British Journal of Cancer (2007) 97, 162–169

www.bjcancer.com

Clinical Studies

Randomised phase III trial of carboplatin plus etoposide *vs* split doses of cisplatin plus etoposide in elderly or poor-risk patients with extensive disease small-cell lung cancer: JCOG 9702

H Okamoto*[,1], K Watanabe[1], H Kunikane[1], A Yokoyama[2], S Kudoh[3], T Asakawa[4], T Shibata[4], H Kunitoh[5], T Tamura[5] and N Saijo[6], on Behalf of the Japan Clinical Oncology Group (JCOG)-Lung Cancer Study Group

[1]Department of Respiratory Medicine, Yokohama Municipal Citizen's Hospital, 56 Okazawa-cho, Hodogaya-ku, Yokohama, Kanagawa 240-8555, Japan; [2]Niigata Cancer Center Hospital, Niigata-city, Japan; [3]Osaka City University Medical School, Osaka-city, Japan; [4]National Cancer Center, Tokyo, Japan; [5]National Cancer Center Hospital, Tokyo, Japan; [6]National Cancer Center East Hospital, Kashiwa, Japan

We compared the efficacy and the safety of a carboplatin plus etoposide regimen (CE) *vs* split doses of cisplatin plus etoposide (SPE) in elderly or poor-risk patients with extensive disease small-cell lung cancer (ED-SCLC). Eligibility criteria included: untreated ED-SCLC; age $\geqslant 70$ and performance status 0–2, or age <70 and PS 3. The CE arm received carboplatin area under the curve of five intravenously (IV) on day 1 and etoposide 80 mg m^{-2} IV on days 1–3. The SPE arm received cisplatin 25 mg m^{-2} IV on days 1–3 and etoposide 80 mg m^{-2} IV on days 1–3. Both regimens were given with granulocyte colony-stimulating factor support in a 21–28 day cycle for four courses. A total of 220 patients were randomised. Median age was 74 years and 74% had a PS of 0 or 1. Major grade 3–4 toxicities were (%CE/%SPE): leucopenia 54/51, neutropenia 95/90, thrombocytopenia 56/16, infection 7/6. There was no significant difference (CE/SPE) in the response rate (73/73%) and overall survival (median 10.6/9.9 mo; $P=0.54$). Palliation scores were very similar between the arms. Although the SPE regimen is still considered to be the standard treatment in elderly or poor-risk patients with ED-SCLC, the CE regimen can be an alternative for this population considering the risk–benefit balance.
British Journal of Cancer (2007) **97,** 162–169. doi:10.1038/sj.bjc.6603810 www.bjcancer.com
Published online 19 June 2007

Keywords: small-cell lung cancer; carboplatin; cisplatin; etoposide; elderly; poor-risk

Approximately half of patients with small-cell lung cancer (SCLC) are older than 70 years, and the proportion of elderly SCLC patients is continuously increasing in Japan (Morita, 2002). However, since many investigators have arbitrarily excluded elderly patients from clinical trials, no standard chemotherapeutic regimen has been established for elderly patients with SCLC. The Japan Clinical Oncology Group (JCOG) has reported that carboplatin plus etoposide (CE) is an active and less toxic regimen in elderly patients with SCLC (Okamoto *et al*, 1999). However, other clinical trials have indicated that the combination chemotherapy of reduced (Souhami *et al*, 1997) or split doses of cisplatin plus etoposide (SPE) (Murray *et al*, 1998; Westeel *et al*, 1998) can be safely and effectively administered in elderly or poor-risk patients with SCLC. Therefore, we conducted a phase III trial comparing CE with SPE in elderly or poor-risk patients with SCLC. Although elderly is not the same as poor-risk, many clinical trials for the elderly have included both types of patients. Therefore, we decided to include both elderly and poor-risk patients with SCLC at the time of proposal for this phase III trial.

*Correspondence: Dr H Okamoto;
E-mail address: scyooka@alles.or.jp
Presented in part at the Forty-First Annual Meeting of the American Society of Clinical Oncology, Orlando, FL, May 13-17, 2005.
Received 18 October 2006; revised 25 April 2007; accepted 26 April 2007; published online 19 June 2007

PATIENTS AND METHODS

Patient selection

Eligibility criteria included patients with histologically or cytologically confirmed SCLC who were $\geqslant 70$ years of age and had an Eastern Cooperative Oncology Group performance status (PS) of 0–2, or who were <70 years in age and had a PS of 3. Additional criteria consisted of extensive disease (ED), chemotherapy-naive, evaluable or measurable disease, expected survival $\geqslant 2$ months, adequate organ functions (leucocyte count $\geqslant 4000\,\mathrm{mm}^{-3}$, platelet count $\geqslant 100\,000\,\mathrm{mm}^{-3}$, haemoglobin level $\geqslant 9.0\,\mathrm{g\,dl}^{-1}$, AST/ALT $\leqslant 2\times$ upper limit of normal range, total bilirubin $\leqslant 1.5\,\mathrm{mg\,dl}^{-1}$, creatinine $\leqslant 1.5\,\mathrm{mg\,dl}^{-1}$, 24-h creatinine clearance (Ccr) $\geqslant 50\,\mathrm{ml\,min}^{-1}$, and PaO2 $\geqslant 60$ mmHg), no symptomatic pericardial or pleural effusion requiring drainage, no active concomitant malignancy, no senile dementia, and written informed consent. Exclusion criteria included brain metastases requiring radiotherapy, superior vena cava (SVC) syndrome requiring radiotherapy, serious medical or psychiatric illness, or pregnancy or lactation. Staging procedures included chest X-ray, computed tomography (CT) scan of the chest, CT scan or magnetic resonance

imaging (MRI) of the brain, CT scan or ultrasound of the abdomen, isotope bone scanning, and bone marrow aspiration or biopsy.

Treatment protocol

Patients were randomised to either the CE arm or the SPE arm. The CE regimen consisted of carboplatin area under the curve (AUC) of five intravenously (IV) on day 1 and etoposide 80 mg m^{-2} IV on days 1, 2, and 3. The SPE regimen consisted of cisplatin 25 mg m^{-2} IV on days 1, 2, and 3 and etoposide 80 mg m^{-2} IV on days 1, 2, and 3. Cycles were repeated every 3–4 weeks for up to four courses. In our previous phase II study using the CE regimen for elderly patients with SCLC, carboplatin AUC of 5 on day 1 and etoposide 100 mg m^{-2} on days 1, 2, and 3 were administered every 4 weeks (Okamoto *et al*, 1999). However, because grade 3 or 4 neutropenia occurred in 91% of the patients, in the current phase III trial we decided to reduce the etoposide dosage to 80 mg m^{-2} on days 1, 2, and 3, and repeat the cycle every 3–4 weeks instead of every 4 weeks. Twenty-four-hour Ccr was substituted for glomerular filtration rate (GFR) in Calvert's formula. Antiemetic prophylaxis with 5-HT3 antagonists plus dexamethasone was used at the treating physician's discretion. According to the Japanese approved guideline, prophylactic use of recombinant human granulocyte colony-stimulating factor (G-CSF) was recommended for daily administration after day 4 until the leucocyte (neutrophil) count exceeded 10 000 (5000) mm^{-3}. If the leucocyte (neutrophil) count decreased to less than 3000 (1500) mm^{-3}, then G-CSF was restarted. However, the actual use of G-CSF was left at the discretion of the treating physician. Subsequent courses of chemotherapy were initiated when leucocyte count $\geqslant$3000 mm^{-3}; platelet count $\geqslant$75 000 mm^{-3}; Cr$\leqslant$1.5 mg dl^{-1}; AST/ALT$\leqslant$2.5 $\times$ upper limit of normal range; and either PS$\leqslant$2 and age$\geqslant$70 years, or PS$\leqslant$3 and age$<$70 years were satisfied both after day 21 and two or more days after the discontinuation of G-CSF. If the above criteria were not satisfied by the first day of the next course, treatment was withheld until full recovery. If more than 6 weeks passed from day 1 of the last course, the patient was removed from protocol treatment. Dose modifications were made based only on grade 4 haematologic toxicities. If grade 4 leucopenia or neutropenia lasting 4 days or more was present, or grade 4 thrombocytopenia occurred, the doses for the next course were carboplatin AUC of 4 on day 1, cisplatin 20 mg m^{-2} for 3 days, and etoposide 60 mg m^{-2} for 3 days. If the same haematologic toxicity was observed after dose reduction, the patient was removed from protocol treatment. If grade 3 or 4 non-haematologic toxicities, except for nausea/vomiting and hyponatraemia, occurred, the patient was removed from protocol treatment even if the toxicities improved thereafter.

Responders after four courses were not allowed to receive further chemotherapy until progressive disease (PD) developed. Although post-protocol treatment was left at the discretion of the physician, crossover treatment was prohibited.

Evaluation

Tumour responses were evaluated according to World Health Organization criteria (World Health Organization, 1979). Toxicities were evaluated according to JCOG Toxicity Criteria (Tobinai *et al*, 1993), which are similar to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC ver 1) for the grading of toxicities.

Palliation score

Study-specific eight-item palliation scores were completed by patients before treatment and 3 weeks after the third course of chemotherapy. The attending physicians were not allowed to complete the scores. The items consisted of cough, pain, anorexia, shortness of breath, well-being, nausea, diarrhoea or constipation, and sleep. The items were scored as not at all present (0), a little (1), moderate (2), and very much (3). The sum of the total score for all eight items was compared between the baseline and post-treatment assessments. If the post-treatment score was below the baseline score, the palliation score for that patient was judged as having shown improvement.

Study design and statistics

This trial was designed as a multicentre, prospective, randomised phase III trial. The study protocol was approved by the Clinical Trial Review Committee of JCOG and the institutional review board of each participating institution before the initiation of the study. The primary endpoint was overall survival (OS). In this study, the experimental arm was the CE arm and the control was the SPE arm. The MST of our previous phase II trial for elderly patients with extensive disease small-cell lung cancer (ED-SCLC) using the CE regimen was 10.1 months. The MST of the SPE regimen for a similar population was not available at the time of the study proposal. Although Westeel and co-workers in 1998 and Murray and co-workers in 1998 reported an excellent MST of SPE plus concurrent chest radiotherapy for elderly or frail patients with limited disease (LD)-SCLC, an MST of the SPE regimen for elderly or frail patients with ED-SCLC was not available at that time. The only data available on the CAV/PE regimen for elderly or poor-risk patients with SCLC using reduced cisplatin (60 mg m^{-2} IV on day 1) were reported by Souhami and co-workers in 1997 and the MST of that study was 5.9 months. Therefore, for statistical calculations in the current phase III trial, we used the MST value of the Souhami trial for the control arm instead of the MST of the SPE regimen. In addition, an individualised AUC-based dosing strategy of carboplatin was expected to have greater efficacy and less toxicity compared with the SPE regimen at that time. This trial was designed as a superiority trial and the planned sample size was 110 patients in each arm for 80% power to detect a 0.67 hazard ratio for CE to SPE in OS at an alpha of 0.025 (one sided) (Schoenfeld and Richter, 1982). Patients were randomised to receive either CE or SPE with a minimisation method for balancing centre, PS (0–1 *vs* 2–3) and age ($\geqslant$70 years *vs* $<$70 years).

Survival distributions were compared by unstratified log-rank test. Proportion of improvement in palliation score was evaluated by Fisher's exact test. The change in each symptom score by treatment arm was evaluated by the Wilcoxon rank-sum test. The relationship between the interval of each chemotherapy course and the two regimens was evaluated by the Wilcoxon rank-sum test. Multivariate analysis was performed using Cox's proportional hazards model to evaluate the importance of seven clinically selected variables (treatment arm, PS, age, sex, lactate dehydrogenase level, alkaline phosphatase level, and leucocyte count) as prognostic factors. All *P*-values in this report are two sided, excluding *P*-values for OS and progression-free survival (PFS).

The interim analysis was performed after half of the planned number of patients had been enrolled in March 2002, with adjustment for multiplicity by the alpha-spending function (DeMets and Lan, 1994) with an O'Brien-Fleming type boundary. Because the interim analysis did not meet the prespecified stopping criteria, the study was continued and the planned accrual of 220 patients was randomised in this trial.

