Erlotinib alone or with bevacizumab as first-line therapy in patients with advanced non-squamous non-small-cell lung cancer harbouring *EGFR* mutations (JO25567): an open-label, randomised, multicentre, phase 2 study

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Summary

Background With use of EGFR tyrosine-kinase inhibitor monotherapy for patients with activating *EGFR* mutation-positive non-small-cell lung cancer (NSCLC), median progression-free survival has been extended to about 12 months. Nevertheless, new strategies are needed to further extend progression-free survival and overall survival with acceptable toxicity and tolerability for this population. We aimed to compare the efficacy and safety of the combination of erlotinib and bevacizumab compared with erlotinib alone in patients with non-squamous NSCLC with activating *EGFR* mutation-positive disease.

Methods In this open-label, randomised, multicentre, phase 2 study, patients from 30 centres across Japan with stage IIIB/IV or recurrent non-squamous NSCLC with activating *EGFR* mutations, Eastern Cooperative Oncology Group performance status 0 or 1, and no previous chemotherapy for advanced disease received erlotinib 150 mg/day plus bevacizumab 15 mg/kg every 3 weeks or erlotinib 150 mg/day monotherapy as a first-line therapy until disease progression or unacceptable toxicity. The primary endpoint was progression-free survival, as determined by an independent review committee. Randomisation was done with a dynamic allocation method, and the analysis used a modified intention-to-treat approach, including all patients who received at least one dose of study treatment and had tumour assessment at least once after randomisation. This study is registered with the Japan Pharmaceutical Information Center, number JapicCTI-111390.

Findings Between Feb 21, 2011, and March 5, 2012, 154 patients were enrolled. 77 were randomly assigned to receive erlotinib and bevacizumab and 77 to erlotinib alone, of whom 75 patients in the erlotinib plus bevacizumab group and 77 in the erlotinib alone group were included in the efficacy analyses. Median progression-free survival was 16·0 months (95% CI 13·9–18·1) with erlotinib plus bevacizumab and 9·7 months (5·7–11·1) with erlotinib alone (hazard ratio 0·54, 95% CI 0·36–0·79; log-rank test p=0·0015). The most common grade 3 or worse adverse events were rash (19 [25%] patients in the erlotinib plus bevacizumab group *vs* 15 [19%] patients in the erlotinib alone group), hypertension (45 [60%] *vs* eight [10%]), and proteinuria (six [8%] *vs* none). Serious adverse events occurred at a similar frequency in both groups (18 [24%] patients in the erlotinib plus bevacizumab group and 19 [25%] patients in the erlotinib alone group).

Interpretation Erlotinib plus bevacizumab combination could be a new first-line regimen in *EGFR* mutation-positive NSCLC. Further investigation of the regimen is warranted.

Funding Chugai Pharmaceutical Co Ltd.

Introduction

Lung cancer is a leading cause of death worldwide; it is the primary cause of cancer deaths in men and the secondary cause in women.[1] Most patients with lung cancer have non-small-cell lung cancer (NSCLC) and a clinically significant proportion of patients have activating mutations of *EGFR*.[2] In this subgroup of patients, EGFR tyrosine-kinase inhibitors have consistently led to better outcomes than has standard chemotherapy.[3–6] Erlotinib and gefitinib have been shown to prolong progression-free survival compared with chemotherapy in several phase 3 trials.[7–10] Unfortunately, most patients with NSCLC with activating *EGFR* mutations who are given EGFR tyrosine-kinase inhibitors eventually develop resistance and relapse within about 1 year of initiation of treatment.[5,7–11] To improve outcomes, the foundation treatment of EGFR tyrosine-kinase inhibitors should be built on through investigation of biologically synergistic combinations.

The anti-angiogenic monoclonal antibody bevacizumab targets the VEGF signalling pathway and has been shown to provide additional efficacy when used in combination with first-line platinum-based chemotherapy in several trials in non-squamous NSCLC.[12–14] The combination of erlotinib and bevacizumab has the potential to prolong progression-free survival in unselected populations of patients with NSCLC.[15,16] In a subgroup analysis of *EGFR*

mutation-positive participants in the phase 3 BeTa study of second-line treatment of NSCLC (12 patients treated with erlotinib and bevacizumab and 18 with erlotinib alone), median progression-free survival with erlotinib plus bevacizumab in patients with *EGFR* mutation-positive disease was substantially higher than with erlotinib alone (17·1 months *vs* 9·7 months).[16,17] However, this analysis was post-hoc and *EGFR* mutation status was not a prespecified stratification factor in this trial. Because of this limitation, we undertook this phase 2 trial to examine the combination of erlotinib and bevacizumab in patients with *EGFR* mutation-positive NSCLC.

Methods

Study design and patients

JO25567 was a randomised, open-label, multicentre, phase 2 study in patients with stage IIIB/IV (according to the 7th edition of the General Rule for Clinical and Pathological Record of Lung Cancer[18]) or recurrent NSCLC with activating *EGFR* mutations. Patients were enrolled from 30 centres across Japan.

Eligible patients had histologically or cytologically (excluding sputum cytology) confirmed stage IIIB/IV or postoperative recurrent non-squamous NSCLC with activating *EGFR* mutation (either exon 19 deletion or Leu858Arg mutation). Tumour samples were screened for *EGFR* mutation by PCR-based hypersensitive *EGFR* mutation testing in local laboratories, according to standard testing practices. Other criteria included age 20 years or older when giving informed consent; Eastern Cooperative Oncology Group performance status 0 or 1; adequate haematological, hepatic, and renal function; and life expectancy 3 months or more at the time of registration. No previous chemotherapy for advanced disease was allowed, but postoperative adjuvant or neoadjuvant therapy of 6 months or more previously was allowed. Previous radiotherapy was also allowed, but only for non-lung lesions. Patients had to have one or more measurable lesion based on Response Evaluation Criteria in Solid Tumors (RECIST 1.1).

Major exclusion criteria included confirmation of Thr790Met mutation, presence of brain metastases, history or presence of haemoptysis or bloody sputum, any coagulation disorder, tumour invading or abutting major blood vessels, coexistence or history of interstitial lung disease, and previous receipt of EGFR inhibitors or VEGF receptor inhibitors.

This study was done in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol was reviewed and approved by the institutional review boards of the participating institutions (appendix p 10), and written informed consent was obtained from all patients.

Randomisation and masking

Patients were randomly assigned (1:1) to receive either erlotinib plus bevacizumab or erlotinib alone with a dynamic allocation method. Central randomisation was done by a clinical research organisation (EPS Corporation, Tokyo, Japan). Patients were stratified according to sex (men *vs* women), disease stage (stage IIIB *vs* stage IV *vs* postoperative relapse), smoking history (never smokers or former light smokers *vs* others), and type of *EGFR* mutation (exon 19 deletion *vs* Leu858Arg mutation). All patients and investigators were unmasked to treatment allocation.

Procedures

Patients assigned to the erlotinib plus bevacizumab group received bevacizumab 15 mg/kg by intravenous infusion on day 1 of a 21-day cycle and erlotinib orally once daily at 150 mg/day, starting from day 1 of cycle 1. Patients in the erlotinib alone group received erlotinib orally once a day at 150 mg/day. Patients remained on treatment until disease progression or unacceptable toxicity. Changes to dose of erlotinib or bevacizumab because of adverse events were allowed, as per the protocol. The dose of bevacizumab was not to be reduced except when dose adjustment was needed because of change in bodyweight. Dose reduction of erlotinib was allowed for up to two doses (100 mg/day and 50 mg/day) in a stepwise decrease. After two steps of dose reduction, erlotinib was discontinued. Patients who required suspension of erlotinib for more than 3 weeks consecutively, or of bevacizumab for more than 6 weeks from the date of previous administration, were discontinued from study treatment. In the erlotinib plus bevacizumab group, if either drug was discontinued, the other could be

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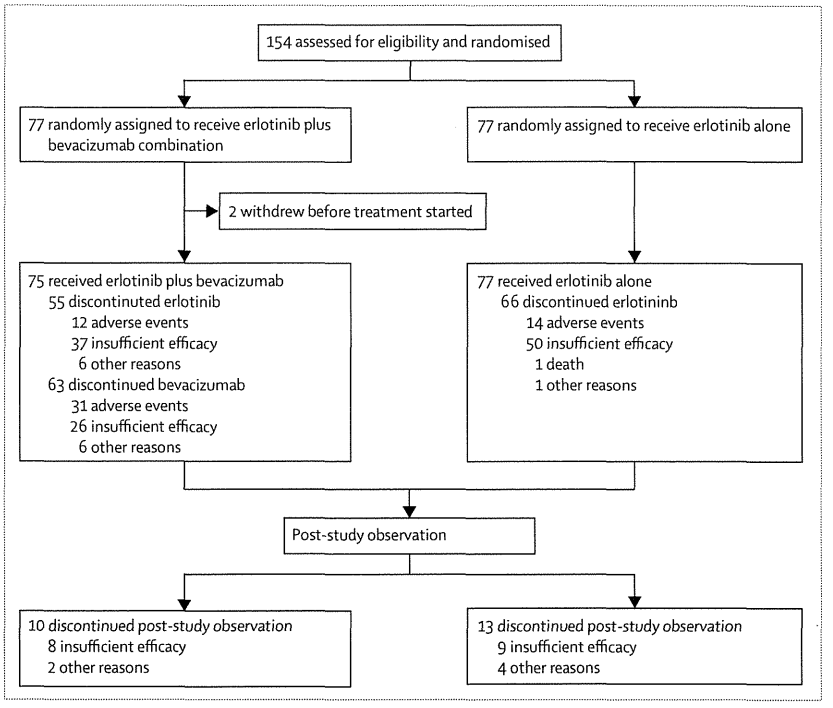

Figure 1: **Trial profile**

continued. Tumour lesions were assessed radiologically at baseline, week 4, week 7, every 6 weeks from week 7 to 18 months, and every 12 weeks thereafter until disease progression according to RECIST 1.1.

	Erlotinib plus bevacizumab group (n=75)	Erlotinib alone group (n=77)
Age (years)		
Median	67·0 (59–73)	67·0 (60–73)
<75	63 (84%)	62 (81%)
≥75	12 (16%)	15 (19%)
Sex		
Male	30 (40%)	26 (34%)
Female	45 (60%)	51 (66%)
Smoking status		
Never smoker	42 (56%)	45 (58%)
Former light smoker	9 (12%)	6 (8%)
Other	24 (32%)	26 (34%)
ECOG performance status		
0	43 (57%)	41 (53%)
1	32 (43%)	36 (47%)
Histopathological classification		
Adenocarcinoma	74 (99%)	76 (99%)
Large-cell carcinoma	0	1 (1%)
Adenosquamous carcinoma	1 (1%)	0
Clinical stage at screening		
IIIB	1 (1%)	0
IV	60 (80%)	62 (81%)
Postoperative recurrence	14 (19%)	15 (19%)
***EGFR* mutation type**		
Exon 19 deletion	40 (53%)	40 (52%)
Exon 21 Leu858Arg mutation	35 (47%)	37 (48%)

Data are n (%) or median (IQR). ECOG=Eastern Cooperative Oncology Group.

