was started from 10 doses of PR-350 in this study. As DLTs, radiation pneumonitis or skin rash of Grade 3 or more was noted in one third or less of 6 to 8 patients at each level, and so 30 daily administrations of PR-350 at 2000 mg/m^2 was determined as the recommended dosage in the Phase II portion of the trial.

A major hematologic toxicity was lymphopenia, although other hematologic toxicities were mild (Table 3). A major nonhematologic toxicity was radiation pneumonitis including two patients with TRD. Grade 3 or higher radiation pneumonitis was observed in 6 patients (16%). A similar rate of radiation pneumonitis is reported by a retrospective study at the National Cancer Center Hospital in Japan (20). In that analysis, severe radiation pneumonitis of Grade 3 or more was noted in 13% of 191 patients with lung cancer treated by CRT or RT alone between 1988 and 1998 (20). On the other hand, a less than 2% incidence of Grade 3 or higher pulmonary toxicity was reported for both sequential and concurrent CRT groups in a Japanese Phase III trial for locally advanced NSCLC using the same eligibility criterion on RT fields (6). It is unclear why pulmonary toxicity in the trial was so low. However the low total RT dose of 56 Gy may have contributed to that.

Because 3D RT planning was not available, it was impossible to correlate toxicity parameters with dose–volume histogram (DVH) information in this study. Although it can not be excluded that PR-350 enhances the effects of radiation on normal lung tissues, we consider that the relatively high incidence of radiation pneumonitis is attributable to our former two-dimensional RT technique. Extramural review of RT films revealed that two TRDs might have been attributable to a violation of protocol guidelines for RT fields or a violation of eligibility criteria on pulmonary disease. To evaluate the effect of PR-350 on radiation pneumonitis, an additional Phase II trial with a three-dimensional RT method may be required.

Neither Grade 3 or more esophageal toxicity, nor Grade 2 or more peripheral neuropathy, was noted. In the PK study, no accumulative effect was observed even after the 30th dose (Fig. 5). The major limitation of 2-nitroimidazoles including misonidazole and ethanidazole is neuropathy (10–12, 21, 22). For head-and-neck cancer, randomized clinical trials comparing RT plus ethanidazole and RT alone have been reported (21, 22). In these trials, ethanidazole at 2000 mg/m^2 given three times weekly for 17 doses was combined with RT, and peripheral neuropathy of Grade 1 to 3 was noted in 24% to 28% of patients. In the present trial, PR-350 at 2000 mg/m^2 was given five times weekly for 10 to 30 doses, and only peripheral neuropathy of Grade 1 was noted in 24% of patients. Thus, PR-350 is apparently less neurotoxic than ethanidazole.

The overall response rate in the RT field was 76% (28/37). For patients who received 21 to 30 doses of PR-350, the overall response rate was as high as 89%. The MST and 2-year survival rate for FAS were 15.9 months and 24%, respectively. This result is well in the range of values for sequential CRT for locally advanced NSCLC (3, 6, 7). In the FAS, patients treated with suboptimal doses of PR-350 (10 or 20 doses) were included in the Phase I portion. Although the analysis according to the intended prescribed doses of PR-350 did not show the difference in survival rate (Fig. 4b), the MST and 2-year survival rate for 18 patients actually receiving 21 to 30 doses of PR-350 were 20.9 months and 33%, respectively (Fig. 4a). These values are well compatible with those for concurrent CRT (6, 7). This Phase II result is promising because a survival rate similar to that for concurrent CRT was obtained by daily administration of PR-350 with an incidence of acute toxicities as low as that for sequential CRT.

At present, concurrent CRT is the standard treatment for locally advanced NSCLC. However, acute toxicities are inevitably more common during concurrent CRT (4–7). So, concurrent CRT is not recommended for elderly patients or patients with a poor performance status. The low incidence of hematologic toxicities and radiation esophagitis in this study has special significance for these patients. The results of this Phase I/II study support the hypothesis that adding PR-350 to sequential CRT may decrease the rate of local recurrence without a significant increase in toxicity. Similarly, a promising clinical result obtained by adding a radiosensitizer, efaproxiral, to sequential CRT has been reported (23). Therefore, the present strategy of sequential CRT combined with PR-350 is a promising approach for locally advanced NSCLC, and a randomized study should be pursued. Furthermore, PR-350 may also be an ideal candidate for incorporation into concurrent CRT, as it could potentially increase the efficacy of concurrent CRT without increasing the toxicities.

REFERENCES

1. Pritchard RS, Anthony SP. Chemotherapy plus radiotherapy compared with radiotherapy alone in the treatment of locally advanced, unresectable, non–small-cell lung cancer. A meta-analysis. *Ann Intern Med* 1996;125:723–729.
2. Non–Small Cell Lung Cancer Collaborative Group. Chemotherapy in non–small cell lung cancer: A meta-analysis using updated data on individual patients from 52 randomised clinical trials. *Br Med J* 1995;311:899–909.
3. Chevalier TL, Arriagada R, Quoix E, *et al.* Radiotherapy alone versus combined chemotherapy and radiotherapy in nonresectable non-small-cell lung cancer: First analysis of a randomized trial in 353 patients. *J Natl Cancer Inst* 1991;83:417–423.
4. Nishimura Y. Rationale for chemoradiotherapy. *Int J Clin Oncol* 2004;9:414–420.
5. Rowell NP, O'Rourke NP. Concurrent chemoradiotherapy in non–small cell lung cancer. *Cochrane Database Syst Rev* 2004;4:CD002140.
6. Furuse K, Fukuoka M, Kawahara M, *et al.* Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable

stage III non–small-cell lung cancer. *J Clin Oncol* 1999;17: 2692–2699.
7. Fournel P, Robinet G, Thomas P, *et al.* Randomized Phase III trial of sequential chemoradiotherapy compared with concurrent chemoradiotherapy in locally advanced non-small-cell lung cancer: Groupe Lyon-Saint-Etienne d'Oncologie Thoracique-Groupe Francais de Pneumo-Cancerologie NPC 95-01 Study. *J Clin Oncol* 2005;23:5910–5917.
8. Tannock IF. Treatment of cancer with radiation and drugs. *J Clin Oncol* 1996;14:3156–3174.
9. Hall EJ, Giaccia AJ. Oxygen effect and reoxygenation. In: Hall EJ, Giaccia AJ, editors. Radiobiology for the Radiologist. 6th ed. St. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 85–105.
10. Hall EJ, Giaccia AJ. Radiosensitizers and bioreductive drugs. In: Hall EJ, Giaccia AJ, editors. Radiobiology for the Radiologist. 6th ed. St. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 419–431.
11. Overgaard J. Clinical evaluation of nitroimidazoles as modifiers of hypoxia in solid tumors. *Oncol Res* 1994;6:509–518.
12. Kaanders JH, Bussink J, van der Kogel AJ. Clinical studies of hypoxia modification in radiotherapy. *Semin Radiat Oncol* 2004;14:233–240.
13. Overgaard J, Hansen HS, Overgaard M, *et al.* A randomized double-blind Phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish Head and Neck Cancer Study (DAHANCA) Protocol 5-85. *Radiother Oncol* 1998;46:135–146.
14. Oya N, Shibamoto Y, Sasai K, *et al.* Optical isomers of a new 2-nitroimidazole nucleoside analog (PR-350 series): Radiosensitization efficiency and toxicity. *Int J Radiat Oncol Biol Phys* 1995;33:119–127.
15. Suzuki Y, Hasegawa M, Hayakawa K, *et al.* In vivo study of radiosensitizing effect of hypoxic cell radiosensitizer PR-350 on a human small cell lung cancer. *Anticancer Res* 1999;19: 3993–4000.
16. Shibamoto Y, Kubota T, Kishii K, Tsujitani M. Radiosensitivity of human pancreatic cancer cells in vitro and in vivo, and the effect of a new hypoxic cell sensitizer, doranidazole. *Radiother Oncol* 2000;56:265–270.
17. Matsuoka H, Shibamoto Y, Kubota T, *et al.* In vivo efficacy and pharmacokinetics of a new hypoxic cell radiosensitizer doranidazole in SUIT-2 human pancreatic cancer xenografted in mouse pancreas. *Oncol Rep* 2000;7:23–26.
18. Nemoto K, Shibamoto Y, Ohmagari J, *et al.* Phase Ia study of a hypoxic cell sensitizer doranidazole (PR-350) in combination with conventional radiotherapy. *Anticancer Drugs* 2001;12: 1–6.
19. Sunamura M, Karasawa K, Okamoto A, *et al.* Phase III trial of radiosensitizer PR-350 combined with intraoperative radiotherapy for the treatment of locally advanced pancreatic cancer. *Pancreas* 2004;28:330–334.
20. Inoue A, Kunitoh H, Sekine I, *et al.* Radiation pneumonitis in lung cancer patients: A retrospective study of risk factors and the long-term prognosis. *Int J Radiat Oncol Biol Phys* 2001; 49:649–655.
21. Eschwege F, Sancho-Garnier H, Chassagne D, *et al.* Results of a European randomized trial of Etanidazole combined with radiotherapy in head and neck carcinomas. *Int J Radiat Oncol Biol Phys* 1997;39:275–281.
22. Riese NE, Buswell L, Noll L, *et al.* Pharmacokinetic monitoring and dose modification of etanidazole in the RTOG 85-27 Phase III head and neck trial. *Int J Radiat Oncol Biol Phys* 1997;39: 855–858.
23. Choy H, Nabid A, Stea B, *et al.* Phase II multicenter study of induction chemotherapy followed by concurrent faproxiral (RSR13) and thoracic radiotherapy for patients with locally advanced non–small-cell lung cancer. *J Clin Oncol* 2005;23: 5918–5928.