RESULTS

Patient characteristics

Between August 1998 and February 2004, a total of 220 patients were registered from 24 institutions. Baseline characteristics were well balanced between the arms. Median age was 74 years, 92% were 70 years or older, 88% were male, and 74% had a PS of 0 or 1 (Table 1). One patient in the CE arm was found to have LD after the completion of protocol chemotherapy due to protocol violation, and this patient was considered ineligible (Figure 1).

Delivery of treatment

Reasons for termination of treatment are listed in Figure 1, and there were no major differences between the arms. Of the patients, 63% in the CE arm and 67% in the SPE arm completed four courses, and 11% in the CE arm and 8% in the SPE arm did not complete treatment because of toxicity or complications. Treatment-related death (TRD) occurred in four patients; three patients in the CE arm and one in the SPE arm. All TRDs of patients who were ⩾70 years old with a good pretreatment PS (all PS 1) were associated with neutropenic infection, which occurred after the first course of chemotherapy. Although the median interval of chemotherapy was slightly more prolonged in the CE arm than in the SPE arm, total delivered courses were similar between the arms (Table 2). One patient in the SPE arm never received chemotherapy due to the occurrence of delirium after registration. Dose reduction was more frequently observed in the CE arm than in the SPE arm: 29% *vs* 10%, $P<0.01$. Course delay, G-CSF delivery and total courses with G-CSF delivery were similar between the arms.

Toxicity and palliation score

Toxicities are listed in Table 3. Grade 3 or 4 leucopenia and neutropenia occurred in 54 and 95% of the CE arm *vs* 51 and 90% of the SPE arm, respectively. Grade 3 or 4 thrombocytopenia occurred more frequently in the CE arm than in the SPE arm: 56 *vs* 16%, $P<0.01$. Gastrointestinal toxicities including nausea or vomiting and diarrhoea were mild in both arms. There were few grade 3 or 4 toxicities and no remarkable differences between the arms. Other non-haematologic toxicities were similarly distributed between the arms. Grade 3–4 hyponatraemia, mainly caused by syndrome of inappropriate antidiuretic hormone (SIADH) secretion, occurred in 14–16% of the patients. More importantly, thrombocytopenia occurred more frequently in the CE arm, but none of the patients in either arm showed grade 3 or 4 bleeding. Only one patient in the CE arm showed grade 2 bleeding. Because no grading of febrile neutropenia was listed in JCOG toxicity criteria, the rate of the toxicity was not investigated in this study.

Baseline and post-treatment palliation scores were evaluated in 220/220 (100%) and 208/220 (95%) patients, respectively. We handled missing values by imputing the worst score. Improvement was achieved in 69 (63%) patients in the CE arm *vs* 61 (56%) patients in the SPE arm, although the difference was not statistically significant ($P=0.34$). Similarly, there were no statistical differences in the change of each symptom score between the arms (Table 4).

Objective tumour response, PFS and OS

The objective response rate of 73% was quite similar between the arms. Five CRs and 75 PRs were observed in each arm (Table 5). Progression-free survival curves and OS curves are shown in Figure 2A and B. Ninety-seven percent of the patients had progressed or died at the time of final analysis. Progression-free survival was quite similar between the arms ($P=0.20$, one sided).

Table 1 Patient characteristics

	CE ($n=110$)	SPE ($n=110$)	*P*-value
Age (years)			
Median (range)	74 (56–86)	73.5 (55–85)	0.34
⩾70 years old (%)	102 (93)	100 (91)	0.81
Sex (male/female)	95/15	98/12	0.68
ECOG PS, 0–1/2/3	81/21/8	81/19/10	0.80
⩾5% weight loss	26	38	0.18
LN metastasis			
Contralateral mediastinum	71	59	0.13
Supraclavicular	89	79	0.15
Distant metastasis			
Liver	30	30	1.0
Lung	31	30	1.0
Brain	18	18	1.0
Bone	25	17	0.23
Adrenal	13	7	0.24
Bone marrow	12	12	1.0

CE, carboplatin plus etoposide; ECOG, Eastern Cooperative Oncology Group; LN, lymph node; PS, performance status; SPE, split doses of cisplatin plus etoposide.

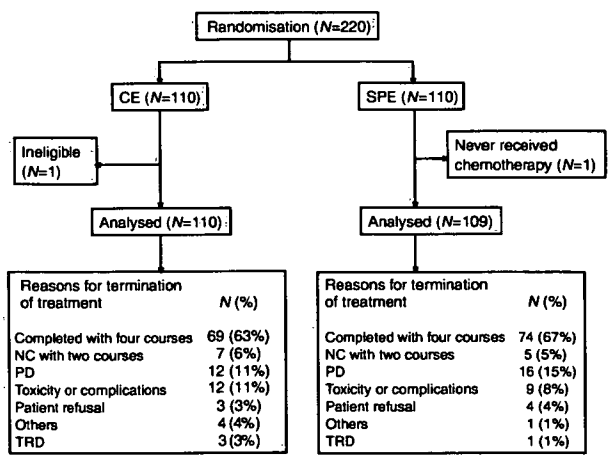

Figure 1 Flow diagram of randomised phase III trial of CE *vs* SPE in elderly or poor-risk patients with extensive disease SCLC.

Table 2 Compliance and drug delivery

	CE ($n=110$)	SPE ($n=109$[a])	*P*-value
Median interval of each chemotherapy (days) (range)			
1–2	27 (14–35)	23 (20–37)	0.02[b]
2–3	25 (21–56)	22 (20–35)	0.07[b]
3–4	27 (21–36)	24 (21–38)	0.05[b]
Total delivered courses/projected courses	353/440 (80%)	360/436 (83%)	
Dose reduction	32 (29%)	11 (10%)	<0.01[c]
Course delay	45 (41%)	40 (37%)	0.58[c]
G-CSF delivery	81 (74%)	84 (77%)	0.64[c]
No. of courses with G-CSF delivery/number of total courses	183/354 (52%)	203/362 (56%)	

CE, carboplatin plus etoposide; G-CSF, granulocyte colony-stimulating factor; SPE, split doses of cisplatin plus etoposide. [a]One patient never received chemotherapy due to delirium after registration. [b]Wilcoxon rank-sum test. [c]Fisher's exact test.

Table 3 Toxicities (JCOG Toxicity Criteria, Worst Grade of Any Course)

	CE					SPE					
	Grade					Grade					
Toxicity	**1**	**2**	**3**	**4**	**3+4 (%)**	**1**	**2**	**3**	**4**	**3+4 (%)**	***P*-value**
Haematologic											
Leucopenia	5	45	46	13	(54)	8	43	49	7	(51)	0.79
Neutropenia	0	5	46	58	(95)	4	7	41	57	(90)	0.22
Anaemia	9	58	32	—	(29)	20	45	27	—	(25)	0.54
Thrombocytopenia	20	18	29	32	(56)	16	15	12	5	(16)	<.01
Non-haematologic											
Nausea/vomiting	40	24	2	—	(2)	46	28	3	—	(3)	0.68
Diarrhoea	8	9	1	0	(1)	11	3	1	0	(1)	1.0
Bilirubin	—	31	0	0	(0)	—	16	1	0	(1)	0.50
AST	47	9	3	0	(3)	30	8	6	0	(6)	0.33
ALT	40	9	2	0	(2)	38	8	4	0	(4)	0.45
Creatinine	10	2	0	0	(0)	27	3	1	0	(1)	0.50
Hyponatraemia	38	11	7	11	(16)	46	20	6	9	(14)	0.58
PaO2	39	21	7	1	(10)	44	23	2	1	(4)	0.22
Fever	15	15	0	0	(0)	21	16	0	0	(0)	—
Infection	12	15	5	3	(7)	16	7	5	1	(6)	0.78
Bleeding	8	1	0	0	(0)	4	0	0	0	(0)	—
Neurologic-sensory	2	1	0	—	(0)	3	2	0	—	(0)	—
Alopaecia	67	22	—	—		66	15	—	—		

CE, carboplatin plus etoposide; JCOG, Japan Clinical Oncology Group; PaO_2, partial pressure of oxygen; SPE, split doses of cisplatin plus etoposide.

Table 4 Palliation score

	CE		SPE		
	Change from baseline		Change from baseline		
Symptom	**Mean (s.d.)**	**Median (range)**	**Mean (s.d.)**	**Median (range)**	***P*[a]**
Cough	−0.38 (1.16)	0 (−3 to 3)	−0.54 (1.06)	0 (−3 to 3)	0.51
Pain	−0.19 (1.00)	0 (−3 to 3)	−0.19 (0.96)	0 (−3 to 3)	0.96
Anorexia	−0.07 (1.16)	0 (−3 to 3)	0.08 (1.22)	0 (−3 to 3)	0.37
Shortness of breath	−0.05 (1.02)	0 (−2 to 3)	−0.31 (0.95)	0 (−3 to 3)	0.12
Well-being	−0.15 (1.13)	0 (−3 to 3)	−0.02 (1.14)	0 (−3 to 3)	0.48
Nausea	0.16 (0.84)	0 (−2 to 3)	0.26 (0.80)	0 (−1 to 3)	0.21
Diarrhoea or constipation	0.05 (1.07)	0 (−3 to 3)	0.04 (0.99)	0 (−3 to 3)	0.69
Sleep	−0.15 (1.08)	0 (−3 to 3)	−0.04 (0.89)	0 (−3 to 2)	0.10
Total	−0.80 (6.04)	−2 (−12 to 22)	−0.71 (5.35)	−1 (−15 to 21)	0.32

CE, carboplatin plus etoposide; s.d., standard deviation; SPE, split doses of cisplatin plus etoposide. [a]Wilcoxon rank-sum test.

The MST was 5.2 months in the CE arm *vs* 4.7 months in the SPE arm. OS was very similar between the arms ($P = 0.54$, one sided). The MST and 1-year survival rate was 10.6 months and 41% in the CE arm *vs* 9.9 months and 35% in the SPE arm.

Second-line chemotherapy

According to an *ad-hoc* survey (not pre-specified in the protocol), 130 (59%) patients (68 (62%) patients in the CE arm and 62 (56%) in the SPE arm) received second-line chemotherapy after relapse and the regimens were almost equally distributed between the arms. The same regimen as the initial chemotherapy, platinum-based combinations, and irinotecan regimens with or without other agents were administered in 17 (15%), 48 (44%), and 40 (36%) patients in the CE arm *vs* 10 (9%), 44 (40%), and 40 (36%) in

Table 5 Therapeutic response (WHO)

	CE	SPE	Total
CR	5	5	10
PR	75	75	150
NC	17	11	28
PD	11	16	27
NE	2	3	5
Total	110	110	220
Response rate	73%	73%	
95% CI	63–81%	63–81%	

CE, carboplatin plus etoposide; CI, confidence interval; CR, complete response; NC, no change; NE, not evaluable; PD, progressive disease; PR, partial response; SPE, split doses of cisplatin plus etoposide; WHO, World Health Organization.

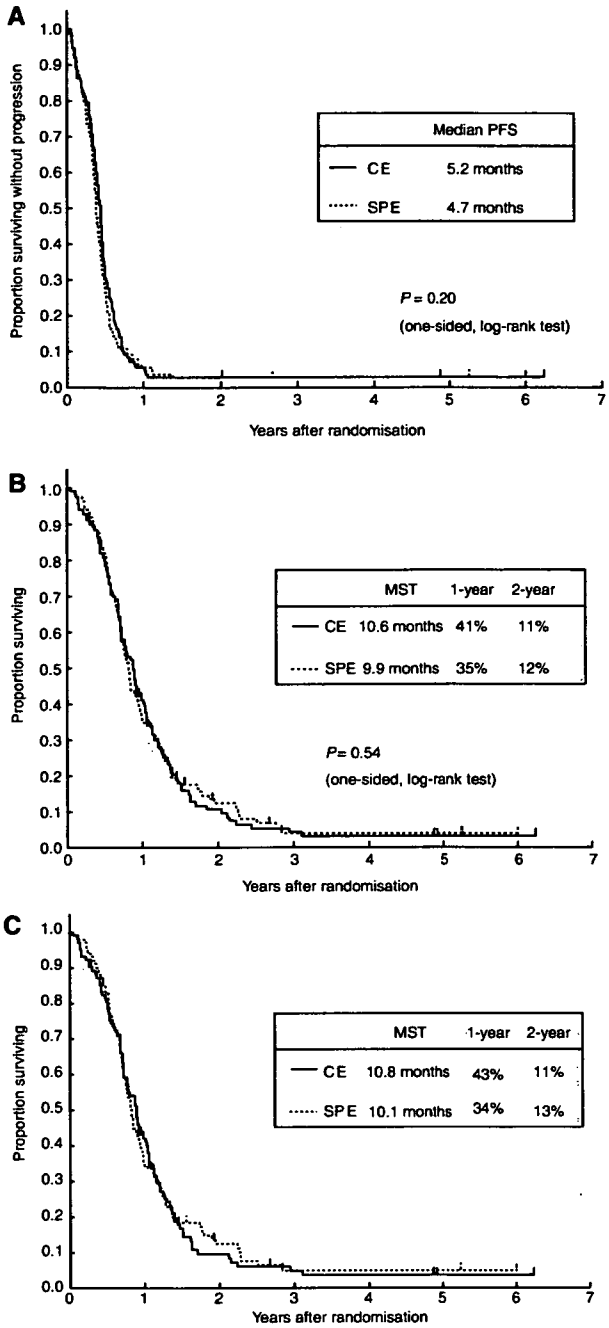

Figure 2 (**A**) PFS curves ($n = 220$). (**B**) OS curves ($n = 220$). (**C**) Survival curves of the patients ⩾70 years of age with a PS of 0–2 ($n = 202$).

the SPE arm. Other chemotherapy regimens included topotecan monotherapy, amrubicin monotherapy, or other regimens.