Table 1: **Baseline demographics and clinical characteristics**

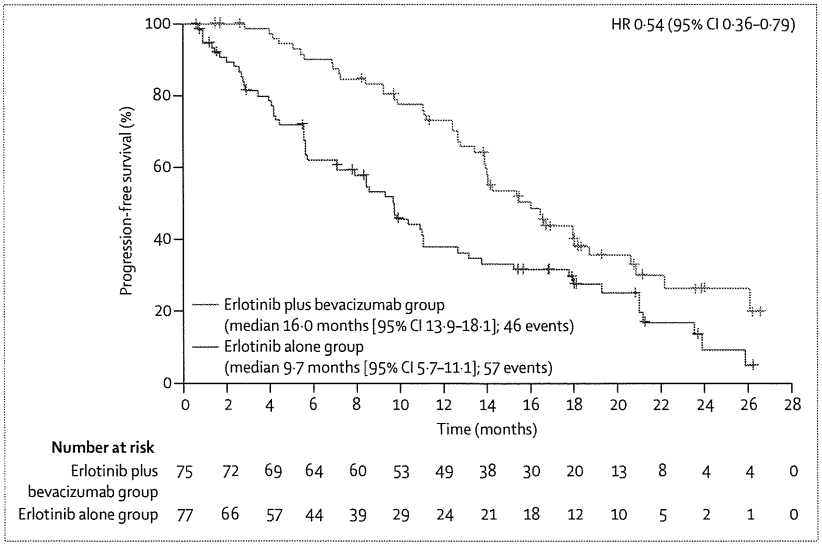

Figure 2: **Progression-free survival, as determined by independent review committee, in the modified intention-to-treat population**
HR=hazard ratio.

Patient-reported outcomes were assessed with the Functional Assessment of Cancer Therapy for patients with Lung cancer (FACT-L) scale until disease progression. An independent review committee of clinicians and radiologists masked to treatment assignment reviewed all tumour images and determined tumour response and progression status. Laboratory studies including blood and urine tests were done at days 1, 8, and 15 in cycles 1 and 2, and day 1 in cycle 3 and thereafter. Adverse events were monitored throughout the study period and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTC-AE) version 4.03.

Outcomes

The primary endpoint was progression-free survival, as determined by an independent review committee. Secondary endpoints were overall survival, tumour response (the proportion of patients with an objective response and disease control, and duration of response) according to RECIST 1.1, quality of life, symptom improvement measured by the FACT-L scale, and safety profile.

Statistical analysis

A median progression-free survival of 13 months was estimated for the erlotinib alone group, and 89 events were deemed necessary to detect a hazard ratio (HR) of 0·7 in favour of erlotinib plus bevacizumab, with a one-sided significance level of 0·2 and a power of 0·8. The target sample size was set at 150 patients (75 patients in both groups), allowing for dropouts. Median progression-free survival was estimated by the Kaplan-Meier method and compared between groups with an unstratified log-rank test. Greenwood's formula was used to calculate 95% CIs. HRs were calculated by unstratified Cox proportional hazard methodology.

In the safety analysis, adverse events were converted to Medical Dictionary for Regulatory Activities (version 14.0) preferred terms, and tabulated by grade. Changes in laboratory test data with time were summarised in tables and graphs.

All patients who received at least one dose of the study treatment were included in the safety analysis population. The modified intention-to-treat population for the efficacy analysis included all patients who received at least one dose of study treatment and had tumour assessment at least once after randomisation. Statistical analyses were done with SAS version 9.2.

The study is registered with the Japan Pharmaceutical Information Center, number JapicCTI-111390.

Role of the funding source

The study was designed and funded by Chugai Pharmaceutical Co Ltd and monitored by a clinical research organisation (Niphix Corp, Tokyo, Japan) who obtained all data and did all initial data analyses; further analysis and interpretation was done by the funder, with

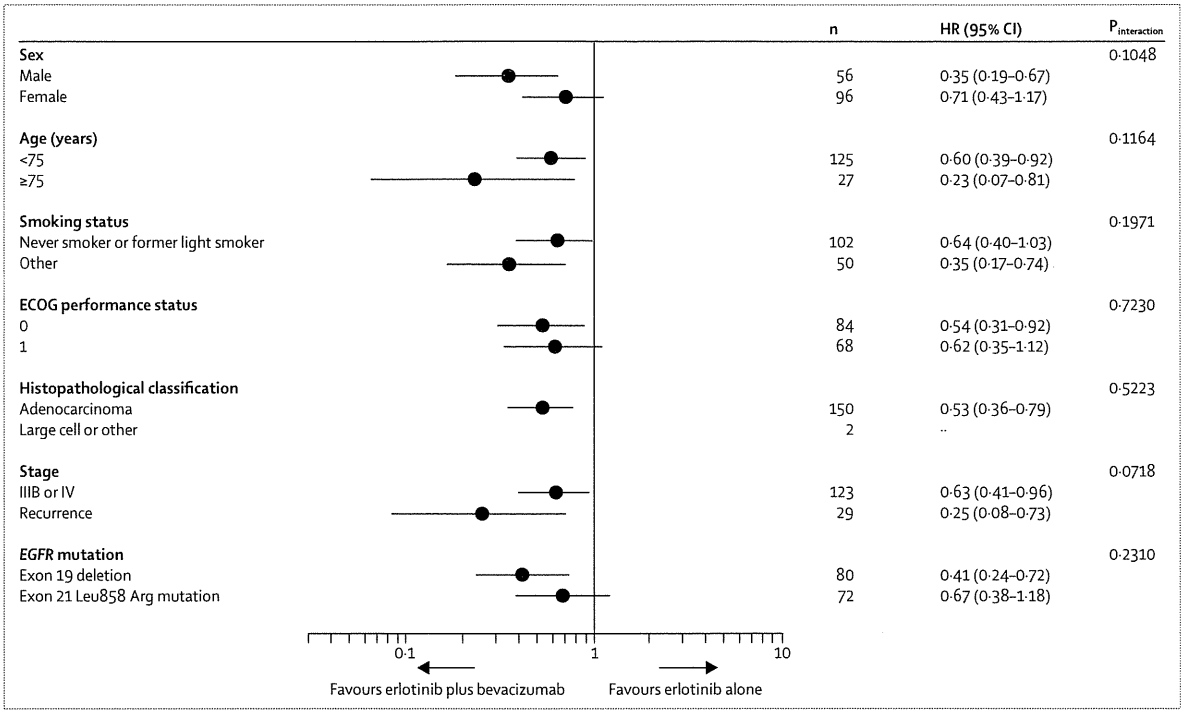

Figure 3: **Forest plot of hazard ratios for progression-free survival by baseline characteristics**
HR=hazard ratio.

	Erlotinib plus bevacizumab group (n=75)	Erlotinib alone group (n=77)
Complete response	3 (4%)	1 (1%)
Partial response	49 (65%)	48 (62%)
Stable disease	22 (29%)	19 (25%)
Progressive disease	0	6 (8%)
Non-evaluable	1 (1%)	3 (4%)

RECIST=Response Evaluation Criteria in Solid Tumors.

Table 2: **Best RECIST response, as determined by independent review committee**

input from the authors and investigators. The initial draft of the report was reviewed and commented on by all authors and by employees of Chugai Pharmaceutical Co Ltd. NobuY had full access to all data, and had final responsibility for the decision to submit the results for publication.

Results

Between Feb 21, 2011, and March 5, 2012, 154 patients were enrolled, of whom 77 were randomly assigned to receive erlotinib plus bevacizumab and 77 to erlotinib alone. Two patients withdrew before treatment started and were excluded (one had multiple thrombosis and the other had increased pleural effusion). Thus, data from 152 patients (75 patients in the erlotinib plus bevacizumab group and 77 in the erlotinib alone group) were included in the analysis population (figure 1). The cutoff date for the primary analysis was June 30, 2013, when 103 progression events had occurred; median follow-up was 20·4 months (IQR 17·4–24·1).

The baseline characteristics of patients were well balanced between the groups (table 1). Median age was 67 years (IQR 60–73), and 27 (18%) patients were aged 75 years or older. *EGFR* mutation subtypes were balanced between the two groups.

Progression-free survival was significantly prolonged with erlotinib plus bevacizumab compared with erlotinib alone (log-rank test p=0·0015; figure 2). When subgroup analyses were done by baseline clinical characteristics, most patient subgroups seemed to have greater benefit from erlotinib plus bevacizumab compared with erlotinib alone. No significant difference was noted between any of the subgroups ($p_{interaction}$>0·05 for all subgroups; figure 3).

Analysis of progression-free survival by mutation subtype showed that in patients whose tumours had an exon 19 deletion (40 [53%] of 75 patients in the erlotinib plus bevacizumab group and 40 [52%] of 77 patients in the erlotinib alone group), median progression-free survival was significantly longer with erlotinib plus bevacizumab than with erlotinib alone (18·0 months [95% CI 14·1–20·6] *vs* 10·3 months [95% CI 8·0–13·1]; HR 0·41 [95% CI 0·24–0·72]; p=0·0011; appendix p 1). In patients whose tumours harboured the Leu858Arg mutation (35 [47%] patients in the erlotinib plus bevacizumab group; 37 [48%] patients in the erlotinib alone group), median progression-free survival was numerically longer with erlotinib plus bevacizumab than with erlotinib alone, but

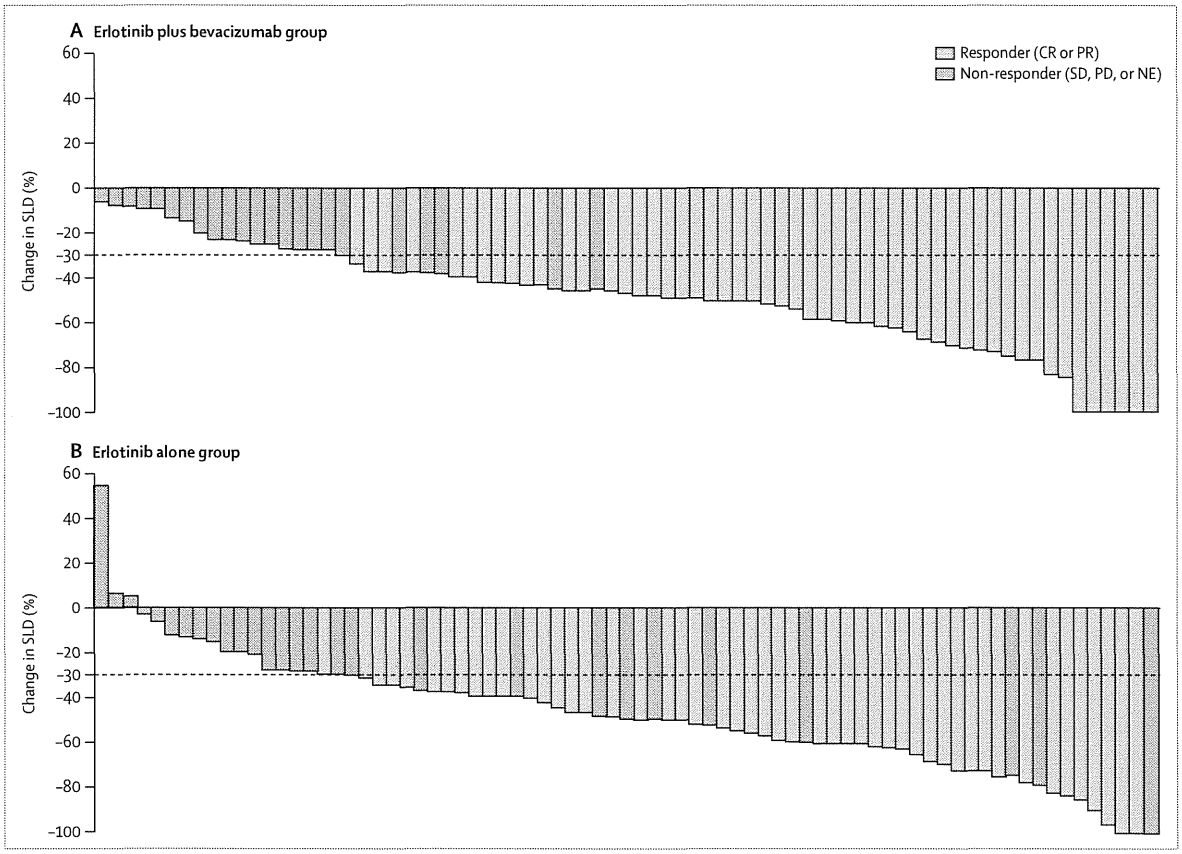

Figure 4: **Waterfall plot of best percentage change from baseline in the sum of longest tumour diameters**
Responders were confirmed by Response Evaluation Criteria in Solid Tumors. CR=complete response. PR=partial response. SD=stable disease. PD=progressive disease. NE=non-evaluable. SLD=sum of longest diameters.

