Table 3. **Multivariate analysis of pretreatment and treatment-related parameters and groups categorized according to worst creatinine grade**

Parameter		Odds ratio (95% confidence interval†)	*P*-value
Sex	Male	1	0.082
	Female	2.34 (0.90–6.10)	
Age	10-year increments	1.55 (0.91–2.64)	0.11
Average daily urine volume†	100-mL increments	0.94 (0.88–1.00)	0.073
Body weight loss	1-kg decrements	1.77 (1.08–2.90)	0.024
Total furosemide dose	10-mg increments	1.21 (1.11–1.33)	<0.001

†The average daily urine volume on days 1–5 of chemotherapy.

a simple and useful indicator of the hydration status of these patients.

The current study also showed that the total furosemide dose was associated with the development of renal toxicity. Vigorous fluid infusion and diuresis with mannitol or furosemide have been used widely for the prevention of cisplatin nephrotoxicity.[11,12] These interventions are thought to reduce the cisplatin concentration in the renal tubules and the time during which this drug and the tubular epithelial cells are in contact.[5] However, numerous experimental studies have provided conflicting results regarding the renal protective effects of these diuretics; cisplatin nephrotoxicity was reduced in some studies but was enhanced in others.[5] A randomized trial of cisplatin at a dose of 100 mg/m^2 and hydration with or without mannitol in patients with malignant melanoma showed that this regimen prevented nephrotoxicity during the first treatment course.[13] Another randomized trial of cisplatin hydration with mannitol or furosemide in patients with advanced solid tumors showed that a serum creatinine elevation of more than 2 mg/dL was observed in 28% of the courses in the mannitol-treated group and 19% of the courses in the furosemide-treated group.[14] A third randomized trial of cisplatin at a dose of 75 mg/m^2 and hydration alone, hydration with mannitol, or hydration with furosemide showed that creatinine clearance did not change before or after cisplatin treatment in the hydration alone and the furosemide-treated groups, but decreased in the mannitol-treated group.[15] However, these randomized trials included only small numbers of patients and therefore are not conclusive. Thus, no reports have convincingly shown any advantage of diuretics in preventing cisplatin nephrotoxicity. These studies differed from the current study, in which furosemide was administered only when fluid retention was suspected based on an increased BW or a decreased UV. Although an association between renal toxicity and the total furosemide dose was observed in this study, patients with fluid retention may be more prone to develop renal toxicity. Another explanation is that furosemide may have a direct toxic effect on the kidney. Thus, the administration of furosemide may be inevitable in some cases to prevent fluid overload during aggressive hydration, but its frequent use should be avoided.

Because renal function decreases physiologically with aging,[16] cisplatin use in elderly patients remains controversial. Some authors of clinical studies for patients aged 70 years or older have concluded that the use of cisplatin at moderate doses (60–100 mg/m^2) should be encouraged in these patients, just as it is in younger patients.[17–19] Studies that evaluated risk factors for cisplatin nephrotoxicity in more than 400 patients showed that an older age was a significant risk factor in two studies[7,20] but not in a third study.[8] In the current study, age was not a risk factor for renal toxicity according to a multivariate analysis, probably because 80 mg/m^2 of cisplatin was administered only to selected elderly patients. In our practice, many elderly patients are treated with cisplatin at a dose of 25 mg/m^2 on three consecutive days or weekly; these patients were excluded from the present study.

In the present study women were more likely to suffer from cisplatin nephrotoxicity than men. Another study also showed that women had a twofold increased risk for renal toxicity compared with men.[7] Although the reason for this difference is not definitely known, it may be explained, at least in part, by a 15% lower unbound cisplatin clearance in women than men,[7,21] because pharmacokinetics of unbound cisplatin have been repeatedly shown to be correlated with cisplatin nephrotoxicity.[22–24]

Although intravenous fluid infusion on the day of cisplatin administration is a well established treatment for preventing nephrotoxicity, the use of subsequent fluid infusions has not been reported. Because the present study showed that dehydration progressed on day 5 in many cases and an elevated serum creatinine level appeared thereafter, maintaining the total body water level during days 1–5 of chemotherapy seems to be important for the prophylaxis of cisplatin nephrotoxicity. For this purpose, a BW measurement carried out before breakfast would be a simple and useful indicator; if oral intake is found to be insufficient, vigorous infusion therapy on days 2–5 may be effective.

In conclusion, the maximum BW loss during days 1–5 of chemotherapy and the total furosemide dose were associated with the development of cisplatin renal toxicity. Maintaining total body water levels during this period seems to be important, and measuring BW would be a simple and useful indicator for this purpose.

Acknowledgment

We thank Mika Nagai for the preparation of this manuscript.