Subset analysis and multivariate analysis

Subset analysis was performed according to PS and age (Table 6). There were no differences in OS between the arms in any subset; thus, an interaction between treatment and PS is unlikely. The survival curves of the patients ⩾70 years of age with a PS of 0–2 are shown in Figure 2C, and the survival curves were very similar with that of original overall populations. Even in the multivariate analysis with seven selected baseline variables, there was no difference in OS between the arms. High lactate dehydrogenase level was most strongly associated with poor prognosis (Table 7).

Table 6 Subset analysis – overall survival

Subgroup	Number of patients (%)	MST (months) CE	MST (months) SPE
PS 0–1	162 (74)	10.9	10.1
PS 2–3	58 (26)	8.3	8.1
<70 years and PS 3	18 (8)	7.1	6.9
⩾70 years and PS 0–2	202 (92)	10.8	10.0

CE, carboplatin plus etoposide; MST, median survival time; PS, performance status; SPE, split doses of cisplatin plus etoposide.

Table 7 Multivariate analysis with baseline prognostic factors

Variables	*P*-value	Hazard ratio	95% CI
Treatment arm (CE vs. SPE)	0.99	0.99	0.75–1.33
Alkaline phosphatase level (normal *vs* abnormal)	0.97	0.99	0.68–1.46
Lactate dehydrogenase level (⩾ × 1.5 *vs* < × 1.5)	<0.001	1.69	1.23–2.26
Leucocyte count (⩾ 10000/mm^3 *vs* < 10000/mm^3)	0.06	1.82	0.99–3.36
Age (⩾75 years *vs* <75 years)	0.77	1.05	0.78–1.41
PS (2–3 *vs* 0–1)	0.41	1.15	0.82–1.61
Sex (female *vs* male)	0.13	0.70	0.45–1.11

CE = carboplatin plus etoposide; SPE = split doses of cisplatin plus etoposide; PS = performance status; CI = confidence interval.

DISCUSSION

Until recently, there was no standard chemotherapeutic regimen for elderly SCLC patients. Two phase III (Medical Research Council Lung Cancer Working Party, 1996; Souhami *et al*, 1997) and two randomised phase II trials (Pfeiffer *et al*, 1997; Ardizzoni *et al*, 2005) have shown that suboptimal chemotherapies, such as oral etoposide monotherapy or attenuated doses of combination chemotherapy, may lead to reduced survival in elderly or poor-risk SCLC patients when compared with standard doses of combination chemotherapies. The CE regimen, which has acceptable toxicities and reproducible efficacy, has been used in elderly or poor-risk patients with SCLC worldwide, although there have been substantial differences in toxicities and efficacy between the reported phase II trials. Four trials demonstrated both favourable toxicities and efficacy (Carney, 1995; Evans *et al*, 1995; Matsui *et al*, 1998; Okamoto *et al*, 1999) and three showed somewhat disappointing results because of suboptimal doses of oral etoposide (Larive *et al*, 2002), greater inclusion of patients with poor prognostic factors (Samantas *et al*, 1999), and deterioration of comorbidities as a result of chemotherapy (Quoix *et al*, 2001). No phase III trial evaluating the role of the CE regimen in this population has been reported until now.

This is the first phase III trial comparing carboplatin-based CE and cisplatin-based SPE regimens in elderly or poor-risk patients with ED-SCLC. In addition, this is also the largest randomised trial specifically designed for elderly or poor-risk SCLC patients. Although there was no significant difference in the palliation scores, response rate, and OS between the arms, the efficacy of

both regimens was promising, as this study included only elderly or poor-risk patients with SCLC. Most toxicities were tolerable and the treatment compliance was also favourable in both arms. Approximately two-thirds of the patients received all four cycles of treatment. The CE arm in the current trial had more pronounced thrombocytopenia, which was considered manageable because none of the patients in the CE arm showed grade 3 or 4 bleeding, and the CE arm had a slightly prolonged course interval and a slightly greater incidence of dose reduction. However, in our opinion, these toxicities are less meaningful in clinical practice. More importantly, the CE regimen does not require hydration and can be given in an outpatient setting. Based on the results of this study, many JCOG members prefer the CE regimen to the SPE regimen and consider it to be more suitable for the control arm of future phase III trials.

The MST of each regimen (10.6 months for CE *vs* 9.9 months for SPE) was promising considering that this study included only elderly or frail patients with ED-SCLC. However, some retrospective studies have shown that fit elderly patients who have adequate organ functions, a good PS, and no comorbidity are able to tolerate intensive chemotherapy well and show a similar therapeutic response and survival rate as younger patients (Siu *et al*, 1996; Yuen *et al*, 2000). In fact, in this trial the MST of fit elderly patients $\geqslant$70 years of age with a PS of 0–1 was 10.9 months for the CE arm and 10.1 months for the SPE arm. In contrast, the MST of patients with a PS of 3 was only approximately 7 months. Furthermore, the group of fit elderly patients comprised 74% of the patients in this study. Therefore, the favourable survival rates in our trial may be attributable to patient selection. In other words, one limitation of this study is that the results of this trial cannot be extrapolated to frail elderly with a poor PS and/or comorbid illness because of the likelihood of greater inclusion of fit elderly patients in this trial.

Although the total dose in both the CE and SPE arms was slightly lower than the standard regimen, 92% of the patients showed grade 3 or 4 neutropenia, and dose reduction and course delay occurred frequently. However, the MST of both regimens was comparable with that of non-elderly or non-selected patients with ED-SCLC in historical reports (Noda *et al*, 2002; Niell *et al*, 2005). These findings suggest that both regimens are not suboptimal, but are near-full and effective doses for elderly or poor-risk patients with ED-SCLC. The CE arm in the current trial had a slightly prolonged course interval and a slightly greater incidence of dose reduction when compared to the SPE regimen. However, 95% of the patients showed grade 3 or 4 neutropenia and 56% showed grade 3 or 4 thrombocytopenia. Therefore, we believe that the dose escalation of the CE regimen may be difficult in this trial.

It remains unclear whether the elderly are able to tolerate a single modest dose of cisplatin (60–80 mg m^{-2} IV) on day 1. We feel that a fit elderly person who passes strict eligibility criteria can receive a modest dose of cisplatin IV on day 1. However, the more common situation is of elderly patients who have comorbidity and a poor PS, and cannot tolerate a standard single dose of cisplatin. Westeel *et al* (1998) and Murray *et al* (1998) reported that split doses of cisplatin were safely and effectively administered in elderly or frail patients with LD-SCLC. The SPE regimen appeared to be an appropriate treatment for elderly patients with SCLC who cannot tolerate a standard single dose of cisplatin. However, it remains unclear whether fit elderly patients in our trial can tolerate a standard single dose of cisplatin, and if so, it also remains unclear whether fit elderly patients who receive a standard single dose of cisplatin are able to achieve a more improved survival than those who receive SPE. Unfortunately, no randomised study comparing a single standard dose of cisplatin with SPE has been reported in fit elderly patients with SCLC.

There are some problems with the design in this study. The hypothesis was that carboplatin would improve survival, and the design of the trial was a superiority design with survival as the primary end point. However, this hypothesis was based on two possible misconceptions. First, carboplatin could be better dosed and might be more efficacious than cisplatin in SCLC. Unfortunately, this hypothesis could not be sustained on the basis of the available literatures. A number of clinical trials have indicated that carboplatin-based combination chemotherapy has a similar or slightly reduced efficacy compared with cisplatin-based combination chemotherapy against various tumours (Go and Adjei, 1999; Hotta *et al*, 2004). Therefore, our trial should have been designed as a non-inferiority trial. However, if this trial were planned as a non-inferiority trial, a total sample size would be about 500 to 1000 patients, with equal expected survival and a non-inferiority margin for hazard ratio ranging from 1.2 to 1.3. Second, the cisplatin dose in the control arm was an attenuated dose. Souhami *et al* (1997) used reduced dose of cisplatin (60 mg m^{-2} IV on day 1) and Murray *et al* (1998) used a single course of a split cisplatin dose in their studies. These regimens were completely different from the control arm in the present study. A standard dose of cisplatin given in 3 days is the best way of giving standard cisplatin (30 mg m^{-2} IV on days 1–3) with etoposide (130 mg m^{-2} IV on days 1–3), according the North Central Cancer Treatment Group (Maksmiuk *et al*, 1994). Had standard SPE been used for the control arm, better survival might have been achieved with increased toxicities. Another problem with the design was the inclusion of patients with a PS of 3, even if they were less than 70 years old. This made the target population heterogeneous. The number of such patients actually recruited was quite small, so emphasising the inappropriateness of their inclusion. A further limitation of this study may be a long accrual period of five-and-a-half years. Because our oncologists might have been afraid of the risk of TRD or increased toxicities in frail elderly with a poor PS and/or comorbid illness, more fit elderly patients were selectively registered and consequently the accrual rate was very slow.

In our trial, although both regimens were well-tolerated and efficacy was promising, over 90% of the patients in both arms showed grade 3 or 4 neutropenia, which may be justified and acceptable for a clinical trial involving elderly or poor risk patients with ED-SCLC, because only 6% of the patients showed grade 3 or 4 infection and TRD occurred in only four (1.8%) patients. Because all TRD occurred after the first course of chemotherapy, careful monitoring and management is necessary, particularly in the first course, if CE or SPE are administered to elderly or frail patients. Several retrospective analyses (Findlay *et al*, 1991; Radford *et al*, 1992) and a prospective study (Timmer-Bonte *et al*, 2005) have shown that standard-dose chemotherapy without G-CSF support causes more risk of early death and sepsis in the older population. Moreover, the American Society of Clinical Oncology (ASCO) guideline recommends the use of prophylactic G-CSF in patients at higher risk for chemotherapy-induced infection, such as those having a poor PS, older age, or comorbid illness (Smith *et al*, 2006). In this trial, the prophylactic use of G-CSF was recommended, but the actual use was left to the discretion of the treating physician because the use of G-CSF leads to increased drug cost. Although G-CSF was administered in only 54% of the total courses, we believe that the prophylactic use of G-CSF with CE regimen should be recommended in a new trial or clinical practice.

In conclusion, although the SPE regimen is still considered to be the standard treatment for elderly or poor-risk patients with ED-SCLC, the CE regimen can be an alternative for this population considering the risk-benefit balance. Based on the results of our trial, a phase III trial of the CE regimen *vs* amrubicin monotherapy, supported by a pharmaceutical company, is now ongoing in elderly patients with ED-SCLC in Japan, and a comparative trial of the CE regimen *vs* carboplatin plus irinotecan regimen (Okamoto *et al*, 2006) is being discussed for a future trial in our group.

ACKNOWLEDGEMENTS

We are indebted to Ms Mieko Imai and Ms Tomoko Yamabe for data management, and to Dr Haruhiko Fukuda for direction of the JCOG data center and oversight of management of the study. This work was supported in part by Grants-in-Aid for Cancer Research and for the Second-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour, and Welfare (Tokyo).