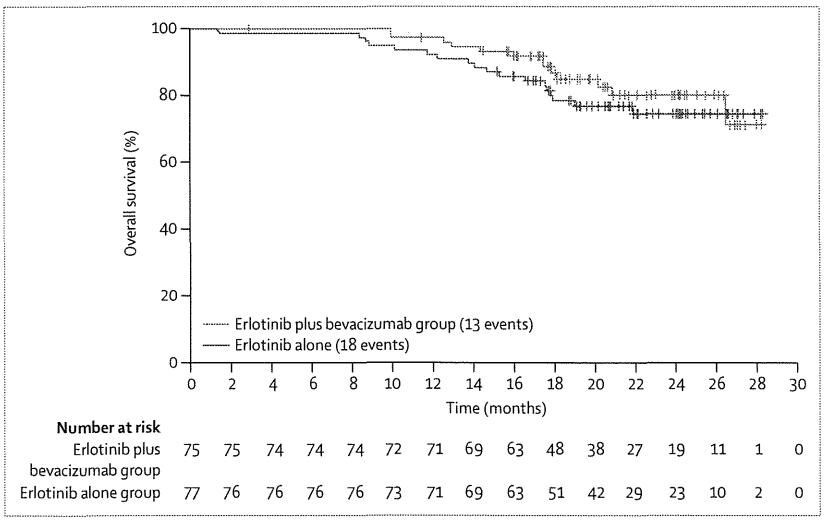

Figure 5: **Overall survival, as determined by independent review committee, in the modified intention-to-treat population**

the difference was not significant (13·9 months [95% CI 11·2–20·9] *vs* 7·1 months [95% CI 4·3–15·2], respectively; HR 0·67 [95% CI 0·38–1·18]; p=0·1653; appendix p 2).

52 (69% [95% CI 58–80]) patients in the erlotinib plus bevacizumab group had an objective response, as did 49 (64% [52–74]) patients in the erlotinib alone group (p=0·4951), although median duration of response was not significantly longer with erlotinib plus bevacizumab than with erlotinib alone (13·3 months [95% CI 11·6–16·5] *vs* 9·3 months [6·9–13·8]; p=0·1118). A greater proportion of patients achieved disease control with erlotinib plus bevacizumab (74 [99%] *vs* 68 [88%]; p=0·0177). Best responses to treatment are shown in table 2.

Figure 4 shows change in tumour size from baseline in the two groups. All patients in the erlotinib plus bevacizumab achieved tumour reduction, but three patients in the erlotinib alone group did not. Of patients who had a 30% or greater reduction in tumour size during treatment, six (8%) patients in the erlotinib plus bevacizumab group and 12 (16%) patients in the erlotinib alone group did not meet the criteria for complete or partial response according to RECIST.

Overall survival data are immature at present and so we cannot present any statistical analyses. At data cutoff, only 13 events (17%) had occurred in the erlotinib plus bevacizumab group and 18 events (23%) in the erlotinib alone group (figure 5).

	Erlotinib plus bevacizumab group (n=75)					Erlotinib alone group (n=77)				
	All	Grade 1–2	Grade 3	Grade 4	Grade 5	All	Grade 1–2	Grade 3	Grade 4	Grade 5
Rash	74 (99%)	55 (73%)	19 (25%)	0	0	76 (99%)	61 (79%)	15 (19%)	0	0
Diarrhoea	61 (81%)	60 (80%)	1 (1%)	0	0	60 (78%)	59 (77%)	1 (1%)	0	0
Paronychia	57 (76%)	55 (73%)	2 (3%)	0	0	50 (65%)	47 (61%)	3 (4%)	0	0
Dry skin	56 (75%)	54 (72%)	2 (3%)	0	0	45 (58%)	45 (58%)	0	0	0
Stomatitis	47 (63%)	46 (61%)	1 (1%)	0	0	46 (60%)	44 (57%)	2 (3%)	0	0
Haemorrhagic event	54 (72%)	52 (69%)	2 (3%)	0	0	22 (29%)	22 (29%)	0	0	0
Liver function disorder or abnormal hepatic function	33 (44%)	27 (36%)	5 (7%)	1 (1%)	0	39 (51%)	25 (32%)	7 (9%)	7 (9%)	0
Hypertension	57 (76%)	12 (16%)	45 (60%)	0	0	10 (13%)	2 (3%)	8 (10%)	0	0
Pruritus	34 (45%)	33 (44%)	1 (1%)	0	0	32 (42%)	32 (42%)	0	0	0
Weight decreased	33 (44%)	33 (44%)	0	0	0	19 (25%)	19 (25%)	0	0	0
Decreased appetite	26 (35%)	25 (33%)	1 (1%)	0	0	26 (34%)	25 (32%)	1 (1%)	0	0
Proteinuria	39 (52%)	33 (44%)	6 (8%)	0	0	3 (4%)	3 (4%)	0	0	0
Dysgeusia	20 (27%)	20 (27%)	0	0	0	17 (22%)	17 (22%)	0	0	0
Nasopharyngitis	20 (27%)	20 (27%)	0	0	0	15 (19%)	15 (19%)	0	0	0
Constipation	17 (23%)	17 (23%)	0	0	0	15 (19%)	14 (18%)	1 (1%)	0	0
Alopecia	13 (17%)	13 (17%)	0	0	0	14 (18%)	14 (18%)	0	0	0
Nausea	12 (16%)	12 (16%)	0	0	0	15 (19%)	15 (19%)	0	0	0
Vomiting	14 (19%)	14 (19%)	0	0	0	7 (9%)	7 (9%)	0	0	0
Malaise	10 (13%)	10 (13%)	0	0	0	10 (13%)	10 (13%)	0	0	0
Insomnia	8 (11%)	8 (11%)	0	0	0	8 (10%)	8 (10%)	0	0	0
Pyrexia	7 (9%)	7 (9%)	0	0	0	9 (12%)	9 (12%)	0	0	0
Upper respiratory tract infection	9 (12%)	9 (12%)	0	0	0	7 (9%)	7 (9%)	0	0	0
Conjunctivitis	8 (11%)	8 (11%)	0	0	0	7 (9%)	7 (9%)	0	0	0
Peripheral oedema	8 (11%)	8 (11%)	0	0	0	6 (8%)	6 (8%)	0	0	0
Fatigue	10 (13%)	9 (12%)	1 (1%)	0	0	3 (4%)	3 (4%)	0	0	0
Nail disorder	9 (12%)	9 (12%)	0	0	0	4 (5%)	4 (5%)	0	0	0
Dry eye	8 (11%)	8 (11%)	0	0	0	3 (4%)	3 (4%)	0	0	0
Dysphonia	8 (11%)	8 (11%)	0	0	0	1 (1%)	1 (1%)	0	0	0

Data are n (%).

Table 3: **Adverse events reported by 10% or more patients for grades 1 and 2 and all adverse events for grades 3–5 (safety population)**

68 (91%) patients in the erlotinib plus bevacizumab group and 41 (53%) patients in the erlotinib group had grade 3 or 4 adverse events. The most common adverse events of any grade in the erlotinib plus bevacizumab group were rash, diarrhoea, hypertension, and paronychia, and in the erlotininb alone group were rash, diarrhoea, and paronychia (table 3). The most common grade 3 or worse adverse events in the erlotinib plus bevacizumab group were hypertension, rash, proteinuria, and liver function disorder or abnormal hepatic function, and in the erlotinib group were rash, liver function disorder or abnormal hepatic function, and hypertension (table 3). Substantially higher (>40%) incidences of hypertension, haemorrhagic events, and proteinuria were noted in the erlotinib plus bevacizumab group compared with the erlotinib alone group (table 3). Serious adverse events were reported by 18 (24%) patients in the erlotinib plus bevacizumab group and 19 (25%) patients in the erlotinib group.

12 (16%) patients in the erlotinib plus bevacizumab group and 14 (18%) patients in the erlotinib group discontinued erlotinib because of adverse events. 31 (41%) patients discontinued bevacizumab because of adverse events (figure 1). Ten patients discontinued both erlotinib and bevacizumab because of adverse events in the erlotinib plus bevacizumab group. Of these patients, seven discontinued erlotinib and bevacizumab simultaneously because of adverse events (liver function disorder or abnormal hepatic function in two patients, and infection, pancreatic cancer, rash, interstitial lung disease, and cerebral infarction in one patient each). In the remaining three patients, bevacizumab was initially discontinued, and patients continued on erlotinib monotherapy, although this was also subsequently discontinued. The dose of erlotinib was reduced to 100 mg for 34 (45%) of 75 patients in the erlotinib plus bevacizumab group and 33 (43%) of 77 patients in the erlotinib alone group; and to 50 mg for 17 (23%) of patients in the erlotinib plus bevacizumab group and eight (10%) patients in the erlotinib alone group.

The major adverse events leading to discontinuation of erlotinib in both groups were liver function disorder or abnormal hepatic function (two [3%] patients in the erlotinib plus bevacizumab group, eight [10%] in the

Panel: Research in context

Systematic review

We searched PubMed for articles published in English until Feb 1, 2014 (with no restrictions for the starting date), using the search terms "bevacizumab", "erlotinib", "NSCLC", and "EGFR". We identified two studies that had assessed the efficacy of erlotinib plus bevacizumab in the first-line setting.[19,20] However, no previous study had assessed the efficacy of the combination of erlotinib and bevacizumab as first-line therapy for patients with activating *EGFR* mutation-positive NSCLC.

Interpretation

To our knowledge, this study is the first to show that the combination of erlotinib and bevacizumab can significantly prolong progression-free survival compared with erlotinib alone in patients with non-squamous *EGFR* mutation-positive NSCLC. Some degree of increased toxicity, particularly hypertension, proteinuria, and haemorrhagic events, was noted with the addition of bevacizumab. Our findings suggest that the combination of erlotinib and bevacizumab could be a new first-line regimen in *EGFR* mutation-positive NSCLC. Two clinical trials, BELIEF (NCT01562028) and ACCRU RC1126 (NCT01532089) are ongoing and the results are awaited to confirm the efficacy and safety shown in our study.

erlotinib alone group), interstitial lung disease (two [3%], three [4%]), and rash (two [3%], none). Major adverse events leading to discontinuation of bevacizumab were proteinuria (11 [15%] patients), haemorrhagic events (nine [12%]), and hypertension (two [3%]). Most haemorrhagic events were low-grade epistaxis or haemorrhoidal bleeding. All of the 11 patients who discontinued bevacizumab because of proteinuria had grade 3 or lower events, and five of these patients recovered during the study period. All of the nine patients who discontinued because of haemorrhagic events had grade 3 or lower events; eight patients improved or recovered during the study period.

