Table 1 Kinetic parameters for ATP

The autophosphorylation reaction was performed using the indicated enzyme and gefitinib (0.5–5 nM). The steady-state kinetic parameters for ATP were determined from the Eadie–Hofstee plot in Figure 5. Results are means ± S.D. for three independent duplicate experiments.

		K_m (μM)				V_{max} (μM · min^{-1})			
		Deletion mutant		Wild-type		Deletion mutant		Wild-type	
Gefitinib (nM)	EGF stimulation...	−	+	−	+	−	+	−	+
0		2.5 ± 0.2	2.2 ± 0.2	4.0 ± 0.3	1.9 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	2.0 ± 0.0	1.9 ± 0.0
0.5		5.6 ± 0.5	5.7 ± 0.4	4.1 ± 0.4	2.3 ± 0.1	1.9 ± 0.1	1.9 ± 0.2	2.0 ± 0.1	1.9 ± 0.1
1		9.8 ± 2.8	10.9 ± 3.0	4.6 ± 1.2	2.5 ± 0.1	2.0 ± 0.1	1.9 ± 0.0	2.0 ± 0.2	1.8 ± 0.1
5		26.1 ± 5.4	30.2 ± 4.2	7.0 ± 2.3	4.9 ± 0.9	1.9 ± 0.1	1.8 ± 0.2	2.0 ± 0.1	1.8 ± 0.2

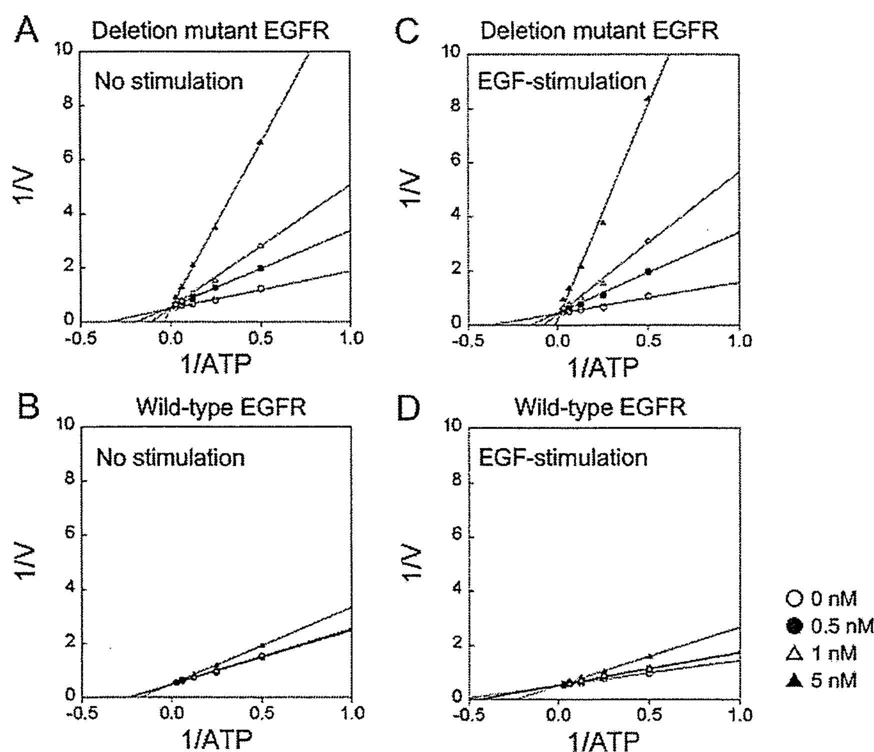

Figure 4 Mechanism of inhibition of deletion mutant EGFR by gefitinib

Autophosphorylation of unstimulated deletion mutant (**A**), unstimulated wild-type (**B**), EGF-stimulated deletion mutant (**C**) and EGF-stimulated wild-type (**D**) EGFR was measured with or without gefitinib at concentrations of 0 (○), 0.5 (●), 1 (△) and 5 (▲) nM. Reciprocal velocity against reciprocal ATP concentrations (0.5–32 μM) were plotted. Data are representative of at least three independent experiments.

low level of EGF-independent basal phosphorylation, whereas autophosphorylation using EGF-stimulated EGFR represents EGF-induced phosphorylation.

Kinetic parameters of autophosphorylation

The deletion mutant EGFR is constitutively phosphorylated under unstimulated conditions. Measuring the autophosphorylation activity of deletion mutant EGFR requires unphosphorylated tyrosine residues of EGFR. An autophosphorylation assay was reconstructed to determine the kinetic parameters of deletion mutant EGFR. The method is summarized in Figure 2. The concentrations of gefitinib used (2 μM) completely inhibited phosphorylation of both the deletion mutant and wild-type EGFR, as demonstrated by immunoblot analysis (Figure 1C). We performed autophosphorylation assays with various amounts of EGFR (results not shown). In our autophosphorylation assay, a constant amount of EGFR (130 ng/well) was adopted to measure its autophosphorylation, because this amount of EGFR was found to be appropriate for detecting changes in the absorbance of both wild-type and deletion mutant EGFR. The autophosphorylation of deletion mutant EGFR and wild-type EGFR was analysed by comparison with unstimulated and EGF-stimulated EGFR (Figure 3). The higher phosphorylation of deletion mutant EGFR shown in Figure 1(A) was lowered by using gefitinib-treated lysates, while the autophosphorylation reaction was initiated by addition of ATP. The ATP-dependent autophosphorylation reactions of deletion mutant EGFR and wild-type EGFR in crude cellular extracts were monitored (Figure 3, insets). The data were transformed into an Eadie–Hofstee plot, and the kinetic parameters were determined as apparent K_m and V_{max} values for ATP (Figure 3 and Table 1). Under unstimulated conditions,

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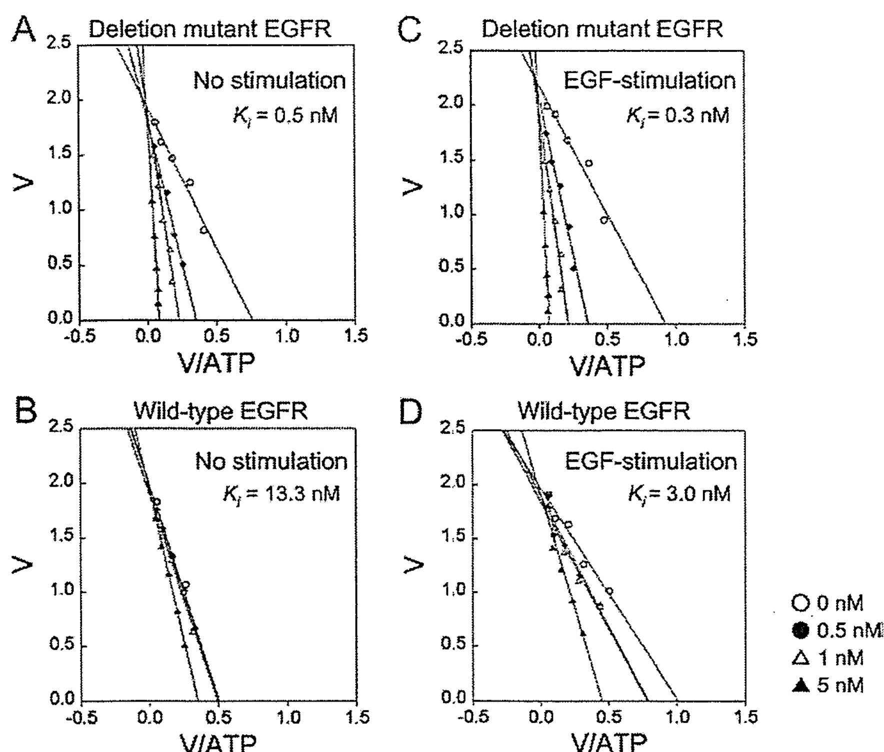

Figure 5 Inhibition constant of gefitinib for autophosphorylation activity of deletion mutant EGFR

The same dataset as shown in Figure 4 was fitted to an Eadie–Hofstee plot, and kinetic parameters from this fit are summarized in Table 1. Shown are the results for the unstimulated (**A**) and EGF-stimulated (**C**) deletion mutant EGFR and unstimulated (**B**) and EGF-stimulated (**D**) wild-type EGFR in response to ATP with or without gefitinib at concentrations of 0 (○), 0.5 (●), 1 (△) and 5 (▲) nM. Results are representative of at least three independent experiments.

differences in activities were seen between unstimulated wild-type (K_m for ATP = 4.0 ± 0.3 μM) and deletion mutant EGFR (K_m for ATP = 2.5 ± 0.2 μM). Under EGF-stimulated conditions, there was no difference in K_m values between EGF-stimulated wild-type EGFR (K_m for ATP = 1.9 ± 0.1 μM) and deletion mutant EGFR (K_m for ATP = 2.2 ± 0.2 μM). The V_{max} values of wild-type EGFR and deletion mutant EGFR were equal under both conditions. These results suggest that the wild-type EGFR is conformationally activated by EGF stimulation, and that the mutant EGFR is active without ligand stimulation.

Gefitinib inhibits autophosphorylation of deletion mutant EGFR

We examined the inhibitory effect of gefitinib (0.5, 1 and 5 nM) on the autophosphorylation of deletion mutant EGFR in comparison with wild-type EGFR under unstimulated and EGF-stimulated conditions. The data were transformed into a Lineweaver–Burk plot for estimation of the mode of inhibition (Figure 4). Lineweaver–Burk plot analysis showed that gefitinib competitively inhibited the autophosphorylation of deletion mutant EGFR as well as that of wild-type EGFR. The data were transformed into an Eadie–Hofstee plot for determination of kinetic parameters (Figure 5). Eadie–Hofstee plot analysis revealed the apparent K_m and V_{max} values for ATP in the presence of various gefitinib concentrations, and the kinetic parameters are summarized in Table 1. The K_i for deletion mutant EGFR and wild-type EGFR was calculated using eqn 1 (see the Materials and methods section). The K_i value of gefitinib for deletion mutant EGFR (K_i for gefitinib = 0.5 ± 0.1 nM) was 26-fold lower than that for wild-type EGFR (K_i for gefitinib = 13.3 ± 5.1 nM) under unstimulated conditions (Figure 5). Under EGF-stimulated conditions, the K_i value of gefitinib for deletion mutant EGFR (0.3 ± 0.1 nM) was 10-fold lower than that for wild-type EGFR (3.0 ± 0.6 nM) (Figure 5). Based on these comparative studies, we concluded that gefitinib binds deletion mutant EGFR more strongly than wild-type EGFR. In addition, we calculated the inhibitory effect of gefitinib for both types of EGFR in the presence of 2 μM ATP (Figure 6). Relatively strong inhibitory activity was detected for deletion mutant EGFR as compared with wild-type EGFR. These results suggest that gefitinib had a high affinity (low K_i value) for deletion mutant EGFR compared with wild-type EGFR.

