NSCLC. In the present study, LCS revealed a significant symptom improvement from the start of gefitinib therapy to 4 weeks. Symptom improvement rate was 49% at 4 weeks of gefitinib therapy, which compares favorably with the improvement rate reported for the overall population in IDEAL-2 (39%).[17]

The toxicity of gefitinib in this study compares favorably with other studies performed in patients with NSCLC older than age 70 years. Fifteen patients (30%) experienced toxicities ≥grade 3. The most frequent AEs (≥G3) were skin disorders and hepatic dysfunction. There were four patients with possible ILD including two treatment-related deaths.

Gefitinib-induced ILD in the Japanese patients, of which multivariate analysis identified male sex, a history of smoking, and coincidence of interstitial pneumonia as significant risk factors, revealed a higher incidence of 3.2%, ranging from 0.4% in female never-smokers to 6.6% in male smokers, than in other ethnicities.[21] Despite strict exclusion of interstitial pneumonia by chest CT, there were 4 patients with possible ILD (8%) in the present study, including 2 treatment-related deaths (4%). One patient with treatment-related death was a male smoker with the higher risk of gefitinib-induced ILD, however, another was a female never-smoker with the lower risk. With strict exclusion of interstitial pneumonia by chest CT, it might be difficult to completely prevent development of gefitinib-induced ILD as it was previously reported that 5 of 34 patients without interstitial shadow by chest CT experienced ILD.[26] Further scientific investigations are required to elucidate gefitinib-induced ILD.

A recent phase III study for elderly patients (≥70) with advanced NSCLC (WJTOG9904) reported that docetaxel monotherapy significantly improved RR, PFS, and overall disease-related symptoms compared with vinorelbine mono-

therapy.[27] The data suggest that docetaxel monotherapy should be considered as an option in the standard treatment in this patient population. More recently, a large second line trial comparing gefitinib with docetaxel (INTEREST trial n = 1440) demonstrated the noninferiority of gefitinib to docetaxel in terms of OS, with a more favorable toxicity profile and QoL score in gefitinib arm.[28] These findings support the notion that EGFR-TKIs may be an ideal agent to investigate in the first line setting in elderly patients with advanced NSCLC. It has been recently reported that another EGFR-TKIs, erlotinib, is active and relatively well tolerated in chemotherapy-naive elderly patients (≥70) with advanced NSCLC.[29]

In conclusion, we showed the effective outcome of gefitinib monotherapy in chemotherapy-naive patients ≥75 years of age with advanced NSCLC. Despite our intentions not to discriminate, a higher percentage of women (65%), never smokers (61%), and patients with adenocarcinoma (82%) were enrolled because of a possible selection bias on the part of treating physicians. Although our present study suggests that gefitinib is a viable option for such selected patients, our data may not be applicable to elderly patients in general. Further studies are required to determine which patients will ultimately benefit from this therapy.

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A Randomized, Double-Blind, Phase IIa Dose-Finding Study of Vandetanib (ZD6474) in Japanese Patients With Non-Small Cell Lung Cancer

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Introduction: Vandetanib (ZACTIMA™) is a once-daily, oral anticancer drug that selectively inhibits vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) signaling. Vandetanib was evaluated as a monotherapy in a randomized, double-blind, dose-finding study in Japan.

Patients and Methods: Eligible patients with locally advanced or metastatic (stage IIIB/IV) or recurrent non-small cell lung cancer, previously treated with chemotherapy, were randomized to receive once-daily oral vandetanib 100, 200, or 300 mg (1:1:1). The primary objective was to determine the objective response rate for each vandetanib dose.

Results: Fifty-three patients received vandetanib (100 mg, $n = 17$; 200 mg, $n = 18$; 300 mg, $n = 18$). The objective response rate in each dose arm was 17.6% (3 of 17; 100 mg), 5.6% (1 of 18; 200 mg), and 16.7% (3 of 18; 300 mg). Common adverse events included rash, diarrhea, hypertension, and asymptomatic QTc prolongation. The adverse event profile was generally consistent with that reported previously for agents that inhibit the VEGFR or EGFR signaling pathways. Among the three responders evaluated for *EGFR* mutation, two had no mutation, and in one case, the *EGFR* mutation status could not be determined by direct DNA sequencing and amplification refractory mutation system assay of *EGFR* exons 19–21. Baseline plasma VEGF levels appeared to be lower in patients who experienced clinical benefit after vandetanib treatment.

Conclusion: In Japanese patients with advanced non-small cell lung cancer, vandetanib monotherapy (100–300 mg/d) demonstrated antitumor activity with an acceptable safety and tolerability profile.

Key Words: Non-small cell lung cancer, Vandetanib, EGFR, VEGFR.

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Non-small cell lung cancer (NSCLC) accounts for approximately 75% of lung cancers and is the leading cause of cancer-related death worldwide.[1] Despite the introduction of more effective chemotherapeutic agents, new approaches are required to further improve patient outcome and survival. A major focus of new anticancer research is the targeting of cell-signaling pathways that contribute to tumor growth and progression.

Vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) are key drivers of tumor angiogenesis and cell proliferation, respectively, and both pathways have been validated as clinically relevant targets in NSCLC. The addition of bevacizumab, a humanized anti-VEGF-A monoclonal antibody, to paclitaxel and carboplatin has demonstrated clinical benefit in patients with NSCLC,[2] and the EGFR inhibitors gefitinib and erlotinib have demonstrated clinical activity as single agents in NSCLC.[3,4] Furthermore, EGFR is known to regulate the production of VEGF and other proangiogenic factors[5] and resistance to EGFR inhibition has been associated with increased expression of VEGF in a human tumor xenograft model of NSCLC.[6] Therefore, targeting the VEGFR and EGFR pathways may be more effective than inhibiting either pathway alone. This hypothesis is supported by the promising results from early clinical evaluation of erlotinib and bevacizumab in combination in patients with recurrent NSCLC.[7]

Vandetanib (ZACTIMA™) is a once-daily, orally available anticancer drug that inhibits VEGFR- and EGFR-dependent signaling,[8] as well as the RET (REarranged during

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Transfection) receptor tyrosine kinase, which is an important growth driver in certain types of thyroid cancer.[9] Early clinical evaluation of vandetanib has demonstrated a promising efficacy and safety profile in a broad population of patients with advanced cancer. Phase I studies in advanced solid tumors conducted in the USA/Australia[10] and Japan[11] showed that once-daily doses of vandetanib (up to and including 300 mg) were generally well tolerated. In the Japanese study, objective tumor responses were observed in 4 of 9 patients with refractory NSCLC. Subsequent phase II studies in advanced NSCLC demonstrated antitumor activity both as a monotherapy and in combination with certain chemotherapy.[12–14] The positive outcome of these phase II trials led to the ongoing phase III evaluation of vandetanib in previously treated advanced NSCLC.

The primary objective of this randomized phase IIa study was to assess the objective response rate (ORR) to vandetanib (100, 200, or 300 mg/d) in Japanese patients with refractory NSCLC. The three doses investigated were selected based on the outcome of the Japanese phase I trial.[11]

PATIENTS AND METHODS

Patients

Patients with histologic or cytologic confirmation of locally advanced/metastatic (stage IIIB/IV) or recurrent NSCLC after failure of 1 or 2 platinum-based chemotherapy regimens were recruited from eight centers in Japan. The main eligibility criteria were age ≥20 years, a WHO performance status of 0 to 2, an estimated life expectancy ≥12 weeks, and completion of prior chemotherapy and/or radiotherapy at least 4 weeks before study entry (8 weeks for chest radiation and 6 weeks for mitomycin C). Patients with squamous cell histology were also eligible, and brain metastases were permitted if patients were asymptomatic and did not require corticosteroid treatment. Key exclusion criteria were a mixed small-cell and non-small cell histology, evidence of severe or uncontrolled systemic diseases, poorly controlled hypertension, a QTc interval ≥460 milliseconds by electrocardiogram during the screening period, and prior treatment with EGFR or VEGFR signaling inhibitors. All patients provided written informed consent. The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki, applicable guidelines on good clinical practice, local Institutional Review Board approval, and the AstraZeneca policy on Bioethics.

Study Design and Treatments

This was a randomized, double-blind, parallel-group, phase IIa dose-finding multicenter study to assess the efficacy and safety of vandetanib. A total of 53 patients were randomized (1:1:1) to receive once-daily oral vandetanib (100, 200, or 300 mg/d; Figure 1). Patients were stratified by histology (adenocarcinoma versus others), gender (male versus female), and smoking history (smoker versus nonsmoker). Treatment continued until a withdrawal or dose-interruption criterion was met. These criteria included progressive disease (PD), unacceptable toxicity, protocol noncompliance, or voluntary discontinuation by the patient.

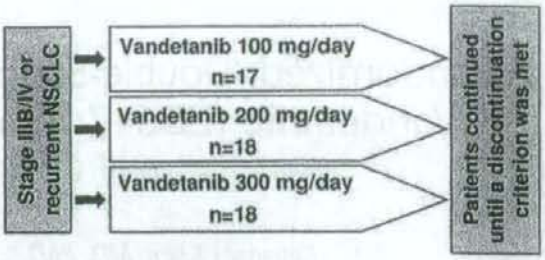

FIGURE 1. Study design.

Efficacy

The primary objective of the study was to determine ORR with vandetanib monotherapy, using the Response Evaluation Criteria in Solid Tumors (RECIST); assessments were performed at baseline and every 4 weeks for the first 24 weeks of treatment, and then every 8 weeks until withdrawal. A confirmed complete response or partial response (PR) was considered to be an objective tumor response. Investigator assessment of best overall tumor response was used for the primary analysis and these assessments were subsequently submitted to AstraZeneca for review by the response evaluation committee. Secondary efficacy endpoints included time to progression (TTP), duration of response (the time interval between the date of first documented objective tumor response until the date of PD or death), and disease control rate (DCR) for each dose of vandetanib. Time to progression was calculated from the date of randomization until the date of PD or death (in the absence of progression) and estimated using the Kaplan–Meier method. DCR was defined as confirmed complete response, PR, or stable disease (SD) ≥8 weeks.