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SHORT COMMUNICATION

Sequential occurrence of non-small cell and small cell lung cancer with the same *EGFR* mutation

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KEYWORDS
EGFR mutation;
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Summary We report a case of small cell lung cancer (SCLC) developing after prolonged treatment (more than 2 years) for primary adenocarcinoma of the lung, and we show that both the SCLC and non-small cell lung cancer (NSCLC) tissues obtained from the same site share the same deletion in exon 19 of *EGFR*. This case suggests that the activating *EGFR* mutations may confer the pathogenesis of a subset of SCLC.

1. Introduction

The identification of somatic mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) in patients with NSCLC and the association of such mutations with the clinical response to EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib have had a substantial impact on the treatment of this disease [1,2]. To date, however, only a few *EGFR* mutations have been detected in other solid tumors including SCLC.

2. Case report

A 46-year-old Japanese woman with no smoking history was diagnosed in July 2003 with stage IIIB adenocarcinoma (acinar type) of the lung, with a primary tumor in the left lower lobe and pleural disseminations. A computed tomography (CT) scan showing the tumor (arrow) and hematoxylin–eosin (HE) staining of a tumor biopsy specimen are shown (Fig. 1A). The patient received first-line treatment with cisplatin and vinorelbine and showed a brief partial response. She

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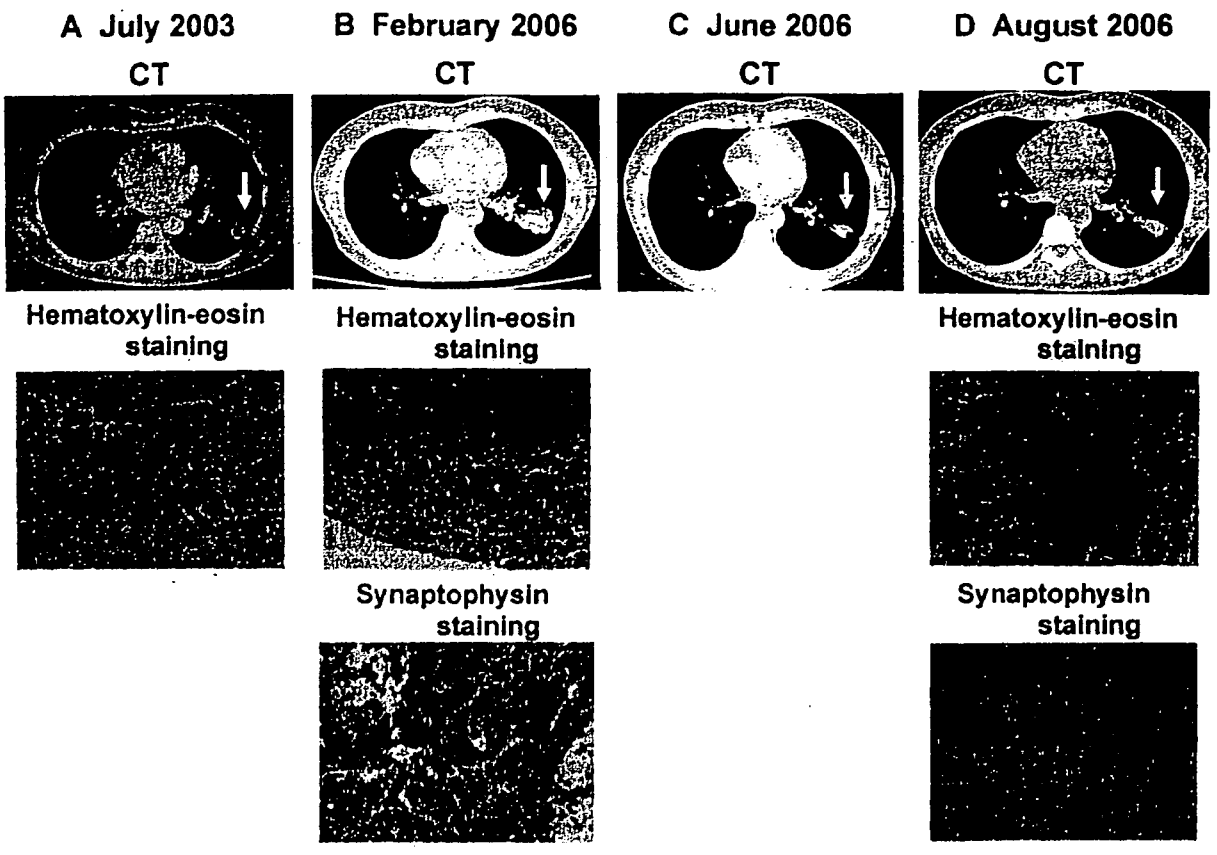

Fig. 1 Chest CT scan: (A) before treatment and HE staining of a tumor biopsy specimen; (B) before second lung biopsy and HE and synaptophysin stainings of a tumor biopsy specimen; (C) after four cycles of cisplatin and irrinotecan; (D) before third lung biopsy and HE and synaptophysin stainings of a tumor biopsy specimen.

subsequently underwent combination chemotherapy with gemcitabine and paclitaxel, manifesting a minor response on radiographic examination. In September 2004, the mass in the left lower lobe had progressed and treatment with gefitinib (250 mg daily) was initiated. After 10 months of treatment with gefitinib alone and transient disease stabilization, a repeat evaluation in July 2005 showed progression of the primary lung tumor. Gefitinib was discontinued, and the patient was enrolled in a phase I clinical trial of new agents. The primary tumor showed no evidence of regression on radiological examination. A magnetic resonance imaging (MRI) scan in December 2005 revealed multiple brain metastases in both hemispheres, which were accompanied by symptoms including headache, nausea, and visual disturbances. After surgical resection of the largest tumor in the right parietal lobe, the patient was exposed to 10 fractions of 3 Gy whole-brain radiotherapy. Her symptoms improved markedly, and MRI scans after radiotherapy revealed almost complete regression of the brain metastases. Histological examination of the resected brain tumor revealed a synaptophysin-positive small cell cancer. The patient provided informed consent to repeated lung biopsies for histological examination. A biopsy specimen of the progressive mass in the left lower lobe in February 2006 revealed SCLC by HE staining and was positive for synaptophysin by immunohistochemical analysis (Fig. 1B). A second lung biopsy specimen was microdissected for extraction of genomic DNA and analysis of *EGFR* mutations. A heterozygous in-frame 15-bp deletion in exon 19 of *EGFR* was detected with the use of the amplification refractory mutation system (ARMS); the genomic DNA of the patient was thus subjected to amplification by the polymerase chain reaction with primers specific for the wild-type (Fig. 2A, left panel) or mutant (Fig. 2A, right panel) versions of exon 19. The deletion was confirmed to be delE746–A750 by nucleotide sequencing. On the basis of the histological diagnosis of SCLC, the patient was treated with four cycles of cisplatin and irrinotecan, and she achieved a partial response (Fig. 1C). A repeat chest CT evaluation in August 2006 showed progression of the primary lung tumor (Fig. 1D). A new lung biopsy specimen revealed nests of adenocarcinoma cells forming small tubular structures, the same subtype of the adenocarcinoma at initial diagnosis on July 2003, and was negative for synaptophysin staining (Fig. 1D). In addition, ARMS analysis of the adenocarcinoma specimen detected the same in-frame 15-bp deletion in exon 19 of *EGFR* that had been identified in the previous SCLC specimen (Fig. 2B).

3. Discussion

EGFR mutations are more frequent in women, Asians, individuals with adenocarcinoma, or those who have never

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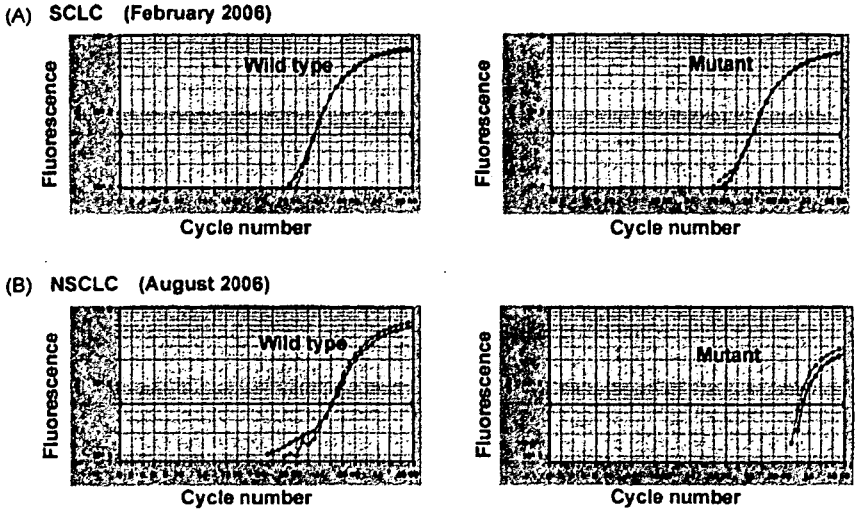

Fig. 2 Results of ARMS analysis of (A) the SCLC. Ascending curves, performed in duplicate (green and red), indicate that wild type (left panel) and deletion mutation in exon 19 (right panel) were detected; (B) the adenocarcinoma. Ascending curves, performed in duplicate (green and red), indicate that wild type (left panel) and deletion mutation in exon 19 (right panel) were detected.