DISCUSSION

Wild-type EGFR is unphosphorylated, being in an inactive form, under unstimulated conditions. The binding of ligands to the extracellular domain of EGFR induces dimerization and phosphorylation of the receptor into the active form [13]. The kinetic parameters of wild-type EGFR in our autophosphorylation assay are consistent with those of previous reports [14,15]. Crystallographic analysis has shown that the structure of the EGFR kinase domain after forming a complex with erlotinib exhibits a conformation consistent with the active form of protein kinases [16,17]. Previously, we reported that the deletion mutant EGFR was dimerized and phosphorylated constitutively without ligand stimulation, suggesting an active conformation [9]. We analysed the enzymatic properties of the deletion mutant EGFR, and

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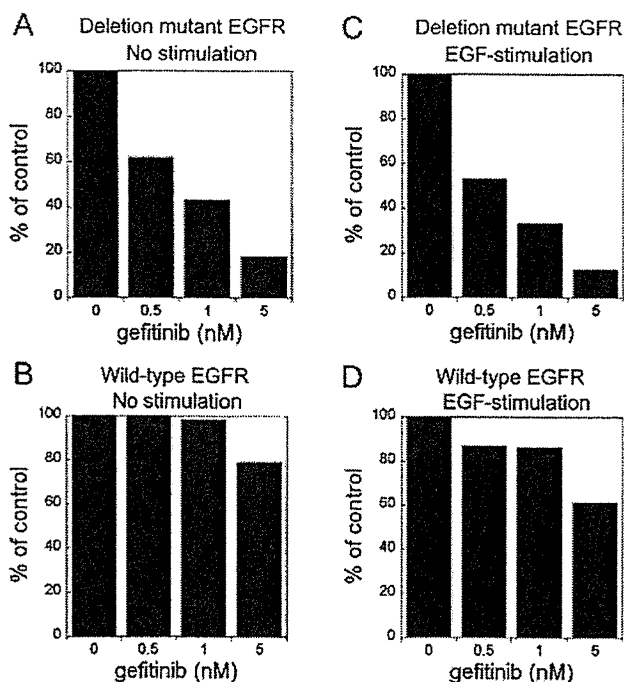

Figure 6 Effects of gefitinib on autophosphorylation of deletion mutant EGFR

The percentage of absorbance compared with the control under conditions of 2 μM ATP was calculated using the same dataset as shown in Figure 4 at a concentration of 2 μM ATP. The results shown are for unstimulated (**A**) and EGF-stimulated (**C**) deletion mutant EGFR and unstimulated (**B**) and EGF-stimulated (**D**) wild-type EGFR in response to ATP with or without gefitinib. Results are representative of at least three independent experiments.

determined the K_i value of gefitinib for deletion mutant EGFR. The inhibition constant of gefitinib for wild-type EGFR was similar to the value reported by Wakeling et al. [18]. We showed that the K_i value of gefitinib for deletion mutant EGFR was much lower than that for wild-type EGFR. The evidence of the decreased K_i value of gefitinib for deletion mutant EGFR means that gefitinib binds deletion mutant EGFR more strongly than wild-type EGFR. The high-affinity interaction between deletion mutant EGFR and gefitinib may be attributable to structural differences between deletion mutant EGFR and wild-type EGFR.

Our conclusion does not contradict the previous report by Stamos et al. [16] on a similar EGFR-targeted tyrosine kinase inhibitor, erlotinib, which binds to the active form of EGFR [14]. This result differs from that reported elsewhere: Fabian et al. [19] reported that there were no differences in the binding affinity of EGFR-targeted tyrosine kinase inhibitors between wild-type EGFR and mutant EGFR, including the deletion mutation. They constructed and expressed the kinase domain of EGFR on a bacteriophage surface, followed by interaction with immobilized inhibitors using biotin–avidin systems. Conversely, in our experiments, we performed autophosphorylation assays with EGFR extracted from 293-pΔ15 and the 293-pEGFR cells overexpressing deletion mutant and wild-type EGFR respectively. We consider our cell-based autophosphorylation assay results to reflect the native state of deletion mutant EGFR and to possibly explain the hypersensitivity of mutant-expressing cells to gefitinib.

We demonstrated that the deletion mutant actually binds gefitinib more strongly than wild-type EGFR. This is likely to be the mechanism of action of other tyrosine kinase inhibitors such as erlotinib, ZD6474 [dual inhibitor targeted to VEGFR2 (vascular endothelial growth factor receptor 2)/KDR (kinase insert domain-containing receptor) and EGFR] and other possible multi-targeted tyrosine kinase inhibitors. Indeed, EGFR-specific tyrosine kinase inhibitors AG1478 and erlotinib, as well as ZD6474, as described in our previous report [7] showed different growth-inhibitory activities against HEK-293 transfected with deletion mutant EGFR (results not shown). Thus it is likely that these (ATP competitive) tyrosine kinase inhibitors have different binding property effects on wild-type and deletion mutant EGFR to those of gefitinib.

In the present study, we focused on the enzymatic properties of in-frame deletion mutant EGFR (delE746–A750). The inhibition of receptor autophosphorylation in deletion mutant EGFR by gefitinib was much greater than that in wild-type EGFR. Next, it is necessary to examine the kinetic properties of other types of EGFR mutants, especially L858R, and these findings may pave the way for the discovery of different kinase inhibitors with different inhibition profiles for EGFR.

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REFERENCES

1 Arteaga, C. L. (2003) ErbB-targeted therapeutic approaches in human cancer. Exp. Cell Res. **284**, 122–130

2 Traxler, P., Furet, P., Mett, H., Buchdunger, E., Meyer, T. and Lydon, N. (1997) Design and synthesis of novel tyrosine kinase inhibitors using a pharmacophore model of the ATP-binding site of the EGF-R. J. Pharm. Belg. **52**, 88–96

3 Shepherd, F. A., Rodrigues Pereira, J., Ciuleanu, T., Tan, E. H., Hirsh, V., Thongprasert, S., Campos, D., Maoleekoonpiroj, S., Smylie, M., Martins, R. et al. (2005) Erlotinib in previously treated non-small-cell lung cancer. N. Engl. J. Med. **353**, 123–132

4 Bell, D. W., Lynch, T. J., Haserlat, S. M., Harris, P. L., Okimoto, R. A., Brannigan, B. W., Sgroi, D. C., Muir, B., Riemenschneider, M. J., Iacona, R. B. et al. (2005) Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. J. Clin. Oncol. **23**, 8081–8092

5 Lynch, T. J., Bell, D. W., Sordella, R., Gurubhagavatula, S., Okimoto, R. A., Brannigan, B. W., Harris, P. L., Haserlat, S. M., Supko, J. G., Haluska, F. G. et al. (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N. Engl. J. Med. **350**, 2129–2139

6 Paez, J. G., Janne, P. A., Lee, J. C., Tracy, S., Greulich, H., Gabriel, S., Herman, P., Kaye, F. J., Lindeman, N., Boggon, T. J. et al. (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science **304**, 1497–1500

7 Arao, T., Fukumoto, H., Takeda, M., Tamura, T., Saijo, N. and Nishio, K. (2004) Small in-frame deletion in the epidermal growth factor receptor as a target for ZD6474. Cancer Res. **64**, 9101–9104

8 Tracy, S., Mukohara, T., Hansen, M., Meyerson, M., Johnson, B. E. and Janne, P. A. (2004) Gefitinib induces apoptosis in the EGFRL858R non-small-cell lung cancer cell line H3255. Cancer Res. **64**, 7241–7244

9 Sakai, K., Arao, T., Shimoyama, T., Murofushi, K., Sekijima, M., Kaji, N., Tamura, T., Saijo, N. and Nishio, K. (2005) Dimerization and the signal transduction pathway of a small in-frame deletion in the epidermal growth factor receptor. FASEB J. **20**, 311–313

10 Nishio, K., Arioka, H., Ishida, T., Fukumoto, H., Kurokawa, H., Sata, M., Ohata, M. and Saijo, N. (1995) Enhanced interaction between tubulin and microtubule-associated protein 2 via inhibition of MAP kinase and CDC2 kinase by paclitaxel. Int. J. Cancer **63**, 688–693

11 Kawamura-Akiyama, Y., Kusaba, H., Kanzawa, F., Tamura, T., Saijo, N. and Nishio, K. (2002) Non-cross resistance of ZD0473 in acquired cisplatin-resistant lung cancer cell lines. Lung Cancer **38**, 43–50

12 Koizumi, F., Shimoyama, T., Taguchi, F., Saijo, N. and Nishio, K. (2005) Establishment of a human non-small cell lung cancer cell line resistant to gefitinib. Int. J. Cancer **116**, 36–44

13 Tanner, K. G. and Kyte, J. (1999) Dimerization of the extracellular domain of the receptor for epidermal growth factor containing the membrane-spanning segment in response to treatment with epidermal growth factor. J. Biol. Chem. **274**, 35985–35990

14 Nair, N., Davis, R. J. and Robinson, H. L. (1992) Protein tyrosine kinase activities of the epidermal growth factor receptor and ErbB proteins: correlation of oncogenic activation with altered kinetics. Mol. Cell. Biol. **12**, 2010–2016

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15 Wood, E. R., Truesdale, A. T., McDonald, O. B., Yuan, D., Hassell, A., Dickerson, S. H., Ellis, B., Pennisi, C., Horne, E., Lackey, K. et al. (2004) A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. Cancer Res. **64**, 6652–6659
16 Stamos, J., Sliwkowski, M. X. and Eigenbrot, C. (2002) Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. J. Biol. Chem. **277**, 46265–46272
17 Noble, M. E., Endicott, J. A. and Johnson, L. N. (2004) Protein kinase inhibitors: insights into drug design from structure. Science **303**, 1800–1805
18 Wakeling, A. E., Guy, S. P., Woodburn, J. R., Ashton, S. E., Curry, B. J., Barker, A. J. and Gibson, K. H. (2002) ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. Cancer Res. **62**, 5749–5754
19 Fabian, M. A., Biggs, 3rd, W. H., Treiber, D. K., Atteridge, C. E., Azimioara, M. D., Benedetti, M. G., Carter, T. A., Ciceri, P., Edeen, P. T., Floyd, M. et al. (2005) A small molecule-kinase interaction map for clinical kinase inhibitors. Nat. Biotechnol. **23**, 329–336

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Original Articles

Pharmacokinetics and Pharmacodynamics of Weekly Epoetin Beta in Lung Cancer Patients

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Background: To assess the pharmacokinetic profile and time-course of trough concentrations and hemoglobin levels associated with subcutaneous weekly administration of epoetin beta in lung cancer patients with chemotherapy-induced anemia.
Methods: Epoetin beta was subcutaneously administered to 15 anemic lung cancer patients once weekly for 8 weeks at doses of 9000, 18 000 and 36 000 IU. Pharmacokinetic parameters (C_{max}, AUC_{inf} and $T_{1/2}$) were determined after the first single dose administration on a model-independent basis, and the relationship between the dose and these parameters was examined for linearity.
Results: Weekly administration of epoetin beta at 9000, 18 000 and 36 000 IU produced C_{max} values of 308 ± 117 (mean ± standard deviation), 678 ± 86.7 and 1316 ± 766 mIU/ml, and AUC_{inf} values of 15 300 ± 9524, 54 574 ± 16 265 and 88 501 ± 55 687 hr mIU/ml, respectively, showing dose-proportional increases. Trough concentrations tended to increase in the presence of severe bone marrow suppression induced by chemotherapy or other factors. Extremely high values were seen in three patients, but there was no apparent trend toward an increase with repeated doses. After 8 weeks' administration at 9000, 18 000 and 36 000 IU, hemoglobin levels were changed by −0.37 ± 1.26, 2.15 ± 1.36 and 2.82 ± 2.17 g/dl, respectively.
Conclusions: Epoetin beta exhibited linear pharmacokinetics when administered to anemic cancer patients at weekly doses of 9000–36 000 IU and did not cause drug accumulation. Hemoglobin levels increased with weekly doses of 18 000 or 36 000 IU.

Key words: anemia – epoetin beta – pharmacokinetics

INTRODUCTION

Cancer patients receiving multicycle chemotherapy and radiotherapy frequently develop anemia, with one clinical study reporting that hemoglobin levels fell to 8–12 g/dl in 75% of patients undergoing these therapies (1). Among patients undergoing chemotherapy, anemia with hemoglobin levels of <8.0 g/dl reportedly occurs in 50–60% of ovarian cancer, lung cancer, non-Hodgkin's malignant lymphoma or multiple myeloma patients (2).

The etiology of chemotherapy-induced anemia includes the following: myelosuppression of chemotherapy or radiotherapy, reduced production of the bone-marrow-stimulating hormone erythropoietin (EPO), diminished bone marrow response to EPO and cancer cell-induced immune system activation resulting in reduced iron availability (3).

EPO, a hematopoietic hormone mainly produced in the kidneys, acts on erythroblastic precursor cells to promote differentiation and proliferation of erythrocytes and disappears in the bone marrow and spleen. Epoetin beta is a human EPO preparation that is mass-produced by recombinant gene technology and is commonly used in treatment of patients with renal failure-induced anemia. In Europe and the United States, it has already been approved and has also been administered to cancer patients with anemia with demonstrated effects in reducing required blood transfusion volumes, elevating hemoglobin concentrations and improving quality of life (QOL) (4,5). Furthermore, in the US, the American Society of

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Hematology and the American Society of Clinical Oncology jointly issued clinical practice guidelines in 2002 for the use of EPO preparations (6). Thus, the general use of epoetin in anemic cancer patients has been advocated. Meanwhile, in Japan, EPO preparation has not been approved for cancer patients with anemia, but clinical trials are now in progress.