Safety and Tolerability

Safety was assessed by monitoring for adverse events (AEs) and collecting laboratory data. All AEs were collected for up to 30 days after the last dose of vandetanib and were graded according to Common Terminology Criteria for Adverse Events (CTCAE, version 3). Unless otherwise clinically indicated, 12-lead electrocardiograms were performed twice at screening, weekly for the first 8 weeks of treatment, and then once every 4 weeks thereafter. Vandetanib treatment was interrupted following: a single QTc measurement ≥550 milliseconds; 2 consecutive QTc measurements ≥500 milliseconds but <550 milliseconds; an increase of ≥100 milliseconds from baseline; or an increase of ≥60 milliseconds from baseline QTc to a QTc value ≥460 milliseconds. Upon resolution of QTc prolongation, vandetanib treatment was recommenced at a reduced dose.

Pharmacokinetics

To investigate the pharmacokinetic (PK) profile of vandetanib, blood samples were collected on the same days as scheduled electrocardiogram measurements. Plasma concentrations of vandetanib were determined using reversed-phase liquid chromatography–mass spectrometry. The col-

lected data were related to a nonlinear mixed effects model to estimate population PK using NONMEM V (v 1.1).

Tumor Biomarkers

An exploratory objective of this study was to investigate how variations in copy number or mutational status of the *EGFR* gene affect tumor response in advanced NSCLC patients receiving vandetanib treatment. Tumor biopsy samples were obtained from consenting patients, formalin-fixed, and embedded in paraffin. Gene copy number was investigated by fluorescence in situ hybridization using the LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis, Abbott Laboratories, IL) according to a previously published method.[15] Tumor samples had a high *EGFR* gene copy number if there was high gene polysomy (≥4 *EGFR* gene copies in ≥40% of tumor cells) or gene amplification (presence of tight *EGFR* gene clusters, an *EGFR* gene to chromosome 7 ratio of ≥2, or ≥15 copies of the *EGFR* gene per tumor cell in ≥10% of analyzed cells).

EGFR mutations were analyzed by DNA sequencing of exons 19–21, and additionally by using the amplification refractory mutation system (ARMS) assay to detect the exon 21 L858R point mutation and the most common exon 19 deletion (del G2235–A2249).[16]

Plasma Biomarkers

Plasma samples were collected from patients at baseline, day 29, and day 57, and stored at −70°C. The concentrations of the following angiogenic markers were determined by colorimetric Sandwich ELISA (R&D Systems, Minneapolis, USA): VEGF (Cat. #DVE00), the soluble angiopoietin receptor Tie-2 (Cat. #DTE200), and VEGFR-2 (Cat. #DVR200).

RESULTS

Patient Characteristics

Fifty-three patients were recruited from eight centers in Japan between December 27, 2004, and September 30, 2005. All were randomized on this study and received study drug. Patient characteristics and baseline demographics were generally similar in the three arms, and the patient populations were considered to be appropriate for the dose-finding objectives of this study (Table 1). At the time of data cut-off (23 January 2006), 11 patients were ongoing; PD was the most common reason for discontinuation ($n = 35$). Other reasons for discontinuation were AEs ($n = 6$) and withdrawal of consent ($n = 1$).

Efficacy

The overall ORR was 13.2% (95% CI: 5.5–25.3%) (7 of 53 patients), and all 7 responders were PRs (Table 2). According to vandetanib dose received, the ORRs were 17.6% (95% CI: 3.8–43.4%) (3 of 17 patients; 100 mg), 5.6% (95% CI: 0.1–27.3%) (1 of 18 patients; 200 mg), and 16.7% (95% CI:3.6–41.4%) (3 of 18 patients; 300 mg). In all cases, the response evaluation committee assessment of tumor responses was similar to the investigator assessments. The characteristics of those patients who achieved a PR are described in Table 3. Secondary efficacy assessments are presented in Table 2 and Figure 2.

Safety

Overall, the most common AEs were rash, diarrhea, hypertension, and QTc prolongation (Table 4). In general, no major differences were observed in the incidences of

TABLE 1. Patient Demographic and Baseline Characteristics (Full Analysis Set)

	Vandetanib 100 mg/d ($n = 17$)	Vandetanib 200 mg/d ($n = 18$)	Vandetanib 300 mg/d ($n = 18$)	Total ($n = 53$)
Median age, yr (range)	58 (30–78)	61 (43–77)	61 (44–77)	60 (30–78)
Male (%)	11 (64.7)	12 (66.7)	11 (61.1)	34 (64.2)
Female (%)	6 (35.3)	6 (33.3)	7 (38.9)	19 (35.8)
Smoking history[a]				
No (%)	5 (29.4)	8 (44.4)	7 (38.9)	20 (37.7)
Yes (%)	12 (70.6)	10 (55.6)	11 (61.1)	33 (62.3)
WHO performance status 0/1/2	5/12/0	7/11/0	6/12/0	18/35/0
Previous chemotherapy				
One regimen (%)	13 (76.5)	9 (50.0)	14 (77.8)	36 (67.9)
Two regimens (%)	4 (23.5)	9 (50.0)	4 (22.2)	17 (32.1)
Staging (%)				
IIIB	2 (11.8)	3 (16.7)	1 (5.6)	6 (11.3)
IV	14 (82.4)	12 (66.7)	15 (83.3)	41 (77.4)
Recurrent	1 (5.9)	3 (16.7)	2 (11.1)	6 (11.3)
Histology (%)				
Squamous	5 (29.4)	6 (33.3)	4 (22.2)	15 (28.3)
Adenocarcinoma	11 (64.7)	12 (66.7)	12 (66.7)	35 (66.0)
Other	1 (5.9)	0	2 (11.1)	3 (5.7)
Brain metastasis at study entry (%)	4 (23.5)	3 (16.7)	5 (27.8)	12 (23.6)

[a] No, patients who have smoked <100 cigarettes in their lifetime; Yes, patients who have smoked >100 cigarettes in their lifetime.