The median duration of erlotinib treatment was 431 days (range 21–837) in the erlotinib plus bevacizumab group and 254 days (18–829) in the erlotinib group, whereas median duration of bevacizumab was 325 days (1–815). The median duration of bevacizumab in patients who discontinued treatment because of proteinuria was 329 days (113–639) and because of haemorrhagic events was 128 days (23–357).

The relative dose intensity of erlotinib (calculated as [totally administered dose / total treatment duration] / 150×100) was similar in both groups (95·3% [range 34·7–100·0] in the erlotinib plus bevacizumab group and 98·7% [33·3–100·0] in the erlotinib alone group), whereas that of bevacizumab (calculated as totally administered dose/planned dose×100) was 93·9% (72·4–99·7).

Haemoptysis was reported in six (8%) patients in the erlotinib plus bevacizumab group (five [7%] patients had grade 1 events and one [1%] had a grade 2 event); one patient (1%) had a grade 1 event in the erlotinib alone group. Interstitial lung disease was reported for five (3%) of all patients. One patient in the erlotinib alone group had grade 3 interstitial lung disease, but all other cases were grade 1 or 2, and all patients recovered. During the study period, one patient in the erlotinib group died by drowning, and a potential association with the study drug was confirmed.

No significant difference was noted between the two groups in terms of quality of life, including total FACT-L score, trial outcome index score, and all other subscores, since the standard deviations at each time point overlapped (appendix pp 3–9).

Discussion

In this study, the addition of bevacizumab to erlotinib significantly prolonged progression-free survival in patients with NSCLC with activating *EGFR* mutation-positive disease compared with erlotinib alone. To our knowledge, this is the first randomised study to show a clinically significant treatment effect of combining an EGFR tyrosine-kinase inhibitor with another biological drug in patients with activating *EGFR* mutation-positive NSCLC (panel). We noted clear separation of the Kaplan-Meier survival curves from the start of treatment, despite the use of erlotinib in both groups.

Multivariate analysis according to baseline patient characteristics showed a consistent treatment benefit, with longer progression-free survival noted with erlotinib plus bevacizumab across most subgroups of patients. Previous studies have reported that erlotinib tends to be more effective in tumours with *EGFR* exon 19 deletions versus those with Leu858Arg mutations,[7,8,21] which is consistent with our results.

No new safety signals were identified and the incidence of adverse events (any grade) and serious adverse events was similar between the two groups. There were more grade 3 or worse adverse events in the erlotinib plus bevacizumab group. Discontinuation of bevacizumab because of adverse events was more common than that reported in previous studies.[13,14] One possible reason for this discrepancy could be the longer duration of treatment than in previous studies: the median treatment duration of bevacizumab was 325 days (16 cycles), which is substantially longer than that in previous studies. Furthermore, proteinuria was one of the major adverse events that led to discontinuation of bevacizumab, and the time to onset of bevacizumab discontinuation because of proteinuria tended to be in the later treatment phase (median 329 days [range 113–639]). Nevertheless, despite the high incidence of bevacizumab discontinuation because of adverse events, most of these events (mainly proteinuria and haemorrhagic events) were deemed non-serious and reversible.

The incidence of grade 3 or greater hypertension and proteinuria were higher than those in previous studies, again possibly related to the prolonged duration of treatment. Another potential factor that could explain the difference in the incidence of hypertension is in the definition of grading used; we used CTC-AE version 4.03, whereas previous studies[14,16] used CTC-AE version 3. Akhtar and colleagues[22] showed that the change in CTC-AE version from 3 to 4 could lead to a significant

shift in the severity of adverse events in clinical trials. Furthermore, despite the somewhat higher incidence of hypertension observed in this study, only two (3%) of 75 patients discontinued bevacizumab administration because of hypertension.

Although we noted no significant difference in the proportion of patients achieving an objective response between the erlotinib plus bevacizumab group and erlotinib alone groups, all patients in the erlotinib plus bevacizumab group had a reduction in tumour size. Of those patients who had a greater than 30% reduction in the sum of longest diameter of their target lesions from baseline, more patients in the erlotinib alone group failed to meet the criteria for complete or partial response. These findings suggest that the addition of bevacizumab to erlotinib might help to maintain the tumour-suppressing effect after reduction in tumour size, which might explain the difference in progression-free survival between the two groups.

One possible mechanism to explain this effect could be improved drug delivery. Bevacizumab changes tumour vessel physiology, resulting in increased intratumoral uptake of drugs.[23,24] The results of a preclinical study suggested that patients on lower doses of EGFR tyrosine-kinase inhibitors tend to develop treatment resistance earlier than those who receive higher doses.[25,26] Therefore, achieving a higher intratumoral concentration of erlotinib could delay the appearance of resistant cells. Another possible mechanism that could explain these findings is the effective blocking of angiogenesis signalling via the VEGF receptor and EGFR signalling pathways, which is thought to promote tumour growth.[27,28] In addition to synergistic inhibition of tumour growth signalling, VEGF signal inhibition is still effective for tumours harbouring EGFR tyrosine-kinase inhibitor resistance mutations. In preclinical studies, blocking the VEGF receptor signalling pathway overcame resistance for EGFR signalling blockage by Thr790Met EGFR mutation in vivo.[29,30]

Another treatment strategy that has been recently investigated is the combination of an EGFR tyrosine-kinase inhibitor with chemotherapy. Wu and colleagues[31] reported that platinum doublet chemotherapy with intercalated erlotinib increased progression-free survival compared with platinum doublet chemotherapy alone. In a subset analysis of the *EGFR* mutation-positive population in this study, progression-free survival was 16·8 months. In our study, median progression-free survival with erlotinib and bevacizumab was 16·0 months. The first-line use of erlotinib and bevacizumab could allow chemotherapy to be reserved for subsequent lines of treatment, which might further improve survival outcomes in these patients.

Our study has several limitations. First, the analysis of *EGFR* mutations was not done at a central laboratory and various methods were used, including the peptide nucleic acid, locked nucleic acid PCR clamp method, the PCR invader method, and the cycleave method. However, on the basis of previous evidence, these methods are generally judged to provide consistent results.[32] Second, because some patients are still receiving the first-line treatment and overall survival data are still immature, assessment of subsequent treatment effects after progression is not possible. Data relating to post-study treatment will be reported in due course with updated overall survival results. Third, we did not use the EQ-5D questionnaire developed by the EuroQol group for quality-of-life assessment. Therefore, we could not formally estimate quality-adjusted life-years for a cost-effectiveness analysis. The health economics related to the combined use of erlotinib and bevacizumab remains unclear and should be discussed in future studies. Additionally, follow-up for overall survival is still ongoing and these results are needed before the clinical value of this combination can be determined.

In summary, our study provides, to the best of our knowledge, the first evidence that the addition of bevacizumab to erlotinib confers a significant improvement in progression-free survival when used as first-line treatment for patients with non-squamous NSCLC with activating *EGFR* mutation-positive disease. Some degree of increased toxicity, particularly hypertension, proteinuria, and haemorrhagic events, seems to be associated with the addition of bevacizumab. Our findings suggest that the combination of erlotinib and bevacizumab could be a new first-line regimen in *EGFR* mutation-positive NSCLC, and that further investigation of the regimen is warranted. Two clinical trials, BELIEF (NCT01562028) and ACCRU RC1126 (NCT01532089), are ongoing and the results are awaited to confirm the efficacy and safety shown in our study.

Contributors

NobuY was the principal investigator. TS, TK, MN, KG, NoboY, IO, TY, KT, RH, MF, and NobuY contributed to the study design and data analysis and data interpretation. TS, TK, MN, KG, SA, YH, NoboY, TH, MM, KN, SN, IO, and NobuY contributed to patient recruitment and data collection. NobuY, TS, KT, and RH prepared the initial draft of the report input from other authors. All authors approved the final version of the report.

Declaration of interests

TS received research grants and honoraria from Chugai Pharmaceutical. TK received research grants and honoraria from Chugai Pharmaceutical; honoraria from Eli Lilly, Ono Pharmaceutical, Novartis Pharma, Taiho Pharmaceutical, and AstraZeneca; and research grants from Nippon Boehringer Ingelheim, Kyowa Hakko Kirin, Pfizer, and Shionogi. MN received research grants and honoraria from Chugai Pharmaceutical, Pfizer, Novartis Pharma, Taiho Pharmaceutical, Nippon Boehringer Ingelheim, and AstraZeneca; research grants from MSD and Bristol-Myers Squibb. KG received research grants and honoraria from Chugai Pharmaceutical, Taiho Pharmaceutical and Nippon Boehringer Ingelheim; honoraria from AstraZeneca, Sanofi, Novartis Pharma, Pfizer, Yakult Honsha, Ono Pharmaceutical and Eli Lilly. SA received honoraria from Chugai Pharmaceutical, Eli Lilly, Taiho Pharmaceutical, Sawai Pharmaceutical, and Novartis Pharma. YH received research grants and honoraria from Chugai Pharmaceutical, Ono Pharmaceutical, and Taiho Pharmaceutical; honoraria from AstraZeneca, Eli Lilly, Novartis Pharma, and Takeda Pharmaceutical; research grants form Yakult Honsha, MSD, Kyowa Hakko Kirin, and Daiichi Sankyo. NoboY received research grants form Chugai Pharmaceutical, Pfizer, Takeda Bio, Astellas Pharma, Taiho Pharmaceutical, and Bristol-Myers Squibb. TH received research grants form Chugai Pharmaceutical, AstraZeneca, Nippon Boehringer Ingelheim, Pfizer, Eli Lilly, Takeda Bio, Novartis Pharma, Ono Pharmaceutical, Daiichi Sankyo, Merck Serono, Kyowa Hakko Kirin, Dainippon Sumitomo Pharma, Bristol-Myers Squibb, and Esai.

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Phase II study of zoledronic acid combined with docetaxel for non-small-cell lung cancer: West Japan Oncology Group

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Key words

Chemotherapy, docetaxel, non-small-cell lung cancer, phase II, zoledronic acid

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The aim of this open-label, multicenter, randomized phase II trial was to evaluate the efficacy and safety of zoledronic acid in combination with docetaxel in previously treated patients with non-small-cell lung cancer (NSCLC) and bone metastases. In this study, patients randomly received docetaxel (60 mg/m^2) with (group DZ) or without (group D) zoledronic acid every 21 days. There were 50 patients in each group, and the primary endpoint was progression-free survival. In an efficacy analysis of 94 patients (DZ, 48; D, 46), the median progression-free survival was 2.7 months (95% confidence interval [CI], 1.5–3.5 months) for the DZ group and 2.6 months (95% CI, 1.5–3.4 months) for the D group (stratified log-rank test, $P = 0.89$). The median overall survival was 10.4 months (95% CI, 7.0–15.8 months) for the DZ group and 9.7 months (95% CI, 6.1–12.5 months) for the D group (stratified log-rank test, $P = 0.62$). There were no clinically relevant differences in the frequencies of grade 3 or 4 adverse events between the two groups. No treatment-related deaths occurred in the DZ group. Zoledronic acid combined with docetaxel was well tolerated but did not meet the primary endpoint of demonstrating a longer progression-free survival in advanced NSCLC patients with bone metastases compared with docetaxel alone. This trial was registered with the University Hospital Medical Information Network (UMIN000001098).