Despite the increasing usage of epoetin, its pharmacokinetics have not been adequately investigated at high, once-weekly doses of 30 000 or 40 000 IU that are typically administered subcutaneously to cancer patients with anemia (7). To the best of our knowledge, the literature contains no pharmacokinetic data for epoetin beta in patients with cancer-related or chemotherapy-induced anemia, and the effect of the chemotherapy on serum EPO concentrations was not clear. We therefore studied the pharmacokinetic profile and time-course of trough concentrations and hemoglobin levels associated with subcutaneous weekly administration of epoetin beta in lung cancer patients with chemotherapy-induced anemia.

PATIENTS AND METHODS

Patients

Inclusion criteria were as follows: (i) histological or cytological confirmation of lung cancer diagnosis; (ii) treated with cyclic chemotherapy; (iii) aged between 20 and 79 years; (iv) life expectancy of at least 2 months; (v) anemia (hemoglobin level of ≤11.0 g/dl) considered to be primarily chemotherapy-induced; and (vi) adequate renal and hepatic function.

Exclusion criteria included (i) iron deficiency (Mean corpuscular volume ≤80μm^3 or iron saturation [{Fe/(Fe+ Unsaturated iron-binding capacity)} × 100] ≤ 15.0%); (ii) blood cell transfusion in the 4 weeks prior to the study; (iii) rHuEPO therapy in the 4 weeks prior to the study; (iv) documented hemorrhagic lesion; (v) pregnancy, breastfeeding or not using adequate birth control measures; (vi) history of myocardial, pulmonary or cerebral infarction, serious drug allergy, uncontrolled hypertension, hypersensitivity to any EPO preparation, any serious complication; and (vii) a primary hematologic disorder as the cause of the present anemia.

The protocol was approved by the institutional review board of the National Cancer Center Hospital, and written informed consent was obtained from all patients who participated in the study.

Study Design

This was an open-label, single-arm, dose-escalation study. Patients were assigned sequentially to one of three groups, receiving epoetin beta at either 9000, 18 000 or 36 000 IU per patient. This was administered by weekly subcutaneous injection for 8 weeks. If the patient's hemoglobin level recovered to 14 g/dl or higher, the treatment was stopped. Chemotherapy and radiotherapy were not performed from 7 days prior to until 4 days following the initial dose, and blood transfusion was not performed until 4 days after the initial dose. Oral iron supplementation (200 mg of ferrous sulfate) was administered daily. Blood samples for detection of epoetin beta antibody were collected before the first administration and 7 days after the last administration. Patients were followed for 1 week after the end of drug administration. Granulocyte colony-stimulating factor administration was allowed to the patients whose neutrophils count was <500 per cubic millimeter or those with neutropenic fever whose neutrophils count was <1000 per cubic millimeter.

Serum Assay

To determine the pharmacokinetic parameters, blood samples were collected immediately prior to and 6, 10, 24, 34, 48, 72, 96 and 168 h after the initial dose of epoetin beta. To investigate the time-course of trough concentrations, samples were also collected immediately prior to the administration of each dose.

Blood samples were allowed to stand at room temperature for ~30 min and then centrifuged at 4°C and 3000 rpm for ~10 min to separate the serum. The resulting serum was stored frozen at below −20°C until used for measurement of serum EPO concentrations.

Serum EPO concentrations were measured by the RIA method developed and validated by Chugai Pharmaceutical Co., Ltd., Tokyo, Japan. Validation of this assay revealed the following: quantification range, 6–384 mIU/ml; intra-assay precision (repeatability) and accuracy of 2.7–6.3% and −22.1 to −5.5%, respectively; and inter-assay precision (reproducibility) and accuracy of 2.4–7.6% and −18.1–3.0%, respectively. If the assayed value exceeded the upper limit of the quantification range (378 mIU/ml), the sample was diluted for re-measurement.

Pharmacokinetic Analysis

Since EPO is an endogenous substance, measurements of serum EPO concentration following the first administration were baseline corrected to account for the presence of endogenous EPO. The corrected values were then used to determine descriptive statistics for drug concentration at each blood sampling time-point and the pharmacokinetic parameters.

The following pharmacokinetic parameters were determined after the initial dose by using WinNonlin Pro v.3.3 (Pharsight Corporation, Mountain View, CA) in a model-independent manner: C_{max}, AUC_{inf} and $T_{1/2}$.

C_{max} was observed values. AUC_{inf} was calculated by the trapezoidal method with infinite extrapolation by dividing the last plasma concentration by the elimination rate constant (K_{el}). $T_{1/2}$ was calculated as $0.693/K_{el}$.

Trough concentrations were not baseline corrected.

Pharmacodynamic Analysis

Hemoglobin levels and platelet counts were assessed weekly.

Statistical Analysis

All statistical analyses were performed using SAS v. 8.2 (SAS Institute, Cary, NC). Descriptive statistics were not calculated if they were to be based on available data from less than half the subjects.

Analyses of dose linearity were performed for C_{max} and AUC_{inf}. Each analysis used the power model: $\log y = \alpha + \beta \cdot \log \text{dose}$, where β is the slope and y represents the pharmacokinetic parameter. Fitting a linear relationship between log y and log dose is an extension of the analysis of variance model. The key feature of the power model is the assumption of linearity between the log-transformed values of parameters and doses. The 95% confidential interval (CI) of the slope of the log-transformed parameters plotted against log dose was estimated, and dose-proportionality was concluded to be present if the 95% CI contained a slope with a value of 1.

RESULTS

Patients' Characteristics

Fifteen patients were enrolled in the study. Their characteristics are shown in Table 1. Participants were 8 men and 7 women, aged 30–78 years (median age, 69.0 years), who were being treated with chemotherapy (containing platinum in 12 cases). Four patients received prior radiation therapy (brain radiation in four cases and thoracic radiation in three cases). Ten patients had small cell carcinoma, four had adenocarcinoma and one had large cell carcinoma. Doses of 9000, 18 000 and 36 000 IU were administered to 3, 6 and 6 patients, respectively. Data from all 15 patients were included for evaluation of pharmacokinetic analysis and hemoglobin response. In all patients, the hemoglobin levels at the time of registration were <11.0 g/dl. Five patients discontinued this study for the following reasons: recovery of hemoglobin level to 14 g/dl or higher, $n = 1$ (36 000 IU); adverse effects (rotary vertigo), $n = 1$ (36 000 IU); withdrawal of consent, $n = 1$ (9000 IU); and disease progression, $n = 2$ (18 000 IU, 36 000 IU).

Pharmacokinetics Analysis

The mean baseline serum EPO concentration across all patients was 77.3 mIU/ml, with a median value of 59.9 mIU/ml, a minimum of 23.6 mIU/ml and a maximum of 301 mIU/ml. The 9000 IU group showed the highest mean, attributable to an extremely high value of 301 mIU/ml in one patient (Table 1).

The time-courses of the mean serum drug concentrations by dose group are shown in Fig. 1, and a summary of the pharmacokinetic parameters are given in Table 2.

The power model gave 95% CI of the slope (β) of the C_{max}-dose and AUC_{inf}-dose curves of 0.551–1.388 and 0.532–1.753, respectively, both including '1'.

Table 1. Patients' characteristics

Characteristic	Item	Total	9000 IU	18 000 IU	36 000 IU
Sex	Male	8	1	3	4
	Female	7	2	3	2
Histology	Small cell	10	3	4	3
	Large cell	1	0	1	0
	Adenocarcinoma	4	0	1	3
ECOG* performance status	0	3	1	1	1
	1	12	2	5	5
Prior chemotherapy	None	2	0	1	1
	Non plantinum based	1	0	0	1
	Plantinum based	12	3	5	4
Age (years)	Median	69.0	78.0	69.5	68.0
	Range	30–78	53–78	54–75	30–71
Hemoglobin** (g/dl)	Mean	9.4	9.1	9.2	9.8
	Range	6.8–11.4	6.8–11	7.5–10.3	7.1–11.4
Serum Fe (μg/dl)	Mean	76.8	111.3	69.5	66.8
	Range	17–154	45–154	37–148	17–106
Serum ferritin (ng/ml)	Mean	371.9	533.8	254.8	408.0
	Range	68.3–786	68.3–786	99.7–509.7	79.6–608.8
Serum endogenous erythropoietin (mIU/ml)	Mean	77.3	122.7	70.9	60.1
	Range	23.6–301	26.9–301	23.6–158	41.5–74.1

*Eastern Cooperative Oncology Group.
**The hemoglobin levels show the values just before the first administration of erythropoietin.

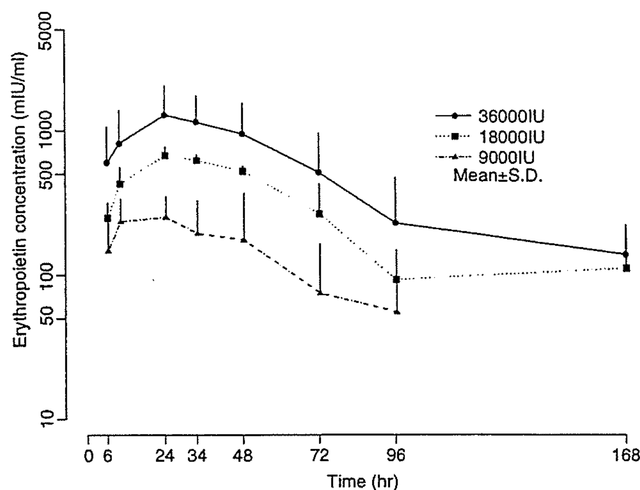

Figure 1. Time-course of mean serum drug concentrations of erythropoietin in each dose group following first dose. The mean drug concentrations for each group changed in a parallel manner up to 96 h.

Table 2. Summary of descriptive statistics for pharmacokinetic parameters of erythropoietin following the first dose

PK parameter	Unit	9000 IU	18 000 IU	36 000 IU
		$n = 3$ (Mean ± SD)	$n = 6$ (Mean ± SD)	$n = 6$ (Mean ± SD)
C_{max}	mIU/ml	308 ± 117	678 ± 86.7	1316 ± 766
AUC_{inf}	hr·mIU/ml	15 300 ± 9524	54 574 ± 16 265	88 501 ± 55 687
$T_{1/2}$	hr	24.5 ± 18.1	43.6 ± 22.0	30.4 ± 22.1

C_{max} and AUC_{inf} increased in an almost dose-proportional manner, whereas $T_{1/2}$ was constant.

Trough Concentrations

Time-courses of trough concentrations are shown by dose group in Fig. 2. Considerable variations in trough concentration occurred over the 8 week period. EPO concentration did not increase with repeated doses of epoetin beta, suggesting that drug accumulation did not occur. In some patients, trough concentrations were extremely high after chemotherapy (Fig. 3).

Relationship of Trough Concentration with Bone Marrow Suppression

Time-courses of trough concentrations, hemoglobin levels and platelet counts in the three patients with markedly elevated trough concentrations are shown in Fig. 3. In these patients, hemoglobin level and platelet count fell during the period in which trough concentration increased rapidly.

Pharmacodynamics Results

The time-course of mean hemoglobin levels is shown in Fig. 4. Hemoglobin levels were unchanged at a dose of 9000 IU, but tended to increase at doses of 18 000 and 36 000 IU. At 8 weeks, the change of hemoglobin levels from baseline was −0.37 ± 1.26 g/dl in the 9000 IU group, 2.15 ± 1.36 g/dl in the 18 000 IU group and 2.82 ± 2.17 g/dl in the 36 000 IU group. One patient receiving 9000 IU and two patients receiving 18 000 IU underwent blood cell transfusion. Only one patient (who received 36 000 IU weekly) exceeded predetermined threshold levels of hemoglobin for discontinuation of the study.

Safety

Once-weekly dosing of epoetin beta was well tolerated in all study patients, with no life-threatening toxic effects occurring during the trial. Leucopenia was the most frequent adverse event (13 of 15), followed by nausea (9 of 15). Other frequent adverse events were anorexia (7 of 15), diarrhea (7 of 15), thrombocytopenia (6 of 15), alopecia (5 of 15), fatigue (5 of 15), constipation (4 of 15), elevated serum lactate dehydrogenase (4 of 15), insomnia (3 of 15), dizziness (3 of 15), vomiting (3 of 15), back pain (3 of 15) and elevated aspartate aminotransferase (3 of 15). These adverse events are typical for this patient population receiving chemotherapy, and none occurred in an epoetin dose-dependent manner. Adverse events possibly associated with epoetin beta occurred in six patients, and these events were manageable. These adverse events consisted of grade 3 hypertension and vertigo, grade 2 increased bilirubin, constipation and hyperkalemia and grade 1 headache, nausea, vomiting, insomnia, diarrhea, mouth dryness, fatigue, neck pain, rash, hyperventilation, cardiomegaly, hyperkalemia, hyponatremia, increased phosphorus and increased asparatate aminotransferase. Only one patient in the 9000 IU cohort showed grade 3 hypertension from the 7th day of the first administration to the 65th day. One serious adverse event (rotary vertigo) occurred in a patient (a 31-year-old woman); it remitted after around 2 weeks and resolved after 5 weeks. This event was considered by the investigator to be related to epoetin beta, and the patient therefore discontinued the study. No antibodies to epoetin beta were detected.