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doi: 10.1111/j.1349-7006.2007.00532.x

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

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(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* anti-tumor 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

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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

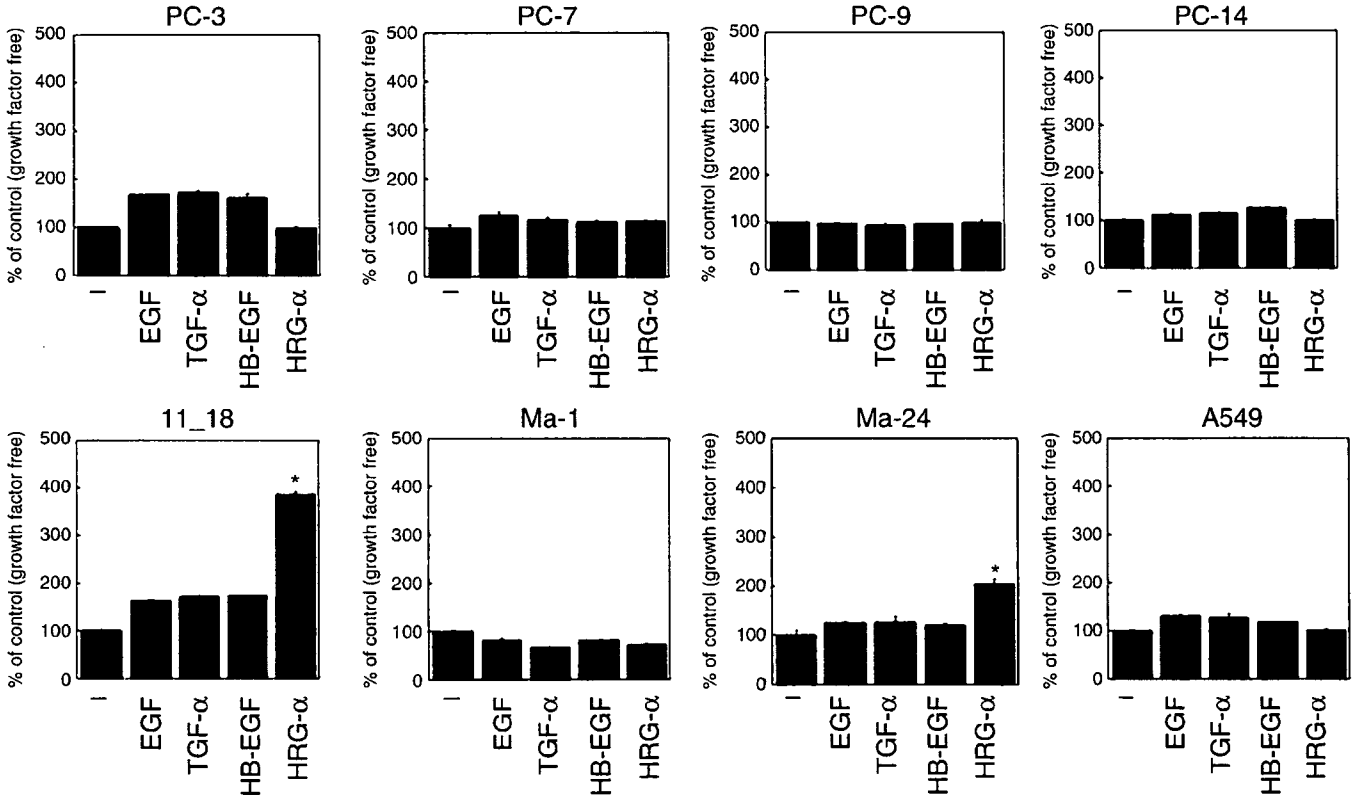

Fig. 1. Ligand-dependent cell growth in the lung cancer cells. Non-small cell lung cancer cells were stimulated with or without 100 ng/mL of epidermal growth factor (EGF), transforming growth factor (TGF)-α, heparin-binding epidermal growth factor (HB-EGF), and heregulin (HRG)-α. After incubation for 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.

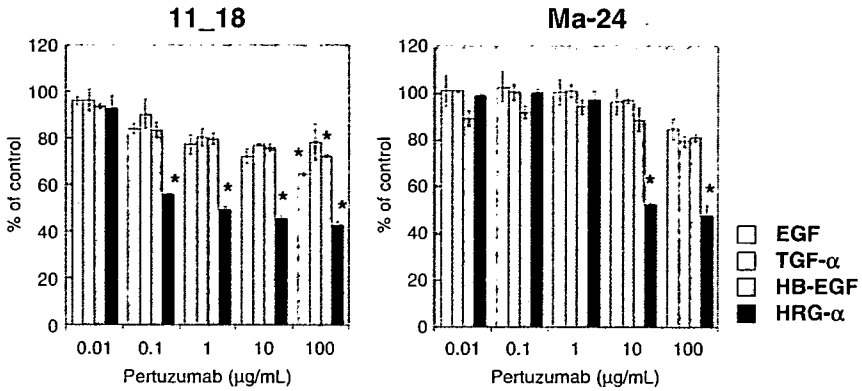

Fig. 2. Growth inhibitory effect of pertuzumab in the lung cancer cells. The lung cells were exposed to pertuzumab (0.01–100 μg/mL) for 72 h in serum free medium with or without 100 ng/mL of epidermal growth factor (EGF), transforming growth factor (TGF)-α, heparin-binding epidermal growth factor (HB-EGF), 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.