smoked [3–5]. However, EGFR expression has been shown to be low or undetectable in SCLC, and screening of SCLC for *EGFR* mutations has yielded negative results [5]. We previously described the first case of SCLC with a deletion in exon 19 of *EGFR* in a nonsmoking Japanese woman [6]. Another case of SCLC with an 18-bp deletion in exon 19 of *EGFR* in a nonsmoking woman was also recently reported [7]. All reported cases of SCLC with *EGFR* mutations, including the present case, have thus been in women who have never smoked, even though SCLC occurs almost exclusively in smokers. Furthermore, all three of these SCLC cases were initially diagnosed as adenocarcinoma. In the present case, SCLC developed after prolonged treatment (>2 years) for primary adenocarcinoma, and both SCLC and NSCLC (adenocarcinoma) tissues obtained from the same site shared the same *EGFR* mutation. Small cell carcinoma of the prostate, which shares histological similarities with SCLC, has been shown to arise during the course of treatment for prostatic adenocarcinoma, suggesting that prostatic small cell carcinoma may originate from multipotent stem cells of the prostate that have the ability to differentiate into either epithelial or neuroendocrine type carcinoma [8–10]. It remains unclear whether the primary tumor of the present patient originally had a minor SCLC component or whether SCLC arose from transdifferentiation of the adenocarcinoma. Our finding that SCLC and NSCLC developed at the same site in the lung and shared the same somatic *EGFR* mutation suggests, however, that different types of lung cancer may arise from a common stem cell with multiple potential pathways of differentiation.

Conflict of interest

We, all authors, indicate no potential conflicts of interest.

References

[1] Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, 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;350:2129–39.
[2] Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, 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 USA 2004;101:13306–11.
[3] Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
[4] Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919–23.
[5] Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339–46.
[6] Okamoto I, Araki J, Suto R, Shimada M, Nakagawa K, Fukuoka M. EGFR mutation in gefitinib-responsive small-cell lung cancer. Ann Oncol 2006;17:1028–9.
[7] Zakowski MF, Ladanyi M, Kris MG. EGFR mutations in small-cell lung cancers in patients who have never smoked. N Engl J Med 2006;355:213–5.
[8] Valle J, von Boguslawsky K, Stenborg M, Andersson LC. Progression from adenocarcinoma to small cell carcinoma of the prostate with normalization of prostate-specific antigen (PSA) levels. Scand J Urol Nephrol 1996;30:509–12.
[9] Miyoshi Y, Uemura H, Kitami K, Satomi Y, Kubota Y, Hosaka M. Neuroendocrine differentiated small cell carcinoma presenting as recurrent prostate cancer after androgen deprivation therapy. BJU Int 2001;88:982–3.
[10] Trotz C. Prostate cancer with a normal PSA: small cell carcinoma of the prostate—a rare entity. J Am Board Fam Pract 2003;16:343–4.

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PHASE II STUDIES

Phase II study of amrubicin, 9-amino-anthracycline, in patients with advanced non-small-cell lung cancer: a West Japan Thoracic Oncology Group (WJTOG) study

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Summary *Purpose:* We conducted a multicenter phase II study of amrubicin, a novel 9-aminoanthracycline, to evaluate its efficacy and safety in patients with non-small-cell lung cancer (NSCLC). *Patients and methods:* Entry requirements included cytologically or histologically proven measurable NSCLC, stage III or IV, no prior therapy, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, and adequate organ function.

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Amrubicin was given by daily intravenous injection at 45 mg/m^2/day for three consecutive days, repeated at 3 week intervals. Each patient received at least three treatment cycles. *Results:* Sixty-two patients were enrolled in this study. Of the 62 registered patients, 60 were eligible and assessable for efficacy, and 59 for toxicity. Overall response rate was 18.3% (95% confidence interval [CI], 9.5 to 30.4%) and median survival time was 8.2 months (95% CI, 6.7 to 10.4 months). Major toxicity was myelosuppression, with incidences of grade 3 or 4 toxicity of 78.0% for neutropenia, 54.2% for leukopenia, 30.5% for anemia, and 28.8% for thrombocytopenia. Non-hematological toxicities with a greater than 50% incidence were anorexia (69.5%), nausea/vomiting (55.9%), and alopecia (75.9%), but were relatively mild, with grade 3 toxicities observed in only one patient each (1.7%). *Conclusion:* Amrubicin was an active, well-tolerated agent in the treatment of NSCLC.

Keywords Amrubicin · Anthracycline · Non-small-cell lung cancer · Phase II study

Introduction

Non-small-cell lung cancer (NSCLC) is already a leading cause of cancer-related deaths worldwide, with an incidence which is increasing. Current therapeutic options are unsatisfactory, however, and development of novel, more effective antitumor agents has been sought.

Amrubicin is a novel, totally synthetic 9-aminoanthracycline, (+)-(7S,9S)-9-acetyl-9-amino-7-[(2-deoxy-β-D-*erythro*-pentopyranosyl)oxy]-7,8,9,10-tetrahydro-6,11-dihydroxy-5,12-naphthacenedione hydrochloride, with a similar structure to doxorubicin (Fig. 1) [1].

An important characteristic of amrubicin is that it is a prodrug which is converted to the active metabolite, amrubicinol, via reduction of its C-13 ketone group to a hydroxy group by carbonyl reductase [2]. *In vitro* studies have shown that the cytotoxic activity of amrubicinol is 20 to 220 times more potent than that of its parent compound, amrubicin, and has closely similar potency to doxorubicin [3]. The efficacy and toxicity of amrubicin is therefore largely dependent on the tissue distribution of amrubicinol. Among results to date, amrubicin showed more potent antitumor activity than doxorubicin in several human tumor xenografts implanted in nude mice [4], and antitumor activity was closely reflective of the tumor concentration of amrubicinol [5]. The acute toxicity profile of this agent is qualitatively comparable to that of doxorubicin [6], but it has rarely been shown to cause the delayed-type toxicity observed with doxorubicin, particularly cardiotoxicity [7, 8], nor did it exacerbate doxorubicin-induced myocardial toxicity in dogs [8]. Amrubicin and amrubicinol are weak DNA intercalaters and potent inhibitors of topoisomerase II [9].

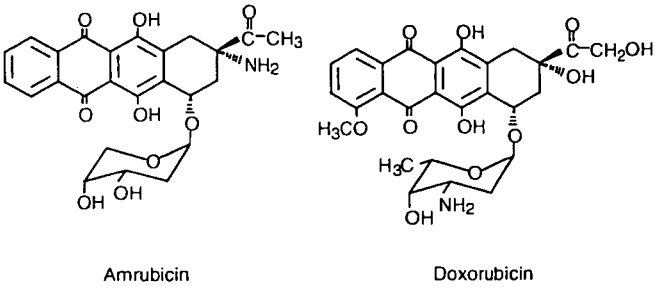

Fig. 1 Chemical structures of amrubicin and doxorubicin

Clinically, amrubicin showed substantial activity against NSCLC in an early phase II study of single intravenous injection of 120 mg/m^2 every 3 weeks, with a partial response (PR) rate in 5 of 20 previously untreated patients (25%; 95% CI, 8.7 to 49.1%) [10]. An additional phase I–II study for NSCLC was conducted by daily intravenous administration for three consecutive days [11], on the basis of experimental findings that amrubicin showed better efficacy in a divided treatment schedule than in a single injection [12]. The maximum tolerated dose was set at 50 mg/m^2/day and the recommended dose for the phase II study was 45 mg/m^2/day. Overall response rate in the phase I–II study was 25.0% (95% CI, 10.7 to 44.9%), with seven PRs in 28 previously untreated patients. These reproducible response rates of more than 20% in two clinical studies suggest that amrubicin may be a promising agent in the treatment of NSCLC, in contrast to doxorubicin which shows only marginal activity against NSCLC [13].

Here, we conducted one of two phase II studies with an identical protocol and monitoring to assess the efficacy and safety of amrubicin by daily intravenous administration for three consecutive days in previously untreated patients with advanced NSCLC.

Patients and methods

Eligibility

This study investigated patients with histologically or cytologically confirmed unresectable NSCLC in stages IIIA, IIIB, and IV. Eligibility criteria included no prior treatment, measurable lesions, an ECOG performance status of 0 to 2, an estimated life expectancy of at least 2 months, and age less than 75 years. Adequate organ function was also required, with a WBC count ≥4,000/μL, platelet count ≥100,000/μL, hemoglobin level ≥10 g/dL, AST and ALT< 100 U/L, total bilirubin level ≤1.5 mg/dL, serum creatinine level ≤1.2 mg/dL, ECG within normal limits, and left ventricular ejection fraction (LVEF, echocardiogram) ≥60%.

Exclusion criteria included symptomatic brain metastasis, bone metastasis requiring radiation treatment, accumulation of plural fluid requiring treatment like drainage, continuous long-term treatment with non-steroidal anti-inflammatory agents, glucocorticoids, or morphine derivatives, serious complications or other active cancer, and those judged by the investigators to be inappropriate for the study. Patients who were pregnant, breast-feeding, or taking inadequate contraceptive precautions were also ineligible. Further, the protocol was amended during the course of the study to exclude patients with confirmed or suspected interstitial pneumonitis owing to the exacerbation of asymptomatic interstitial pneumonitis, identified by chest X-ray or computed tomographic (CT) scan before treatment, in three patients in an identical study, of whom two died [14]. The study protocol was approved by the institutional review board at each hospital, and written informed consent was obtained from all patients prior to participation.