DISCUSSION

Serum EPO levels are reported to be higher in cancer patients than in healthy adults (8). The results of this study were in accordance with this, showing higher baseline serum EPO concentrations in patients than in healthy adults (8.40 ± 3.82, 8.62 ± 5.83 mIU/ml) (9) or renal anemia patients (23.05 ± 16.63 mIU/ml) (10). In addition, serum EPO concentrations in cancer patients exhibited wide variation, from typical levels in healthy adults to extremely high levels. Overall, this suggests that the predose endogenous EPO exhibited high mean serum levels and wide individual differences in cancer patients with anemia.

In the present study, we have investigated the pharmacokinetic characteristics of epoetin beta after the initial dose of 9000, 18 000 and 36 000 IU and have studied the time-course

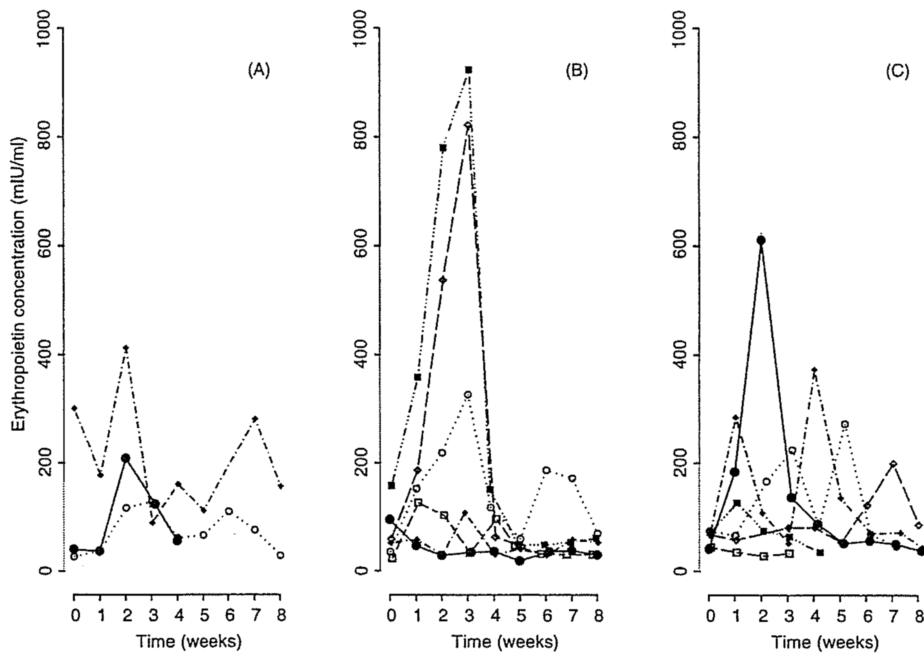

Figure 2. Time-course of trough concentrations of erythropoietin in each dose group. (A) 9000 IU, (B) 18 000 IU, (C) 36 000 IU. Trough concentrations of erythropoietin did not increase with repeated doses of epoetin beta, suggesting that drug accumulation did not occur.

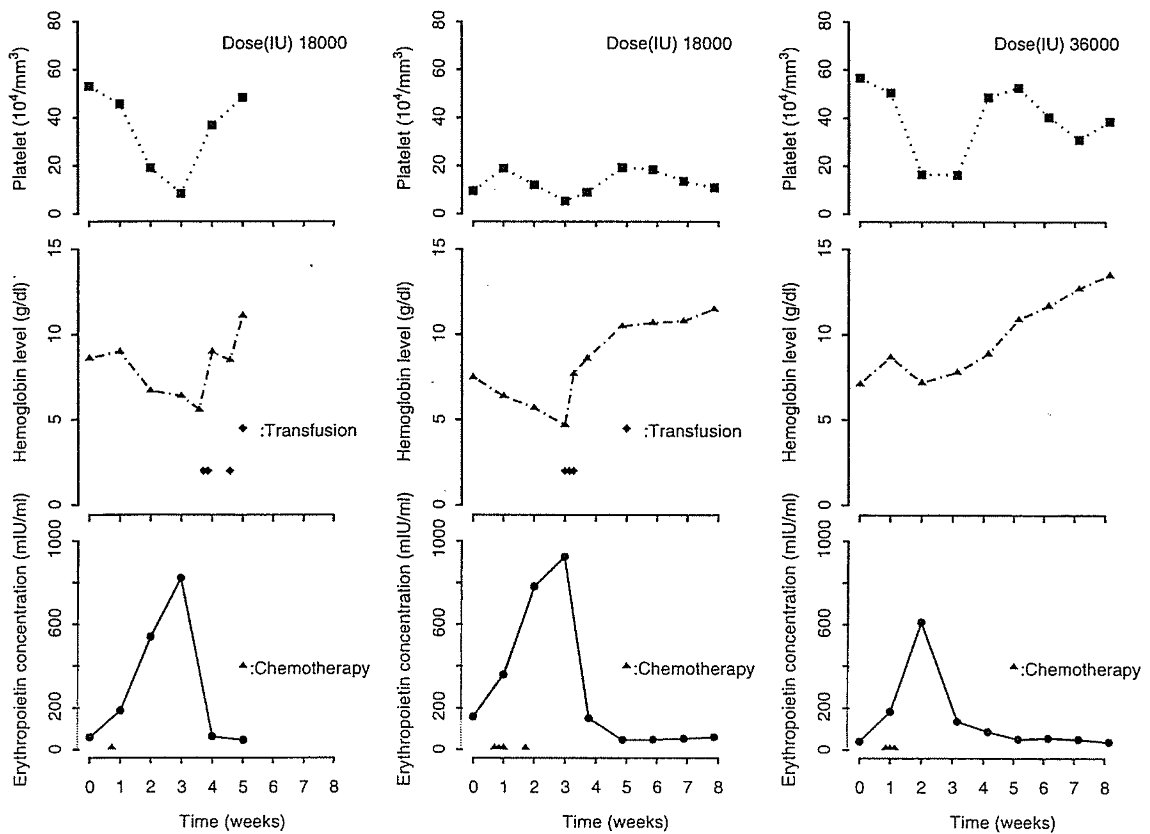

Figure 3. Time-course of trough concentrations of erythropoietin, hemoglobin levels and platelet counts in three patients with extremely high trough concentrations. The elevation of trough concentration is correlated with decrease of platelet counts and Hb levels, which may be associated with bone marrow suppression.

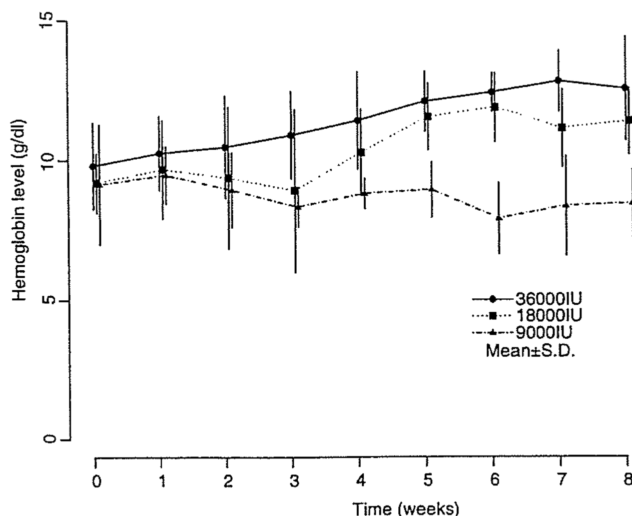

Figure 4. Time-course of mean hemoglobin levels in each dose group. Hemoglobin levels were unchanged at a dose of 9000 IU, but tended to increase at doses of 18 000 and 36 000 IU.

of trough concentrations after once-weekly repeated dose subcutaneous administration in anemic lung cancer patients. The study provides evidence that epoetin beta has almost linear, dose-dependent pharmacokinetics following subcutaneous administration at doses of 9000–36 000 IU in cancer patients.

During the period of once-weekly administrations of epoetin beta, trough concentrations transiently increased after cancer chemotherapy in many patients, but did not appear to continue to increase with repeated administration of epoetin beta. Some patients showed extremely high trough concentrations that were correlated with periods of marked thrombocytopenia. Increases in trough concentrations may be associated with bone marrow suppression, and this finding is in agreement with reports showing that busulfan-induced bone marrow ablation increases serum EPO concentrations (11) and that chemotherapy increases EPO concentrations in patients with leukemia (12,13). Jelkmann reported that elimination of EPO occurs mainly in bone marrow (14). It is conceivable that the function of bone marrow could be damaged by chemotherapeutic agents after chemotherapy. Elimination of EPO could decrease in the damaged bone marrow, thereby the trough levels of EPO could increase.

At 8 weeks, mean changes in hemoglobin levels from baseline were -0.37 ± 1.26, 2.15 ± 1.36 and 2.82 ± 2.17 g/dl for 9000, 18 000 and 36 000 IU, respectively. Hemoglobin levels increased with repeated doses of 18 000 IU or more. A dose-finding study conducted by Sakai et al. (15) in Japanese patients with lung cancer or malignant lymphoma revealed a similar pattern of hemoglobin change (0.04 ± 1.98, 1.04 ± 1.75 and 1.75 ± 2.15 g/dl for 9000, 18 000 and 36 000 IU doses of epoetin beta) and concluded that the recommended dose was 36 000 IU in chemotherapy-induced anemic patients. Taken together, these results suggest that epoetin beta is sufficiently effective for cancer patients with anemia.

In conclusion, subcutaneous administration of epoetin beta at doses of 9000–36 000 IU in cancer patients with anemia yielded pharmacokinetic linearity, with no drug accumulation caused by repeated doses. Epoetin beta administration at 18 000 IU or higher is therefore anticipated to raise hemoglobin levels without compromising safety.

References

1. Groopman JE, Itri LM. Chemotherapy-induced anemia in adults: incidence and treatment. *J Natl Cancer Inst* 1999;91:1616–34.
2. Ludwig H. Epoetin in cancer-related anaemia. *Nephrol Dial Transplant* 1999;14[Suppl 2]:85–92.
3. Beguin Y. Prediction of response and other improvements on the limitations of recombinant human erythropoietin therapy in anemic cancer patients. *Haematologica* 2002;87:1209–21.
4. Demetri GD, Kris M, Wade J, Degos L, Cella D. Quality-of-life benefit in chemotherapy patients treated with epoetin alfa is independent of disease response or tumor type: results from a prospective community oncology study. *Procrit Study Group J Clin Oncol* 1998;16:3412–25.
5. Cella D, Zagari MJ, Vandoros C, Gagnon DD, Hurtz HJ, Nortier JWR. Epoetin alfa treatment results in clinically significant improvements in quality of life in anemic cancer patients when referenced to the general population. *J Clin Oncol* 2003;21:366–73.
6. Rizzo JD, Lichtin AE, Woolf SH, Seidenfeld J, Bennett CL, Cella D, et al. Use of epoetin in patients with cancer: evidence-based clinical practice guidelines of the American Society of Clinical Oncology and the American Society of Hematology. *J Clin Oncol* 2002;20:4083–107.
7. Gabrilove JL, Cleeland CS, Livingston RB, Sarokhan B, Winer E, Einhorn LH. Clinical evaluation of once-weekly dosing of epoetin alfa in chemotherapy patients: improvements in hemoglobin and quality of life are similar to three-times-weekly dosing. *J Clin Oncol* 2001;19:2875–82.
8. Ozguroglu M, Arun B, Demir G, Demirelli F, Mandel NM, Buyukunal E, et al. Serum erythropoietin level in anemic cancer patients. *Medical Oncology* 2000;17:29–34.
9. Charuruks N, Limpanasithikul W, Voravud N, Sutheesophon K. Erythropoietin level and hematologic parameters in healthy adults. *J Med Assoc Thai* 2000;83:1267–73.
10. Yamazaki C, Watanabe Y, Sakamoto N. Pharmacokinetic study of recombinant human erythropoietin treatment in pre-dialysis end stage renal disease patients. *Japanese J Nephrol* 1993;35:1233–42.
11. Chapel S, Veng-Pedersen P, Hohl RJ, Schmidt RL, Mcguire EM, Widness JA. Changes in erythropoietin pharmacokinetics following busulfan-induced bone marrow ablation in sheep: evidence for bone marrow as a major erythropoietin elimination pathway. *J Pharamcol Exp Ther* 2001;298:820–4.
12. Piroso E, Allan JE, Jaime C. Inappropriate increase in erythropoietin titers during chemotherapy. *Am J Hematol* 1989;32:248–54.
13. Sawabe Y, Kikuno K, Iseki T, Iida S, Tabata Y, Yonemitsu H. Changes in serum erythropoietin and the reticulocyte count during chemotherapy for leukemias. *Eur J Haematol* 1996;57:384–8.
14. Jelkmann W. The enigma of the metabolic fate of circulating erythropoietin (Epo) in view of the pharmacokinetics of the recombinant drugs rhEpo and NESP. *Eur J Haematol* 2002;69:265–74.
15. Sakai H, Ohashi Y, Hirashima K, Saijo N. Japan Erythropoietin Study Group. Once weekly epoetin beta to increase hemoove quality of life in anemic cancer patients receiving chemotherapy. Meeting proceedings of the American Society of *Clinical Oncology* 2004;23:767.