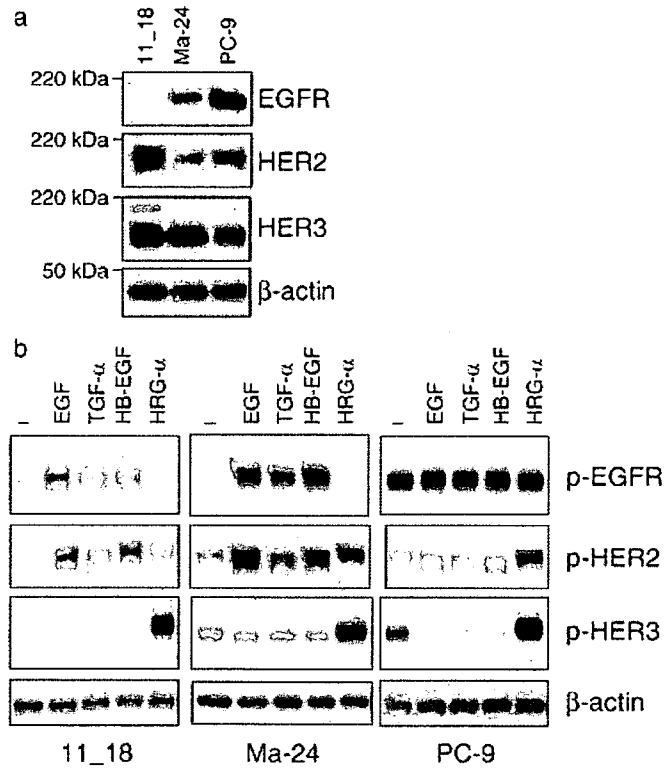

Fig. 3. Expression and phosphorylation of HER receptors in non-small cell lung cancer cells. (a) Expression of epidermal growth factor receptor (EGFR), HER2, and HER3 was detected using western blot analysis. Each lane contained 20 μg protein. β-Actin was used as a loading control. (b) The cells were 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

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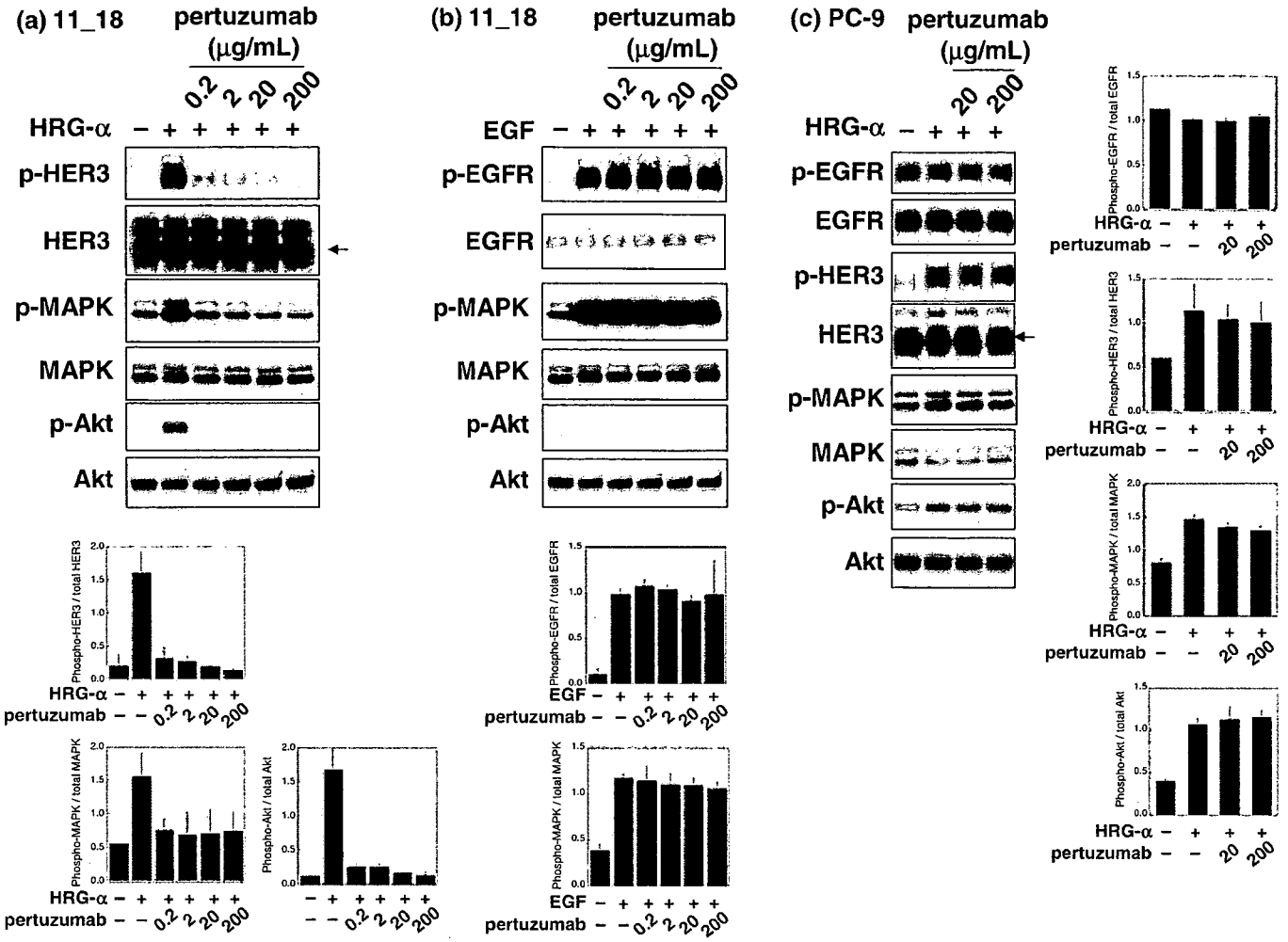