TABLE 2. Efficacy Summary

	Vandetanib 100 mg/d (n = 17)	Vandetanib 200 mg/d (n = 18)	Vandetanib 300 mg/d (n = 18)
Primary efficacy assessment			
Best response (RECIST)			
Partial response, n (%)	3 (17.6)	1 (5.6)	3 (16.7)
Stable disease ≥8 wk, n (%)	5 (29.4)	6 (33.3)	8 (44.4)
Disease progression, n (%)	9 (52.9)	10 (55.6)	7 (38.9)
Not evaluable, n (%)	0	1 (5.6)	0
Secondary efficacy assessments			
Disease control ≥8 wk, n (%)	8 (47.1)	7 (38.9)	11 (61.1)
Duration of response (wk)			
Median (range)[a,b]	na	na	15.9 (7.3–20.1)
Time to progression (wk)			
Median (range)[a]	8.3 (4.0–40.7)	12.3 (0–40.3)	12.3 (1.4–32.7)
No. of events	12	13	13

na, not applicable; RECIST, Response Evaluation Criteria in Solid Tumors.
[a] Median estimated using the Kaplan–Meier method.
[b] This parameter could not be estimated in the 100 and 200 mg/d arms owing to the lack of progressions by the date of data cut-off.

TABLE 3. Characteristics of Patients Who Were Partial Responders

Treatment (initial dose)	Gender	Age (yr)	Smoking History[a]	Histology	Previous Chemotherapy Regimens	Time to PR (d)	Duration of Response (d)
100 mg	Male	65	Yes	Adenocarcinoma	1	28	204[b]
100 mg	Female	72	No	Adenocarcinoma	1	78	141[b]
100 mg	Male	52	No	Adenocarcinoma	1	143	141[b]
200 mg	Female	69	No	Adenocarcinoma	1	26	140[b]
300 mg[c]	Male	69	Yes	Adenocarcinoma	2	31	51
300 mg	Female	68	No	Adenocarcinoma	1	28	81[b]
300 mg	Female	55	No	Adenocarcinoma	1	82	141

[a] No, patients who have smoked <100 cigarettes in their lifetime; Yes, patients who have smoked >100 cigarettes in their lifetime.
[b] Censored on the day of last tumor evaluation due to absence of disease progression (response ongoing at data cut-off).
[c] Patient started study treatment with 300 mg and the treatment was stopped 29 d after the start due to QTc prolongation. The patient re-started at a reduced dose level (200 mg) 35 d after the start.

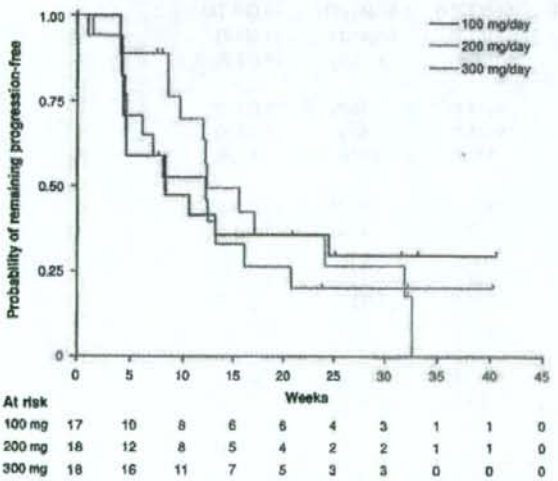

FIGURE 2. Kaplan–Meier curve for time to progression.

the common AEs across the three vandetanib arms, although the incidences of diarrhea, constipation, and abnormal hepatic function were numerically higher in the vandetanib 300 mg arm compared with the 100 or 200 mg arms. A dose-dependent increase in the incidence of CTC grade 3 and 4 events was observed; the incidence of these events in the 100, 200, and 300 mg dose arms were 29.4% (5 of 17 patients), 38.9% (7 of 18 patients), and 66.7% (12 of 18 patients), respectively. Of the 24 CTC grade 3 or 4 AEs considered by the investigator to be vandetanib-related, hypertension (100 mg, n = 4; 200 mg, n = 3; 300 mg, n = 3), and asymptomatic QTc prolongation (200 mg, n = 1; 300 mg, n = 1) were reported in more than one patient. Across the three dose levels, the AEs in this study were generally manageable with symptomatic treatment, dose interruption, or reduction.