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Appendix

This study was coordinated by the Japan Clinical Oncology Group (N Saijo, Chairperson) and was performed with the cooperation of the following institutions and investigators: Tochigi Cancer Center Hospital, Tochigi (K Mori, M Noda, T Kondo, and Y Kamiyama); National Nishi-Gunma Hospital, Gunma (S Tsuchiya, Y Koike, K Satoh, A Tohi, and K Kaira); Gunma Cancer Center Hospital, Gunma (K Minato); Saitama Cancer Center Hospital, Saitama (H Sakai, K Kobayashi, and R Kuroki); National Cancer Center, Central Hospital, Tokyo (T Tamura, Y Ohe, H Kunitoh, I Sekine, H Nokihara, and H Murakami); National Cancer Center Hospital East, Chiba (R Kakinuma, K Kubota, H Ohmatsu, K Gotoh, and S Niho); National International Medical Center, Tokyo (Y Takeda, S Izumi, A Kawana, M Kamimura, and M Iikura); Toranomon Hospital, Tokyo (K Kishi, and M Kawabata); Kanagawa Cancer Center Hospital, Kanagawa (K Yamada, I Nomura, F Oshita, and M Ikehara), Yokohama Municipal Citizen's Hospital, Kanagawa (K Watanabe, H Kunikane, H Okamoto, A Nagatomo, and H Aono); Niigata Cancer Center Hospital, Niigata (A Yokoyama, H Tsukada, M Makino, T Shinbo, S Kinebuchi, J Tanaka, M Tango, and T Ohara); Gifu City Hospital, Gifu (T Sawa, M Miwa, T Ishiguro, M Sawada, and T Yoshida); Aichi Cancer Center Central Hospital, Aichi (K Yoshida, and T Hida); Aichi Cancer Center Aichi Hospital, Aichi (H Saitoh, and M Okuno); Osaka City University Medical School, Osaka (S Kudoh, S Kyoh, H Kamoi, N Yoshimura, T Kodama, K Ohtani, S Shiraishi, S Nomura, S Enomoto, H Matsuura, and R Wake); Kinki University Medical School, Osaka (T Nogami, N Yamamoto, S Sakai, K Kodama, K Akiyama, J Tsurutani, K Tamura); Osaka Prefectural Adult Disease Center, Osaka (S Nakamura, F Imamura, M Yoshimura, S Yamamoto, K Ueno, H Ohmiya, H Matsuoka, and H Uda); Osaka Prefectural Respiratory and Allergy Medical Center, Osaka (M Furukawa, T Yamadori, T Takimoto, and T Hirashima); National Kinki Central Thoracic Disease Center, Osaka (S Minami, N Naka, T Kawaguchi, and H Ishikawa); National Toneyama Hospital, Osaka (Y Okano); Osaka City General Medical Center, Osaka (N Takifuji, and M Miyazaki); Kobe City Central Hospital, Kobe (T Nishimura, Y Okazaki, D Kinose, H Fujii, S Takakura, and M Hayashi); Sasebo City General Hospital, Nagasaki (J Araki); Kumamoto Regional Medical Center, Kumamoto (H Senba, T Seto, and S Fujii).

Lung Cancer (2007) 57, 328–333

available at www.sciencedirect.com

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LUNG CANCER

Detection of unsuspected distant metastases and/or regional nodes by FDG-PET in LD-SCLC scan in apparent limited-disease small-cell lung cancer

Seiji Niho[a,*], Hirofumi Fujii[b], Koji Murakami[b,c], Seisuke Nagase[a], Kiyotaka Yoh[a], Koichi Goto[a], Hironobu Ohmatsu[a], Kaoru Kubota[a], Ryuzo Sekiguchi[d], Shigeru Nawano[d], Nagahiro Saijo[a], Yutaka Nishiwaki[a]

[a] *Division of Thoracic Oncology, National Cancer Center Hospital East, Kashiwanoha 6-5-1, Kashiwa, Chiba 277-8577, Japan*
[b] *Functional Imaging Division, National Cancer Center Research Center for Innovative Oncology, Chiba, Japan*
[c] *PET Center, Dokkyo Medical University, Tochigi, Japan*
[d] *Department of Radiology, National Cancer Center Hospital East, Chiba, Japan*

Received 12 January 2007; received in revised form 30 March 2007; accepted 6 April 2007

KEYWORDS
Small-cell lung cancer;
Limited-disease;
FDG-PET;
CT;
Staging;
Occult distant metastasis

Summary We retrospectively investigated the clinical usefulness of fluorodeoxyglucose positron emission tomography (FDG-PET) for evaluation of patients with limited-disease small-cell lung cancer (LD-SCLC) diagnosed by conventional staging procedures. Sixty-three patients received whole body FDG-PET scans after routine initial staging procedures. The findings of FDG-PET scans suggesting extensive-stage disease were confirmed by other imaging tests or by the patient's clinical course. FDG-PET scan findings indicated distant metastases in 6 of 63 patients. Metastatic disease was confirmed in five of these six patients (8%, 95% confidence interval: 3–18%). FDG-PET scan also detected regional lymph node metastases even in nine patients (14%) in whom computed tomography images had been negative, including contralateral lymph node metastasis in three patients. FDG-PET scan detected additional lesions in patients diagnosed as having LD-SCLC by conventional staging procedures. The therapeutic strategies were changed in 8% of patients based on the results of FDG-PET. FDG-PET scan is recommended as an initial staging tool for patients with this disease.

1. Introduction

Small-cell lung cancer (SCLC) accounts for 15–20% of all lung cancers. SCLC shows more aggressive biological behaviour than non-small cell lung cancer (NSCLC). A clinical two-stage system proposed by the Veterans Administration Lung

* Corresponding author. Tel.: +81 4 7133 1111; fax: +81 4 7131 4724.
E-mail address: siniho@east.ncc.go.jp (S. Niho).

0169-5002/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2007.04.001

Study Group (VALSG) distinguishes limited-disease (LD) and extensive-disease (ED) in SCLC [1]. LD is defined as limited to one hemithorax, including mediastinal, contralateral hilar and ipsilateral supraclavicular lymph nodes, while ED represents tumour spread beyond these regions. Approximately two-thirds of patients with SCLC are diagnosed as having ED at the initial staging. The current standard care for LD-SCLC is a combination of chemotherapy and chest irradiation. With current treatment, patients with LD have a median survival of 23–27 months [2,3], compared to 10–12 months for those with ED [4]. Therefore, accurate pretreatment staging is important for patients with SCLC in order to determine the appropriate therapy.

Conventional staging procedures for lung cancer consist of computed tomography (CT) of the chest and upper abdomen, bone scan, and CT scan or magnetic resonance imaging (MRI) of the brain. Recently, fluorodeoxyglucose positron emission tomography (FDG-PET) was introduced as a staging tool for NSCLC. According to the guidelines of the American Society of Clinical Oncology, PET scan is recommended for survey occult locoregional lesions and distant metastases in patients with NSCLC [5]. Two separate prospective studies demonstrated that FDG-PET detected unsuspected distant metastases in 24% of patients with apparent stage III NSCLC [6,7]. Another study showed that FDG-PET changed or influenced management decisions in 67% of patients with NSCLC. PET plays an important role in staging of NSCLC [8]. However, previous PET studies of SCLC involved only a relatively small number of patients [9–17]. In a prospective study, FDG-PET was performed for 24 patients diagnosed as having LD-SCLC by conventional staging procedures [9]. Based on FDG-PET findings, two of these 24 patients were upstaged to ED. Bone metastases were found in one patient, and contralateral supraclavicular lymph node metastasis in another. Larger studies are required to confirm the role of FDG-PET in the staging of LD-SCLC. In this study, we retrospectively investigated the usefulness of FDG-PET to detect distant metastases or unsuspected regional nodal metastases in patients with LD-SCLC diagnosed by conventional staging procedures.

2. Patients and methods

2.1. Patients

Seventy patients were newly diagnosed as having LD-SCLC by conventional staging procedures at the National Cancer Center Hospital East between July 2003 and December 2006. Conventional staging procedures included history and physical examination, chest radiography, CT scan of the chest, CT scan or ultrasound (US) of the abdomen, bone scan, and CT scan or MRI of the brain. CT scan and MR images were enhanced with contrast media. LD is defined in this study as disease limited to one hemithorax, including mediastinal, contralateral hilar and supraclavicular lymph nodes, ipsilateral pleural effusion, and pericardial effusion, while ED represents tumour spread beyond these manifestations [18]. This study included 63 patients who received whole body FDG-PET scan after the routine initial staging procedures. Fifty-seven were male and the remaining 6 were female. Median age was 64 years, range 48–80 years. Forty-two patients received FDG-PET before commencement of chemotherapy. The remaining 21 patients received FDG-PET 1 to 11 days (median: 4 days) after commencement of chemotherapy. Forty-four and 19 patients received CT scan and US of the abdomen, respectively.

2.2. FDG-PET scan

FDG-PET scans were performed before March 2005 (patients No. 1–25), and FDG-PET/CT scans were performed after April 2005 (patients No. 26–63). Three hundred MBq of F-18 FDG were intravenously injected after at least 6 h of fasting. Acquisition was initiated 60 min after the injection. FDG-PET imaging was performed using a GE Advance Scanner (General Electric Medical System, Milwaukee, WI), whose axial field of view was 15.2 cm and spatial resolution 4.9 mm of full-width-half-maximum. Scans were performed using two-dimensional acquisition mode from the thigh to the skull base with seven bed positions. Each bed position was composed of 1 min of transmission scanning and 5 min of emission scanning.

FDG-PET/CT imaging was performed using a GE Discovery LS Scanner (General Electric Medical System, Milwaukee, WI) or a GE Discovery ST Scanner (the same manufacturer). The PET component of the GE Discovery LS Scanner was the same as that of the GE Advance Scanner. For the PET component of the GE Discovery ST Scanner, the axial field of view was 15.7 cm and the spatial resolution was 6.2 mm of full-width-half-maximum. PET scans were performed with both scanners using 2-dimensional acquisition mode from the thigh to the skull base with 7 bed positions. Each bed position was composed of 4 min of emission scanning. The CT component of both PET/CT scanners was a 16-row multi-detector CT scanner and CT images were acquired with a tube voltage of 140 kV, and the tube current was automatically set using the auto-exposure control function so that the number of standard deviations of noise was limited to 10. Attenuation correction of PET images was performed using the data from CT images.

Image reconstruction was performed using an ordered subsets expectation maximization (OSEM) algorithm with subset and iteration values of 14 and 2, respectively.

2.3. Image interpretation

All PET and CT images were interpreted by experienced radiologists and physicians. The 4.25 mm-thick images of axial, coronal and sagittal planes on hard copy films were reviewed. Uptake stronger than mediastinal blood pool activity was diagnosed as malignancy by the visual estimation. Symmetrical activities observed in both hilar regions were considered to be benign reactive changes. Any discrepancies between the radiologist and physician were resolved by discussion. The findings detected by FDG-PET were confirmed by other image tests or observation of the clinical course. FDG-PET was conducted after conventional staging procedures. CT, US and bone scans were interpreted without the FDG-PET findings. However, FDG-PET scan was interpreted in comparison with CT findings, while PET/CT findings were interpreted independently.

Table 1 Discrepancy between FDG-PET and conventional staging procedures (distant metastases)

Patient no.	Age (years)	Gender	CT N	PET N	PET M	Interval between conventional staging procedures and FDG-PET (days)	Comments
2	61	Male	2	2	1	20	Multiple bone metastases (PET)
6	68	Male	2	2	1	7	Lymph* node metastasis around the cardia (PET)
47	61	Male	3	3	1	28	Multiple bone metastases (PET)
55	68	Male	2	2	1	20 (CT) and 14 (bone scan)	Liver, axillary lymph node, and iliac bone metastases (PET)
59	52	Male	3	3	1	13	Adrenal, cervical and mandibular lymph node metastases (PET)
63	59	Male	3	3	1	18 (CT) and 11 (bone scan)	Multiple bone and liver metastases (PET)

FDG, fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; N, node; M, metastasis.
* Diagnosis of lymph node metastasis was not confirmed by other imaging modalities or observation of the clinical course.