Fig. 4. Effect of pertuzumab on epidermal growth factor receptor (EGFR) and HER3 phosphorylation and their downstream signaling pathways. The 11_18 and PC-9 cells were exposed to pertuzumab for 6 h and stimulated with either heregulin (HRG)-α or epidermal growth factor (EGF) for 10 min. Cell lysate were 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.

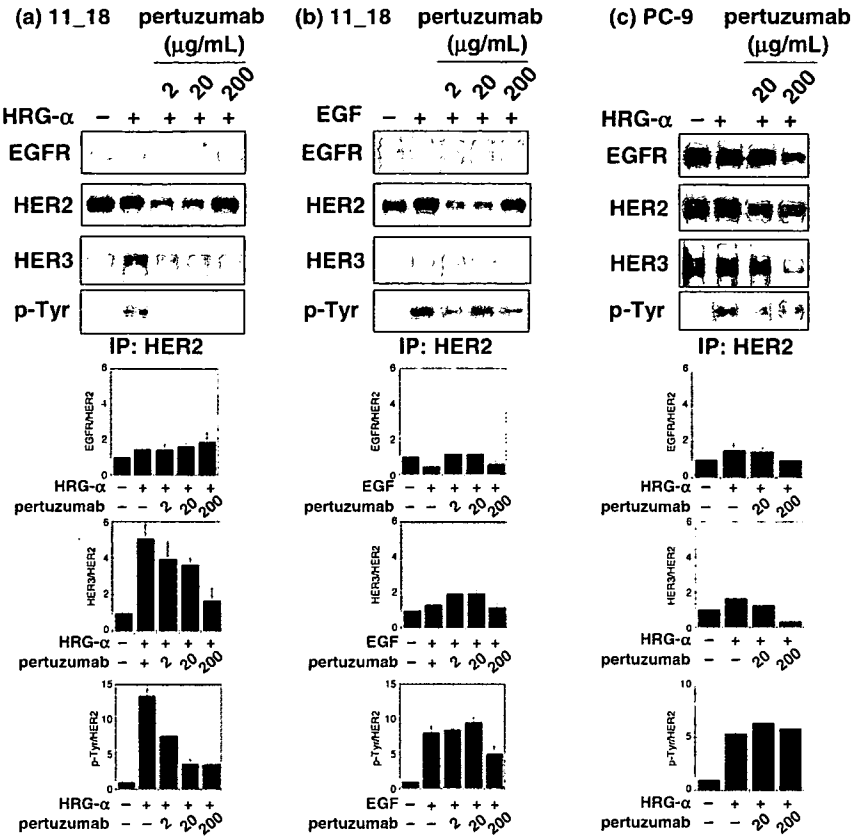

Fig. 5. Effect of pertuzumab on heterodimer formation. The 11_18 and PC-9 cells were exposed to pertuzumab for 6 h and stimulated with either heregulin (HRG)-α or epidermal growth factor (EGF) for 10 min. Cell lysates were immunoprecipitated with anti-HER2 antibody, separated using sodium dodecyl sulfate–polyacrylamide 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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Jpn J Clin Oncol 2007;37(7)482–486
doi:10.1093/jjco/hym053