Molecular Biology, Genomics, and Proteomics in Bronchioloalveolar Carcinoma

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Abstract: The charge of the Molecular Biology, Genomics, and Proteomics in Bronchioloalveolar Carcinoma Committee was to evaluate the molecular biology, genomic changes, and proteomic findings in patients with bronchioloalveolar carcinoma compared with other types of lung cancer. The literature was reviewed and unpublished information was presented by the committee members at the session. The molecular biology studies have included findings on epidermal growth factor receptor (*EGFR*) mutations, p53 mutations, K-*ras* mutations, and loss of heterozygosity. The genomic changes have mostly focused on the mRNA expression arrays as well as protein studies. The current state of knowledge was reviewed, the missing information was acknowledged, and proposals for future research were identified.

Key Words: Lung neoplasm, Adenocarcinoma, Bronchioloalveolar, Adenocarcinoma, Carcinoma, Non-small cell lung cancer.

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Little information is available about p53 mutations and p53 protein overexpression detected by immunohistochemistry, microsatellite loss of heterozygosity (LOH), and K-*ras* mutations in adenocarcinoma of the bronchioloalveolar subtype, according to the last World Health Organization (WHO) pathological classification proposed in 1999. However, the frequency of these molecular abnormalities seems to increase during the multistep process of carcinogenesis of peripheral adenocarcinoma going from atypical alveolar hyperplasia adenocarcinoma to bronchioloalveolar carcinoma (BAC) and to invasive adenocarcinoma.

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ATYPICAL ADENOMATOUS HYPERPLASIA

There is an increasing body of evidence to support the concept of atypical adenomatous hyperplasia (AAH) as the precursor of at least a subset of adenocarcinomas.[1] AAH is most frequently detected in lungs from patients bearing lung cancers (9–20%), especially adenocarcinomas (up to 40%) compared with squamous cell carcinomas (11%).[2] Several molecular changes frequently present in lung adenocarcinomas are also present in AAH lesions, and there is further evidence that AAH may represent true preneoplastic lesions.[1] The most important findings are the presence in AAHs of K-*ras* (codon 12) mutations (40%),[3] loss of *LKB1* function (20%),[4] allelic losses in chromosomes 3p (20%), 9p ($p16^{INK4a}$, 10%), 9q (50%), 17q, and 17p (*TP53*, 5%),[5,6] and overexpression of cyclin D1 (70%), p53 (ranging from 10 to 60%),[7] and survivin (50%).[8] Despite the evidence that AAH is a precursor lesion for a subset of lung adenocarcinomas, there is general consensus that the pathogenesis of most adenocarcinomas is still unknown. The findings of relatively infrequent tyrosine kinase domain epidermal growth factor receptor (*EGFR*) mutations in AAH lesions (three out of 40 examined)[9,10] and no *EGFR* mutation[11,12] or relatively low frequency in true BACs of the lung[9] support the concept that genetic abnormalities of *EGFR* are not relevant in the pathogenesis of alveolar types of lung neoplasia. In addition, Tang et al.[13] recently reported that *EGFR* mutation is an early event in the pathogenesis of lung cancer, being identified in histologically normal epithelium of small bronchi and bronchioles adjacent to *EGFR* mutant lung adenocarcinomas in nine out of 21 (43%) patients examined, but in none of the patients without mutation in the tumor. These data further support the notion than AAH lesions are not involved in the pathogenesis of *EGFR* mutant lung adenocarcinomas.

BAC, ADENOCARCINOMA WITH BRONCHIOLOALVEOLAR FEATURES, AND ADENOCARCINOMA OF THE LUNG

The frequency of *EGFR* mutations has also been studied in patients with BAC, adenocarcinoma with BAC features, and adenocarcinomas of the lung. Although responses to EGFR tyrosine kinase inhibitors have been reported to be higher[14] and EGFR mutations were preferentially observed in tumors having BAC features,[12,15] we did not find association with the BAC subtype of adenocarcinoma in 97 cases from

the United States[11] using the criteria stated by the 1999 WHO classification of lung tumors.[16,17]

In addition to the WHO system, Noguchi et al.[18,19] have classified adenocarcinomas into different categories that have different frequencies of genetic changes. Koga et al.[20] reported that p53 mutations were present in approximately 0% of 17 pure BAC, 11% of 27 mixed adenocarcinoma with BAC features, and 48% of 101 invasive adenocarcinomas. Similar to the frequency of mutations, the frequency of p53 protein overexpression detected by immunohistochemistry increased from 6% (2/32 tumors) in pure BAC to 28% (27/133) in BAC with foci of active fibroblastic proliferation (Noguchi type C) and to 40% (14/35) in adenocarcinoma.[21] p53 mutation and protein overexpression were also correlated with the size and invasive component of small peripheral adenocarcinomas (≥5 mm: 41%; <5 mm: 20%).[22,23]

The frequency of allelic losses also increased significantly during malignant progression. According to Noguchi's classification,[18,19] frequencies of allelic losses at chromosomal loci 3p, 17p, 18q, and 22q were significantly lower in BAC with or without alveolar collapse (Noguchi types A and B, respectively) than in BAC with active fibroblastic proliferation (Noguchi type C) in a series of 66 small peripheral adenocarcinomas.[24]

The frequency and type of K-*ras* mutation in BAC are related to the cytological features (mucinous versus nonmucinous). This raises the question of whether the mucinous form might represent a biological entity separate from the nonmucinous form. Small series of tumors (all <50) from patients with adenocarcinoma of the lung show that the K-*ras* mutation is present in 73 to 100% of the mucinous types and that the type of the mutation was usually G to A (codon 12), whereas it was seen in 10 to 43% in the nonmucinous types, usually in G to T transversions.[25–27] Mutations at codon 12 of the K-*ras* oncogene were found in 39% of 41 AAH, 42% of 18 adenocarcinomas, and none of five lung neoplasms that were not adenocarcinomas. Of the patients with both an AAH and a synchronous adenocarcinoma, more than half did not have the mutation in both the AAH and the synchronous lung adenocarcinoma, suggesting that peripheral adenocarcinomas arise not always from AAH but sometimes directly from a background of field cancerization.[27]

Adenocarcinomas with BAC features are also characterized by an intense inflammatory reaction especially containing alveolar neutrophils and macrophages. Increased numbers of tumor-infiltrating neutrophils are linked to poorer outcomes in these patients.[28] Tumor environment drives local neutrophil recruitment and activation via C-X-C chemokine release such as interleukin-8 and epithelial cell–derived neutrophil activating protein 78 but also prolongs alveolar neutrophil survival through the production of soluble antiapoptotic factors (granulocyte–macrophage colony–stimulating factor and granulocyte colony–stimulating factor).[29,30] The mechanisms by which neutrophils influence the prognosis of adenocarcinoma with BAC features could be multiple. It has been postulated that the persistence of neutrophil alveolitis would result in persistent release of proinflammatory mediators such as cytokines, proteases, and reactive oxygen and nitrogen species that can damage DNA and activate oncogenes.[31,32] Among these factors released by neutrophils, hepatocyte growth factor seems to be particularly involved in the progression of these types of tumors, especially through its mitogenic and scattering properties, favoring c-Met expressing tumor-cell migration along the alveolar basal membrane.[33] Lastly, neutrophils might be involved in luminal tumor spread by promoting tumor-cell shedding (M. Wislez, AACR 2004), described pathologically as the presence of micropapillary clusters that are also involved in the mechanism of aerogenous progression.[34]

GENOMIC AND PROTEOMIC STUDIES OF BAC

As mentioned before, BAC is thought to arise from AAH and is potentially an intermediate to invasive adenocarcinoma. Extensive analyses of BAC using gene-expression profiling and proteomic-based studies have not yet been performed and are only available for limited numbers of these cancers. These types of studies may have the potential to define similarity or differences in the observed types of adenocarcinoma of the lung. Of particular interest is the potential regulatory pathway involved in the lepidic growth patterns of BAC, which is different from most other adenocarcinomas of the lung. The observation that some adenocarcinomas can exhibit regions of BAC provides complexity and has resulted in multiple pathological-based classifications.[14,16–19] Genomic studies have the potential to define the similarities as well as key differences between BAC, adenocarcinomas with BAC features, and adenocarcinomas of the lung.

Recent studies examining individual genes have hinted at differences between BAC and adenocarcinomas. The tumor suppressor in the lung cancer-1 gene encodes an adhesion molecule and is frequently associated with LOH at that locus in non–small-cell lung cancer. Both normal lung cells and BAC retain expression of tumor suppressor in lung cancer-1, whereas 63% of adenocarcinomas demonstrated decreased expression detected by immunohistochemistry.[35] BACs have very low p53 DNA mutation frequencies compared with adenocarcinomas of the lung.[20] LOH at the 3p FHIT loci was observed in 43% of BAC, and 12th codon K-*ras* mutations are detected in the mucinous form of BAC.[36] A comparative LOH study between 14 BAC and 20 stage I lung adenocarcinomas using nine chromosomal regions revealed that the most frequently affected chromosomal regions in BAC were 8q and 17p.[37] In adenocarcinomas of the lung, LOH at 1p, 3p, 7q, and 18q was more frequent than in BAC, and fractional allele loss was greater in adenocarcinomas of the lung than BAC.

Using immunocytochemistry to examine protein expression, detection of the thyroid transcription factor-1 (TTF-1), cytokeratin 7, and cytokeratin 20 were measured in both mucinous and nonmucinous BAC.[38] TTF-1 was detected in 17% of mucinous and 94% of nonmucinous BAC, cytokeratin 7 was detected in 100% of mucinous and 23% of nonmucinous BAC, and cytokeratin 20 was detected in 60% of mucinous and 0% of nonmucinous BAC.[38] In a study that examined MUC protein expression in AAH, BAC, and adenocarcinomas with BAC features, MUC1 decreased from

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AAH to BAC and from BAC to adenocarcinoma, whereas MUC2, MUC5AC, MUC6, and depolarized MUC6 increased.[39] Alterations in p53 and the increased expression of MUC1, MUC5AC, and MUC6 were noted.