3. Results

3.1. Detection of distant metastasis

FDG-PET showed results different from those of conventional staging procedures in 17 of 63 patients. PET scan demonstrated findings suggesting distant metastases in 6 of 63 patients (Table 1). The median interval between conventional staging procedures and FDG-PET was 16 days (range: 7–28). Abnormal uptake was observed around the cardia in one of these six patients (No.6). A repeat FDG-PET study demonstrated a longer uptake stripe indicating radiation-induced oesophagitis and the diagnosis could not be established, as there was a remaining possibility of physiological uptake in the oesophagus. The diagnosis of metastatic disease was confirmed in the remaining five patients (8%, 95% confidence interval (CI): 3–18%). Among these five patients, four had bone metastases, two had liver metastases, one had adrenal metastasis, and two had lymph node metastases in the cervical or axillary region. The therapeutic strategy for these five patients was changed and they received only chemotherapy without thoracic radiotherapy. One patient (No. 47) had shown negative findings on bone scintigraphy four weeks before the FDG-PET study, but PET scan demonstrated increased FDG uptake in bones throughout the body. MRI of the spine confirmed the diagnosis of multiple bone metastases (Fig. 1). A repeat bone scan after three months detected obvious multiple bone metastases in No. 2 patient. Two hepatic lesions, as well as the primary tumour, mediastinal and hilar lymph nodes, had all increased in size after two cycles of chemotherapy in patient No. 55. A hepatic lesion, as well as the primary tumour, had decreased in size after two cycles of chemotherapy in patient No. 63. These hepatic lesions were compatible with liver metastases. Abnormal uptake by the right adrenal gland disappeared on repeat PET/CT after four cycles of chemotherapy in patient No. 59. Abnormal uptake in primary and mediastinal lesions was extremely decreased in this patient. The right adrenal gland lesion was compatible with metastasis.

FDG-PET detected liver metastasis in one of 44 patients staged by CT scan of the abdomen (No. 55), and liver or adrenal metastasis in two of 19 patients staged by US (Nos. 59 and 63). Liver and adrenal metastases not detected by US were small, such that the CT part of PET/CT could not detect them as metastases. Ratios of upstaging by FDG-PET between initial CT scan and US of the abdomen were not statistically significant (1/44 versus 2/19, $P = 0.214$).

3.2. Detection of regional lymph node metastases

FDG-PET scans detected regional lymph node metastases that had been negative on CT scans in nine patients (14%) (Table 2). The median interval between CT of the chest and FDG-PET was 19 days (range: 7–34). FDG-PET scans newly detected ipsilateral supraclavicular lymph node metastasis in four patients, contralateral lymph node metastasis in three, and mediastinal lymph node metastasis in two. These nine patients all underwent curative chemoradiotherapy, and abnormal FDG uptake in mediastinal and/or supraclavicular lymph nodes disappeared or decreased on repeat PET scans after chemoradiotherapy. These lymph nodes were considered positive for metastasis.

CT scan detected swollen mediastinal lymph nodes without abnormal FDG uptake in two patients. One patient had a past history of pulmonary tuberculosis complicated by pulmonary fibrosis. The swollen pretracheal lymph node was considered negative for metastasis because the node size remained unchanged after four cycles of chemotherapy although the primary tumour shrank. This case showed false positive findings on CT whereas FDG-PET correctly diagnosed the extent of disease (No. 43). The other patient had atelectasis of the right middle lobe due to the primary tumour. Superior mediastinal and subcarinal lymph nodes were considered to be metastatic on CT, but abnormal FDG uptake was absent. After three cycles of chemotherapy the

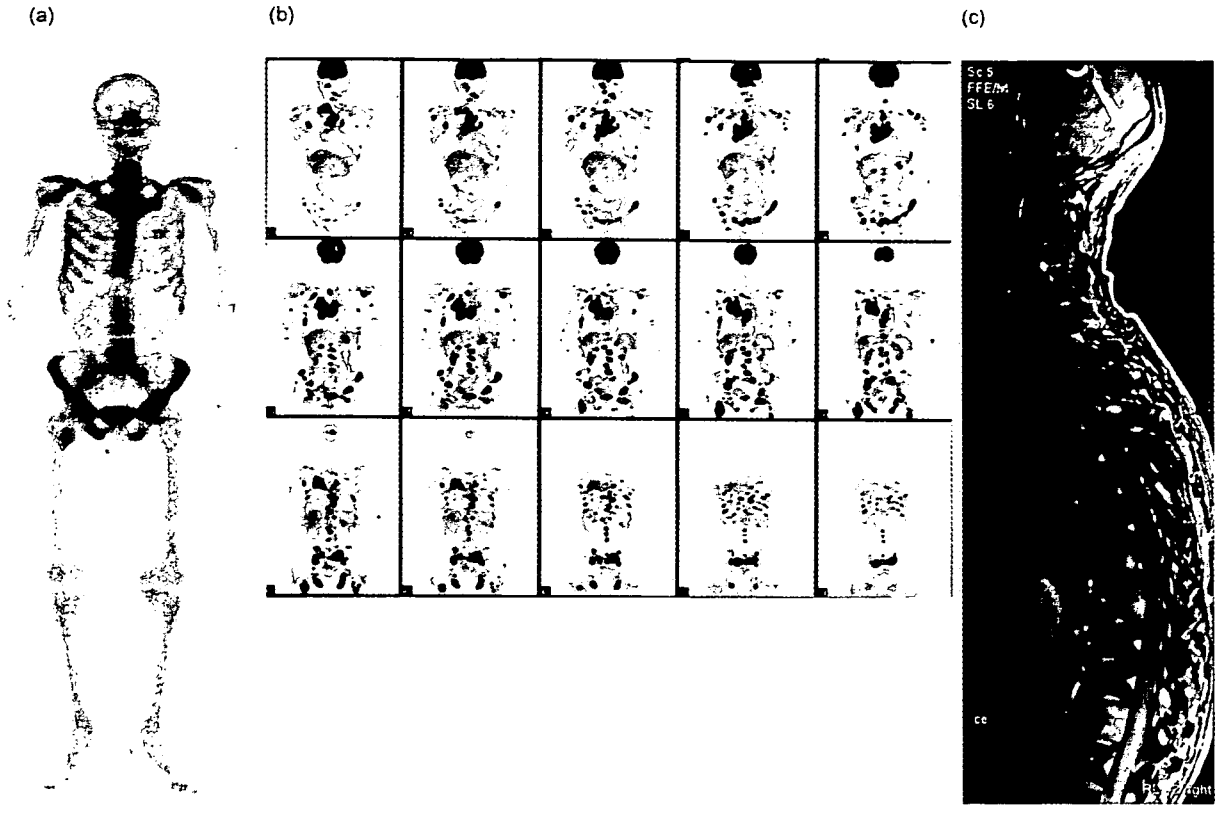

Fig. 1 A 61-year-old man with small-cell lung cancer. Bone scintigraphy was negative for osseous metastasis (a). However, PET scan demonstrated increased FDG uptake in bones throughout the body (b). MRI of the spine confirmed multiple bone metastases (c).

mediastinal lesion showed no change although the primary tumour had decreased in size and atelectasis of the right middle lobe was improved. The mediastinal lymph nodes were considered negative for metastasis (No. 61).

4. Discussion

SCLC tends to disseminate early in the disease course and displays a more aggressive clinical behaviour than NSCLC. Local treatment modalities alone such as radiotherapy or surgery are not effective in prolonging survival beyond a few weeks. Systemic chemotherapy is the mainstay of treatment for patients in all stages of SCLC. A combination of chemotherapy and thoracic irradiation can promote long-term survival for patients diagnosed as having limited disease and recent clinical trials of chemoradiotherapy for LD-SCLC obtained 5-year survival rates of 24–26% [2,3]. However, thoracic irradiation might cause severe radiation pneumonitis, resulting in respiratory failure and/or treatment-related death. Furthermore, thoracic irradiation might also cause oesophagitis which worsens patient quality of life. Accurate clinical staging is important to determine the indications for chemoradiotherapy in SCLC. Our study demonstrated that FDG-PET scan detected unsuspected distant metastases in 8% of patients with LD-SCLC based on conventional staging procedures and that the detection of these new lesions changed their therapeutic strategies. Furthermore, FDG-PET scan detected regional lymph node metastases which had not been visualized on CT scan in 14% of patients. The radiation field could be appropriately set to cover the positive nodes based on the PET study results. Our results reconfirmed those of a previous preliminary study with a smaller number of patients [9].

Is the rate of the detection of unsuspected distant metastases (8%) clinically significant? Previous studies demonstrated that FDG-PET scan detected unsuspected distant metastases in 24% of patients with stage III NSCLC [6,7]. Compared to this result, the impact of FDG-PET on the staging of SCLC seems to be weaker. SCLC tends to have more obvious distant metastases than NSCLC, because of the aggressive biological behaviour of SCLC. Therefore, FDG-PET might detect unsuspected distant metastases at a relatively low rate. The most common region for unsuspected PET-detected metastasis in NSCLC was the abdomen, with 53% of pateints having adrenal, liver, and other lesions [6]. In our study, FDG-PET detected bone metastases in four of five patients who were upstaged from LD to ED. These lesions might reflect metastasis to the bone marrow, although no pathological evidence was obtained, because neither bone marrow biopsy nor aspiration cytology was routinely conducted for the initial clinical staging.

Our retrospective analyses have several limitations. We did not confirm histologically regional lymph node or distant metastases detected by FDG-PET or CT. These lesions were not routinely biopsied and most metastatic lesions were chemosensitive and radiosensitive. Our confirmation was inevitably based on observation of the clinical course.

Table 2 Disagreement between FDG-PET and conventional staging procedures (regional lymph node metastases)

Patient no.	Age (years)	Gender	CT N	PET N	PET M	Interval between CT scan of the chest and FDG-PET (days)	Comments
1	63	Male	3	3	0	8	Contralateral supraclavicular lymph node metastasis (PET)
5	64	Female	1	2	0	34	Subcarinal lymph node metastasis (PET)
16	71	Male	3	3	0	7	Contralateral supraclavicular lymph node metastasis (PET)
20	69	Male	3	3	0	20	Ipsilateral supraclavicular lymph node metastasis (PET)
25	60	Male	3	3	0	27	Ipsilateral supraclavicular lymph node metastasis (PET)
30	66	Male	2	2	0	7	Pretracheal lymph node metastasis (PET)
33	72	Male	3	3	0	13	Ipsilateral supraclavicular lymph node metastasis (PET)
41	49	Female	3	3	0	19	Contralateral supraclavicular lymph node metastasis (PET)
43	73	Male	2	0	0	34	False-positive pretracheal lymph node metastasis (CT)
56	48	Female	3	3	0	11	Ipsilateral supraclavicular lymph node metastasis (PET)
61	74	Male	2	0	0	27	False-positive superior mediastinal and subcarinal lymph nodes (CT).

FDG, fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; N, node; M, metastasis.

We employed no special strategies to reduce the bias of PET readers. PET readers might have reported in such a way as to reduce or increase the impact of PET. One-third of patients received FDG-PET after commencement of chemotherapy. However, the median interval between commencement of chemotherapy and FDG-PET was 4 days (range: 1–11 days). We considered the chemotherapy to have had no effects on the findings of FDG-PET in such a short time after the initiation of chemotherapy.

FDG-PET is expected to have the potentially to both up- and downstage patients with SCLC as well as NSCLC. A previous study demonstrated that FDG-PET correctly downstaged ED to LD in three of 120 patients with SCLC [10]. These three patients had adrenal swelling on CT scan, but these lesions were negative on FDG-PET. On the other hand, FDG-PET correctly upstaged LD to ED in 10 of 120 patients with SCLC. It seems that SCLC seldom has a solitary distant metastasis because of its aggressive clinical behaviour. Most ED-SCLC has multiple, not solitary, or obvious distant metastasis. Furthermore, the health insurance system does not allow patients who obviously have metastatic lung cancer to receive FDG-PET in Japan. Therefore, we did not include patients with ED-SCLC in our analysis. Needless to say, FDG-PET is considered to be useful in patients with possible, but not evident, distant metastasis on other imaging tests, such as a solitary adrenal swelling.