Effect of Platinum Combined with Irinotecan or Paclitaxel against Large Cell Neuroendocrine Carcinoma of the Lung

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Received August 16, 2006; accepted March 3, 2007; published online July 24, 2007

Background: The efficacy of chemotherapy in patients with large cell neuroendocrine carcinoma of the lung (LCNEC) remains unclear.
Methods: Of 42 consecutive patients with LCNEC, 22 with measurable disease receiving chemotherapy were enrolled as the subjects of this study. The clinical characteristics and objective responses to chemotherapy in these patients were analysed retrospectively.
Results: The distribution of the disease stage in the patients consisting of 21 males and one female (median age: 67 years; range: 47–78 years) was as follows: stage IIB ($n = 1$), stage IIIA ($n = 1$), stage IIIB ($n = 5$), stage IV ($n = 8$), and post-operative recurrence ($n = 7$). Chemotherapy consisted of cisplatin and irinotecan ($n = 9$), a platinum agent and paclitaxel ($n = 6$), paclitaxel alone ($n = 1$), cisplatin and vinorelbine ($n = 1$), cisplatin and docetaxel ($n = 1$), and a platinum and etoposide ($n = 4$). The objective response rate in the 22 patients was 59.1% (95% CI, 38.1–80.1). An objective response was obtained in five of the nine patients receiving irinotecan and five of the seven patients receiving paclitaxel. The progression-free survival, median overall survival and 1-year survival rates were 4.1 months (95% CI, 3.1–5.1), 10.3 months (95% CI, 5.8–14.8) and 43.0% (95% CI, 20.7–65.3), respectively. The median overall survival of the patients treated with irinotecan or paclitaxel was 10.3 months (95% CI, 0–21.8), and the 1-year survival rate of these patients was 47.6% (95% CI, 20.4–74.8).
Conclusion: Our results suggest that irinotecan and paclitaxel may be active against LCNEC.

Key words: lung cancer – large cell neuroendocrine carcinoma – chemotherapy – irinotecan – paclitaxel

INTRODUCTION

Neuroendocrine tumors of the lung can be placed in the biological spectrum ranging from typical to atypical carcinoid, which are tumors of low to intermediate grade malignancy, to large cell neuroendocrine carcinomas (LCNEC) and small-cell lung carcinomas (SCLC), which are high-grade malignant tumors. LCNEC was proposed as a separate category by Travis et al. in 1991, who recognized a type of poorly differentiated high-grade carcinoma exhibiting features of neuroendocrine appearance on light microscopy, immunohistochemistry, and/or electron microscopy (1). Several different terminologies and classifications have been proposed to date, and this class of tumors is likely to become widely recognized and included in the updated histological classification of the World Health Organization (2).

The clinical features of LCNEC have not yet been completely clarified. The prognosis of patients with surgically resected LCNEC is intermediate between that of an atypical carcinoid and SCLC, and is the same as that of resected non-small-cell lung carcinoma (NSCLC), except for stage I LCNEC, which has a poorer prognosis than that of stage I NSCLC (3–6). In a multi-institutional study in Japan, it was found that both LCNEC and SCLC were similarly aggressive and that there was no survival difference between the two types of lung cancer (7). In a small case series of LCNEC, we reviewed the records of patients with surgically resected,

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and patients treated medically who were autopsied before 1995, and determined that the chemosensitivity of LCNEC to cisplatin-based regimens may be intermediate between that of NSCLC and SCLC (8). Third generation cytotoxic agents developed in the 1990s, such as paclitaxel, docetaxel, gemcitabine, vinorelbine and irinotecan, have been shown to be active agents against advanced lung cancer, and combinations of platinum and one of the third generation cytotoxic agents have been shown to be superior in terms of prolonging the survival to the existing platinum-based combinations in both patients with NSCLC and those with SCLC (9–14). In the present study, we conducted a retrospective review of the records of our patients with LCNEC who had been treated with chemotherapy, and analysed the efficacy of the chemotherapy regimens.

PATIENTS AND METHODS

From April 1999 to January 2006, 42 patients were diagnosed as having LCNEC at our institution. Of these, one patient underwent surgery, four were treated with radiation therapy alone, and three received only supportive care. Of the 34 patients who had received chemotherapy, four who had also received concurrent radiotherapy and two without evaluable lesions were excluded from this study. In addition, six patients who entered a phase II trial of cisplatin and irinotecan combination for LCNEC were also excluded from this study, because their results will be published elsewhere. Thus, 22 patients were finally enrolled as the subjects of this study.