Treatment

Amrubicin (Sumitomo Pharmaceuticals Co., Ltd, Osaka, Japan) was supplied as a freeze-dried powder in vials containing 20 mg each. It was reconstituted in 20 mL of physiological saline or 5% glucose solution and given by intravenous infusion at 45 mg/m^2/day over 5 min on three consecutive days, with the cycle repeated every 3 weeks. A minimum of three cycles was undertaken except in the occurrence of disease progression, unacceptable toxicity or patient noncompliance.

Before treatment, all patients underwent medical history review, physical examination, hematology and serum biochemistry tests, urinalysis, electrocardiography (ECG), echocardiogram for left ventricular ejection fraction (LVEF), and baseline tumor measurements (e.g. chest radiography, CT scans, bone scintigraphy). Measurable and assessable lesions were evaluated within 2 weeks of the start of treatment, and ECG and LVEF within 1 month.

Laboratory variables were measured weekly as a rule, including complete differential blood cell counts, platelet counts, hematocrit, blood biochemistry, and urinalysis. Complete differential blood cell and platelet counts were obtained at least twice weekly when myelosuppression was observed. The ECG was measured with every treatment cycle, and the LVEF test every second cycle. Chest radiography and CT scans were carried out every cycle as a rule.

Subjective symptoms and objective signs were observed and recorded as required

Adjustment of dosage and schedule modification

Treatment was repeated when the WBC count recovered to ≥3,000/μL and the platelet count recovered to ≥100,000/μL. Treatment was delayed when recovery was incomplete until these values were reached, and withdrawn if they were not reached within 5 weeks. Dosage was maintained as in the previous course if the WBC nadir was <1,000/μL for ≤3 days, or ≥1,000/μL and the platelet nadir was ≥50,000/μL, and reduced by 5 mg/m^2/day from the previous dosage if the respective values were <1,000/μL for ≥4 days and/or <50,000/μL.

Response and toxicity evaluation

Response was assessed in accordance with the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [15], which are virtually identical to those of the World Health Organization [16], namely with a complete response (CR) defined as the disappearance of all lesions for a minimum of 4 weeks; a partial response (PR) as a 50% or greater decrease in the sum of the products of the diameters of the measurable lesions for a minimum period of 4 weeks and no new lesions; no change (NC) as a decrease in the tumor mass of less than 50% or any increase of less than 25%; and progression disease (PD) as an increase in the size of any measurable lesion by 25% or the appearance of new lesions.

Toxicity was graded based on the side effect record form of the Japan Society for Cancer Therapy criteria [15]. Toxicity items not included on the record form were recorded as present or absent without grading.

Table 1 Patient characteristics

Patient characteristics	No. of patients	Percent
No. of enrolled patients	62	
No. of eligible patients	60	
Age, years		
Median	65.5	
Range	41–75	
Gender		
Male	37	61.7
Female	23	38.3
Performance status (ECOG scale)		
0	8	13.3
1	41	68.3
2	11	18.3
Histology		
Squamous cell carcinoma	24	40.0
Adenocarcinoma	29	48.3
Large cell carcinoma	7	11.7
Stage		
IIIA	5	8.3
IIIB	14	23.3
IV	41	68.3

ECOG Eastern Cooperative Oncology Group

Table 2 Response to amrubicin

	No. of patients	Response (No. of patients)					Response rate, % [95%CI]
		CR	PR	NC	PD	NE	
Eligible patients	60	0	11	30	16	3	18.3 [9.5–30.4]
Histology:							
Squamous cell carcinoma	24	0	6	9	7	2	25.0
Adenocarcinoma	29	0	5	17	6	1	17.2
Large cell carcinoma	7	0	0	4	3	0	0
Stage							
IIIA	5	0	2	3	0	0	40.0
IIIB	14	0	3	7	3	1	21.4
IV	41	0	6	20	13	2	14.6
Performance status (ECOG):							
0	8	0	2	4	2	0	25.0
1	41	0	8	22	10	1	19.5
2	11	0	1	4	4	2	9.1

Abbreviations: CR, complete response; PR, partial response; NC, no change; PD, progressive disease; NE, not evaluated; ECOG, Eastern Cooperative Oncology Group

Statistical analyses

Primary endpoint was response rate. In this study, the number of patients was estimated as 60 to guarantee at least 10% response rate with a probability of 95% at 20% of expected response rate. Secondary endpoints were overall survival and safety. The time frame for overall survival was defined as the time from treatment until onset of the event. Kaplan–Meier life table was constructed for patient survival, 1-year survival, 2-year survival and median survival time [17]. All analyses were done using SAS, version 8.2 (SAS Institute Inc., Cary, North Carolina).

Results

Patient characteristics

Of 62 patients registered between April 1995 and September 1997 through 14 participating institutions in Japan, 60 patients were eligible and assessable for efficacy and 59 were assessable for safety (Table 1). Two patients were ineligible due to the protocol deviation in the inclusion criteria, not NSCLC in one patient and receiving prior chemotherapy in a second patient. Another patient was not safety-assessable due to a withdrawal of informed consent soon after the completion of first cycle treatment. By stage, 41 patients had stage IV disease, 14 had stage IIIB, and 5 had stage IIIA. Histologically, 29 patients had adenocarcinoma, 24 squamous cell carcinoma, and only 7 large cell carcinoma. Most patients had a good performance status (PS) of 0 or 1, but 11 (18.3%) had PS of 2. No patient had received any prior treatment, including radiotherapy.

Response and survival

Response among the 60 eligible patients was 11 PRs, giving an overall response rate of 18.3% (95% CI, 9.5 to 30.4%) (Table 2). Responders were 6 (25.0%) of 24 patients with squamous cell carcinoma and 5 (17.2%) of 29 with adenocarcinoma.

Regarding the overall survival curve, median survival time was 8.2 months (95% CI, 6.7 to 10.4 months), and 1- and 2-year survival rates were 34.9% and 7.6%, respectively (Fig. 2).

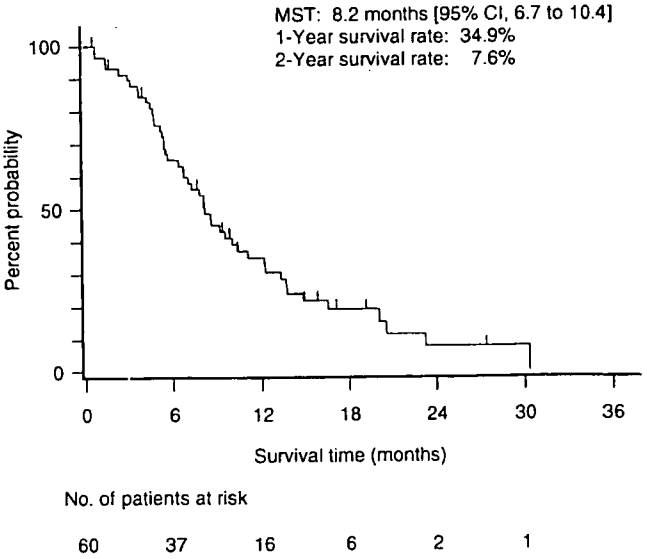

Fig. 2 Overall survival of patients with advanced non-small-cell lung cancer following treatment with amrubicin. Median survival time was 8.2 months (95% confidence interval, 6.7–10.4 months)

Table 3 Major treatment-related hematologic toxicity of amrubicin

Toxicity	No. of assessable patients	Toxicity grade					
		1	2	3	4	≥1	≥3
		(No. of patients)				Frequency (%)	
Anemia (hemoglobin)	59	16	17	16	2	86.4	30.5
Leukopenia	59	5	16	21	11	89.8	54.2
Neutropenia	59	0	7	12	34	89.8	78.0
Thrombocytopenia	59	12	3	9	8	54.2	28.8

Safety

Hematologic toxicities observed throughout the present clinical trial for which a causal relationship to amrubicin could not be denied are shown in Table 3. The most common was myelosuppression, particularly neutropenia, leukopenia and anemia (hemoglobin decrease) with frequencies of 89.8, 89.8 and 86.4%, respectively. Thrombocytopenia was somewhat lower frequent (54.2%). Among these, the incidence of grade 3 or 4 toxicity was 78.0% for neutropenia, 54.2% for leukopenia, 30.5% for anemia, and 28.8% for thrombocytopenia.

Although mild, non-hematologic toxicities included stomatitis, anorexia, nausea/vomiting, diarrhea, fever, alopecia, and AST/ALT elevation were each seen in more than 10% of the patients (Table 4). Grade 3/4 episodes were seen only for anorexia, nausea/vomiting, and alopecia with frequencies of each 1.7%. ECG abnormalities for which a relationship to amrubicin was unknown were seen in two patients, one with transient negative T and the second with ST depression, but were judged not to be clinically significant on review by a cardiologist. A decrease in LVEF for which a causal relation to amrubicin could not be denied was seen in two patients, one with a decrease from 73% at base line to 53% after three cycles of treatment and in the second from 69 to 52% after two cycles. LVEF values fluctuate readily under the influence of various factors, and these changes are not particularly abnormal. Moreover, no accompanying changes in ECG or symptoms were seen, and thus the medical significance was not clear. However, given that amrubicin is an anthracycline derivative, like doxorubicin, the cardiotoxicity of which is well known, treatment was discontinued as precaution.