Six patients discontinued vandetanib because of an AE considered by the investigator to be vandetanib-related: cryptogenic organizing pneumonia (COP), hepatic steatosis, and photosensitivity reaction (each n = 1, 200 mg arm); QTc prolon-

TABLE 4. Number of Patients With Most Commonly Reported Adverse Events (Occurring in ≥10% Across all Treatment Groups), Regardless of Causality

MedDRA Preferred Term[a]	Vandetanib 100 mg/d (n = 17)	Vandetanib 200 mg/d (n = 18)	Vandetanib 300 mg/d (n = 18)	Total (n = 53)
Rash (%)	10 (59)	9 (50)	9 (50)	28 (53)
CTC grade 3/4	0/0	1/0	0/0	1/0
Diarrhea (%)	8 (47.1)	8 (44)	11 (61)	27 (51)
CTC grade 3/4	0/0	1/0	1/0	2/0
Hypertension (%)	8 (47)	10 (56)	7 (39)	25 (47)
CTC grade 3/4	4/0	3/0	3/0	10/0
ECG QTc prolonged (%)	4 (24)	9 (50)	8 (44)	21 (40)
CTC grade 3/4	0/0	1/0	1/0	2/0
Photosensitivity reaction (%)	2 (12)	5 (28)	5 (28)	12 (23)
CTC grade 3/4	0/0	0/0	0/0	0/0
Nasopharyngitis (%)	3 (18)	4 (22)	4 (22)	11 (21)
CTC grade 3/4	0/0	0/0	0/0	0/0
Dry skin (%)	2 (12)	4 (22)	5 (28)	11 (21)
CTC grade 3/4	0/0	0/0	0/0	0/0
Nausea (%)	3 (18)	3 (17)	4 (22)	10 (19)
CTC grade 3/4	0/0	0/0	0/0	0/0
Constipation (%)	2 (12)	1 (6)	6 (33)	9 (17)
CTC grade 3/4	0/0	0/0	0/0	0/0
Fatigue (%)	4 (24)	1 (6)	2 (11)	7 (13)
CTC grade 3/4	0/0	0/0	0/0	0/0
ECG QT prolonged (%)	1 (6)	2 (11)	4 (22)	7 (13)
CTC grade 3/4	0/0	0/0	0/0	0/0
Hepatic function abnormal (%)	1 (6)	1 (6)	4 (22)	6 (11)
CTC grade 3/4	0/0	0/0	1/0	1/0
Hematuria (%)	2 (12)	2 (12)	2 (12)	6 (11)
CTC grade 3/4	0/0	0/0	0/0	0/0

[a] MedDRA version 8.1.

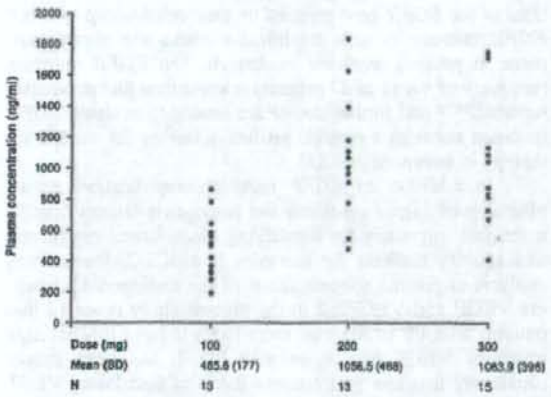

FIGURE 3. Observed maximum vandetanib plasma concentration at day 28. Patients who received dose reduction within the first 28 days were excluded.

gation, alanine aminotransferase increased, and erythema multiforme (each n = 1, 300 mg arm). Only COP was classed as a serious AE. Six patients had vandetanib dose reductions due to AEs (100 mg, n = 1; 200 mg, n = 1; 300 mg, n = 4).

Seven patients experienced eight respiratory-related events (COP, dyspnoea, interstitial lung disease [ILD], hypoxia, pneumonitis [all n = 1], and pneumonia [n = 3]). The incidence of these events in the three dose levels was 5.9% (1 of 17 patients; 100 mg), 11.1% (2 of 18 patients; 200 mg) and 22.2% (4 of 18 patients; 300 mg), respectively. Four of these events were considered to be related to vandetanib (COP, ILD, pneumonia [n = 2]). The ILD event was reported in a 64-year-old male patient in the 300 mg arm and resulted in patient death. This event was reported 8 days after vandetanib 300 mg was discontinued because of disease progression. No postmortem examination was performed and the investigator and a third-party physician considered the cause of death to be ILD.

All QTc prolongation was asymptomatic and manageable with dose interruption and/or reduction. The incidence of QTc prolongation was lower in the vandetanib 100 mg (24%) arm compared with the 200 mg (50%) and 300 mg (44%) arms. The mean change in QTc interval from baseline to week 3 (when maximum prolongation was observed) in the 100, 200, and 300 mg arms was +14 milliseconds (range, −25 to 29 milliseconds), +16.5 milliseconds (range, −36 to 49 milliseconds), and +27.6 milliseconds (range, 4 to 51 milliseconds), respectively. Protocol-defined QTc prolongation determined at the treatment site resulted in dose reduc-

come. In contrast, plasma levels of VEGFR-2 showed a trend to decrease over the same period, whereas plasma Tie-2 levels did not seem to change (Table 6). Baseline plasma VEGF levels appeared to be lower in patients who experienced clinical benefit following vandetanib treatment: PR (median 22.3 pg/ml, $n = 6$) and SD (median 37.0 pg/ml, $n = 16$) versus PD (median 63.7 pg/ml, $n = 21$). Patients with a low (below median) baseline plasma VEGF level had a longer TTP (median, 24.1 week) than those with a high (above median) baseline VEGF level (median, 8.3 weeks) (Figure 4). No clear relationship was apparent between baseline levels of plasma Tie-2 and VEGFR-2 and tumor response.