According to the VALSG system, LD-SCLC is defined as a tumour confined to one hemithorax and regional lymph nodes [1]. Contralateral hilar or contralateral supraclavicular nodal involvement was classified as ED. According to the International Association for the Study of Lung Cancer (IASLC) consensus report, the classification of LD-SCLC includes bilateral hilar and/or supraclavicular nodal involvement, and ipsilateral pleural effusion [18]. A previous retrospective study demonstrated that the IASLC staging criteria for SCLC patients had a higher prognostic impact than VALSG criteria [19]. Therefore, we adopted the IASLC staging criteria for SCLC in our study.

In conclusion, FDG-PET scans detected unsuspected distant metastases in five of 63 patients with LD-SCLC (95% CI: 3–18%) and these findings resulted in a change of therapeutic strategies in these five patients. FDG-PET scans also detected contralateral supraclavicular lymph node metastases that had been negative on CT scans in three other

patients. These additional findings facilitated setting appropriate irradiation fields. FDG-PET scan is recommended as an initial staging tool in patients with apparent LD-SCLC.

Conflict of interest

The authors certify that there are no potential conflicts of interest.

Acknowledgments

We thank Takashi Sato, R.T., Hideaki Kitamura, R.T., Kazumasa Inoue, R.T. and Akira Hirayama, R.T. and Yoshiki Kojima, Ph.D. for technical assistance. This work was supported in part by a Grant from the Ministry of Health, Labour, and Welfare for the third term Comprehensive Strategy for Cancer Control and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour, and Welfare, Japan.

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Pertuzumab, a novel HER dimerization inhibitor, inhibits the growth of human lung cancer cells mediated by the HER3 signaling pathway

Kazuko Sakai,[1,3] Hideyuki Yokote,[1,4] Kimiko Murakami-Murofushi,[3] Tomohide Tamura,[2] Nagahiro Saijo[2] and Kazuto Nishio[1,4,5]

[1]Shien-Laboratory,[2]Medical Oncology, National Cancer Center Hospital; Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045; [3]Department of Biology, Faculty of Science, Ochanomizu University, Ohtsuka 2-1-1, Bunkyo-ku, Tokyo 112-8610; [4]Depatment of Genome Biology, Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan

(Received December 18, 2006/Revised April 4, 2007/Accepted May 10, 2007/Online publication July 9, 2007)

A humanized anti-HER2 monoclonal antibody pertuzumab (Omnitarg, 2C4), binding to a different HER2 epitope than trastuzumab, is known as an inhibitor of heterodimerization of the HER receptors. Potent antitumor activity against HER2-expressing breast and prostate cancer cell lines has been clarified, but this potential is not clear against lung cancers. The authors investigated the *in vitro* antitumor activity of pertuzumab against eight non-small cell lung cancer cells expressing various members of the HER receptors. A lung cancer 11_18 cell line expressed a large amount of HER2 and HER3, and its cell growth was stimulated by an HER3 ligand, heregulin (HRG)-α. Pertuzumab significantly inhibited the HRG-α-stimulated cellular growth of the 11_18 cells. Pertuzumab blocked HRG-α-stimulated phosphorylation of HER3, mitogen-activated protein kinase (MAPK), and Akt. In contrast, pertuzumab failed to block epidermal growth factor (EGF)-stimulated phosphorylation of EGF receptor (EGFR) and MAPK. Immunoprecipitation showed that pertuzumab inhibited HRG-α-stimulated HER2/HER3 heterodimer formation. HRG-α-stimulated HER3 phosphorylation was also observed in the PC-9 cells co-overexpressing EGFR, HER2, and HER3, but the cell growth was neither stimulated by HRG-α nor inhibited by pertuzumab. The present results suggest that pertuzumab is effective against HRG-α-dependent cell growth in lung cancer cells through inhibition of HRG-α-stimulated HER2/HER3 signaling. (*Cancer Sci* 2007; 98: 1498–1503)

The HER family of receptor tyrosine kinases consists of four members: EGFR (also termed HER1/ErbB-1), HER2/ErbB-2/Neu, HER3/ErbB-3, and HER4/ErbB-4.[1] Binding of ligands leads to the homo- and heterodimer formation of the receptor tyrosine kinase.[2] There are numerous HER-specific ligands that generate signaling diversity within the cell.[3] EGF, amphiregulin, and TGF-α are known as a specific ligand of EGFR. HB-EGF, β-cellulin, and epiregulin have dual specificity for binding to EGFR and HER4. HRG-α binds HER3 and HER4.[4] No direct ligand for HER2 has been discovered. Dimerization consequently stimulates the intrinsic tyrosine kinase activity of receptors, and activates the downstream-signaling molecules such as MAPK, Akt, JAK, and STAT.[5,6]

Pertuzumab is a humanized monoclonal antibody and binds to the dimerization domain of HER2 distinct from the domain that trastuzumab binds to.[7] Therefore, pertuzumab is known as a dimerization inhibiter between HER2 and the other HER family receptors. A phase I trial of pertuzumab has been performed for advanced tumors,[8] and phase II studies of pertuzumab are underway. Two members of the HER family, HER2 and HER3, act as key oncogenes in breast cancer cells.[9,10] *In vitro* and *in vivo* anti-tumor activities of pertuzumab have been reported in breast tumors through the inhibition of the HER2/HER3 heterodimer formation.[11,12] In lung cancer cells, EGFR plays a crucial role in their biological behavior, but it is unclear whether pertuzumab inhibits the growth of the lung cancer cells mediated by HER family receptors.

The authors have focused on the growth inhibitory effect of pertuzumab against NSCLC cells expressing different types of HER receptors, and analyzed the mechanism of action of pertuzumab in response to the HER receptor ligand.

Materials and Methods

Reagents. Pertuzumab (Omnitarg, 2C4) was provided in sterile water at 25 mg/mL by Genentech, Inc. (South San Francisco, CA, USA) before use. All chemicals and reagents were purchased from Sigma (St Louis, MO, USA) unless noted otherwise.

Cell lines. The human NSCLC cell lines PC-7, PC-9, and PC-14 (Tokyo Medical University, Tokyo, Japan),[13,14] A549 (American Type Culture Collection, Manassas, VA, USA), and PC-3, Ma-1, Ma-24, and 11_18,[15] were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FBS (Life Technologies, Rockville, MD, USA).

Cell stimulation and lysis. Cells were starved in serum free RPMI 1640 medium for 24 h and treated with EGF, TGF-α, HB-EGF, and HRG-α at 100 ng/mL for 10 min. Cells were washed twice with ice-cold PBS, and lysed with lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 5 mM sodium pyrophosphate, 50 mM NaF, 1 mM sodium vanadate, 4 mg/mL leupeptin, 4 mg/mL apoprotein, 1 mM PMSF). Protein concentration of the supernatants was determined by the BCA protein assay (Pierce, Rockford, IL, USA).

Immunoprecipitation. Cell lysates (1000 μg) were incubated with the anti-HER2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight. Protein G magnetic beads (New England BioLabs, Beverly, MA, USA) were added for 2 h. Beads were washed three times with lysis buffer, resuspended in SDS sample buffer with 2% β-mercaptoethanol, boiled, and separated using SDS-PAGE.

Western blotting. Cell lysates were electrophoretically separated on SDS-PAGE and transferred to a polyvinylidene difluoride

[5]To whom correspondence should be addressed. E-mail: knishio@med.kindai.ac.jp

Abbreviations: BCA, bicinchoninic acid; ECL, electrochemiluminescence; EDTA, ethylene diamine tetra-acetic acid; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FBS, fetal bovine serum; HB-EGF, heparin-binding epidermal growth factor; HRG-α, heregulin-α; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NSCLC, non-small cell lung cancer; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; RPMI, Roswell Park Memorial Institute; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; STAT, signal transducer and activator of transcription; TGF-α, transforming growth factor-α.

doi: 10.1111/j.1349-7006.2007.00553.x

membrane (Millipore, Bedford, MA, USA). The membrane was probed with each antibody against EGFR and HER2 (Transduction Laboratory, San Diego, CA, USA), HER3 (Santa Cruz Biotechnology), phospho-EGFR (Tyr1068), phospho-HER3 (Tyr1289), MAPK, phospho-MAPK (Thr202/204), Akt, phospho-Akt (Ser473) (Cell Signaling, Beverly, MA, USA), phosphotyrosine (PY-20, Transduction Laboratory), and β-actin (Sigma) as the first antibody, followed by detection using a horseradish peroxidase-conjugated secondary antibody. The bands were visualized with ECL (Amersham, Piscataway, NJ, USA), and images of blotted patterns were analyzed with NIH image software (National Institutes of Health, Bethesda, MD, USA).

Growth inhibition assay. A 100-μL volume of cell suspension (5000 cells/well) in serum-free RPMI 1640 medium was seeded into a 96-well plate and 50 μL of each drug at various concentrations and 50 μL of EGF, TGF-α, HB-EGF, and HRG-α, at 100 ng/mL was added. Human IgG1 (Calbiochem, Cambridge, MA, USA) was used as isotype control. After incubation for 72 h at 37°C, 20 μL of MTS solution (Promega, Madison, WI, USA) was added to each well and the plates were incubated for a further 2 h at 37°C. The absorbance readings for each well were determined at 490 nm with a Delta-soft on a Macintosh computer (Apple, Cupertino, CA, USA) interfaced to a Bio-Tek Microplate Reader EL-340 (BioMetallics, Princeton, NJ, USA). For ligand-stimulated growth of cells, the experiment was performed in six replicate wells for each ligand and carried out independently three times. For growth inhibition of pertuzumab, the experiment was performed in three replicate wells for each drug concentration and carried out independently three times as described elsewhere.[16]

Results

HRG-α dependent cell growth in lung cancer cells. Ligand-dependent cell growth of lung cancer cells was examined (Fig. 1). The addition of EGF, TGF-α, and HB-EGF increased the cell growth of the PC-3, 11_18, and A549 cells, but not that of the PC-7, PC-9, PC-14, Ma-1, and Ma-24 cells. HRG-α addition significantly increased the growth of the 11_18 cells (390% of control, $P < 0.01$ by t-test) and Ma-24 cells (204% of control, $P < 0.01$ by t-test), but did not influence the growth of any other cells. These findings suggest that the growth of the 11_18 and Ma-24 cells is depending upon HRG-α.

Pertuzumab inhibits HRG-α-dependent cell growth of the 11_18 and Ma-24 cells. Pertuzumab inhibited cell growth stimulated by HRG-α ($IC_{50} = 0.12$ μg/mL) but not stimulated by EGF, TGF-α, and HB-EGF in the 11_18 cells ($IC_{50} > 100$ μg/mL; Fig. 2). Pertuzumab also inhibited HRG-α dependent cell growth in the Ma-24 cells ($IC_{50} = 39.8$ μg/mL). Isotype control human IgG1 had no effect on ligand-dependent growth in the 11_18 and Ma-24 cells (data not shown). The growth of the other cells was not affected by exposure to pertuzumab (data not shown). This finding suggests that pertuzumab selectively inhibits HRG-α-dependent cell growth.