Discussion

This present study indicates that amrubicin is an active agent in the treatment of patients with NSCLC. Overall response rate was 18.3% (95% CI, 9.5 to 30.4%) and median survival time was 8.2 months (95% CI, 6.7 to 10.4 months). In an identical study, which included 61 patients, amrubicin achieved overall response rate of 27.9%, with 1 CR and 16 PRs, and median survival was 9.8 months [14]. Thus, the overall response rate for amrubicin in these two studies with an identical protocol was 23.1% (95% CI, 16.0 to 31.7%).

Table 4 Major treatment-related non-hematologic toxicities of amrubicin

Toxicity	No. of assessable patients	Toxicity grade					
		1	2	3	4	≥1	≥3
		(No. of patients)				Frequency (%)	
Stomatitis	59	7	2	0	0	15.3	0
Anorexia	59	20	20	1	–[a]	69.5	1.7
Nausea and vomiting	59	21	11	1	–[a]	55.9	1.7
Diarrhea	59	9	0	0	0	15.3	0
Fever	59	8	7	0	–[a]	25.4	0
Phlebitis	59	2	0	0	0	3.4	0
Alopecia	58	27	16	1	–[a]	75.9	1.7
Total bilirubin elevation	58	4	0	0	0	6.9	0
AST elevation	59	10	1	0	0	18.6	0
ALT elevation	59	9	4	0	0	22.0	0
ALP elevation	59	3	1	0	0	6.8	0
BUN elevation	59	4	0	0	0	6.8	0
Others[b]	LVEF decrease, 2/42[c]; ECG abnormality, 2/54[c]						

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urine nitrogen; LVEF, left ventricular ejection fraction; ECG, electrocardiogram

[a] Toxicity grade not defined.

[b] Toxicities not graded.

[c] Ratio of number of reported patients to number of observed patients.

NSCLC is known to have poor sensitivity to chemotherapy [18–20], but the recent development of newer agents such as paclitaxel, docetaxel, gemcitabine, vinorelbine, and irinotecan has seen considerable improvements in therapeutic outcomes [21, 22], with response rates of more than 20% when used as single agents in previously untreated patients with advanced NSCLC. The present results indicate that amrubicin which is different from these newer agents in mode of action [9], namely the inhibition of topoisomerase II, is comparable to these newer agents in efficacy for NSCLC.

The major toxicity of amrubicin was hematologic, particularly neutropenia and leukopenia. In contrast, no febrile neutropenia was observed. Non-hematologic toxicity was relatively mild, with the only grade 3/4 episodes being seen for anorexia, nausea/vomiting, and alopecia with frequencies of each 1.7%. These safety results are supported by those from an identical study [14]. In that study, interstitial pneumonitis developed in three patients, of whom two died [14]. So the protocol was revised to exclude patients with confirmed or suspected interstitial pneumonitis. In the present study, interstitial pneumonitis was not seen.

Among cardiotoxicity, abnormalities in ECG and a decrease in LVEF were seen in two patients each. These changes were asymptomatic and did not overlap in the same patients. These findings suggest that unlike the case of cardiomyopathy caused by doxorubicin, the effect of amrubicin on cardiac function is neither serious nor definite. It is well known that doxorubicin experimentally and clinically causes cardiomyopathy which is cumulative toxicity caused by long-term treatment. In contrast, amrubicin on repeated administration did not cause cardiotoxicity or aggravate doxorubicin-induced cardiotoxicity in rabbits and dogs [7, 8]. Although cardiomyopathy has not been clinically observed to date, careful observation on the effects of amrubicin on the heart is required in further clinical studies, especially for patients on long-term treatment.

In conclusion, amrubicin showed promising activity against NSCLC in the present study. In a previous study, moreover, the combination of amrubicin and cisplatin demonstrated an impressive response rate and median survival time for extensive-stage SCLC (87.8% and 13.6 months, respectively) [23]. We are presently planning a phase II study of the combination of amrubicin and cisplatin for advanced NSCLC.

References

1. Ishizumi K, Ohashi N, Tanno N (1987) Stereospecific total synthesis of 9-aminoanthracyclines: (+)-9-amino-9-deoxydaunomycin and related compounds. J Org Chem 52:4477–4485
2. Tani N, Yabuki M, Komuro S, Kanamaru H (2005) Characterization of the enzymes involved in the in vitro metabolism of amrubicin hydrochloride. Xenobiotica 35:1121–1133
3. Yamaoka T, Hanada M, Ichii S, Morisada S, Noguchi T, Yanagi Y (1998) Cytotoxicity of amrubicin, a novel 9-aminoanthracycline, and its active metabolite amrubicinol on human tumor cells. Jpn J Cancer Res 89:1067–1073
4. Morisada S, Yanagi Y, Noguchi T, Kashiwazaki Y, Fukui M (1989) Antitumor activities of a novel 9-aminoanthracycline (SM-5887) against mouse experimental tumors and human tumor xenografts. Jpn J Cancer Res 80:69–76
5. Noguchi T, Ichii S, Morisada S, Yamaoka T, Yanagi Y (1998) In vivo efficacy and tumor-selective metabolism of amrubicin to its active metabolite. Jpn J Cancer Res 89:1055–1060
6. Morisada S, Yanagi Y, Kashiwazaki Y, Fukui M (1989) Toxicological aspects of a novel 9-aminoanthracyline, SM-5887. Jpn J Cancer Res 80:77–82
7. Suzuki T, Minamide S, Iwasaki T, Yamamoto H, Kanda H (1997) Cardiotoxicity of a new anthracycline derivative (SM-5887) following intravenous administration to rabbits: Comparative study with doxorubicin. Invest New Drugs 15:219–225
8. Noda T, Watanabe T, Kohda A, Hosokawa S, Suzuki T (1998) Chronic effects of a novel synthetic anthracycline derivative (SM-5887) on normal heart and doxorubicin-induced cardiomyopathy in dogs. Invest New Drugs 16:121–128
9. Hanada M, Mizuno S, Fukushima A, Saito Y, Noguchi T, Yamaoka T (1998) A new antitumor agent amrubicin induces cell growth inhibition by stabilizing topoisomerase II-DNA complex. Jpn J Cancer Res 89:1229–1238
10. Hiraki S, Shinkai T, Furuse K, Fukuoka M, Ohnoshi T, Kimura I (1993) A phase II study of SM-5887, a novel 9-aminoanthracycline, for non-small-cell lung cancer. Proceedings of the 18th international congress of chemotherapy 866–867
11. Sugiura T, Ariyoshi Y, Negoro S, Nakamura N, Ikegami H, Takada M, Yana T, Fukuoka M (2005) Phase I/II study of amrubicin, a novel 9-aminoanthracycline, in patients with advanced non-small-cell lung cancer. Invest New Drugs 23:331–337
12. Noguchi T, Ichii S, Morisada S, Yamaoka T, Yanagi Y (1999) Evaluation of amrubicin with a 5 day administration schedule in a mouse model. Jpn J Cancer Chemother 26:1305–1312
13. Cortes EP, Takita H, Holland JF (1974) Adriamycin in advanced bronchogenic carcinoma. Cancer 34:518–525
14. Sawa T, Yana T, Takada M, Sugiura T, Kudoh S, Kamei T, Isobe T, Yamamoto H, Yokota S, Katakami N, Tohda Y, Kawakami A, Nakanishi Y, Ariyoshi Y (2006) Multicenter phase II study of amrubicin, 9-amino-anthracycline, in patients with advanced non-small-cell lung cancer (Study 1): West Japan Thoracic Oncology Group (WJTOG) trial. Invest New Drugs 24:151–158
15. Japan Society for Cancer Therapy (1993) Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. J Jpn Soc Cancer Ther 28:101–130
16. World Health Organization (1979) Handbook for Reporting Results of Cancer Treatment (WHO Offst Publication No. 48). World Health Organization, Geneva, Switzerland
17. Kaplan WH, Meier P (1952) Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:583–612
18. Grilli R, Oxman AD, Julian JA (1993) Chemotherapy for advanced non-small cell lung cancer: how much benefit is enough? J Clin Oncol 11:1866–1872
19. Souquet PJ, Chauvin F, Boissel JP, Cellerino R, Cormier Y, Ganz PA, Kaasa S, Pater JL, Quoix E, Rapp E, Tumarello D, Williams J, Woods BL, Bernard JP (1993) Polychemotherapy in advanced non-small cell lung cancer: a meta-analysis. Lancet 342:19–21
20. Non-small Cell Lung Cancer Collaborative Group (1995) Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. BMJ 311:899–909
21. Ginsberg RJ, Vokes EE, Raben A (2001) Non-small cell lung cancer. In: DeVita VTJ, Hellman S, Rosenberg SA (eds) Cancer:

principles and practice of oncology, 6th edn. Lippincott Williams and Wilkins, Philadelphia, pp 925–983
22. Bunn PA, Kelly K (1998) New chemotherapeutic agents prolong survival and improve quality of life in non-small cell lung cancer: a review of the literature and future directions. Clin Cancer Res 5:1087–1100
23. Ohe Y, Negoro S, Matsui K, Nakagawa K, Sugiura T, Takada Y, Nishiwaki Y, Yokota S, Kawahara M, Saijo N, Fukuoka M, Aritoshi Y (2005) Phase I–II study of amrubicin and cisplatin in previously untreated patients with extensive-stage small-cell lung cancer. Ann Oncol 16:430–436

Combination of SN-38 with gefitinib or imatinib overcomes SN-38-resistant small-cell lung cancer cells