The histological confirmation of the diagnosis of LCNEC in the medically treated patients was based on examination of biopsy and/or cytology specimens. The histological or cytological diagnosis was reviewed by one of the authors (K.T.). We classified LCNEC according to the histopathological criteria proposed in the WHO classification. Immunohistochemical analysis was performed to confirm the neuroendocrine differentiation of the tumor cells (2).

Clinical information about the cases was obtained from medical records. All patients underwent a chest and abdominal computed tomography, a head computed tomography or magnetic resonance imaging and a bone scintigraphy in clinical disease staging before chemotherapy. The clinical disease staging was reassessed according to the latest International Union Against Cancer (UICC) staging criteria (15). The response to chemotherapy and the survival were assessed retrospectively. The objective tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumor guidelines (16). The survival distributions for overall survival (OS) and progression-free survival (PFS) were estimated according to the Kaplan–Meier method (17). The OS was measured from the date of start of chemotherapy to the date of death or the last follow-up. For PFS, documented disease recurrence was scored as an event. All analyses were performed using the SPSS statistical software (SPSS version 11.0 for Windows; SPSS Inc, Chicago, IL).

RESULTS

The clinical characteristics of the 22 patients are summarized in Table 1. Surgical resected primary tumor, incisional biopsy of metastatic lesion, exploratatory thoracotomy, transbronchial or percutaneous biopsy and cytological examination were positive in seven, five, two, six and two patients, respectively. Thus, the histological diagnosis was made based on examination of a large tumor sample in 14 (63.6%) of the 22 patients. The marked predominance of men and smokers in this study was consistent with the demographic features of our previous LCNEC studies (6–8). One patient with stage IIB received chemotherapy and was enrolled to this study, because surgical resection and definitive radiotherapy were not indicated in this patient because of his poor pulmonary function. Abnormally high serum levels of CEA, NSE and proGRP at the start of chemotherapy were found in 52.4% (11/21), 72.7% (16/22) and 52.4% (11/21) of the patients, respectively.

Table 1. Patient characteristics

Characteristics		*n*	%
Gender	Male	21	95
	Female	1	5
Age	Median (range)	67 (47–78)	
Smoking history	Yes	21	95
	No	1	5
Performance status	0	7	32
	1	14	64
	2	1	5
Clinical stage	IIB	1	4
	IIIA	1	5
	IIIB	5	23
	IV	8	36
	Post-operative recurrence	7	32
Prior treatment	None	14	64
	Surgery	7	32
	Surgery for brain metastasis	1	5
	Radiotherapy	3	14
Site of metastasis	None	7	32
	Brain	2	9
	Lung	3	14
	Liver	5	23
	Bone	4	18
	Lymph node	6	27
	Others	3	14

The chemotherapy regimens used were as follows: cisplatin (80 mg/m^2, day 1) and irinotecan (60 mg/m^2, days 1 and 8) ($n = 6$); cisplatin (60 mg/m^2, day 1) and irinotecan (60 mg/m^2, days 1, 8 and 15) ($n = 3$); carboplatin (AUC = 6, day 1) and paclitaxel (200 mg/m^2, day 1) ($n = 5$); cisplatin (80 mg/m^2, day 1) and paclitaxel (175 mg/m^2, day 1) ($n = 1$); paclitaxel alone (80 mg/m^2, weekly) ($n = 1$); cisplatin (80 mg/m^2, day 1) and vinorelbine (20 mg/m^2, days 1, 8 and 15) ($n = 1$); cisplatin (25 mg/m^2, days 1, 8 and 15) and docetaxel (20 mg/m^2, days 1, 8 and 15) ($n = 1$); carboplatin (AUC = 5, day 1) and etoposide (100 mg/m^2, days 1–3) ($n = 3$); cisplatin (80 mg/m^2, day 1) and etoposide (100 mg/m^2, days 1–3) ($n = 1$). The median number of chemotherapy cycles was three (range, 1–5). One complete response and 12 partial responses were noted in the 22 patients, yielding an overall response rate of 59.1% (95% CI, 38.1–80.1) (Table 2). An objective response was obtained in five of the nine patients (55.6%) receiving irinotecan and five of the seven patients (71.4%) receiving paclitaxel. The toxicities related to these treatments were, in general, acceptable. Two patients received gefitinib after failure of the first-line chemotherapy, but none of them achieved an objective response. The overall PFS, median OS and 1-year survival rate of all the patients were 4.1 months (95% CI, 3.1–5.1), 10.3 months (95% CI, 5.8–14.8) and 43.3% (95% CI, 21.0–65.6), respectively (Fig. 1). The median OS of the patients treated with irinotecan or paclitaxel was 10.3 months (95% CI, 0–21.8), and the 1-year survival rate of these patients was 47.6% (95% CI, 20.4–74.8).