DISCUSSION

The primary objective of this phase IIa study was to assess the ORR to three doses of vandetanib (100, 200, and 300 mg/d) in Japanese patients with advanced or recurrent NSCLC. These doses of vandetanib were selected based on the outcomes of a Japanese phase I study where it was observed that vandetanib was well tolerated up to a dose of 300 mg and objective tumor responses were observed in 4 of 9 patients with NSCLC at doses of either 200 or 300 mg.[11]

In this study, objective tumor responses were observed at all three doses of vandetanib. The ORR in the 100, 200, and 300 mg arms was 17.6% (3 of 17 patients), 5.6% (1 of 18 patients), and 16.7% (3 of 18 patients), respectively. The DCR and TTP were similar across the three dose arms. It was noted that 50% (9 of 18) of the patients in the 200 mg arm had failed two previous chemotherapy regimens, compared with 23.5% (4 of 17 patients) and 22.2% (4 of 18 patients) in the 100 and 300 mg arms, respectively. It is possible that these differences contributed to the lower ORR observed in the 200 mg arm, although the number of patients in each dose arm was too small to allow any definitive conclusions to be made.

Vandetanib was well tolerated at 100, 200, and 300 mg dose levels in this study. Overall, AEs were generally mild

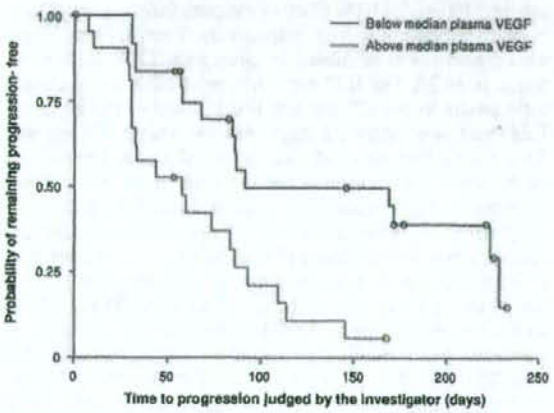

FIGURE 4. Kaplan–Meier curve of low (below median) versus high (above median) baseline plasma VEGF and time to progression.

and manageable with symptomatic treatment, dose interruption or reduction. In addition, the AE profile was consistent with that determined during phase I evaluation in patients with advanced solid tumors[10,11] and phase II monotherapy data in NSCLC.[12] Furthermore, the AE profile was also consistent with that reported previously for agents that inhibit the VEGFR[17,18] or EGFR[4,19] signaling pathways. In general, no apparent dose dependence was noted in the incidence of the common AEs in this study except for asymptomatic QTc prolongation (24%, 56%, and 44% for the 100, 200, and 300 mg dose arms, respectively), an event that was manageable by dose interruption/reduction.

A notable feature of this study, and the phase II program for vandetanib in NSCLC, is that patients with squamous cell histology or stable brain metastases were permitted to enter the trials. Both of these factors have been associated with an increased risk of bleeding, including severe life-threatening hemoptysis in NSCLC patients with squamous histology in a randomized phase II study of bevacizumab with carboplatin and paclitaxel.[20] These events have also been reported with other inhibitors of VEGF/VEGFR signaling, such as sunitinib and sorafenib.[17,18] Importantly, no CNS hemorrhage AEs or hemoptysis attributable to vandetanib were reported in this study.

The PK profile in this NSCLC patient population was consistent with that seen previously during Phase I evaluation in Japanese and USA/Australian patients with a range of solid tumors.[10,11]

In patients with NSCLC, specific *EGFR* mutations are associated with increased sensitivity to EGFR tyrosine kinase inhibitors,[21,22] and a better survival outcome with gefitinib has been shown to correlate with high *EGFR* gene copy number.[23] In this study, an exploratory analysis of tumor samples for amplification of *EGFR* gene copy number and somatic mutations of the *EGFR* gene revealed no clear relationship between *EGFR* mutation or gene amplification status and clinical outcome in patients receiving vandetanib. The *EGFR* mutation frequency of 4% (1 of 27 patients) is lower than that previously reported,[24,25] and further studies are needed to evaluate *EGFR* mutation status as a possible predictive marker for vandetanib therapy in advanced NSCLC.

In addition to *EGFR* mutation/amplification status, plasma profiling of cytokines and angiogenic factors may be a feasible approach for identifying blood-based prognostic and activity markers for therapies in NSCLC. Preliminary analysis of plasma concentrations of the angiogenesis markers VEGF and VEGFR-2 in the present study revealed that patients with PR or SD were more likely to have low baseline levels of VEGF than those with PD. It has been shown previously that low pretreatment levels of circulating VEGF correlated with a good response to gefitinib treatment in patients with NSCLC.[26] The significance of the relationship between these biomarkers and clinical outcome requires further investigation.