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Abstract. Irinotecan is one of the effective anticancer agents for small-cell lung cancer (SCLC) and 7-ethyl-10-hydroxy-campthothecin (SN-38) is an active metabolite of irinotecan. Gefitinib and imatinib are tyrosine kinase inhibitors which have clinical activities in several malignancies and they are also potent inhibitors of breast cancer resistance protein (BCRP) transporter, which confers the resistance of topoisomerase I inhibitors including SN-38 and topotecan. The cytotoxicity of SN-38, gefitinib and imatinib for the SN-38-resistant cells (SBC-3/SN-38) from human SCLC cells, SBC-3, was evaluated using AlamarBlue assay. The drug concentration required to inhibit the growth of tumor cells by 50% (IC_{50}) for 96-h exposure was used to evaluate the cytotoxicity. BCRP expression was determined by Western blotting and immunofluorescence staining. Intracellular topotecan accumulation was evaluated by flow cytometry. No differences were observed in the IC_{50} values (mean ± SD) of the tyrosine kinase inhibitors between the SBC-3 cells and the SBC-3/SN-38 cells: 15±1.6 and 12±2.8 μM of gefitinib, respectively; 15±0.51 and 14±3.9 μM of imatinib, respectively. The SBC-3/SN-38 was 9.5-fold more resistant to SN-38 than the parental SBC-3. The SBC-3/SN-38 restored sensitivity to SN-38 when combined with 8 μM gefitinib or 8 μM imatinib, even though the IC_{50} values of SN-38 combined with gefitinib or imatinib in the SBC-3 cells did not change. BCRP was equally overexpressed in the SBC-3/SN-38 with and without gefitinib or imatinib. In addition, the BCRP expression on the SBC-3/SN-38 cell membrane with and without gefitinib seemed to be equal. Gefitinib increased intracellular accumulation of topotecan in the SBC-3/SN-38 cells. Gefitinib or imatinib reversed SN-38-resistance in these SCLC cells, possibly due to intracellular accumulation of SN-38 without any change in BCRP quantity. Irinotecan with gefitinib or imatinib might be effective for SCLC refractory to irinotecan.

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Key words: gefitinib, imatinib, irinotecan, topotecan, small-cell lung cancer

Introduction

Gefitinib, an epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI), showed antitumor activity in several cancers, especially in non-small-cell lung cancer (NSCLC) (1). Imatinib is also a TKI and it has demonstrated clinical efficacy in Bcr-Abl-expressing chronic myeloid leukemia and c-Kit-expressing gastrointestinal stromal tumors (2). Breast cancer resistance protein (BCRP) is a transporter, which contributes to a reduced accumulation of topoisomerase I inhibitors in the cells by an enhanced efflux of them (3,4). Recently, gefitinib and imatinib have been reported to be potent inhibitors of BCRP and reverse the BCRP-mediated resistance (5).

A combination of irinotecan and cisplatin is one of the standard chemotherapy regimens in the treatment of extensive disease small-cell lung cancer (SCLC) (6). 7-ethyl-10-hydroxy-campthothecin (SN-38) is an active metabolite of irinotecan. We have already established an SN-38-resistant subline (SBC-3/SN-38) from a human SCLC cell line, SBC-3 (7). In the present study, the usefulness and the mechanism of the combination of either SN-38 with gefitinib or imatinib for the SBC-3/SN-38 cells were evaluated.

Materials and methods

Chemicals and reagents. SN-38 and topotecan were provided by Yakult Honsha Co., and SmithKline Beecham Co., Tokyo, Japan, respectively. Gefitinib and imatinib were purchased from AstraZeneca, Osaka and Novartis Pharma, Tokyo, Japan,

respectively. The drugs were dissolved in dimethylsulfoxide and the drug solutions were stored at -20°C. AlamarBlue (UK-Serotec Ltd., Oxford, UK) was purchased from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan.

Cell culture. The parent cell line, SBC-3 was established from bone marrow aspirate of a previously untreated patient with SCLC (8). The growth medium (RPMI-FBS) was RPMI-1640 supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). The SN-38-resistant subline (SBC-3/SN-38) was established by the continuous exposure of the SBC-3 cells to increasing concentrations of SN-38 (7).

Assay of drug sensitivity. Drug sensitivity was determined using an AlamarBlue assay (9). Briefly, 50 μl of RPMI-FBS containing serial concentrations of each chemotherapeutic agent was prepared in 96-well flat-bottomed microplates (Coster 3596, Corning Inc., Corning, NY, USA). Next, 50 μl of RPMI-FBS containing 500 cells for SBC-3 and 1500 cells for SBC-3/SN-38 were added to each well. The cells were then incubated at 37°C for 96 h in a highly humidified incubator with 5% CO_2 and 95% air. Next, 10 μl of AlamarBlue was added to each well. After incubation at 37°C for 5 h, the fluorescence of each well was measured using Fluoroskan Ascent (Labsystems Inc., Franklin, MA, USA) with 544-nm excitation and 590-nm emission. The fluorescence of a well without chemotherapeutic agents was used as the control and a well containing only RPMI-FBS and AlamarBlue was used to determine the background. The percentage of surviving cells was calculated using the following formula: [(mean fluorescence in 4 test wells - fluorescence in background wells)/(mean fluorescence in control wells - fluorescence in background wells)] x 100. The drug concentration required to inhibit the growth of tumor cells by 50% (IC_{50}) was determined by plotting the logarithm of the drug concentration versus the percentage of surviving cells. Determinations were carried out in quadruplicate in each experiment, and the results were confirmed by 3 or more separate experiments.

Western blotting. The cells were cultured for 96 h in the absence or presence of 2 or 8 μM of gefitinib or imatinib in RPMI-FBS. The cells were lysed in a radioimmunoprecipitation assay buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1% SDS, 0.5% deoxycholate, 1% NP-40, 1 mM EDTA and ß-mercaptoethanol plus protease and phosphatase inhibitors. Aliquots of cell lysates (14 μg protein per lane) were electrophoresed on a 10% Readygels J (Bio-Rad, Tokyo, Japan) and then were transferred to PVDF membrane. The membrane was blocked in 5% non-fat dry milk in 20 mM Tris-HCl, pH 8.0, 150 mM and 0.05% Tween-20 at room temperature for 1 h. The membrane was then incubated with an appropriate dilution of the primary antibody at 4°C overnight. Following washing, a secondary antibody, was diluted at 10000-fold for 1 h at room temperature. Anti-BCRP monoclonal antibody (BXP-21) from Kamiya Co. (Seatle, WA, USA) (1:500) and anti-actin monoclonal antibody (MAB1501) from Chemicon International Inc. (Temecula, CA, USA) (1:1000) as the primary antibodies and the enhanced chemiluminescence detection system (Amersham Co., Bucks, UK) were used.

Table I. IC_{50} values (μM; mean ± SD) of gefitinib and imatinib in the parent (SBC-3) and SN-38-resistant subline (SBC-3/SN-38).

	IC_{50}	
	Gefitinib	Imatinib
SBC-3	15±1.6	15±0.51
SBC-3/SN-38	12±2.8	14±3.9

IC_{50}, 50% inhibitory concentration; SD, standard deviation.

Immunofluorescence. The cells were incubated in RPMI-FBS with and without 8 μM gefitinib for 1 and 4 h at a cell density of 1x10^6/ml in a 37°C/5% CO_2 incubator. At the end of each time period, the cells were collected and washed twice with phosphate-buffered saline (PBS) at 4°C. The location of BCRP was visualized by staining the cells using anti-BCRP monoclonal antibody (sc-18841) (1:50) and goat anti-mouse IgG-FITC (sc-2781) (1:100) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) using a confocal laser-scanning microscope (Zeiss LSM 510, Tokyo, Japan). The excitation of fluorescent dye was performed at 488 nm for IgG-FITC.

Intracellular topotecan accumulation. The cells were incubated in RPMI-FBS with drugs (50 or 100 μM topotecan with and without 8 μM gefitinib) at a cell density of 1x10^6/ml in a 37°C/5% CO_2 incubator for 15 min. At the end of each time, the cells were collected and washed twice with PBS at 4°C. Topotecan was detected with 488-nm excitation and 585-nm emission by FACS Calibur (Becton-Dickinson Immunocytometry Systems, San Jose, CA, USA). The data were analyzed according to the ModFit LT software (Verity Software House, Inc., Topsham, ME, USA).

Results

The mean values for IC_{50} of gefitinib and imatinib for SBC-3 and SBC-3/SN-38 cells ranged from 12 to 15 μM (Table I). The resistant cells retained their sensitivity to gefitinib and imatinib at the same level as that observed in the parent cells. The combination effect of SN-38 with gefitinib or imatinib is shown in Table II. When the SBC-3 cells were simultaneously treated with gefitinib or imatinib (0.5, 2 and 8 μM), the IC_{50} values of SN-38 were approximately 9.4-11 μM. In contrast, the IC_{50} values of SN-38 for the SBC-3/SN-38 declined from 95 to 12 or 13 μM with gefitinib or imatinib, respectively, in a dose-dependent manner. SN-38 sensitivity in the SBC-3/SN-38 cells was restored by adding 8 μM gefitinib or imatinib.

The overexpression of BCRP in SBC-3/SN-38 is shown in lanes 2 and 8 in Fig. 1. Neither imatinib nor gefitinib affected the BCRP levels in SBC-3/SN-38 (lanes 4, 6, 10 and 12). The BCRP was located on cell membrane in SBC-3/SN-38 and seemed equivalent both with and without gefitinib (Fig. 2). There was no difference in the expression on the cell membrane between 1- and 4-h treatment of gefitinib. Fig. 3 shows the

Table II. IC_{50} values (nM; mean ± SD) for SN-38 with several concentrations of gefitinib or imatinib in the parent (SBC-3) and SN-38-resistant subline (SBC-3/SN-38).