Lung cancer is the leading cause of cancer death worldwide and non–small-cell lung cancer (NSCLC) accounts for more than 80% of all cases of lung cancer.[1] For individuals with advanced NSCLC, first-line treatment with platinum-based chemotherapy offers only a moderate improvement in survival and quality of life over best supportive care (BSC) alone.[2,3] Second-line treatment with docetaxel, despite a low tumor response rate, is a standard treatment option on the basis of phase III studies comparing docetaxel with ifosfamide, vinorelbine or BSC alone.[4,5] Thus, there is a need for new treatment options to prolong the survival of patients with advanced NSCLC. Approximately 30–40% of patients with NSCLC develop bone metastases, which often cause skeletal-related events (SRE) such as pathologic fracture, spinal cord compression, or the need for palliative radiation or surgery to the bone.[6] SRE are associated with decreased quality of life, increased health-care costs and poor survival; therefore, it is clinically imperative to prevent SRE during the treatment of advanced NSCLC.[7–10]

Zoledronic acid, a nitrogen-containing bisphosphonate, significantly delays the appearance of SRE and reduces the incidence of SRE compared with a placebo in patients with cancer and bone metastases, including those with NSCLC.[11,12] Furthermore, several preclinical and clinical studies provide evidence supporting the use of zoledronic acid for the treatment of patients with advanced NSCLC.[13–16] The inclusion of zoledronic acid in chemotherapy regimens has an additive and/or synergistic antitumor effect on NSCLC cell lines and may prolong survival and delay disease progression in patients with advanced NSCLC.[17–19] However, whether the inclusion of zoledronic acid in such regimens has clinically meaningful survival benefits in patients with NSCLC and bone metastases is uncertain. Therefore, we

conducted this study to evaluate the efficacy and safety of zoledronic acid in combination with docetaxel in previously treated patients with NSCLC and bone metastases.

Patients and Methods

Study design. We conducted an open-label, multicenter, randomized phase II study in Japan. The study protocol was approved by the West Japan Oncology Group (WJOG) Protocol Review Committee and the institutional review board of each participating institution. This trial was registered with the University Hospital Medical Information Network (UMIN000001098).

Eligibility criteria. Patients were required to be histologically or cytologically diagnosed with NSCLC and bone metastases (at least one bone metastasis that had not been treated with radiation therapy) and to have had previous treatment with one or two chemotherapy regimens. Other inclusion criteria included an age of ≥20 years, Eastern Cooperative Oncology Group performance status of 0–2, measurable disease, no history of chemotherapy with docetaxel, no history of prior treatment with zoledronic acid, adequate baseline organ function (leukocyte count ≥3500/mm^3; absolute neutrophil count ≥2000 /mm^3; hemoglobin ≥9.0 g/dL; platelet count ≥100 000/mm^3; total bilirubin ≤2.0 mg/dL; aspartate aminotransferase and alanine aminotransferase [ALT] levels ≤100 IU/L; creatinine clearance, ≥30 mL/min; and SpO_2 under room air, ≥90%). Written informed consent was obtained from all patients. Patients were ineligible if they had active concomitant malignancy, third-space fluid collection requiring drainage, radiographic signs of interstitial pneumonia or pulmonary fibrosis, active SRE at the time of registration, hypercalcemia requiring prompt treatment, active periodontal disease or severe comorbidities (active infectious disease, severe heart disease, uncontrolled diabetes mellitus, gastrointestinal bleeding, intestinal paralysis, bowel obstruction or psychiatric disease), or a history of drug allergy. Patients receiving systemic steroid medication and pregnant or breast-feeding women were also excluded.

Treatment. Equal numbers of patients randomly received 60 mg/m^2 docetaxel intravenously for 1 h with (DZ group) or without (D group) intravenous zoledronic acid for 15 min. Random assignment was stratified by institution, gender and performance status (0–1 or 2). The dose of zoledronic acid for each patient was based on his or her creatinine clearance (>60 mL/min, 4 mg; 50–60 mL/min, 3.5 mg; 40–49 mL/min, 3.3 mg; 30–39 mL/min, 3.0 mg). Zoledronic acid was administered to patients in the DZ group immediately after docetaxel administration. Patients were treated every 3 weeks until their disease progressed, toxicity became intolerable or they refused additional treatment. The dose of docetaxel was decreased to 50 mg/m^2 if any of the following was observed: leukocyte count <1000/mm^3, platelet count <25 000/mm^3, grade 3 febrile neutropenia or grade 3 nonhematological toxicity (with the exception of hyponatremia, hypocalcaemia and alopecia). In cases of grade 4 nonhematological toxicity or continued toxicity requiring a second dose reduction, the protocol treatment was terminated. Other criteria for protocol treatment termination included use of excluded concomitant therapy and physician recommendation.

Patients received full supportive care as required, including transfusion of blood products. Granulocyte colony-stimulating factor was administered as needed. There was no restriction on subsequent chemotherapy after disease progression in this study.

Evaluation. Patient assessment, including physical examination, complete blood count and biochemistry, was performed every 1–2 weeks. Bone markers and levels of urinary N-terminal telopeptide of type I collagen (NTX) and serum C-terminal telopeptide of type I collagen (I-CTP) were evaluated every 4 weeks. SRE included pathologic fracture, spinal cord compression and need for palliative radiation or surgery to the bone, and were assessed every 6 weeks.

Patients who received one or more protocol treatment were evaluated for safety during treatment. Adverse events were recorded and graded using the Common Terminology Criteria for Adverse Events, Version 3.0. The Response Evaluation Criteria in Solid Tumors guideline version 1.0 was used to evaluate tumor response.[20] Computed tomography was performed at baseline and every 6 weeks. A complete response (CR) or a partial response (PR) was confirmed at least

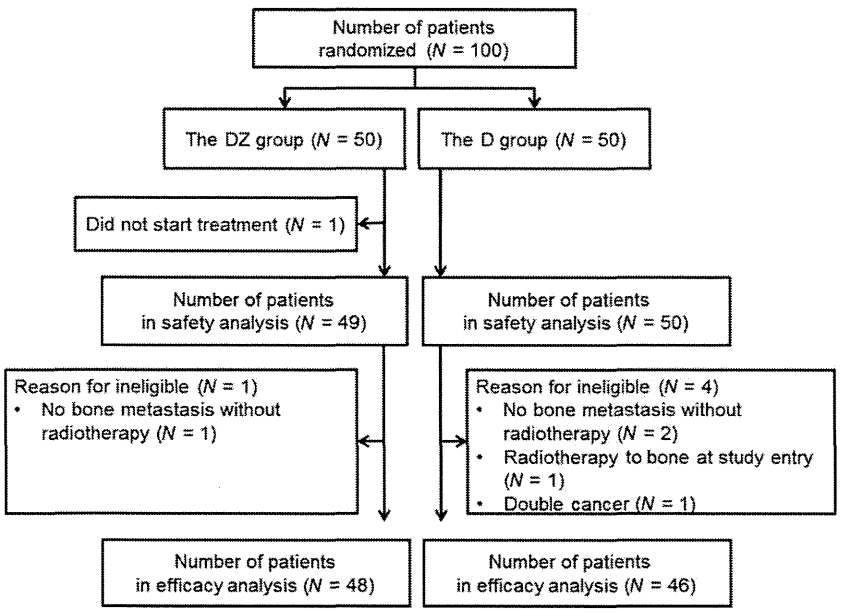

Fig. 1. Patient disposition. D, docetaxel alone; DZ, docetaxel with zoledronic acid.

4 weeks after the first documentation of the response. Stable disease (SD) was defined as either sufficient tumor shrinkage to qualify as a CR or a PR or sufficient increase in tumor mass to qualify as progressive disease (PD) after at least 6 weeks. Progression-free survival (PFS) was defined as the time from patient registration to objective tumor progression or patient death. Patients whose disease had not progressed at the time of termination of protocol treatment were assessed until progression or death was documented. SRE-free survival was defined as the time from patient registration to the appearance of SRE or the death of the patient. Patients who had not experienced SRE at the time of termination of protocol treatment were assessed until SRE or death was documented. Overall survival (OS) was defined as the time from patient registration to death from any cause. All patients were followed up for 1 year after the last patient had enrolled.

Study endpoints and statistical analyses. The primary endpoint in this study was PFS. The secondary endpoints included OS, overall response rate (ORR), SRE rate, SRE-free survival and safety. This randomized phase II study was designed to detect a 1-month improvement in PFS, with an assumed PFS of 2 months in the D group and 3 months in the DZ group, with a two-sided alpha error of 20% and a power of approximately 80%. A total of 100 patients were registered over 2 years with a 1-year follow-up period after the last enrollment. Survival curves were estimated using the Kaplan–Meier method and compared by log-rank test. Fisher's exact test was used for categorical data. All analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics. From May 2007 to March 2010, 100 patients from 15 Japanese institutions were enrolled in this study: 50 patients were randomly assigned to the DZ group and 50 to the D group (Fig. 1). Patient demographics and baseline disease characteristics were well-balanced between the two treatment groups (Table 1). While one patient in the DZ group did not receive any protocol treatment, 99 patients (49 for DZ and 50 for D) were assessable for safety. In the DZ group 1 patient and in the D group 4 patients were ineligible, and 94 patients (48 for DZ and 46 for D) were included in the efficacy analysis (Fig. 1). The median number of treatment cycles was three for the DZ group (range, 1–19 cycles) and three for the D group (range, 1–17 cycles). The median number of administered doses of zoledronic acid was 3 (range, 1–19), with a median drug exposure of 12.0 mg (range, 3.5–76.0 mg). Reasons for going off protocol included disease progression (37 for DZ and 33 for D), patient refusal (eight for DZ and eight for D), unacceptable toxicity (two for DZ and five for D) and others (two for DZ and four for D).

Safety. Adverse events for the 99 patients included in the safety analysis are summarized in Table 2. The occurrence of adverse events was similar in the two groups, with the exception of any grade of hypocalcemia (76% *vs* 30%) and pyrexia (39% *vs* 10%), which were more frequent in the DZ group compared with the D group. One patient in the DZ group experienced periodontal disease, but no cases of osteonecrosis of the jaw (ONJ) were observed in either group. The most common adverse events worse than grade 3 were leukopenia (63% and 56% for DZ and D, respectively), neutropenia (78% and 80% for DZ and D, respectively), febrile neutropenia (4% and 12% for DZ and D, respectively) and elevated ALT level (27% and 30% for DZ and D, respectively). There were no clinically relevant differences in the frequencies of adverse events of grade 3 or higher between the two groups. The protocol treatment was terminated in seven patients because of unacceptable toxicity levels, including grade 3 nail change ($N = 1$) and grade 2 periodontal disease ($N = 1$) in the DZ group, and required a second dose reduction because of grade 4 leukopenia ($N = 1$) or grade 3 febrile neutropenia ($N = 1$), grade 4 infection ($N = 1$), grade 3 allergic reaction ($N = 1$) and grade 1 pneumonitis ($N = 1$) in the D group. No treatment-related deaths were observed in the DZ group, while two treatment-related deaths were observed in the D group (infection, $N = 1$; gastrointestinal perforation, $N = 1$).