In conclusion, vandetanib monotherapy (100–300 mg/d) demonstrated antitumor activity with an acceptable safety and tolerability profile in Japanese patients with advanced NSCLC. Based only on this study, there is no com-

pelling evidence to identify the optimal dose of vandetanib monotherapy in this population of patients; further investigation of vandetanib doses in the range 100 to 300 mg is warranted in Japanese patients with advanced NSCLC. Other randomized phase II studies of vandetanib in advanced NSCLC have demonstrated improvements in progression-free survival with vandetanib 300 mg as a monotherapy versus gefitinib[12] and with the combination of vandetanib 100 mg and docetaxel.[14] Phase III evaluation of vandetanib in a broad population of patients, both as monotherapy at 300 mg (versus placebo in patients previously treated with anti-EGFR therapy [ZEPHYR]; versus erlotinib [ZEST]) and at 100 mg in combination with docetaxel (ZODIAC) or pemetrexed (ZEAL), has been initiated in global trials.

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ORIGINAL ARTICLE

Aberrant expression of Fra-2 promotes CCR4 expression and cell proliferation in adult T-cell leukemia

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Adult T-cell leukemia (ATL) is a mature CD4+ T-cell malignancy etiologically associated with human T-cell leukemia virus type 1 (HTLV-1). Primary ATL cells frequently express CCR4 at high levels. Since HTLV-1 Tax does not induce CCR4 expression, transcription factor(s) constitutively active in ATL may be responsible for its strong expression. We identified an activator protein-1 (AP-1) site in the CCR4 promoter as the major positive regulatory element in ATL cells. Among the AP-1 family members, Fra-2, JunB and JunD are highly expressed in fresh primary ATL cells. Consistently, the Fra-2/JunB and Fra-2/JunD heterodimers strongly activated the CCR4 promoter in Jurkat cells. Furthermore, Fra-2 small interfering RNA (siRNA) or JunD siRNA, but not JunB siRNA, effectively reduced CCR4 expression and cell growth in ATL cells. Conversely, Fra-2 or JunD overexpression promoted cell growth in Jurkat cells. We identified 49 genes, including c-Myb, BCL-6 and MDM2, which were downregulated by Fra-2 siRNA in ATL cells. c-Myb, BCL-6 and MDM2 were also downregulated by JunD siRNA. As Fra-2, these proto-oncogenes were highly expressed in primary ATL cells but not in normal CD4+ T cells. Collectively, aberrantly expressed Fra-2 in association with JunD may play a major role in CCR4 expression and oncogenesis in ATL.
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Keywords: adult T-cell leukemia; CCR4; Fra-2; JunD; c-Myb; MDM2; BCL-6

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Introduction

Adult T-cell leukemia (ATL) is a highly aggressive malignancy of mature CD4+CD25+ T cells etiologically associated with human T-cell leukemia virus type 1 (HTLV-1; Yamamoto and Hinuma, 1985). HTLV-1 encodes a potent viral transactivator Tax that activates the HTLV-1 long terminal repeat (LTR) and also induces the expression of various cellular target genes, including those encoding cytokines, cytokine receptors, chemokines, cell adhesion molecules and nuclear transcriptional factors, collectively leading to the strong promotion of cell proliferation (Yoshida, 2001; Grassmann *et al.*, 2005). However, ATL develops after a long period of latency, usually several decades, during which oncogenic progression is considered to occur through the accumulation of multiple genetic and epigenetic changes (Matsuoka, 2003). Furthermore, circulating ATL cells usually do not express Tax and are considered to be independent of Tax (Matsuoka, 2003). Previously, Mori *et al.* have demonstrated the strong constitutive activation of nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) in primary ATL cells (Mori *et al.*, 1999, 2000). However, the molecular mechanisms of ATL oncogenesis still remain largely unknown.

CCR4 is a chemokine receptor known to be selectively expressed by Th2 cells, regulatory T cells (Treg) and skin-homing effector/memory T cells (Imai *et al.*, 1999; Iellem *et al.*, 2001; Yoshie *et al.*, 2001). Previously, we and others showed that ATL cells in the majority of cases are strongly positive for surface CCR4 (Yoshie *et al.*, 2002; Ishida *et al.*, 2003; Nagakubo *et al.*, 2007). Ishida *et al.* have also demonstrated a significant correlation of CCR4 expression with skin involvement and poor prognosis in ATL patients (Ishida *et al.*, 2003). Furthermore, several groups have reported that FOXP3, a forkhead/winged helix transcription factor and a specific marker of Treg (Hori *et al.*, 2003), is frequently expressed in ATL (Karube *et al.*, 2004; Matsubara *et al.*, 2005), supporting the notion that at least a fraction of ATL cases are derived from Treg.

It is also notable that primary ATL cells express CCR4 at levels much higher than normal resting CD4+CD25+ T cells (Nagakubo *et al.*, 2007). Given

that CCR4 is not inducible by Tax (Yoshie *et al.*, 2002), transcription factor(s) constitutively active in ATL cells may be responsible for CCR4 expression. Here, we demonstrate that Fra-2, one of the AP-1 family members (Shaulian and Karin, 2002; Eferl and Wagner, 2003), is aberrantly expressed in primary ATL cells. We further demonstrate that the Fra-2/JunD heterodimer plays a major role in both CCR4 expression and cell proliferation in ATL cells. Furthermore, we demonstrate that the proto-oncogenes c-Myb, BCL-6 and MDM2 (Oh and Reddy, 1999; Pasqualucci *et al.*, 2003; Vargas *et al.*, 2003) are the downstream target genes of the Fra-2/JunD heterodimer and are highly expressed in primary ATL cells. Thus, aberrantly expressed Fra-2 in association with JunD may be involved in ATL oncogenesis.