	Gefitinib (μM)	IC_{50} for SN-38	Imatinib (μM)	IC_{50} for SN-38
SBC-3	0	10±0.11		
	0.5	10±0.48	0.5	9.6±0.35
	2	9.4±0.30	2	11±0.35
	8	10±1.4	8	11±0.38
SBC-3/SN-38	0	95±4.3		
	0.5	40±1.5	0.5	42±0.83
	2	22±2.7	2	22±2.0
	8	12±0.52	8	13±1.3

IC_{50}, 50% inhibitory concentration; SD, standard deviation.

effects of gefitinib on the intracellular accumulation of topotecan. In a dose-dependent manner, topotecan was accumulated in the SBC-3 cells equally irrespective of adding gefitinib. There were no differences in the cellular fluorescence of SBC-3/SN-38 cells without gefitinib. However, gefitinib increased the intracellular accumulation of topotecan in the SBC-3/SN-38 in a dose-dependent manner.

Discussion

Gefitinib and imatinib reversed SN-38-resistance in the SBC-3/SN-38 overexpressing BCRP. Previous studies have indicated that gefitinib or imatinib reversed topoisomerase I inhibitor-resistance (10-14), while we showed that both TKIs were equally effective. Imatinib reversed BCRP-mediated resistance to SN-38 while also increasing the accumulation of topotecan in osteosarcoma cells and breast cancer cells overexpressing BCRP (10,11). The mechanism for overcoming resistance, however, remains unclear. Houghton *et al* showed that imatinib inhibited the function of BCRP but was not a substrate for the protein (10), while Burger *et al* revealed that it was a competitive substrate for BCRP (11). Other investigators showed that gefitinib reversed topoisomerase I inhibitor-resistance (12-14). Nakamura *et al* (13) and Yanase *et al* (12) suggested that the mechanism was not the competitive inhibition but the inhibition of the pump function of BCRP using an intravesicular transport assay. Recently, Nakanishi

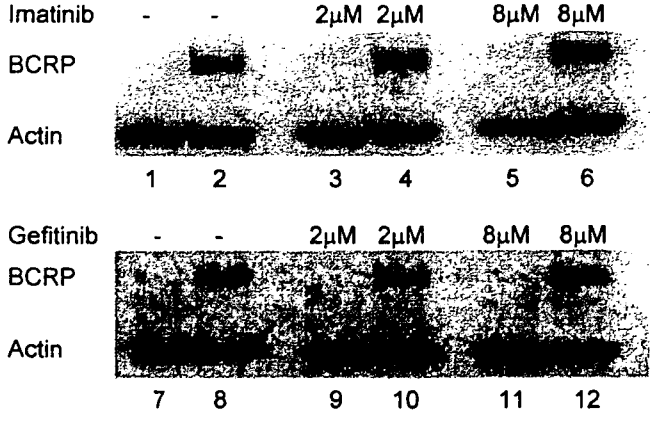

Figure 1. The expression of BCRP in SBC-3 and SBC-3/SN-38 cells treated with gefitinib or imatinib. The overexpression of BCRP in SBC-3/SN-38 is shown in lanes 2 and 8. Imatinib or gefitinib did not affect the BCRP levels in SBC-3/SN-38 (lanes 4, 6, 10 and 12). SBC-3 cells did not have any detectable BCRP with and without imatinib or gefitinib.

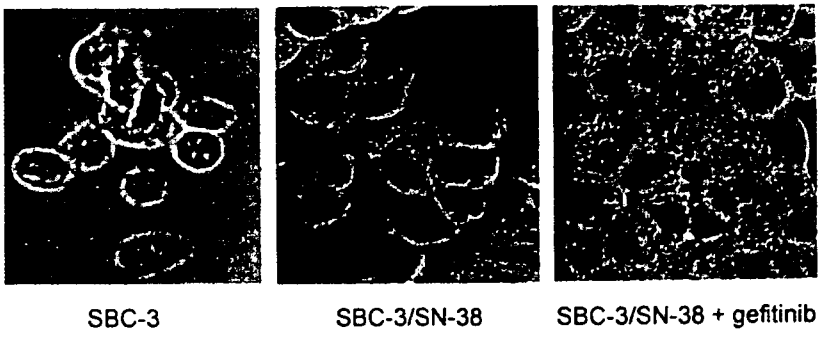

Figure 2. The expression of BCRP in SBC-3/SN-38 cells with 1-h treatment of gefitinib. The BCRP was located on cell membrane in SBC-3/SN-38 and seemed equivalent with and without gefitinib. There was no detectable BCRP expression in SBC-3.

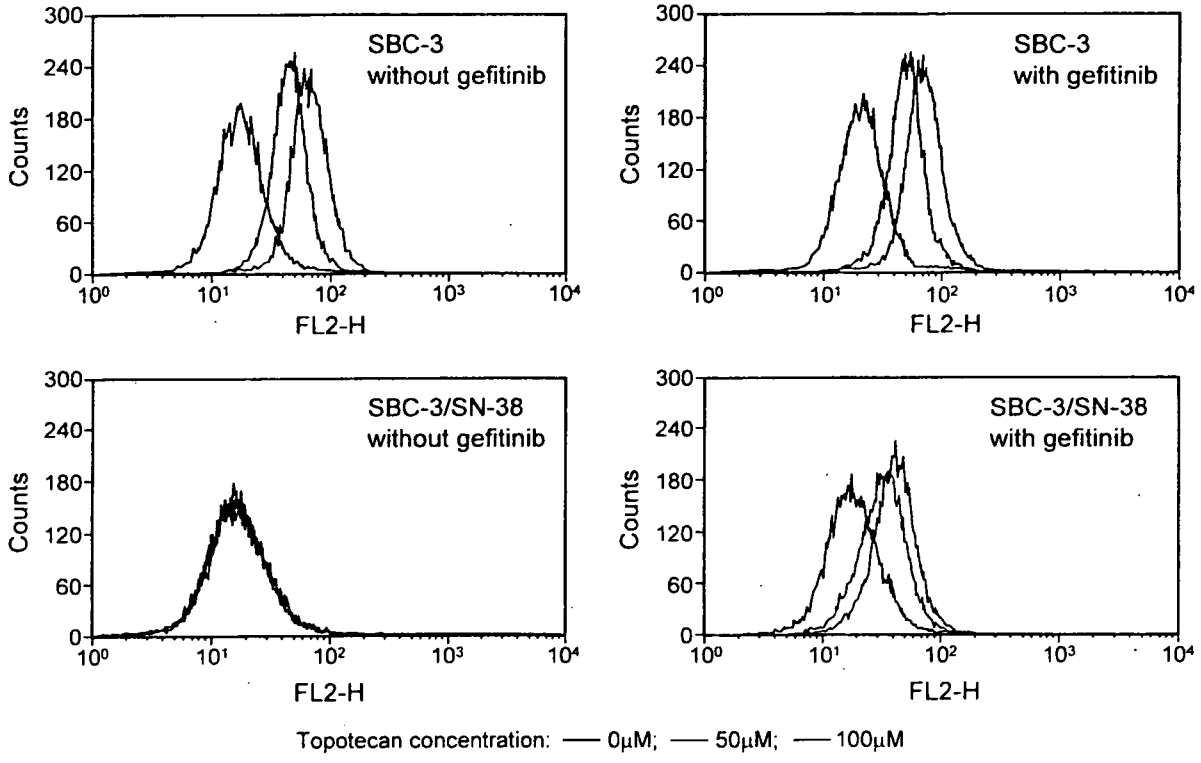

Figure 3. Effect of gefitinib on the intracellular topotecan accumulation. In a dose-dependent manner, topotecan was accumulated in the SBC-3 cells equally despite the addition of gefitinib. There were no differences in the cellular fluorescence of SBC-3/SN-38 cells without gefitinib. However, gefitinib increased the intracellular accumulation of topotecan in SBC-3/SN-38 in a dose-dependent manner.

et al reported that imatinib decreased the BCRP level in the mitoxantrone-resistant K562/BCRP-MX10 cells overexpressing BCRP (15). To our knowledge, there have been no reports regarding the change of the BCRP expression level by gefitinib. We experimented using Western blotting and immunofluorescence in order to determine whether gefitinib could either decrease the total BCRP or induce an internalization of BCRP. As a result, gefitinib did not affect the BCRP expression level either in the cells or on the cell membrane. Meanwhile, the intracellular accumulation of topotecan increased in the SBC-3/SN-38 cells in a dose-dependent manner. Although we could not determine from our study whether gefitinib is a competitive inhibitor or not, it might therefore increase the SN-38 sensitivity in the SBC-3/SN-38 cells, not due to a decrease in BCRP but to pump dysfunction of BCRP.

The concentration of 8 μM of imatinib or gefitinib was considered to be relatively high in terms of the clinical settings. In the case of imatinib, this was a clinically achievable serum concentration with and without chemotherapeutic agents (16,17). Meanwhile, the pharmacologically achievable gefitinib concentration was 1 μM at most (18), although the maximum plasma concentration was 3.875 μg/ml (8.67 μM) in the child treated with 500 mg/m^2 of gefitinib (19). The mean concentration in breast tumor tissues was 16.7 μM (median, 14.3 μM; range, 0.2-25.8 μM) in the 19 breast cancer patients, which was 42 times higher than plasma (20). Eight μM of gefitinib may therefore be an achievable concentration in lung tissue.