Table 1. Patient demographics and baseline disease characteristics

Characteristic	DZ group ($N = 50$)		D group ($N = 50$)	
	Number	%	Number	%
Age, years				
Median	62		63	
Range	34–77		45–79	
Sex				
Female	19	38	18	36
Male	31	62	32	64
ECOG performance status				
0–1	47	94	47	94
2	3	6	3	6
Smoking status				
Smoker	19	38	15	30
Never smoked	31	62	35	70
Histological subtype				
Adenocarcinoma	39	78	38	76
Squamous cell carcinoma	5	10	7	14
Others	6	12	5	10
Number of prior chemotherapies				
1	34	68	39	78
2	15	30	11	22
No data	1	2	0	0
Number of bone metastases				
Single	11	22	12	24
Multiple	39	78	38	76
Prior SRE				
No	41	82	42	84
Yes	8	16	8	16
No data	1	2	0	0
Urinary NTX				
High level (≥64 nmol/mmol creatinine)	20	40	22	44
Normal level (<64 nmol/mmol creatinine)	23	46	22	44
No data	7	14	6	12
Serum I-CTP				
High level (≥4.5 ng/mL)	35	70	35	70
Normal level (<4.5 ng/mL)	8	16	9	18
No data	7	14	6	12

D, docetaxel alone; DZ, docetaxel with zoledronic acid; ECOG, Eastern Cooperative Oncology Group; I-CTP, C-terminal telopeptide of type I collagen; NTX, N-terminal telopeptide of type I collagen; SRE, skeletal-related event.

Efficacy. For the 94 patients included in the efficacy analysis, the ORR was 8% for the DZ group (CR, $N = 0$; PR, $N = 4$;

Table 2. Summary of adverse events (CTCAE)

Adverse event	DZ group (N = 49)				D group (N = 50)			
	All		≥Grade 3		All		≥Grade 3	
	Number	%	Number	%	Number	%	Number	%
Leukopenia	45	92	31	63	47	94	28	56
Neutropenia	45	92	38	78	46	92	40	80
Anemia	33	67	3	6	31	62	3	6
Thrombocytopenia	2	4	0	0	5	10	0	0
Elevated ALT level	24	49	13	27	21	42	15	30
Elevated AST level	19	39	4	8	16	32	3	6
Elevated creatinine level	7	14	1	2	13	26	2	4
Hypercalcemia	2	4	0	0	8	16	1	2
Hypocalcemia	37	76	3	6	15	30	0	0
Febrile neutropenia	2	4	2	4	6	12	6	12
Infection	13	27	5	10	5	10	3	6
Sensory neuropathy	12	24	2	4	11	22	1	2
Fatigue	33	67	2	4	33	66	2	4
Anorexia	30	61	2	4	30	60	1	2
Nausea	20	41	1	2	23	46	0	0
Vomiting	8	16	1	2	8	16	0	0
Allergic reaction	3	6	0	0	2	4	1	2
Gastrointestinal perforation	0	0	0	0	1	2	1	2
Pyrexia	19	39	0	0	5	10	0	0
Periodontal disease	1	2	0	0	0	0	0	0

ALT, alanine transaminase; AST, aspartate aminotransferase; CTCAE, Common Terminology Criteria for Adverse Events, version 3.0; D, docetaxel alone; DZ, docetaxel with zoledronic acid.

SD, N = 18; PD, N = 25; not evaluable, N = 1) and 4% for the D group (CR, N = 0; PR, N = 2; SD, N = 20; PD, N = 23; not evaluable, N = 1). The difference in ORR between the two groups was not statistically significant (P = 0.88). Median PFS was 2.7 (95% CI, 1.5–3.5) months for the DZ group and 2.6 (95% CI, 1.5–3.4) months for the D group (stratified log-rank test, P = 0.89; Fig. 2a). Median OS was 10.4 (95% CI, 7.0–15.8) months for the DZ group and 9.7 (95% CI, 6.1–12.5) months for the D group (stratified log-rank test, P = 0.62; Fig. 2b). No remarkable difference in PFS (Fig. 3a) or OS (Fig. 3b) was observed according to demographic characteristics (number of bone metastases, prior SRE, baseline urinary NTX and baseline serum I-CTP).

For the 94 patients included in the efficacy analysis, the cumulative incidence rates of an SRE at 3, 6, 9 and 12 months were 17%, 20%, 27% and 30%, respectively, for the DZ group, and 16%, 27%, 39% and 39%, respectively, for the D group (Fig. 4a). Median SRE-free survival was 7.2 (95% CI, 4.9–10.7) months for the DZ group and 6.0 (95% CI, 4.4–8.5) months for the D group (stratified log-rank test, P = 0.84). In subset analyses of the SRE rate according to baseline bone marker levels (Fig. 4b), the cumulative incidence rates of SRE at 12 months were 44% for the DZ group (N = 19) and 48% for the D group (N = 19) in patients with high baseline urinary NTX levels, 24% for the DZ group (N = 29) and 30% for the D group (N = 27) in patients with normal or unknown baseline urinary NTX levels, 43% for the DZ group (N = 34) and 38% for the D group (N = 32) in patients with high baseline serum I-CTP levels, and 7% for the DZ group (N = 14) and 37% for the D group (N = 14) in patients with normal or unknown baseline serum I-CTP levels.

Discussion

This is the first prospective, randomized, phase II study to evaluate the efficacy and safety of zoledronic acid in combination with docetaxel in previously treated advanced NSCLC

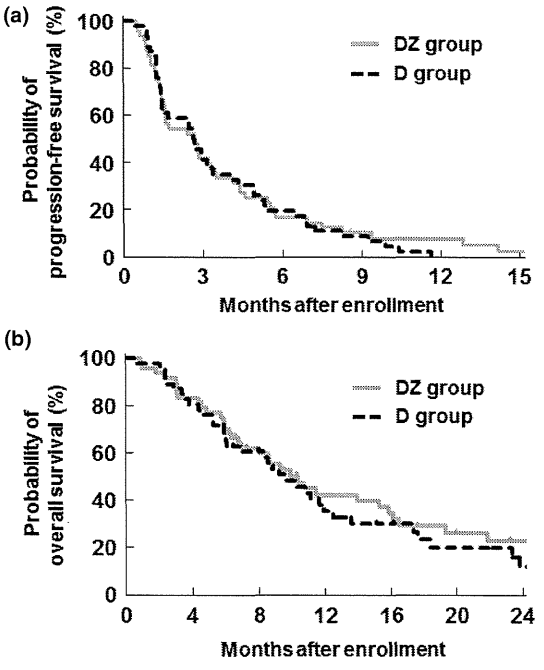

Fig. 2. (a) Progression-free survival and (b) overall survival in the DZ and D groups. D, docetaxel alone; DZ, docetaxel with zoledronic acid.

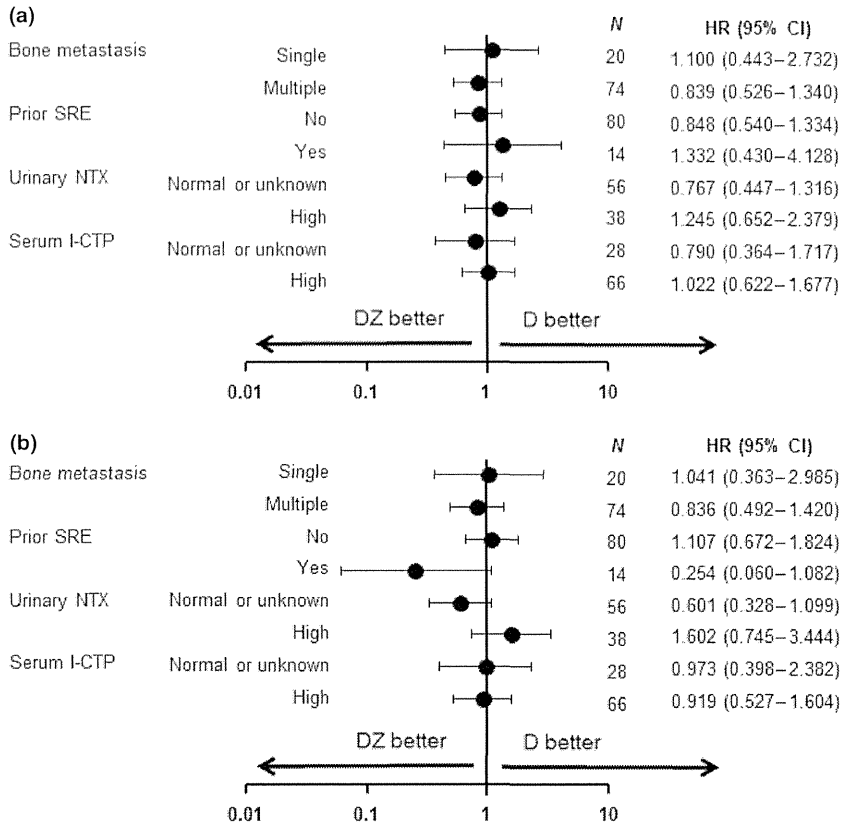

Fig. 3. (a) Subgroup analyses of hazard ratio for progression-free survival and (b) overall survival in the DZ and D groups. D, docetaxel alone; DZ, docetaxel with zoledronic acid; I-CTP, C-terminal telopeptide of type I collagen; NTX, N-terminal telopeptide of type I collagen; SRE, skeletal-related event.

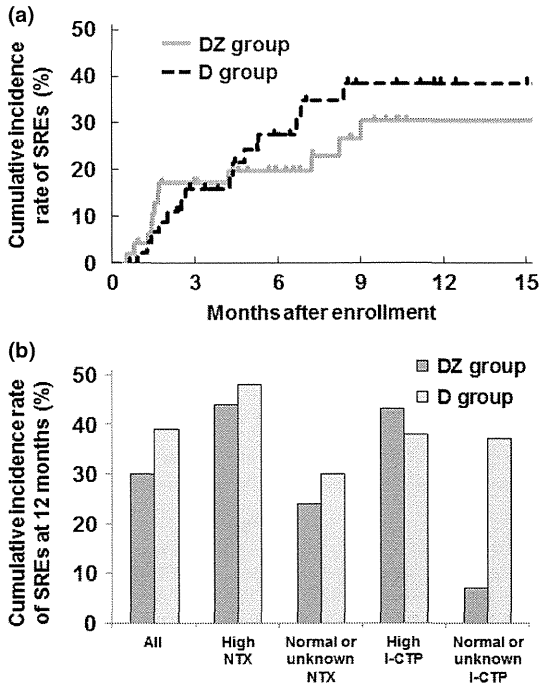

Fig. 4. (a) Cumulative incidence rate of SRE in the DZ and D groups. (b) Subgroup analyses of SRE rate according to baseline bone marker levels in the DZ and D groups. D, docetaxel alone; DZ, docetaxel with zoledronic acid; I-CTP, C-terminal telopeptide of type I collagen; NTX, N-terminal telopeptide of type I collagen; SRE, skeletal-related event.

patients with bone metastases. The similarity in the median PFS and OS of patients in the DZ and D groups suggests that the combination of zoledronic acid and docetaxel might not provide survival benefits to patients with NSCLC and bone metastases compared with docetaxel alone. In a previous randomized phase III study, a subgroup analysis of patients with NSCLC (N = 382) revealed that zoledronic acid significantly reduced the risk of a first on-study SRE compared with a placebo. However, there was no significant difference in OS between the two groups (median 187 days for zoledronic acid *vs* 157 days for placebo; P = 0.539).[11,12,14] Two randomized studies in which zoledronic acid was combined with standard treatment also showed no survival benefits for patients with NSCLC who had no bone involvement.[21,22] These results are consistent with our observation that zoledronic acid failed to prolong the survival of NSCLC patients with bone metastases. In a recent subgroup analysis of a randomized phase III study, denosumab significantly improved OS, whereas zoledronic acid did not. This analysis was conducted on a group of 811 patients with lung cancer and bone metastases (median 8.9 *vs* 7.7 months for denosumab and zoledronic acid, respectively; hazard ratio for death, 0.80; 95% CI, 0.67–0.95; P = 0.01) and 702 patients with NSCLC and bone metastases (median 9.5 *vs* 8.0 months for denosumab and zoledronic acid, respectively; hazard ratio for death, 0.78; 95% CI, 0.65–0.94; P = 0.01).[23,24] Denosumab, a human anti-RANKL monoclonal antibody, is a potential anticancer therapy for patients with NSCLC and bone metastases and should be evaluated further in future studies.