ADDITIONAL GENOMIC AND PROTEOMIC STUDIES

A comparison of normal lung tissue and BAC using oligonucleotide arrays was reported by Goodwin et al.[40] and identified 12 up-regulated and six down-regulated genes in the BAC tumors. Although this analysis provides some information, a comparison of BAC and adenocarcinomas was not included, which may be most relevant in defining critical genes involved in the development of these cancers. We used oligonucleotide arrays to examine gene expression in 14 BAC and 73 adenocarcinomas.[41] The most highly expressed genes that were significantly different between the BAC tumors and adenocarcinomas and higher in BAC included the surfactant pulmonary-associated proteins A1, A2, C and D, MUC1, TTF-1 and TTF-3, villin 2, and prostaglandin D2 synthetase. Interestingly, higher mRNA expression for both fos and jun B were detected in BAC, which may reflect an elevated AP-1 activity and upstream signaling events in these tumors. The higher level of expression of surfactant genes is consistent with the well-differentiated phenotypic characteristics of BAC. TTF-1 was the most differentially expressed gene between BAC and adenocarcinomas, consistent with the high TTF-1 protein expression reported in BAC.[38] Because of the small numbers of tumors for our analyses, it was not possible to divide the BAC tumors into separate categories such as mucinous, nonmucinous, and mixed histology. Although we found MUC1 mRNA present in both BAC and adenocarcinomas of the lung, the significantly increased expression in BAC is consistent with the higher MUC1 protein levels that have been reported in these tumors.[39]

Analysis of survival-related genes revealed prostaglandin D2 synthetase and neutrophil elastase 2 to be more highly expressed in BAC than the other adenocarcinomas. In contrast, much lower levels of vascular endothelial growth factor were detected in the BAC, possibly reflecting a lesser level of angiogenesis and hypoxia in these tumors relative to the adenocarcinomas. Adenocarcinomas also expressed increased levels of metallothionein 2A and thioredoxin reductase mRNA. We speculate that these genes may correspond to smoking-related alterations because these genes may change in response to reactive oxygen species originating from tobacco smoking or in response to inflammatory cells. Alternately, the expression of thioredoxin reductase and metallothionein 2 may reflect the higher rates of cell proliferation in the lung adenocarcinomas relative to BAC.

Few, if any, large-scale proteomic analyses of BAC have been reported. We examined the same BAC and lung adenocarcinomas for mRNA using oligonucleotide arrays and also at the protein level with two-dimensional gel electrophoresis and mass spectrometry.[42] A total of 682 protein spots were quantified, and 75 proteins were found to differ significantly ($p < 0.05$) between BAC and lung adenocarcinomas. Thirty-eight protein spots were successfully identified using mass spectometry. Of interest were the relatively higher expression of the ras-related protein RAB-14, glutathione-s-transferase-pi, cytokeratin 7, and three isoforms of the selenium-binding protein 1 in BAC compared with adenocarcinomas of the lung. Adenocarcinomas expressed higher levels of phosphoglycerate kinase 1, pyruvate kinase M1/M2, and stathmin (OP-18) compared with BACs. Increased phosphoglycerate kinase 1 is consistent with higher hypoxia-induced glycolysis in the adenocarcinomas of the lung relative to BAC.[42]

Future studies that include sufficient numbers of the various histological subtypes of BAC are needed to provide insight into the similarities and differences among these tumors and as compared with lung adenocarcinomas. The NCI Director's Challenge: Validation Study of Lung Adenocarcinomas will examine gene expression using Affymetrix 133A oligonucleotide arrays among approximately 500 tumors. Thus, a relatively large number of BACs will be included in this study, allowing potential gene pathways to be defined that may be relevant to our understanding of the growth- and cell-signaling systems in BAC. These analyses will also incorporate detailed pathologic assessment of each tumor so that the subtypes of each BAC can be compared. It is expected that these data, made available to the research community, will then stimulate further research into potential new markers for early diagnosis and possible therapeutic intervention strategies that may be effective for BAC.

FUTURE DIRECTIONS

The Committee responsible for Molecular Biology, Genomics, and Proteomics in Bronchioloalveolar Carcinoma outlined studies that will provide further insights into BAC. The most important part of the meeting was partial agreement and understanding about the interpretation of the pathological classification. The participants in the meeting agreed on a common set of descriptors for the pathological interpretation of BAC that will be used more consistently in the future.

TABLE 1. Different Biological Properties in Atypical Adenomatous Hyperplasia, Pure Bronchioloalveolar Cancer, Adenocarcinoma with Bronchioloalveolar Cancer Features, and Adenocarcinoma

	Atypical Adenomatous Hyperplasia	Bronchioloalveolar Carcinoma	Adenocarcinoma with Bronchioloalveolar Carcinoma Features	Adenocarcinoma of the Lung
EGFR mutation	↓ <5%	10%		↑ 40%
TP53 mutations	Not reported	↓ 0%	↓ 10%	↑ 50%
p53 by immunohistochemistry	Not reported	↓ 5%	↑ 30%	↑ 50%

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Upcoming technological improvements will provide additional insights into the biology of BAC. These will include the increasing ability to detect genetic changes in BAC and adenocarcinomas including, but not be limited to, *EGFR*, HER-2/neu, B-raf, K-*ras*, and *TP53*. In addition, there is the ability to detect genetic loss in the whole genome using studies with single-polynucleotide polymorphisms or array chromosomal genomic hybridization. There is increasing ability to use small and smaller amounts of DNA and DNA from paraffin-embedded tissues. Future studies will provide information on the degree of genetic changes seen in early lesions (<1cm) that are being detected more often as computerized tomographic scanning of the chest is becoming more widely used. These findings can be compared with the more advanced lesions. The genetic changes can also provide insights into the clonality of the BACs to determine whether the multiple lesions in the lungs arise from single or multiple clones. Table 1

REFERENCES

1. Wistuba II, Gazdar A. Lung cancer preneoplasia. *Ann Rev Pathol Mech Dis* 2005;1:331–348.
2. Kerr KM. Pulmonary preinvasive neoplasia. *J Clin Pathol* 2001;54:257–271.
3. Westra WH. Early glandular neoplasia of the lung. *Respir Med* 2000;1: 163–169.
4. Ghaffar H, Sahin F, Sanchez-Cepedes M, et al. LKB1 protein expression in the evolution of glandular neoplasia of the lung. *Clin Cancer Res* 2003;9:2998–3003.
5. Kitaguchi S, Takeshima Y, Nishisaka T, et al. Proliferative activity, p53 expression and loss of heterozygosity on 3p, 9p and 17p in atypical adenomatous hyperplasia of the lung. *Hiroshima J Med Sci* 1998;47: 17–25.
6. Takamochi K, Ogura T, Suzuki K, et al. Loss of heterozygosity on chromosomes 9q and 16p in atypical adenomatous hyperplasia concomitant with adenocarcinoma of the lung. *Am J Pathol* 2001;159:1941–1948.
7. Tominaga M, Sueoka N, Irie K, et al. Detection and discrimination of preneoplastic and early stages of lung adenocarcinoma using hnRNP B1 combined with the cell cycle-related markers p16, cyclin D1, and Ki-67. *Lung Cancer* 2003;40:45–53.
8. Nakanishi K, Kawai T, Kumaki F, et al. Survivin expression in atypical adenomatous hyperplasia of the lung. *Am J Clin Pathol* 2003;120:712–719.
9. Yoshida Y, Shibata T, Kokubu A, et al. Mutations of the epidermal growth factor receptor gene in atypical adenomatous hyperplasia and bronchioloalveolar carcinoma of the lung. *Lung Cancer* 2005;50:1–8.
10. Yatabe Y, Kosaka T, Takahashi T, et al. EGFR mutation is specific for terminal respiratory unit type adenocarcinoma. *Am J Surg Pathol* 2005; 29:633–639.
11. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339–346.
12. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* 2004;101:13306–13311.
13. Tang X, Shigematsu H, Bekele BN, et al. EGFR tyrosine kinase domain mutations are detected in histologically normal respiratory epithelium in lung cancer patients. *Cancer Res* 2005;65:7568–7572.
14. Miller VA, Kris MG, Shah N, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:1103–1109.
15. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.
16. Travis WD, Brambilla E, Muller-Hermelink HK, et al. Tumours of the lung. In Travis WD, Brambilla E, Muller-Hermelink HK, et al (Eds.), Pathology and Genetics: Tumours of the Lung, Pleura, Thymus and Heart. World Health Organization Classification of Tumours. Pathology & Genetics. Lyon: IARC Press, 2004, pp 9–124.
17. Travis WD, Garg K, Franklin WA, et al. Evolving concepts in the pathology and computed tomography imaging of lung adenocarcinoma and bronchioloalveolar carcinoma. *J Clin Oncol* 2005;23:3279–3287.
18. Noguchi M, Minami Y, Iijima T, et al. Reproducibility of the diagnosis of small adenocarcinoma of the lung and usefulness of an educational program for the diagnostic criteria. *Pathol Int* 2005;55:8–13.
19. Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis. *Cancer* 1995;75: 2844–2852.
20. Koga T, Hashimoto S, Sugio K, et al. Clinicopathological and molecular evidence indicating the independence of bronchioloalveolar components from other subtypes of human peripheral lung adenocarcinoma. *Clin Cancer Res* 2001;7:1730–1738.
21. Kawasaki M, Noguchi M, Morikawa A, et al. Nuclear p53 accumulation by small-sized adenocarcinomas of the lung. *Pathol Int* 1996;46:486–490.
22. Slebos RJ, Baas IO, Clement MJ, et al. p53 alterations in atypical alveolar hyperplasia of the human lung. *Hum Pathol* 1998;29:801–808.
23. Terasaki H, Niki T, Matsuno Y, et al. Lung adenocarcinoma with mixed bronchioloalveolar and invasive components: clinicopathological features, subclassification by extent of invasive foci, and immunohistochemical characterization. *Am J Surg Pathol* 2003;27:937–951.
24. Aoyagi Y, Yokose T, Minami Y, et al. Accumulation of losses of heterozygosity and multistep carcinogenesis in pulmonary adenocarcinoma. *Cancer Res* 2001;61:7950–7954.
25. Maeshima AM, Niki T, Maeshima A, et al. Modified scar grade: a prognostic indicator in small peripheral lung adenocarcinoma. *Cancer* 2002;95:2546–2554.
26. Marchetti A, Buttitta F, Pellegrini S, et al. Bronchioloalveolar lung carcinoma: K-ras mutations are constant events in the mucinous subtype. *J Pathol* 1996;179:254–259.
27. Westra WH, Baas IO, Hruban RH, et al. K-ras oncogene activation in atypical alveolar hyperplasias of the human lung. *Cancer Res* 1996;56: 2224–2228.
28. Bellocq A, Antoine M, Flahault A, et al. Neutrophil alveolitis in bronchioloalveolar carcinoma: induction by tumor-derived interleukin-8 and relation to clinical outcome. *Am J Pathol* 1998;152:83–92.
29. Wislez M, Fleury-Feith J, Rabbe N, et al. Tumor-derived granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor prolong the survival of neutrophils infiltrating bronchoalveolar subtype pulmonary adenocarcinoma. *Am J Pathol* 2001;159:1423–1433.
30. Wislez M, Philippe C, Antoine M, et al. Upregulation of bronchioloalveolar carcinoma-derived C-X-C chemokines by tumor infiltrating inflammatory cells. *Inflamm Res* 2004;53:4–12.
31. Jackson JH, Vollenweider M, Hill J, et al. Stimulated human leukocytes cause activating mutations in the K-ras protooncogene. *Oncogene* 1997; 14:2803–2808.
32. Weitberg AB, Weitzman SA, Destrempes M, et al. Stimulated human phagocytes produce cytogenetic changes in cultured mammalian cells. *N Engl J Med* 1983;308:26–30.
33. Wislez M, Rabbe N, Marchal J, et al. Hepatocyte growth factor production by neutrophils infiltrating bronchioloalveolar subtype pulmonary adenocarcinoma: role in tumor progression and death. *Cancer Res* 2003;63:1405–1412.
34. Hoshi R, Tsuzuku M, Horai T, et al. Micropapillary clusters in early-stage lung adenocarcinomas: a distinct cytologic sign of significantly poor prognosis. *Cancer* 2004;102:81–86.
35. Ito A, Okada M, Uchino K, et al. Expression of the TSLC1 adhesion molecule in pulmonary epithelium and its down-regulation in pulmonary adenocarcinoma other than bronchioloalveolar carcinoma. *Lab Invest* 2003;83:1175–1183.
36. Marchetti A, Pellegrini S, Bertacca G, et al. FHIT and p53 gene abnormalities in bronchioloalveolar carcinomas. Correlations with clinicopathological data and K-ras mutations. *J Pathol* 1998;184:240–246.
37. Sasatomi E, Johnson LR, Aldeeb DN, et al. Genetic profile of cumulative mutational damage associated with early pulmonary adenocarcinoma: bronchioloalveolar carcinoma vs. stage I invasive adenocarcinoma. *Am J Surg Pathol* 2004;28:1280–1288.