Ligand-stimulated phosphorylation of HER receptors. The expression levels of the HER receptors in the pertuzumab-sensitive (11_18 and Ma-24 cells) and pertuzumab-resistant cell (PC-9 cells) lines were determined using western blotting (Fig. 3a). Comparison of the protein expression levels of EGFR revealed high to moderate expression in the PC-9 and Ma-24 cells. EGFR was also detected in the 11_18 cells, although the expression in this

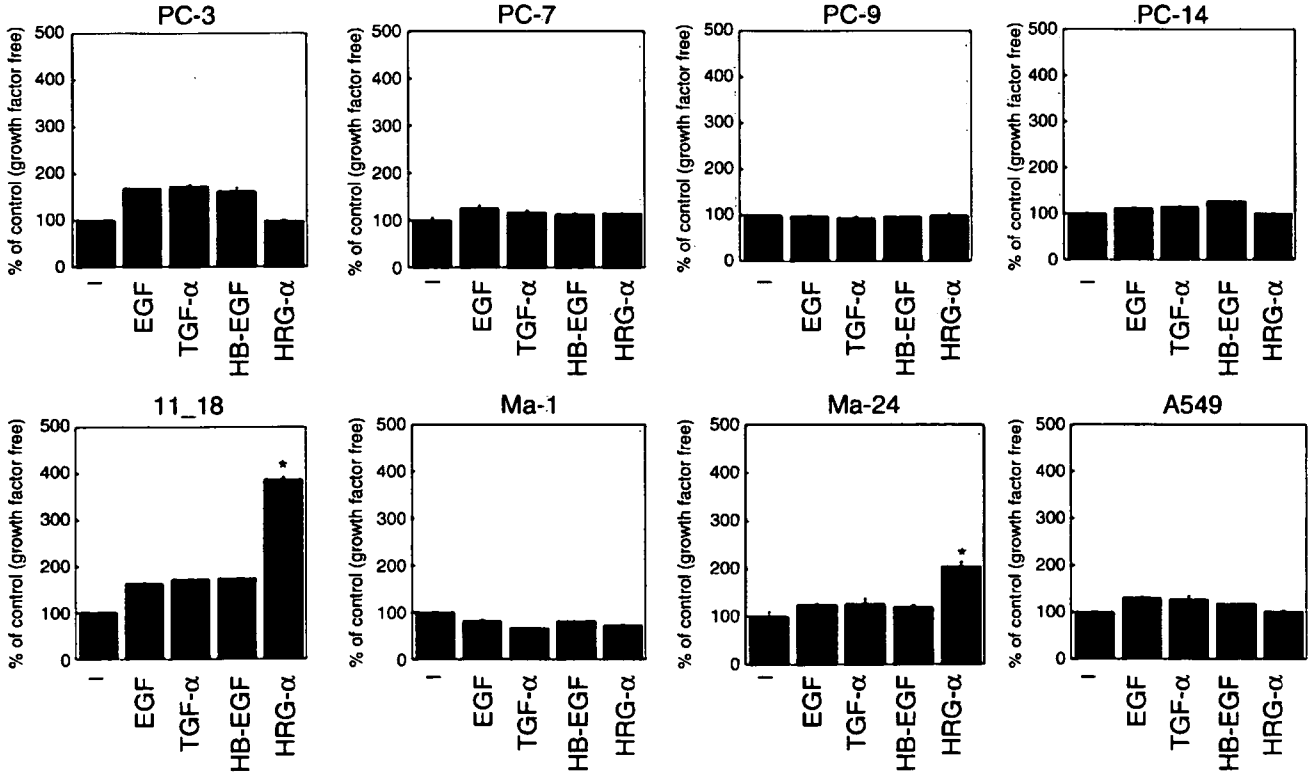

Fig. 1. Ligand-dependent cell growth in the lung cancer cells. Non-small cell lung cancer cells were stimulated with or without 100 ng/mL of epidermal growth factor (EGF), transforming growth factor (TGF)-α, heparin-binding epidermal growth factor (HB-EGF), and heregulin (HRG)-α. After incubation for 72 h, cell growth was determined using the MTS assay. The growth of cells was presented as the percentage of absorbance compared with ligand-untreated cells. Error bars represent SE. *Significant difference ($P < 0.01$; t-test) compared to the ligand-non-stimulated cells. Data shown are representative of at least three independent experiments with similar results.

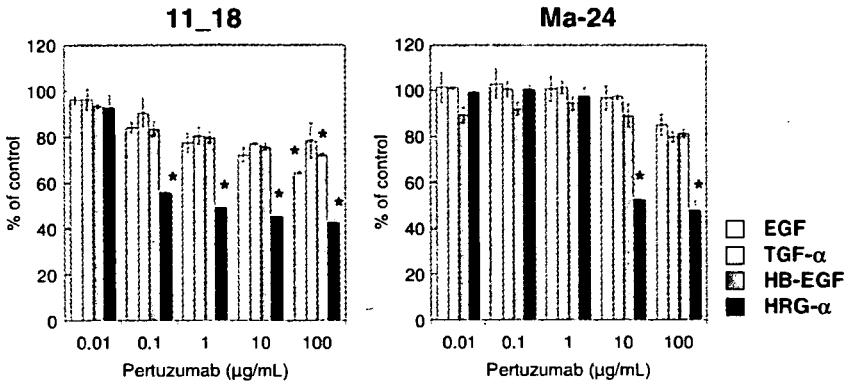

Fig. 2. Growth inhibitory effect of pertuzumab in the lung cancer cells. The lung cells were exposed to pertuzumab (0.01–100 μg/mL) for 72 h in serum free medium with or without 100 ng/mL of epidermal growth factor (EGF), transforming growth factor (TGF)-α, heparin-binding epidermal growth factor (HB-EGF), or heregulin (HRG)-α. The viability was determined using the MTS assay. Result are presented as the percentage of absorbance compared with pertuzumab-untreated cells. Error bars represent SE. *Significant difference ($P < 0.01$; *t*-test) compared to pertuzumab-untreated cells. Data shown are representative of at least three independent experiments with similar results.

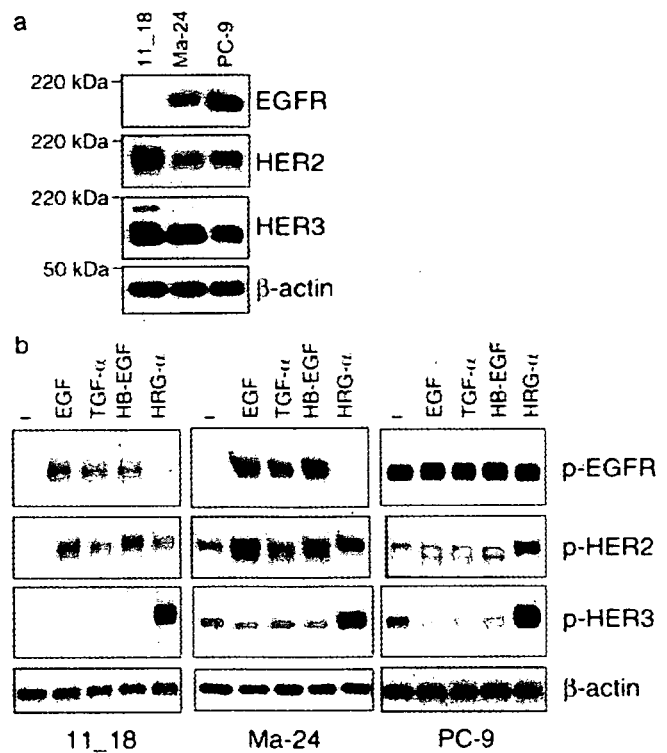

Fig. 3. Expression and phosphorylation of HER receptors in non-small cell lung cancer cells. (a) Expression of epidermal growth factor receptor (EGFR), HER2, and HER3 was detected using western blot analysis. Each lane contained 20 μg protein. β-Actin was used as a loading control. (b) The cells were stimulated with or without 100 ng/mL of epidermal growth factor (EGF), transforming growth factor (TGF)-α, heparin-binding epidermal growth factor (HB-EGF), and heregulin (HRG)-α for 10 min. Phosphorylation of EGFR and HER3 was detected using western blot analysis. Phosphorylation of HER2 was detected using immunoprecipitation followed by western blotting. β-Actin was used as a loading control. Data shown are representative of at least two independent experiments with similar results.

cell line was weak. The expression levels of HER2 were higher in the PC-9 and 11_18 cells than in the Ma-24 cells, which only expressed moderate levels of this receptor. All three cell lines showed strong expression of HER3. HER4 could not be detected in any of the three cell lines (data not shown). In contrast, these lung cancer cell lines expressed different types of EGFR mutations; the PC-9 cells had a 15-base deletion mutant (delE746-A750, exon 19), the 11_18 cells had a L858R point mutation (exon 21) of EGFR, and the Ma-24 cells had a E709G point mutation (exon 18) of EGFR. No mutations were detected in exons 19–21 of HER2 (data not shown).

Next, the ligand-stimulated phosphorylation of the HER receptors in the lung cancer cells after serum starvation was examined (Fig. 3b). While the ligands for EGFR (EGF, TGF-α, and HB-EGF) phosphorylated cellular EGFR in the 11_18 and Ma-24 cells, the EGFR in the PC-9 cells was hyperphosphorylated even under the non-stimulated condition, because PC-9 cells express an active mutant of EGFR. These results suggest that the EGF/TGF-α or HB-EGF–EGFR signals are active in lung cancer cells. The ligands for HER3 (HRG-α) specifically phosphorylated HER3 in the 11_18, Ma-24, and PC-9 cells. Phosphorylation of HER2 was analyzed by immunoprecipitation using an anti-HER2 antibody followed by western blotting for phosphotyrosine. The ligands for EGFR and HER3 phosphorylated HER2 in the 11_18 and Ma-24 cells, whereas only HRG-α but not the other ligands specifically phosphorylated HER2 in the PC-9 cells. These findings also suggest that the HRG-α–HER3 signal is active in lung cancer cells.

Pertuzumab blocks HRG-α but not EGF-stimulated signals. An inhibitory effect of pertuzumab on HRG-α-dependent cell growth in the 11_18 cells was demonstrated. To examine the effect of pertuzumab on signal transduction of both EGFR and HER3 in this cell line, the 11_18 cells were exposed to pertuzumab (0.2–200 μg/mL for 6 h) (Fig. 4a,b). HRG-α-stimulated phosphorylation of HER3 was dose-dependently inhibited by exposure to pertuzumab in the 11_18 cells, whereas EGFR phosphorylation was not stimulated by HRG-α stimulation (data not shown). MAPK and Akt were phosphorylated by HRG-α stimulation and these were inhibited by pertuzumab dose-dependently in the 11_18 cells. In contrast, EGF-stimulated phosphorylation of EGFR and MAPK was not inhibited by pertuzumab in the 11_18 cells. Phosphorylation of Akt was not detected by addition of EGF in the 11_18 cells. EGF did not phosphorylate HER3 and pertuzumab did not affect it (data not shown). Taken together, these results showed that pertuzumab inhibited HRG-α-stimulated phosphorylation of HER3, MAPK, and Akt, but not EGF-stimulated EGFR phosphorylation signaling.

HER3 is phosphorylated in response to HRG-α in the PC-9 cells as observed in the 11_18 cells, but the growth of the PC-9 cells was not increased by HRG-α (Figs 1,3b). To clarify the phosphorylation-inhibitory potential of pertuzumab, the effect of pertuzumab on signal transduction of the PC-9 cells was examined (Fig. 4c). When the PC-9 cells were stimulated by the addition of HRG-α, HER3 was phosphorylated in the PC-9 cells, but phosphorylation of HER3 was not inhibited by pertuzumab (20 and 200 μg/mL for 6 h). EGFR expressed in the PC-9 cells is constitutively active and pertuzumab failed to affect