For patients with NSCLC and bone metastases, increased SRE risk correlated with a history of SREs, multiple bone metastases, and bone turnover markers.[25–27] Significantly high levels of urinary NTX, a sensitive bone resorption marker, were also associated with increased SRE risk and poor survival prognosis.[27] In agreement, the cumulative incidence rates of SRE were high in patients with high baseline urinary NTX levels in our study. A retrospective analysis of a phase III study revealed that zoledronic acid significantly reduces the risk of death compared with a placebo in 144 patients with NSCLC and high baseline NTX levels (hazard ratio for death, 0.65; 95% CI, 0.45–0.95; $P = 0.025$).[15] In our study, for 38 patients (19 for DZ and 19 for D) with NSCLC and high baseline NTX levels, the median OS was 8.6 months for the DZ group and 11.2 months for the D group (hazard ratio for death, 1.60; 95% CI, 0.75–3.44). Therefore, combination treatment with zoledronic acid and docetaxel did not improve OS in previously treated patients with NSCLC and bone metastases in addition to high baseline NTX levels. However, the number of patients in our study was small; as such, this study was not powered to detect differences in secondary variables, and statistical testing was performed for exploratory purposes.

The most common severe toxicities in the present study were leukopenia, neutropenia, febrile neutropenia and elevated ALT levels, which were similar in the two groups. No treatment-related deaths were observed in the DZ group. Although hypocalcemia and pyrexia were more frequent in the DZ group than in the D group, they were mild and manageable in most cases. A possible reason for the high incidence of hypocalcemia in this study was underuse of calcium supplements and vitamin D. Prophylactic oral administration of daily calcium supplements and vitamin D should be considered during treatment with zoledronic acid. No patient experienced ONJ in this study, although it may be argued that the duration of zoledronic acid treatment was too short for this to occur. No additional adverse events were observed in the present study compared with previous studies.[11,12,23,24]

The present study demonstrated the safety and tolerability of the combination of zoledronic acid and docetaxel but did not meet the primary endpoint of PFS in advanced NSCLC patients with bone metastasis. Based on these results, we abandoned assessment of the survival benefits of adding zoledronic acid to docetaxel treatment in a larger phase III study. There are potential limitations to our study. First, we used an open-label study design despite the use of PFS as the primary endpoint. Second, the sample size of the present study was relatively small. Third, we did not collect data regarding post-study treatment with zoledronic acid. New treatment options are still needed to prolong the survival of advanced NSCLC patients with bone metastasis.

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Molecular profiling of small cell lung cancer in a Japanese cohort

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ABSTRACT

Objectives: Advances in the molecular profiling of lung adenocarcinoma over the past decade have led to a paradigm shift in its diagnosis and treatment. However, there are very few reports on the molecular profiles of small cell lung cancers (SCLCs). We therefore conducted the present Shizuoka Lung Cancer Mutation Study to analyze genomic aberrations in patients with thoracic malignancies.
Materials and methods: We collected samples of SCLC from a biobank system and analyzed their molecular profiles. We assessed 23 mutations in nine genes (*EGFR, KRAS, BRAF, PIK3CA, NRAS, MEK1, AKT1, PTEN,* and *HER2*) using pyrosequencing plus capillary electrophoresis. We also amplified *EGFR, MET, PIK3CA, FGFR1,* and *FGFR2* using quantitative real-time polymerase chain reaction (PCR) and the fusion genes *ALK, ROS1,* and *RET* using reverse transcription PCR.
Results: Between July 2011 and January 2013, 60 SCLC patients were enrolled in the study. Samples included eight surgically resected snap-frozen samples, 50 formalin-fixed paraffin-embedded samples, and seven pleural effusion samples. We detected 13 genomic aberrations in nine cases (15%), including an *EGFR* mutation ($n = 1$, G719A), a *KRAS* mutation ($n = 1$, G12D), *PIK3CA* mutations ($n = 3$, E542K, E545K, E545Q), an *AKT1* mutation ($n = 1$, E17K), a *MET* amplification ($n = 1$), and *PIK3CA* amplifications ($n = 6$). *EGFR* and *KRAS* mutations were found in patients with combined SCLC and adenocarcinoma. No significant differences were detected in the characteristics of patients with and without genomic aberrations. However, serum neuron-specific enolase and progastrin-releasing peptide levels were significantly higher in patients without genomic aberrations than in those with aberrations ($p = 0.01$ and 0.04, respectively).
Conclusion: Genomic aberrations were found in 15% SCLC patients, with *PIK3CA* amplifications most frequently observed. To further our understanding of the molecular profiles of SCLC, comprehensive mutational analyses should be conducted using massive parallel sequencing.

1. Introduction

Lung cancer is the most common cause of cancer-related deaths, and small cell lung cancer (SCLC) accounts for approximately 12% of all lung cancers [1]. It follows a very aggressive course, with approximately 60–70% patients having disseminated disease at diagnosis. Although SCLC shows high sensitivity to chemotherapy and radiotherapy, the median survival time for extended-disease SCLC is 8–13 months, and the 2-year survival rate is only 5% [2].

Molecular abnormalities have been discovered in patients with non-SCLC over the last decade, and these discoveries have led to a paradigm shift in its diagnosis and treatment. For example, a relationship between activating epidermal growth factor receptor (*EGFR*) mutations and response to gefitinib was reported in 2004 [3,4]. Subsequently, a number of randomized studies showed that patients with activating *EGFR* mutations were highly responsive to

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EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib [5–8]. Currently, it is essential that lung adenocarcinomas are classified on the basis of genomic aberrations to ensure that patients are treated with the appropriate molecular-targeted drugs [9,10]. Analyses of genomic aberrations and the development of new molecular-targeted drugs are ongoing for lung adenocarcinoma. In contrast, there have been few innovations in the treatment of SCLC, despite extensive basic and clinical research over the past 30 years.

There have been few molecular profiles of SCLC, and, till date, no molecular-targeted drugs have shown clinical activity against SCLC [11]. Identification of genomic aberrations linked to SCLC would facilitate the identification of potential therapeutic targets.

We conducted the present Shizuoka Lung Cancer Mutation Study to assess genomic aberrations in patients with thoracic malignancies. A biobank system was established in collaboration with a clinic pathology lab in July 2011. Mutational data were communicated to clinicians and utilized for assigning patients to appropriate therapy and/or enrolling them in clinical trials. Here we report the genomic aberrations identified in patients with SCLC in the Shizuoka study.

2. Materials and methods

2.1. Patients

We collected samples of SCLC from a biobank system and analyzed these to determine their molecular profiles. To evaluate the relationships between any genomic aberrations and patient characteristics, we collected patient demographic and clinical data from medical records. All patients who participated in this study provided their written informed consent.

Pathological diagnoses were made by institutional pathologists according to the 2004 World Health Organization classification based on morphology (uniform round to spindle-shaped small cells, sparse cytoplasm, high mitotic index, and necrotic areas). The diagnosis of SCLC was confirmed when necessary by immunohistochemical analyses of neuroendocrine markers (synaptophysin, chromogranin A, and CD56). And when it is difficult to diagnose samples as SCLC, we additionally performed immunohistochemistry with makers, such as CAM5.2, TTF-1 and Keratin. If more than 10% of a sample comprised adenocarcinoma, the patient was diagnosed with combined SCLC and adenocarcinoma. Surgically resected samples were macrodissected before nucleic acid extraction and tumor biopsy samples with 10% or more tumor cell component were tested for mutational profiling [12]. All of pleural effusion samples were confirmed that malignant cells were present in each pleural effusion by cytology and we analyzed the cytologically confirmed pleural effusion specimens subsequently.

Smokers were defined according to the Brinkman index (BI) as light (BI value < 600) or heavy (BI value ≥ 600) smokers. Limited stage-disease was defined as disease confined to one hemithorax, the ipsilateral supraclavicular fossa, or both. Disease not meeting these criteria was defined as extended-stage disease. Serum neuron-specific enolase (NSE) levels were measured using a solid-phase radioimmunoassay (RIA) method (SRL Inc., Tokyo, Japan), and progastrin-releasing peptide (Pro-GRP) levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (FUJIREBIO Inc., Tokyo, Japan).

2.2. Clinical genotyping

We developed a multiplexed tumor genotyping platform to assess 23 mutations in nine genes (*EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *MEK1*, *AKT1*, *PTEN*, and *HER2*), *EGFR*, *MET*, *PIK3CA*, *FGFR1*, and *FGFR2* amplifications, and *EML4-ALK*, *KIF5B-RET*, *CD74-ROS1*, and *SLC34A2-ROS1* fusion genes (Table 1).

Table 1
Multiplexed tumor genotyping panel.

Gene name	Position	AA mutant	Nucleotide mutant
EGFR	G719	G719	2155G>T/A
		G719A	2156G>C
	exon 19	Deletion	
	T790	T790M	2369C>T
	exon 20	Insertion	
	L858	L858R	2573T>G
	L861	L861Q	2582T>A
KRAS	G12	G12C/S/R	34G>T/A/C
		G12V/A/D	35G>T/C/A
	G13	G13C/S/R	37G>T/A/C
		G13D/A	38G>A/C
	Q61	Q61K	181C>A
		Q61R/L	182A>G/T
		Q61H	183A>T/C
BRAF	G466	G466V	1397G>T
	G469	G469A	1406G>C
	L597	L597V	1789C>G
	V600	V600E	1799T>A
PIK3CA	E542	E542K	1624G>A
	E545	E545K/Q	1633G>A/C
	H1047	H1047R	3140A>G
NRAS	Q61	Q61K	181C>A
		Q61L/R	182A>T/G
MEK1 (MAP2K1)	Q56	Q56P	167A>C
	K57	K57N	171G>T
	D67	D67N	199G>A
AKT1	E17	E17K	49G>A
PTEN	R233	R233*	697C>T
HER2	exon 20	Insertion	

2.3. Nucleic acid sample preparation

DNA samples were extracted from surgically resected tissues, body cavity fluids, and tumor biopsy sections using a QIAamp DNA mini kit (QIAGEN, Hilden, Germany) or a QIAamp DNA formalin-fixed paraffin-embedded (FFPE) tissue kit (QIAGEN). The DNA concentration was measured using a Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA). Total RNAs were isolated with an RNeasy Mini kit (QIAGEN) and measured using a spectrophotometer (NanoDrop 2000C; Thermo Scientific, Wilmington, DE).