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38. Saad RS, Cho P, Silverman JF, et al. Usefulness of Cdx2 in separating mucinous bronchioloalveolar adenocarcinoma of the lung from metastatic mucinous colorectal adenocarcinoma. *Am J Clin Pathol* 2004;122: 421–427.
39. Awaya H, Takeshima Y, Yamasaki M, et al. Expression of MUC1, MUC2, MUC5AC, and MUC6 in atypical adenomatous hyperplasia, bronchioloalveolar carcinoma, adenocarcinoma with mixed subtypes, and mucinous bronchioloalveolar carcinoma of the lung. *Am J Clin Pathol* 2004;121:644–653.
40. Goodwin LO, Mason JM, Hajdu SI. Gene expression patterns of paired bronchioloalveolar carcinoma and benign lung tissue. *Ann Clin Lab Sci* 2001;31:369–375.
41. Beer DG, Kardia SL, Huang CC, et al. Gene-expression profiles predict survival of patients with lung adenocarcinoma. *Nat Med* 2002;8:816–824.
42. Chen G, Gharib TG, Wang H, et al. Protein profiles associated with survival in lung adenocarcinoma. *Proc Natl Acad Sci U S A* 2003;100: 13537–13542.

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Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan

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Background: To compare the efficacy and toxicity of three platinum-based combination regimens against cisplatin plus irinotecan (IP) in patients with untreated advanced non-small-cell lung cancer (NSCLC) by a non-inferiority design.
Patients and methods: A total of 602 patients were randomly assigned to one of four regimens: cisplatin 80 mg/m^2 on day 1 plus irinotecan 60 mg/m^2 on days 1, 8, 15 every 4 weeks (IP) carboplatin AUC 6.0 min × mg/mL (area under the concentration–time curve) on day 1 plus paclitaxel 200 mg/m^2 on day 1 every 3 weeks (TC); cisplatin 80 mg/m^2 on day 1 plus gemcitabine 1000 mg/m^2 on days 1, 8 every 3 weeks (GP); and cisplatin 80 mg/m^2 on day 1 plus vinorelbine 25 mg/m^2 on days 1, 8 every 3 weeks (NP).
Results: The response rate, median survival time, and 1-year survival rate were 31.0%, 13.9 months, 59.2%, respectively, in IP; 32.4%, 12.3 months, 51.0% in TC; 30.1%, 14.0 months, 59.6% in GP; and 33.1%, 11.4 months, 48.3% in NP. No statistically significant differences were found in response rate or overall survival, but the non-inferiority of none of the experimental regimens could be confirmed. All the four regimens were well tolerated.
Conclusion: The four regimens have similar efficacy and different toxicity profiles, and they can be used to treat advanced NSCLC patients.
Key words: carboplatin, cisplatin, gemcitabine, irinotecan, non-small-cell lung cancer, paclitaxel, randomized phase III study, vinorelbine

introduction

Nearly 60 000 patients in Japan died of lung cancer in 2004, and the mortality rate is still increasing [1]. Even old-generation cisplatin-based chemotherapy provides a survival benefit and symptom relief in patients with inoperable non-small-cell lung cancer (NSCLC) [2]. Several anticancer agents including irinotecan, paclitaxel, docetaxel, gemcitabine, and vinorelbine, were developed in the 1990s and most of them have mechanisms of action that differ from those of the old-generation agents [3–7]. The combinations of platinum and these new agents developed in the 1990s are more useful against advanced NSCLC than old-generation combination chemotherapy, and doublets of platinum and new-generation anticancer agents are considered standard chemotherapy regimens for advanced NSCLC, although no consistent standard regimens have yet been established [8–17].

Two phase III studies comparing cisplatin plus irinotecan (IP) with cisplatin plus vindesine for advanced NSCLC have been conducted in Japan [18, 19]. Fukuoka et al. [20] reported the results of a combined analysis of the 358 eligible stage IV patients in these studies. They carried out a multivariate analysis using the Cox regression model with adjustment for well-known prognostic factors, and the Cox regression analysis demonstrated that treatment with IP was one of significant independent favorable factor. Based on their data, we selected IP for the reference arm in our study.

The Ministry of Health, Labour and Welfare of Japan approved the prescription of paclitaxel, gemcitabine, and

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vinorelbine for NSCLC in 1999 and requested a phase III study to confirm the efficacy and safety of these agents. The Japanese investigators and the pharmaceutical companies decided to conduct a four-arm randomized phase III study for NSCLC, the so-called FACS, Four-Arm Cooperative Study. The purpose of the study was to compare the efficacy and toxicity of three platinum-based combination regimens, carboplatin plus paclitaxel (TC), cisplatin plus gemcitabine (GP), cisplatin plus vinorelbine (NP), with IP as the reference arm.

patients and methods

patient selection

Patients with histologically and/or cytologically documented NSCLC were eligible for participation in the study. Each patient had to meet the following criteria: clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy, such as malignant pleural effusion, pleural dissemination, malignant pericardiac effusion, or metastatic lesion in the same lobe), at least one target lesion >2 cm, no prior chemotherapy, no prior surgery and/or radiotherapy for the primary site, age 20–74 years, Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, adequate hematological, hepatic and renal functions, partial pressure of arterial oxygen (paO_2) ≥60 torr, expected survival >3 months, able to undergo first course treatment in an inpatient setting, and written informed consent. The study was approved by the Institutional Review Board at each hospital. Written informed consent was obtained from every patient.

treatment schedule

All patients were randomly assigned to one of the four treatment groups by the central registration office by means of the minimization method. Stage, PS, gender, lactate dehydrogenase (LDH) and albumin values, and institution were used as adjustment variables. The first group received the reference treatment, 80 mg/m^2 of cisplatin on day 1 and 60 mg/m^2 of irinotecan on days 1, 8, and 15, and the cycle was repeated every 4 weeks. The second group received 200 mg/m^2 of paclitaxel (Bristol-Myers K.K., Tokyo, Japan) over a 3-h period followed by carboplatin at a dose calculated to produce an area under the concentration–time curve of 6.0 min × mg/mL on day 1 and the cycle was repeated every 3 weeks. The third group received 80 mg/m^2 of cisplatin on day 1 and 1000 mg/m^2 of gemcitabine (Eli Lilly Japan K.K., Kobe, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. The fourth group received 80 mg/m^2 of cisplatin on day 1 and 25 mg/m^2 of vinorelbine (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. Each treatment was repeated for three or more cycles unless the patient met the criteria for progressive disease or experienced unacceptable toxicity.

response and toxicity evaluation

Response was evaluated according to the Response Evaluation Criteria in Solid Tumors, and tumor markers were excluded from the criteria [21]. Objective tumor response in all responding patients was evaluated by an external review committee with no information on the treatment group. Toxicity grading criteria in National Cancer Institute Common Toxicity Criteria Ver 2.0 were used to evaluate toxicity.

quality of life assessment

Quality of life (QoL) was evaluated by means of the Functional Assessment of Cancer Therapy—Lung (FACT-L) Japanese version and the QoL Questionnaire for Cancer Patients Treated with Anticancer Drugs (QoL-ACD), before treatment, immediately before the second cycles of chemotherapy, and 3 and 6 months after the start of treatment [22–24].

statistical analysis and monitoring

The primary end point of this study was overall survival (OS), and the secondary end points were response rate, response duration, time to progressive disease (TTP), time to treatment failure (TTTF), adverse event, and QoL. The 1-year survival rate of the control group in this study was estimated to be 43% based on the data in published papers, and the 1-year survival rate in the other treatment group was expected to be 50%. The lower equivalence limit for 1-year survival rate was set as '−10%'. The criterion for the non-inferiority of each treatment was a lower limit of the two-sided 95% confidence interval (CI) of the 1-year survival rate of treatment minus that of control larger than the lower equivalence limit. Because the non-inferiority of each treatment versus the control was to be evaluated independently, a separate null hypothesis was stated for each treatment, and for that reason no multiple comparison adjustment was included in the study. Based on the above conditions and binomial distribution, 135 patients were needed per arm for a one-sided Type I error of 2.5% and 80.0% power. In view of the possibility of variance inflation due to censoring, the sample size was set at 600 (150 per arm).

Central registration with randomization, monitoring, data collection, and the statistical analyses were independently carried out by a contract research organization (EPS Co., Ltd, Tokyo, Japan).

results

patient characteristics

From October 2000 to June 2002, a total of 602 patients were registered by 44 hospitals in Japan. All patients had been followed up for >2 years, and 447 patients had died as of June 2004. Of the 602 patients registered, 151 were allocated to the reference treatment, IP, and 150, 151, and 150 patients were allocated to TC, GP, and NP, respectively. Since 10 patients did not receive chemotherapy and 11 patients were subsequently found to be ineligible, 592 patients were assessable for toxicity and 581 patients were assessable for efficacy. Four patients did not receive chemotherapy due to electrolytic disorder, fever, symptomatic brain metastases, and rapid tumor progression in IP, two patients due to refusal and pneumonia in TC, four patients due to lower WBC counts (two patients), rapid tumor progression, and nephritic syndrome in NP. Two patents were ineligible due to wrong stage in IP, two patients were wrong stage and one patient had double cancer in TC, two patients were wrong diagnosis, one patient had massive pleural effusion, one patient received prior chemotherapy in GP, one patient had no target lesions in NP. Age, gender, PS, stage, and LDH and albumin values were well balanced in each arm (Table 1). Fewer patients with adenocarcinoma and more patients with squamous cell carcinona were, however, entered in three experimental arms than in IP.

objective tumor response and response duration

Objective tumor response is shown in Table 2. Forty-five partial responses occurred in the 145 assessable patients in the reference arm, IP, for an objective response rate of 31.0% with a median response duration of 4.8 months. The response rate and median response duration were 32.4% and 4.0 months in TC, 30.1% and 3.5 months in GP, and 33.1% and 3.4 months in NP. The response rates in TC, GP, and NP were not statistically different from the rate in IP according to the results of the χ^2 test.

Table 1. Patient characteristics and treatment delivery

	Cisplatin + irinotecan	Carboplatin + paclitaxel	Cisplatin + gemcitabine	Cisplatin + vinorelbine
Assessable patients	145	145	146	145
Gender (male/female)	97/48	99/46	101/45	101/44
Age, median (range)	62 (30–74)	63 (33–74)	61 (34–74)	61 (28–74)
PS (0/1)	44/101	44/101	45/101	45/100
Histology				
Adenocarcinoma	121	104	108	109
Squamous cell carcinoma	16	31	29	29
Others	8	10	9	7
Stage (IIIB/IV)	31/114	28/117	30/116	26/119
No. of cycles				
Mean ± SD	3.0 ± 1.3	3.5 ± 1.5	3.2 ± 1.2	3.1 ± 1.3
Median	3	3	3	3
Range	1–7	1–10	1–7	1–8

PS, performance status; SD, standard deviation.

Table 2. Survival, TTP, TTTF, response rate, and response duration

	N	Median survival, months	1-year survival (%)	Difference in 1-year survival from IP	2-year survival (%)	TTP (median), months	TTTF (median), months	Response rate (%)	Response duration (median), months
Cisplatin + irinotecan	145	13.9	59.2	–	26.5	4.7	3.3	31.0	4.8 (n = 45)
Carboplatin + paclitaxel	145	12.3	51.0	−8.2% (95% CI −19.6% to 3.3%)	25.5	4.5 (P = 0.355)[a]	3.2 (P = 0.282)[a]	32.4 (P = 0.801)[b]	4.0 (n = 47)
Cisplatin + gemcitabine	146	14.0	59.6	0.4% (95% CI −10.9% to11.7%)	31.5	4.0 (P = 0.170)[a]	3.2 (P = 0.567)[a]	30.1 (P = 0.868)[b]	3.5 (n = 44)
Cisplatin + vinorelbine	145	11.4	48.3	−10.9% (95% CI −22.3% to 0.5%)	21.4	4.1 (P = 0.133)[a]	3.0 (P = 0.091)[a]	33.1 (P = 0.706)[b]	3.4 (n = 48)

[a]Compared with IP by the generalized Wilcoxon test.
[b]Compared with IP by the χ^2 test.
CI, confidence interval; IP, cisplatin plus irinotecan; TTP, time to progressive disease; TTTF, time to treatment failure.