doi: 10.1111/j.1349-7006.2007.00553.x

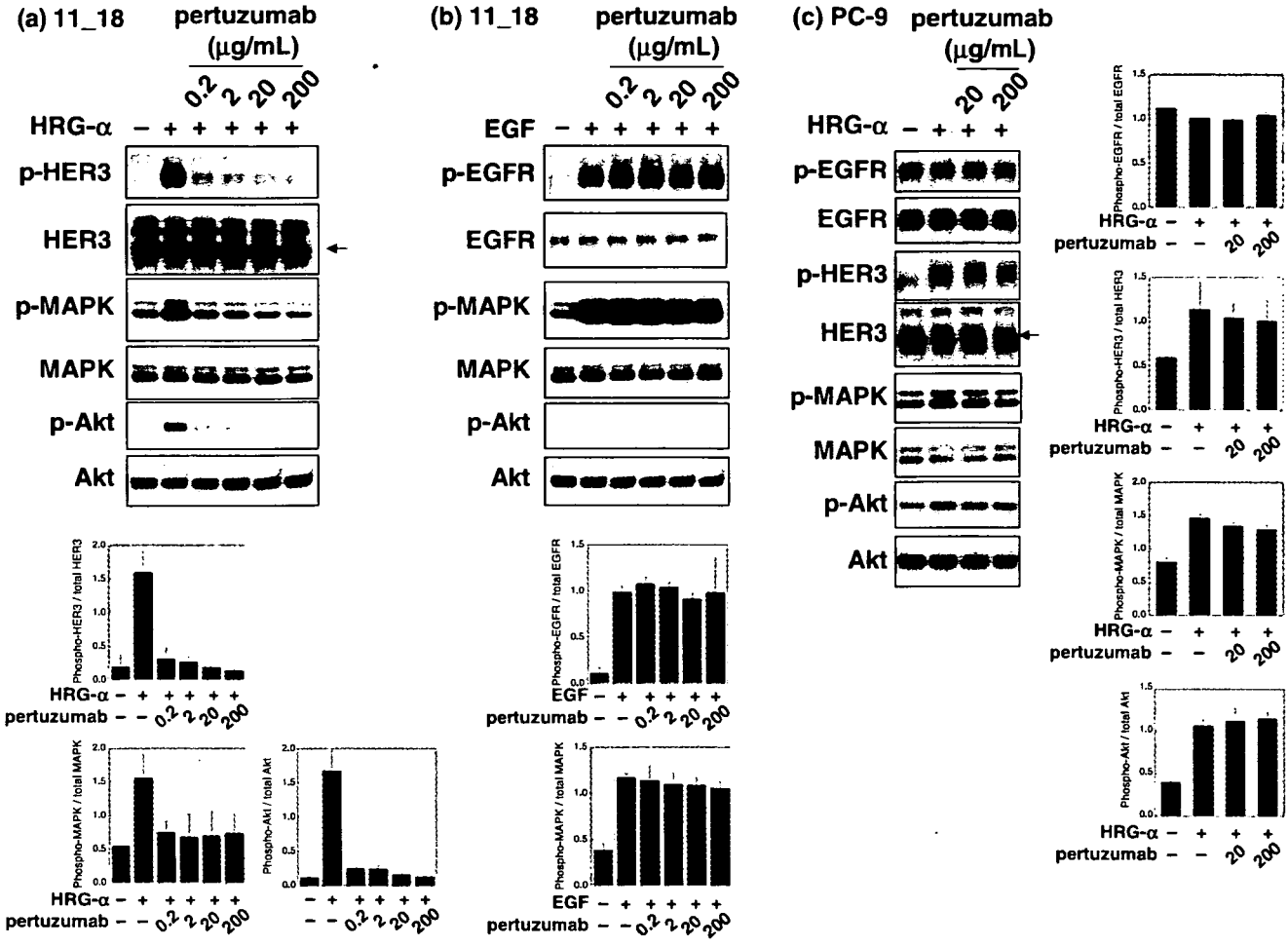

Fig. 4. Effect of pertuzumab on epidermal growth factor receptor (EGFR) and HER3 phosphorylation and their downstream signaling pathways. The 11_18 and PC-9 cells were exposed to pertuzumab for 6 h and stimulated with either heregulin (HRG)-α or epidermal growth factor (EGF) for 10 min. Cell lysate were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotted for indicated antibodies. The intensities of bands were quantified by densitometer. (a) HRG-α-stimulated 11_18 cells. (b) EGF-stimulated 11_18 cells. (c) HRG-α-stimulated PC-9 cells. Data shown are representative of at least two independent experiments with similar results. MAPK, mitogen-activated protein kinase.

the phosphorylation level of the EGFR. Phosphorylation of MAPK and Akt was detected by the addition of HRG-α, but these were not inhibited by pertuzumab. These results suggest that pertuzumab is unable to affect HRG-α-stimulated phosphorylation of HER3 in the PC-9 cells.

To clarify the effect of pertuzumab on HER2 phosphorylation and HER2/HER3 heterodimer formation, cell lysates were immunoprecipitated with anti-HER2 antibody (Fig. 5a,b). HRG-α stimulation increased HER2/HER3 heterodimer formation in the 11_18 cells, and pertuzumab decreased HRG-α-stimulated heterodimer formation. EGFR/HER2 heterodimer formation could be barely detected by HRG-α stimulation because of slight expression of EGFR in the 11_18 cells. In the case of EGF stimulation, HER2/HER3 heterodimer was not increased in the 11_18 cells. These findings suggest that pertuzumab inhibits HER2/HER3 heterodimerization by HRG-α stimulation. The HRG-α-stimulated phosphorylation of HER2 was inhibited by pertuzumab in the 11_18 cells. In contrast, the EGF-stimulated phosphorylation of HER2 was not inhibited. These data suggest that pertuzumab inhibits HRG-α stimulated phosphorylation in 11_18 cells. In the PC-9 cells, HRG-α stimulated HER2/HER3 heterodimer formation could be detected without any ligand stimulation, and pertuzumab diminished HRG-α-stimulated heterodimer formation (Fig. 5c). Phosphorylation of HER2 was increased by HRG-α stimulation, but not inhibited by pertuzumab in PC-9 cells. EGFR/HER2 heterodimer formation could be detected without any ligand stimulation, but pertuzumab did not affect it. Based on these results, it is speculated that the cell growth of the PC-9 cells is predominantly dependent on active EGFR signaling, and phosphorylation of HER3 is maintained by active mutant EGFR.

Discussion

Overexpression of HER3 was observed in the lung cancer cell lines and the HER3 was phosphorylated by the HER3 ligand in these cells. These results suggest that HER3 signaling is active in some types of lung cancer cells. Recently it was reported that high HER3 expression was associated with decreased survival.[17] A relationship between lung cancer metastasis and the expression of HER3 as well as EGFR and HER2 has been reported.[18] These bodies of evidence suggest that HER2/HER3 signaling is activated in a subpopulation of lung cancers and that HER2 and HER3 play an important role in the biological behavior of these lung cancers. Both HER2 and HER3 are therefore considered as a possible important target in the therapeutic strategy against lung cancer, just as they are in breast cancers.

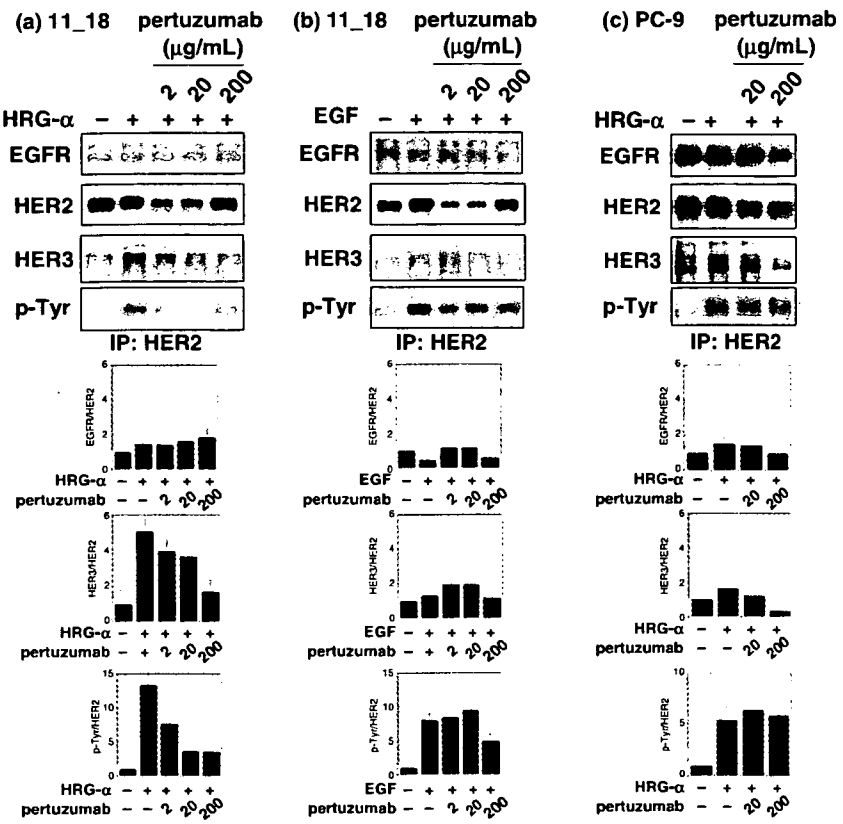

Fig. 5. Effect of pertuzumab on heterodimer formation. The 11_18 and PC-9 cells were exposed to pertuzumab for 6 h and stimulated with either heregulin (HRG)-α or epidermal growth factor (EGF) for 10 min. Cell lysates were immunoprecipitated with anti-HER2 antibody, separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and blotted for indicated antibodies. The intensities of bands were quantified by densitometer. (a) HRG-α-stimulated 11_18 cells. (b) EGF-stimulated 11_18 cells. (c) HRG-α-stimulated PC-9 cells. Data shown are representative of at least two independent experiments with similar results.

HER3 lacks kinase activity because of several base substitutions in motifs that are essential to tyrosine kinase and heterodimerization with HER2 or EGFR is essential for its signal transduction. Therefore co-expression of HER3 and its partners are determinants for the cellular sensitivity against pertuzumab in cancer cells. The present results showed that HER2/HER3 heterodimers are detected by HRG-α stimulation and these data are consistent with previous reports.[19] In contrast, the authors monitored the downstream phosphorylation signal, and demonstrated that HRG-α, but not EGF, phosphorylated Akt in the 11_18 cells. This finding allows us to speculate that HRG-α stimulation leads to Akt phosphorylation through HER2/HER3 heterodimerization.[20–22]

Recently, EGFR mutations have been reported in lung cancers and it was of great interest to clarify the relationship between the EGFR mutation and sensitivity to EGFR-targeted tyrosine kinase inhibitors.[23–25] The PC-9 cells express the deletional mutant EGFR (delE746-A750 in exon 19 of EGFR),[16,23,26,27] and their EGFR was constitutively phosphorylated under non-stimulated conditions (Fig. 3a). The authors speculate that the cell growth of the PC-9 cells is predominantly dependent on active EGFR signaling. In Fig. 3b, treatment with EGF and TGF-α seemed to decrease the phosphorylation of HER3 in PC-9 cells. Unfortunately, we could not conclusively explain this phenomenon. PC-9 cells express deletional EGFR and form EGFR homodimers in the absence of ligand stimulation. At the same time, phospho-HER3 was also detected under these conditions, suggesting that heterodimers of EGFR–HER3 were also formed. Ligand stimulation may alter the balance between homodimers and heterodimers, causing a reduction in HER3 phosphorylation, although there is not any evidence to support this hypothesis. In contrast, the phosphorylation of EGFR in the 11_18 cells that express a different type of mutant EGFR (L858R in exon 21 of EGFR),[26] was not constitutive. This finding may be explained by the differences between deletion mutant EGFR and L858R; constitutive active in the deletion mutant versus hyper-response to ligand stimulation in L858R.[28] Engelman *et al.* suggested that the mutant EGFR is used to couple HER3 in gefitinib-sensitive NSCLC cell lines.[29] The expression level of EGFR in the 11_18 cells was much lower than in the PC-9 cells, and a similar extent of HER3 expression was observed in these cell lines (Fig. 3a). The authors have demonstrated the differential inhibitory effect of pertuzumab against 11_18 and the PC-9 cells. Pertuzumab inhibited HER2/HER3 heterodimer formation and phosphorylation in the 11_18 cells, considering that mutant EGFR do not influence HER3 signals in the 11_18 cells. HER3 phosphorylation in the PC-9 cells was also increased by HRG-α stimulation. Although pertuzumab decreased HER2/HER3 heterodimer formation, it failed to inhibit HRG-α-stimulated HER3 phosphorylation, speculating that an active mutant EGFR transactivates HER3 in the PC-9 cells.

Several EGFR-targeted small inhibitors and antibodies have been under clinical evaluation in the treatment of lung cancer. An EGFR-targeted tyrosine kinase inhibitor, erlotinib, has been clinically applied as a second or third-line single agent therapy in NSCLC patients who have failed standard chemotherapy.[30] Anti-EGFR monoclonal antibodies such as cetuximab and ABX-EGF have been examined in a clinical study.[31] In addition to EGFR, HER2 and HER3 are also considered as important targeting molecules in lung cancers. The present results indicated that pertuzumab effectively inhibited signaling within HER2 and HER3, and may thus be effective in lung cancers expressing HER2 and HER3. To confirm the pertuzumab-sensitive population of lung cancer cells, experiments using small interfering RNA for mutant EGFR will be necessary in future studies.

doi: 10.1111/j.1349-7006.2007.00553.x

In conclusion, the authors have demonstrated that pertuzumab inhibits HRG-α-stimulated cell growth in lung cancer cells through the inhibition of HRG-α-stimulated HER3 signaling. It was further demonstrated that pertuzumab exerts an antiproliferative activity against lung cancer cells expressing HER2 and HER3. The next step will be to examine the clinical relevance of the occurrence of heterodimer formation between HER2 and the other HER receptors in lung cancer.

Acknowledgment

This work was supported by funds for the Third Term Comprehensive 10-Year Strategy for Cancer Control.

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