2.4. Pyrosequencing

Pyrosequencing was used to detect single base substitution-type mutations. An internal fragment of each gene was amplified by polymerase chain reaction (PCR) using primers specific for each gene and a PyroMark PCR kit (QIAGEN). The resulting PCR products were sequenced with the PyroMark Q24 (QIAGEN) pyrosequencer using PyroMark Gold Q96 reagents (QIAGEN) and sequencing primers specific for each gene.

2.5. Fragment analysis

Insertion/deletion-type mutations were identified by sizing the PCR-amplified products using capillary electrophoresis (QIAxcel, QIAGEN).

2.6. Gene copy number analysis

Copy number was evaluated by quantitative real-time PCR (qRT-PCR) performed on a StepOnePlus Real time PCR system (Applied

Biosystems) using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (TAKARA BIO) and PCR primers for each gene. If the gene copy number from the samples was more than double that of the cell line known to be normal human genomic DNA, it was considered as evidence of amplification. Detailed methods are described previously [12].

2.7. Screening for transcripts of fusion genes

Fusion genes were detected by multiplex RT-PCR. Synthesis of cDNA templates was performed with total RNA (1 μg) using Oligo $(dT)_{12-18}$ Primer (Invitrogen) and Omniscript RT (QIAGEN) kits. *EML4-ALK* and *ROS1* fusion genes were detected according to the methods of Sun et al. [13] and Li et al. [14], respectively. Methods for the detection of *KIF5B-RET* fusions were kindly provided by Dr. Takashi Kohno (National Cancer Center, Tokyo).

2.8. Statistical analysis

All categorical variables were analyzed by the chi-square test or Fisher's exact test, as appropriate. Continuous variables, including tumor markers, were analyzed using the Mann–Whitney test. All *p*-values were reported to be two-sided, and values of <0.05 were considered statistically significant. All statistical analyses were performed using JMP version 9.0 software (SAS Institute Inc., Cary, NC, USA). Our study was approved by the Institutional Review Board.

3. Results

3.1. Patient characteristics

Between July 2011 and January 2013, SCLC samples from 60 patients were assessed for genomic aberrations. The patient characteristics are shown in Table 2. The median age (range) was 69 (43–82) years, and most patients were male (83%) and heavy smokers (80%). Only two patients were never-smokers. A total of 57 patients were diagnosed with SCLC, while three were diagnosed with combined SCLC and adenocarcinoma. Thirty-one patients had limited-stage disease and 29 had extended-stage disease. We analyzed eight surgically resected snap-frozen samples, 50 FFPE samples, and seven pleural effusion samples. Five patients provided two specimens: three provided both FFPE and surgically resected snap-frozen samples and two provided both FFPE and pleural effusion samples (Table 3).

Table 2
Patients characteristics that were analyzed in our study (overall, *N* = 60).

	N = 60	%
Median age (years)	69	
Range	43–82	
Gender		
Male	50	83
Female	10	17
Smoking status		
Never	2	3
Light (B.I. < 600)	10	17
Heavy (B.I. ≥ 600)	48	80
Histology		
Small cell carcinoma	57	95
Combined small cell carcinoma with adenocarcinoma	3	5
Disease extent		
Limited stage	31	52
Extended stage	29	48
Samples		
Surgically resected snap-frozen samples	8	
FFPE samples	50	
Pleural effusion	7	

Abbreviation: B.I., Brinkman Index; FFPE, Formalin-fixed paraffin-embedded.

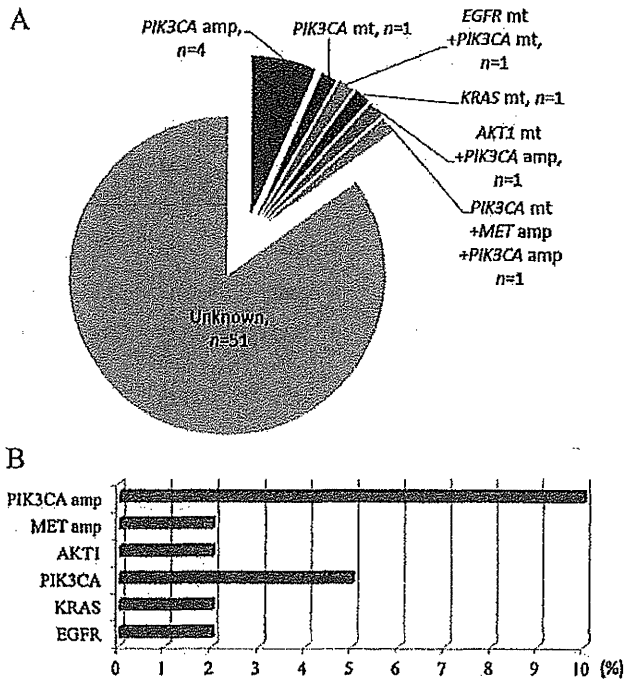

Fig. 1. Relative proportions of genomic aberrations in small cell lung cancer (*N* = 60). (A) Pie chart shows relative proportions of genomic aberrations. (B) Bar chart shows relative proportions of genomic aberrations. *Abbreviations*: mt: mutation; amp: amplification.

3.2. Genomic aberrations

We detected 13 genomic aberrations in nine cases (15%): an *EGFR* mutation (*n* = 1, G719A), a *KRAS* mutation (*n* = 1, G12D), *PIK3CA* mutations (*n* = 3; E542K, E545K, E545Q), an *AKT1* mutation (*n* = 1, E17K), a *MET* amplification (*n* = 1), and *PIK3CA* amplifications (*n* = 6; Fig. 1A and B).

Table 4 shows the individual characteristics of the SCLC patients who harbored genomic aberrations. Eight of the nine patients with genomic aberrations were male, and all were smokers. Two patients were diagnosed with SCLC combined with adenocarcinoma; an *EGFR* mutation was detected in one patient and a *KRAS* mutation in another. The patient with the *EGFR* mutation provided both FFPE and surgically resected snap-frozen samples, but the *EGFR* mutation was detected only in the snap-frozen samples. Genomic aberrations were detected in nine of the 50 FFPE samples, one of eight surgically resected snap-frozen samples, and none of the seven pleural effusion samples.

3.3. Comparison of patient characteristics and genomic aberrations

Patient characteristics are classified by genomic aberration status in Table 4. No significant differences in age, sex, disease extent at diagnosis, or smoking status were found between patients with and without genomic aberrations according to univariate analysis. However, serum NSE and Pro-GRP levels at diagnosis were significantly higher in patients without genomic aberrations than in those with genomic aberrations ($p = 0.02$ and $p = 0.04$, respectively).

Table 3
Patients characteristics that genomic aberrations were detected.

	Age	Gender	B.I.	Disease extent	TNM stage	Samples	Pathology	Genomic aberrations
1	73	Male	2760	LS	IA	FFPE	Small cell carcinoma	PIK3CA amp (3.14)
2	69	Male	1880	LS	IIA	FFPE	Small cell carcinoma	PIK3CA amp (4.42)
3	82	Male	1500	LS	IIIA	FFPE	Small cell carcinoma	PIK3CA amp (2.65)
4	58	Male	1000	ES	IV	FFPE	Small cell carcinoma	PIK3CA (E545K)
5	69	Male	940	LS	IIIA	FFPE	Small cell carcinoma	AKT1 (E17K), PIK3CA amp (2.49)
6	66	Male	840	ES	IIIB	FFPE	Small cell carcinoma	PIK3CA (E542K), MET amp (4.13), PIK3CA amp (3.62)
7	73	Male	795	LS	IIB	FFPE, snap-frozen samples	Small cell carcinoma combined with adenocarcinoma	EGFR (G719A), PIK3CA (E545Q)
8	74	Male	590	ES	IV	FFPE	Small cell carcinoma combined with adenocarcinoma	KRAS (G12D)
9	80	Female	500	LS	IIA	FFPE	Small cell carcinoma	PIK3CA amp (2.78)

Abbreviations: LS, limited stage; ES, extended stage; FFPE, formalin-fixed paraffin-embedded.

Table 4
Patients characteristics classified by genomic aberration status.

	Genomic aberration		*P* value
	Detected	Not detected	
N (%)	9 (15%)	51 (85%)	
Age at diagnosis (years)			0.26
Median	73	69	
Range	58–82	43–82	
Gender, *n* (%)			0.63
Male	8 (89%)	42 (82%)	
Female	1 (11%)	9 (18%)	
Disease extent at diagnosis, *n* (%)			0.32
Limited stage	6 (67%)	25 (49%)	
Extended stage	3 (33%)	26 (51%)	
Smoking status			0.78
Never	0	2	
Light (B.I. < 600)	2	8	
Heavy (B.I. ≥ 600)	7	41	
Serum neuron-specific enolase (NSE) level at diagnosis			0.02
n	9	48	
Median	14	37.1	
Range	7.8–34	6.4–334	
Serum pro-gastrin releasing peptide (Pro-GRP) level at diagnosis			0.04
n	8	47	
Median	75.5	738	
Range	43.1–1500	26.4–65900	

Abbreviation: B.I., Brinkman index.

4. Discussion

As per our knowledge, this was the first molecular profiling report of Asian patients with SCLC, wherein we detected genomic aberrations in 15% patients. *PIK3CA* amplifications were detected in 10% of all samples assessed, while *PIK3CA* mutations were detected in 5%. *PIK3CA genomic aberrations were detected in eight of the* nine patients with genomic aberrations. Recently, two independent comprehensive genomic studies of SCLC were published [15,16]. Peifer et al. [14] analyzed 99 SCLC specimens using 6.0 SNP array analyses and exome, transcriptome, and genome sequencing. They detected *TP53* and *RB1* alterations in 88% and 66% cases, respectively, *MYC* family member and *FGFR1* amplifications in 16% and 6% cases, respectively, and *CREBBP* and *EP300* and *PTEN* mutations in 18% and 10% cases, respectively. They did not detect any *PIK3CA* aberrations. Rudin et al. [15] analyzed 80 SCLC samples, including SCLC cell lines, using multiple exome sequencing, single genome analysis, genome-wide copy-number analysis, and whole-transcriptome sequencing and detected *TP53* and *RB1* mutations in 77% and 31% samples, respectively, a *SOX2* amplification in 27%, and a recurrent *RLF-MYCL1* fusion in 9%. In their study, *PIK3CA* mutation was detected in 2 of 30 primary SCLC tumor samples by exome capture *followed by next generation sequencing (Rudin's report online* methods). Recently, Umemura et al. undertook a comprehensive genomic analysis of SCLC in Japanese patients [17]. They analyzed 51 surgically resected SCLC samples using whole exome sequencing and copy-number analysis. Genetic alterations in the *PI3K* pathway (*PIK3CA, PTEN, AKT2, AKT3, RICTOR, mTOR*) were detected in 17 of 47 samples (36%). *PIK3CA* mutations were detected in three of the 47 samples (6%), which is consistent with the findings from our study.

Okudela et al. reported that *PIK3CA* amplification was detected in 1 of 3 samples (33.3%) and *PIK3CA* gene mutation was detected in