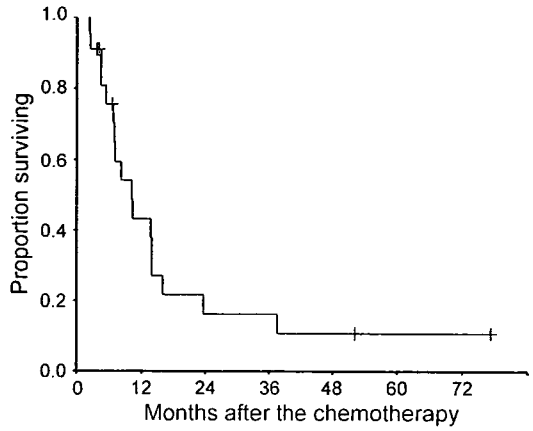

Figure 1. Kaplan–Meier curve for overall survival ($n = 22$). The median survival time was 10.3 months, and the 1- and 2-year survival rates were 43.3 and 16.2%, respectively.

DISCUSSION

In this study, the histological diagnosis of LCNEC was based on examination of a large tumor sample in 14 (63.6%) of the 22 patients, based on biopsies or cytological specimens in the remaining patients (36.4%). Numerous studies have demonstrated that the diagnosis of LCNEC is possible from biopsies or cytological specimens if a sufficient number of tumor cells can be obtained (8,18–21). To establish the pathological diagnosis of LCNEC in this series, we performed a pathological review of the biopsy and cytology specimens, because it was difficult to obtain large specimens of the tumor in these patients with advanced cancer treated medically.

We previously reported a response rate of 64% in 14 chemo-naïve patients with LCNEC who received cisplatin plus mitomycin, vindesine, or etoposide (8). In that study, however, patients with a diagnosis of poorly differentiated adenocarcinoma, poorly differentiated squamous cell carcinoma, large cell carcinoma and small cell carcinoma were selected, and then a diagnosis of LCNEC was made retrospectively by reviewing autopsy or surgically resected specimens. Thus, they were not consecutive, but highly selected patients. This explains, at least partly, the high response rate in the previous study. On the other hand, in the current study we analysed consecutive patients with a diagnosis of LCNEC that is established before treatment.

Rossi et al. showed that objective responses were observed in six (50%) of 12 patients with metastatic LCNEC who received a platinum and etoposide regimen, while no response was obtained in 15 patients receiving regimens for NSCLC treatment (cisplatin and gemcitabine in 10 patients, gemcitabine alone in two patients, and carboplatin and paclitaxel in three patients) (22). In addition, the patients receiving the platinum and etoposide regimen had a significantly better survival than the patients who received the other regimens (median survival time, 51 months versus 21 months). These survival data, however, sound too good for lung cancer patients with a metastatic disease. Neither patient characteristics nor explanation for

Table 2. Chemotherapy regimens and responses

Regimens		No. of patients	CR/PR/SD/PD	Response rate (%)
CPT-11-based	CDDP + CPT-11	9	0/5/3/1	55.6
PTX-based	CBDCA + PTX	5	0/3/2/0	60.0
	CDDP + PTX	1	1/0/0/0	–
	PTX	1	0/1/0/0	–
VNR-based	CDDP + VNR	1	0/1/0/0	–
DTX-based	CDDP + DTX	1	0/1/0/0	–
ETP-based	CBDCA + ETP	3	0/0/3/0	0
	CDDP+ ETP	1	0/1/0/0	–
Total		22		59.1

CPT-11, irinotecan; PTX, paclitaxel; VNR, vinorelbine; DTX, docetaxel; ETP, etoposide; CDDP, cisplatin; CBDCA, carboplatin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

such a long survival was presented in this report (22). Another case series of LCNEC showed that three patients with a stage IV disease received platinum-based chemotherapy (cisplatin and etoposide, carboplatin and gemcitabine, and cisplatin, docetaxel and gemcitabine) but none of them achieved an objective response. Of five patients who received gefitinib as salvage therapy, one achieved a partial response (23).

In this study, the clinical response rates of LCNEC to chemotherapy regimens containing irinotecan or paclitaxel were as high as 70%. The published response rates of NSCLC and SCLC to these regimens are 30–33% and 68–84%, respectively (10–14). The PFS of 4.1 months and median OS of 10.3 months were comparable to the results of previous randomized phase III trials that have reported PFS values of 4.1–6.9 months and median OS values of 9.3–12.8 months in extensive-stage disease SCLC (14). Thus, the response rate and survival of LCNEC were comparable with those of SCLC. Although our retrospective review of clinical data revealed heterogeneous approaches in treatment regimens, our results suggested that irinotecan and paclitaxel may be active agents against LCNEC. LCNEC exhibit both features of NSCLC and SCLC in terms of the morphology and immnohistochemistry, and these anti-cancer agents are effective against both of these types of lung cancer. Considered together, the combinations of cisplatin and irinotecan, and carboplatin and paclitaxel may be promising regimens for LCNEC.