Results

Analysis of CCR4 promoter activity in ATL-derived cell lines

To examine the transcriptional regulation of CCR4 expression in ATL, we constructed a reporter plasmid carrying the CCR4 promoter region from −983 to +25 bp (the major transcriptional initiation site, +1) fused with the luciferase reporter gene. As shown in Figure 1a, pGL3-CCR4 (−983/+25) showed much stronger promoter activities in ATL cell lines (HUT102 and ST1) than in control human T-cell lines (MOLT-4 and Jurkat). We therefore generated a series of 5′-truncated promoter plasmids and examined their activity in ATL cell lines. As shown in Figure 1b, the promoter region from −151 to −96 bp was the major positive regulatory region in both cell lines. The TFSEARCH program (http://mbs.cbrc.jp/research/db/TFSEARCH.html) revealed various potential transcriptional elements in this region (Figure 1c). To identify the actual regulatory elements, we introduced a mutation in each potential element and examined the promoter activity in ATL cell lines. As shown in Figure 1d, a mutation at the AP-1 site or the GATA-3 site significantly reduced the promoter activity. Moreover, double mutations targeting both sites further reduced the promoter activity.

Constitutive expression of Fra-2, JunB and JunD in primary ATL cells

AP-1 is known to be involved in tumorigenesis (Shaulian and Karin, 2002; Eferl and Wagner, 2003), while GATA-3 regulates Th2-type gene expression (Rengarajan *et al.*, 2000). Therefore, we focused on AP-1 in the subsequent study. AP-1 constitutes a heterodimer of a member of the Fos family (c-Fos, FosB, Fra-1 and Fra-2) and a member of the Jun family (c-Jun, JunB and JunD) or a homodimer of the Jun family (Shaulian and Karin, 2002; Eferl and Wagner, 2003). Even though AP-1 was shown to be constitutively active in primary ATL cells (Mori *et al.*, 2000), it has not been clarified which members of AP-1 are actually

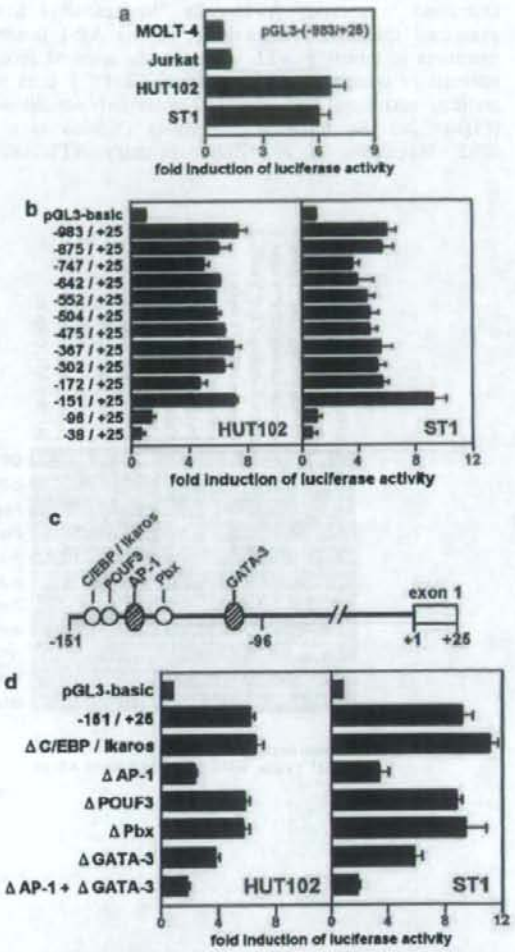

Figure 1 Identification of regulatory elements in the CCR4 promoter. Cells were transfected with pSV-β-galactosidase and pGL3-basic or pGL3-basic inserted with the CCR4 promoter regions as indicated. After 24–27 h, luciferase assays were performed. Promoter activation was expressed by the fold induction of luciferase activity in cells transfected with the CCR4 promoter–luciferase constructs versus cells transfected with the control pGL3-basic. Transfection efficiency was normalized by β-galactosidase activity. Each bar represents the mean ± s.e.m. from three separate experiments. (a) Selective activation of the CCR4 promoter in adult T-cell leukemia (ATL) cell lines. MOLT-4 and Jurkat: control human T-cell lines; HUT102 and ST1: ATL cell lines. (b) Deletion analysis. The promoter region from −151 to −96 bp is necessary and sufficient for reporter gene expression in the two ATL cell lines. (c) The schematic depiction of potential regulatory elements in the promoter region from −151 to −96 bp. (d) Mutation analysis. ΔC/EBP/Ikaros (from TCTTGGGAAA TGA to TCTTGCAAAATGA), ΔAP-1 (from AATGACTAAGA to AATGTCAAAGA), ΔPOUF3 (from CTTGGGAAATGA to CTTGGGAGGTGA), ΔPbx (from AAGAATCAT to AAGA CCCAT) and ΔGATA-3 (from TTCTATCAA to TTCTGACAA). The potential AP-1 and GATA-3 sites present within the −151 to −96 bp region are the major elements for CCR4 promoter activation in the two ATL cell lines.