OS, TTP disease, and TTTF

OS and TTP are shown in Figure 1. Median survival time (MST), the 1-year, and 2-year survival rate in IP were 13.9 months, 59.2%, and 26.5%, respectively. The MSTs, 1-year, and 2-year survival rates were, respectively, 12.3 months, 51.0%, and 25.5% in TC; 14.0 months, 59.6%, and 31.5% in GP; and 11.4 months, 48.3%, and 21.4% in NP. The lower limits of the 95% CI of the difference in 1-year survival rate between IP and TC (−19.6%), GP (−10.9%), and NP (−22.3%) were below −10%, which was considered the lower equivalence limit (Table 2). Thus, the results did not show non-inferiority in three experimental regimens compared with reference treatment. Median TTP and median TTTF were 4.7 and 3.3 months, respectively in IP. Median TTP and TTTF were, respectively, 4.5 and 3.2 months in TC, 4.0 and 3.2 months in GP, and 4.1 and 3.0 months in NP. There were no statistical differences in either TTP or TTTF in TC, GP, or NP, compared with IP according to the results of the generalized Wilcoxon test (Table 2).

hematologic and non-hematologic toxicity

In IP, 47.6% and 83.7% of patients developed grade 3 or worse leukopenia and neutropenia, respectively (Table 3). The incidences of grade 3 or worse leukopenia (33.1%, P = 0.010) and neutropenia (62.9%, P < 0.001) were significantly lower in GP than in IP. The incidence of grade 3 or worse leukopenia (67.1%, P < 0.001) was significantly higher in NP than in IP. Grade 3 or worse thrombocytopenia developed in 5.4% of the patients in IP, and the incidence was significantly higher in GP (35.1%, P < 0.001). The incidence of febril neutropenia in IP was 14.3%, and was significantly lower in GP (2.0%, P < 0.001).

Grade 2 or worse nausea, vomiting, anorexia, and fatigue occurred in 60.5%, 51.0%, 65.3%, and 38.8%, respectively, of the patients in IP. The incidences of grade 2 or worse nausea (TC: 25.0%, P < 0.001, NP: 47.3%, P = 0.022), vomiting (TC: 22.3%, P < 0.001, NP: 36.3%, P = 0.011), and anorexia (TC: 32.4%, P < 0.001, NP: 49.3%, P = 0.005) were significantly lower in TC and NP than in IP. Grade 2 or worse diarrhea was

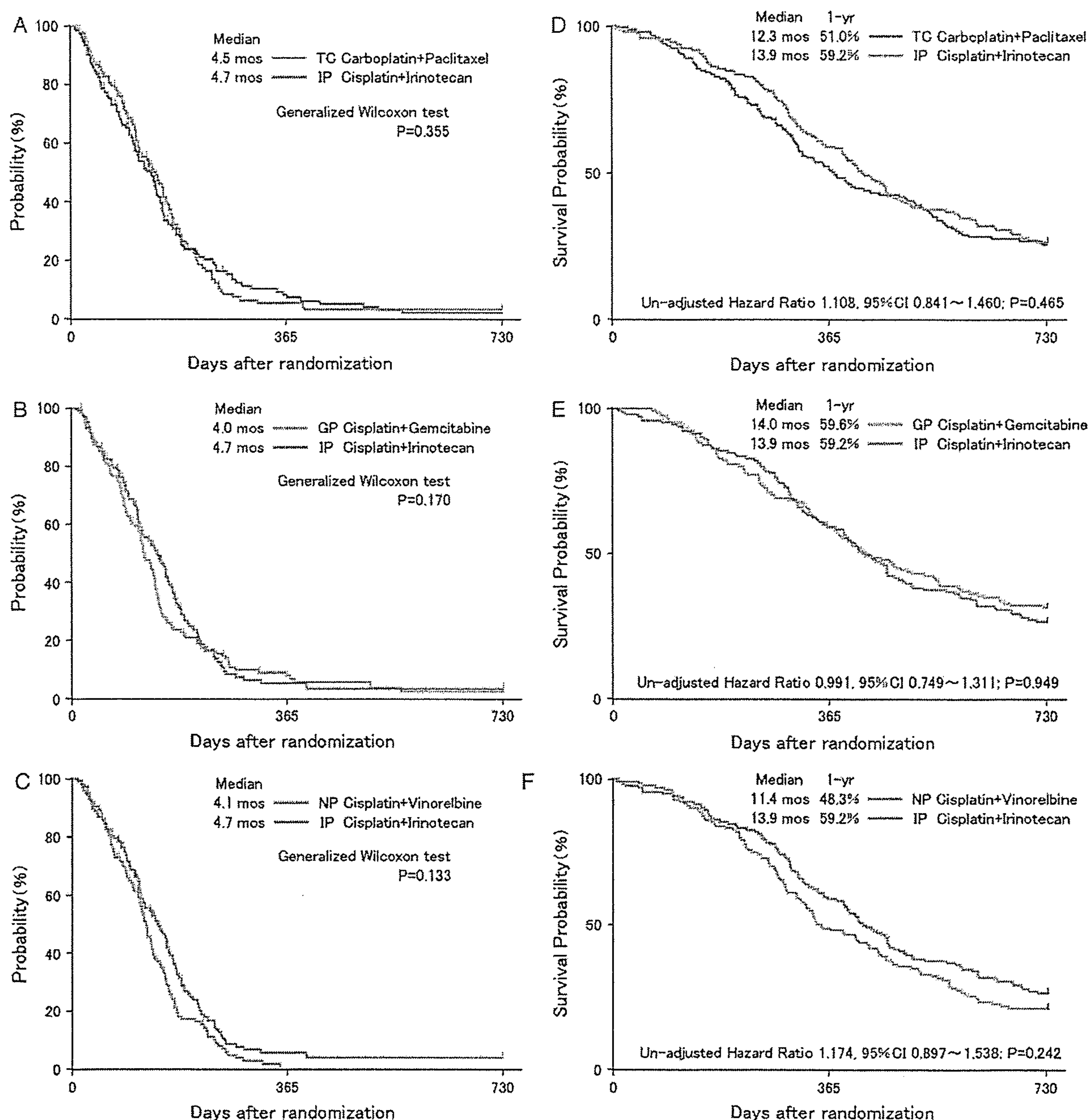

Figure 1. Overall survival (OS) and time to progressive (TTP) disease. TTP and OS in the carboplatin plus paclitaxel (TC) (A, D), cisplatin plus gemcitabine (GP) (B, E), and cisplatin plus vinorelbine (NP) (C, F) were not statistically significantly different from the values in the cisplatin plus irinotecan.

significantly less frequent in TC (6.8%), GP (8.6%), and NP (11.6%) than in IP (48.3%, $P < 0.001$). The incidences of grade 2 or worse sensory neuropathy (16.9%, $P < 0.001$), arthralgia (21.6%, $P < 0.001$), and myalgia (17.6%, $P < 0.001$) were significantly higher in TC than in IP. Grade 2 alopecia occurred in 30.6% of the patients in IP, and its incidence was significantly higher in TC (44.6%, $P = 0.013$) and significantly lower in GP (15.2%, $P = 0.001$) and NP (8.9%, $P < 0.001$). Grade 2 injection site reactions were more frequent in NP (26.7%) than in IP (4.8%, $P < 0.001$).

A total of five patients died of treatment-related toxicity: three in IP (cerebral hemorrhage, interstitial pneumonia, acute circulatory failure/disseminated intravascular coagulation: 2.0%), one in TC (acute renal failure: 0.7%), and one in NP (pulmonary embolism: 0.7%).

second-line treatment

Data on second-line treatment, but not third-line or later treatment, was available in this study, and they showed that

Table 3. Toxicity

	IP (*n* = 147)			TC (*n* = 148)			GP (*n* = 151)			NP (*n* = 146)		
	Grade (%)			Grade (%)			Grade (%)			Grade (%)		
	2	3	4	2	3	4	2	3	4	2	3	4
Leukocytes	42	43	5	39	42	3	40	31[a]	2[a]	25	51[b]	16[b]
Neutrophils	11	39	45	5	19	69	21	40	23[a]	5	16	72
Hemoglobin	42	24	7	42	13[a]	2[a]	44	22	5	43	25	5
Platelets	6	5	1	9	11	0	22	35[b]	0[b]	3	1[a]	0[a]
Febrile neutropenia	–	14	0	–	18	0	–	2[a]	0[a]	–	18	0
Nausea	32	29	–	14[c]	11[c]	–	35	23	–	33[c]	14[c]	–
Vomiting	38	13	0	17[c]	5[c]	0[c]	34	14	0	29[c]	7[c]	0[c]
Anorexia	30	33	2	15[c]	17[c]	1[c]	31	26	1	29[c]	20c	1[c]
Fatigue	27	12	1	26	2	1	17[c]	3[c]	0[c]	23[c]	3[c]	0[c]
Diarrhea	33	15	1	4[c]	3[c]	0[c]	7[c]	2[c]	0[c]	8[c]	4[c]	0[c]
Constipation	27	7	0	30	8	0	33	9	0	40[d]	14[d]	0[d]
Neuropathy, motor	1	0	0	1	1	1	0	0	0	0	0	0
Neuropathy, sensory	1	0	0	14[d]	3[d]	0[d]	0	0	0	0	0	0
Alopecia	31	–	–	45[d]	–	–	15[c]	–	–	9[c]	–	–
Arthralgia	2	0	0	20[d]	2[d]	0[d]	0	0	0	1	0	0
Myalgia	1	0	0	16[d]	2[d]	0[d]	0	0	0	1	1	0
Injection site reaction	5	0	–	5	0	–	5	0	–	27[d]	0[d]	–
Pneumonitis	0	1	1	0	1	0	0	0	0	0	1	0
Creatinine	8	1	0	2[c]	0[c]	0[c]	7	0	0	8	1	0
AST	7	1	1	5	1	0	6	3	0	1	3	0
Fever	2	0	0	5	1	0	1	0	0	1	0	0
Treatment-related death	3 (2.0%)			1 (0.7%)			0			1 (0.7%)		

[a]Incidence of grade 3 or 4 toxicity significantly ($P < 0.05$) lower than that with IP.

[b]Incidence of grade 3 or 4 toxicity significantly ($P < 0.05$) higher than that with IP.

[c]Incidence of grade 2 or worse toxicity is significantly ($P < 0.05$) lower than that with IP.

[d]Incidence of grade 2 or worse toxicity significantly ($P < 0.05$) higher than that with IP.

GP, cisplatin plus gemcitabine; IP, cisplatin plus irinotecan; NP, cisplatin plus vinorelbine; TC, carboplatin plus paclitaxel.

AST, aspartate aminotransferase; –, no category in the criteria.

60%–74% of the patients received chemotherapy and 6%–9% received thoracic irradiation as second-line treatment (Table 4). The percentages of patients in each treatment group who received second-line chemotherapy were not significantly different ($P = 0.081$).

quality of life

The details of the QoL analysis will be reported elsewhere. No statistically significant difference in global QoL was observed among the four treatment groups based on either the FACT-L Japanese version or the QoL-ACD. Only the physical domain evaluated by QoL-ACD was significantly better in TC, GP, and NP than in IP.

discussion

Many randomized phase III studies have compared platinum-plus-new-agent doublets in NSCLC, but, this is the first to evaluate the efficacy of an irinotecan-containing regimen in comparison with other platinum-plus-new-agent doublets in NSCLC [14–17]. Although non-platinum-containing chemotherapy regimens are used as alternatives, doublets of platinum and a new-generation anticancer agent, such as TC, GP, and NP, are considered standard chemotherapy regimens for advanced NSCLC worldwide [13–17, 25]. Although the non-inferiority of none of the three experimental regimens could be confirmed in this study, no statistically significant differences in response rate, OS, TTP, or TTTF were observed between the reference regimen and the experimental regimens. All four platinum-based doublets have similar efficacy against advanced NSCLC but different toxicity profiles. Nevertheless, IP was still regarded as the reference regimen in this study because the non-inferiority of none of the three experimental regimens could be confirmed.

OS in this study was relatively longer than previously reported. The estimated 1-year survival rate in the reference arm was 43%, but the actual 1-year survival rate was 59.2%, much higher than expected. The MSTs reported for patients treated with TC, GP, and NP in recent phase III studies have ranged from 8 to 10 months, and in the present study they were 12.3, 14.0, and 11.4 months, respectively [14–17]. One reason for the good OS in this study was the difference in patient selection criteria, for example exclusion of PS2 patients. Ethnic differences in pharmacogenomics have also been indicated as a possible reason for the good OS in this study [26]. The OS in IP in this study, however, was better than in previous Japanese studies [18, 19]. TTP in this study ranged from 4.0 to 4.7 months, and was similar to the TTP of 3.1–5.5 months reported in the literature [15, 16]. OS not TTP was longer in this study