To evaluate the efficacy of irinotecan- or paclitaxel-based combined chemotherapy for LCNEC, it is necessary to perform prospective phase II trials. However, such trials for LCNEC may be difficult to perform for the following reasons. First, patient accrual is problematic because LCNEC is a relatively rare tumor and accounts for only about 3% of lung cancer patients treated by surgical resection (6). It took us 7 years to accumulate 22 patients with LCNEC treated with chemotherapy. Besides, some studies have revealed the efficacy of adjuvant chemotherapy for both SCLC and NSCLC (24–26). Thus, when patients treated with platinum-based adjuvant chemotherapy regimens are excluded, few subjects with LCNEC with the diagnosis confirmed based on examination of large tumor specimens may remain. Therefore, these trials may only be possible as multi-institutional studies. Second, because it can sometimes be difficult to define the histology of LCNEC without examination of specimens large enough to appreciate the histological architecture and obtain reproducibility, pathological review by experts panel would be needed in these trials.

In conclusion, our results showed that irinotecan- or paclitaxel-based regimens may be as active against LCNEC as that against SCLC. A phase II multi-institutional trial is under way in Japan to elucidate the efficacy of cisplatin- and irinotecan-based therapy regimens against LCNEC.

Acknowledgment

We would like to thank Mika Nagai for her assistance with the preparation of the manuscript.

Conflict of interest statement

None declared.

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Tohoku J. Exp. Med., 2007, **213**, 17-23

Review

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

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

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

Received June 13, 2007; revision accepted for publication July 11, 2007.
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diagnosis or by the time recurrence is detected after surgery for local disease. These patients can be treated with systemic chemotherapy, but the efficacy of currently available anticancer agents is limited to the extent that patients with advanced disease rarely live long. Therefore, new chemotherapeutic agents continue to be developed against lung cancer (Sekine and Saijo 2000).

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

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

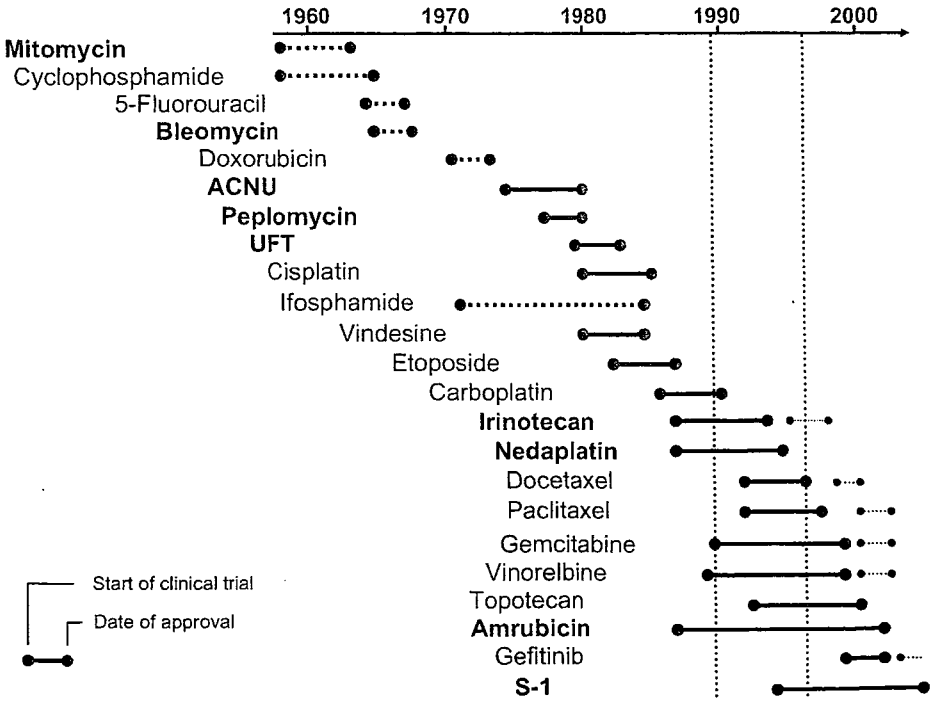

Fig. 1. Anticancer drugs approved for lung cancer in Japan.
Bold: original to Japan. Dotted line: case series studies, solid thick line: investigational new drug phase I-II trials for approval, and dotted thin line: post-marketing sponsored phase III trials. Vertical dotted lines indicate the year when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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The infrastructure and human resources to support clinical trials

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

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

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

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

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

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

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

Acknowledgments

We thank Yuko Yabe and Mika Nagai for kindly preparing this manuscript.

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