The effectiveness of gefitinib for SCLC has only been previously reported in one case report (21). A single agent of gefitinib had effectiveness in NSCLC (1); however, the addition of gefitinib to standard two-drug combinations such as cisplatin plus gemcitabine or carboplatin plus paclitaxel did not produce any survival advantage (22,23). Although imatinib had an antitumor activity for gastrointestinal stromal tumors expressing c-Kit (24), it did not show any effectiveness for SCLC, which commonly expresses c-Kit independently (25-27). In addition, a phase I study of imatinib with cisplatin and irinotecan in patients with untreated extensive SCLC showed increased toxicities (neutropenia, diarrhea and thrombosis) although 5 partial responses of 6 evaluable cases were noted (17). Monoclonal antibody against EGFR, cetuximab, combined with irinotecan was effective for irinotecan-refractory colorectal cancer (28). The two-drug combination of irinotecan with either gefitinib or imatinib may therefore be an interesting regimen for irinotecan-refractory SCLC.

In conclusion, gefitinib and imatinib similarly restore the SN-38 sensitivity in the SBC-3/SN-38 overexpressing BCRP. A combination of irinotecan with gefitinib or imatinib for irinotecan-refractory SCLC might thus be considered in clinical trials.

References

1. Lynch TJ, Adjei AA, Bunn PA Jr, Eisen TG, Engelman J, Goss GD, Haber DA, Heymach JV, Janne PA, Johnson BE, Johnson DH, Lilenbaum RC, Meyerson M, Sandler AB, Sequist LV, Settleman J, Wong KK and Hart CS: Summary statement: novel agents in the treatment of lung cancer: advances in epidermal growth factor receptor-targeted agents. Clin Cancer Res 12: S4365-S4371, 2006.
2. Hochhaus A: Imatinib mesylate (Glivec, Gleevec) in the treatment of chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST). Ann Hematol 83 (suppl 1): S65-S66, 2004.

3. Maliepaard M, van Gastelen MA, De Jong LA, Pluim D, van Waardenburg RC, Ruevekamp-Helmers MC, Floot BG and Schellens JH: Overexpression of the BCRP/MXR/ABCP gene in a topotecan-selected ovarian tumor cell line. Cancer Res 59: 4559-4563, 1999.
4. Garcia-Carbonero R and Supko JG: Current perspectives on the clinical experience, pharmacology, and continued development of the camptothecins. Clin Cancer Res 8: 641-661, 2002.
5. Sugimoto Y, Tsukahara S, Ishikawa E and Mitsuhashi J: Breast cancer resistance protein: molecular target for anticancer drug resistance and pharmacokinetics/pharmacodynamics. Cancer Sci 96: 457-465, 2005.
6. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S and Saijo N: Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 346: 85-91, 2002.
7. Chikamori M, Takigawa N, Kiura K, Tabata M, Shibayama T, Segawa Y, Ueoka H, Ohnoshi T and Tanimoto M: Establishment of a 7-ethyl-10-hydroxy-camptothecin-resistant small cell lung cancer cell line. Anticancer Res 24: 3911-3916, 2004.
8. Miyamoto H: Establishment and characterization of an adriamycin-resistant subline of human small cell lung cancer cells. Acta Med Okayama 40: 65-73, 1986.
9. Ahmed SA, Gogal RM Jr and Walsh JE: A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [^{3}H]thymidine incorporation assay. J Immunol Methods 170: 211-224, 1994.
10. Houghton PJ, Germain GS, Harwood FC, Schuetz JD, Stewart CF, Buchdunger E and Traxler P: Imatinib mesylate is a potent inhibitor of the ABCG2 (BCRP) transporter and reverses resistance to topotecan and SN-38 *in vitro*. Cancer Res 64: 2333-2337, 2004.
11. Burger H, van Tol H, Boersma AW, Brok M, Wiemer EA, Stoter G and Nooter K: Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. Blood 104: 2940-2942, 2004.
12. Yanase K, Tsukahara S, Asada S, Ishikawa E, Imai Y and Sugimoto Y: Gefitinib reverses breast cancer resistance protein-mediated drug resistance. Mol Cancer Ther 3: 1119-1125, 2004.
13. Nakamura Y, Oka M, Soda H, Shiozawa K, Yoshikawa M, Itoh A, Ikegami Y, Tsurutani J, Nakatomi K, Kitazaki T, Doi S, Yoshida H and Kohno S: Gefitinib ('Iressa', ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, reverses breast cancer resistance protein/ABCG2-mediated drug resistance. Cancer Res 65: 1541-1546, 2005.
14. Yang CH, Huang CJ, Yang CS, Chu YC, Cheng AL, Whang-Peng J and Yang PC: Gefitinib reverses chemotherapy resistance in gefitinib-insensitive multidrug resistant cancer cells expressing ATP-binding cassette family protein. Cancer Res 65: 6943-6949, 2005.
15. Nakanishi T, Shiozawa K, Hassel BA and Ross DD: Complex interaction of BCRP/ABCG2 and imatinib in BCR-ABL-expressing cells: BCRP-mediated resistance to imatinib is attenuated by imatinib-induced reduction of BCRP expression. Blood 108: 678-684, 2006.
16. Schmidli H, Peng B, Riviere GJ, Capdeville R, Hensley M, Gathmann I, Bolton AE and Racine-Poon A: Population pharmacokinetics of imatinib mesylate in patients with chronic-phase chronic myeloid leukaemia: results of a phase III study. Br J Clin Pharmacol 60: 35-44, 2005.
17. Johnson FM, Krug LM, Tran HT, Shoaf S, Prieto VG, Tamboli P, Peeples B, Patel J and Glisson BS: Phase I studies of imatinib mesylate combined with cisplatin and irinotecan in patients with small cell lung carcinoma. Cancer 106: 366-374, 2006.
18. Cohen MH, Williams GA, Sridhara R, Chen G, McGuinn WD Jr, Morse D, Abraham S, Rahman A, Liang C, Lostritto R, Bair A and Pazdur R: United States food and drug administration drug approval summary: gefitinib (ZD1839; Iressa) tablets. Clin Cancer Res 10: 1212-1218, 2004.
19. Daw NC, Furman WL, Stewart CF, Iacono LC, Krailo M, Bernstein ML, Dancey JE, Speights RA, Blaney SM, Croop JM, Reaman GH and Adamson PC: Phase I and pharmacokinetic study of gefitinib in children with refractory solid tumors: a Children's Oncology Group Study. J Clin Oncol 23: 6172-6180, 2005.
20. McKillop D, Partridge EA, Kemp JV, Spence MP, Kendrew J, Barnett S, Wood PG, Giles PB, Patterson AB, Bichat F, Guilbaud N and Stephens TC: Tumor penetration of gefitinib (Iressa), an epidermal growth factor receptor tyrosine kinase inhibitor. Mol Cancer Ther 4: 641-649, 2005.
21. Okamoto I, Araki J, Suto R, Shimada M, Nakagawa K and Fukuoka M: EGFR mutation in gefitinib-responsive small-cell lung cancer. Ann Oncol 17: 1028-1029, 2006.
22. Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, Natale RB, Schiller JH, von Pawel J, Pluzanska A, Gatzemeier U, Grous J, Ochs JS, Averbuch SD, Wolf MK, Rennie P, Fandi A and Johnson DH: Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial-INTACT 1. J Clin Oncol 22: 777-784, 2004.
23. Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C, Scagliotti G, Rosell R, Oliff I, Reeves JA, Wolf MK, Krebs AD, Averbuch SD, Ochs JS, Grous J, Fandi A and Johnson DH: Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial-INTACT 2. J Clin Oncol 22: 785-794, 2004.
24. Demetri GD, von Mehren M, Blanke CD, van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD and Joensuu H: Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 347: 472-480, 2002.
25. Johnson BE, Fischer T, Fischer B, Dunlop D, Rischin D, Silberman S, Kowalski MO, Sayles D, Dimitrijevic S, Fletcher C, Hornick J, Salgia R and Le Chevalier T: Phase II study of imatinib in patients with small cell lung cancer. Clin Cancer Res 9: 5880-5887, 2003.
26. Krug LM, Crapanzano JP, Azzoli CG, Miller VA, Rizvi N, Gomez J, Kris MG, Pizzo B, Tyson L, Dunne M and Heelan RT: Imatinib mesylate lacks activity in small cell lung carcinoma expressing c-kit protein: a phase II clinical trial. Cancer 103: 2128-2131, 2005.
27. Dy GK, Miller AA, Mandrekar SJ, Aubry MC, Langdon RM Jr, Morton RF, Schild SE, Jett JR and Adjei AA: A phase II trial of imatinib (ST1571) in patients with c-kit expressing relapsed small-cell lung cancer: a CALGB and NCCTG study. Ann Oncol 16: 1811-1816, 2005.
28. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I and van Cutsem E: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351: 337-345, 2004.

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ORIGINAL ARTICLE

A phase II trial of cisplatin and irinotecan alternating with doxorubicin, cyclophosphamide and etoposide in previously untreated patients with extensive-disease small-cell lung cancer

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Abstract
Purpose The aim of this trial was to investigate the efficacy and safety of cisplatin (P) and irinotecan (I) (PI) alternating with doxorubicin (A), cyclophosphamide (C) and etoposide (E) (ACE) in patients with extensive-disease small-cell lung cancer (ED-SCLC).
Patients and Methods Patients with previously untreated ED-SCLC were enrolled in this trial. In the first, third and fifth cycles, PI (P: 60 mg/m^2 on day 1; I: 60 mg/m^2/day on days 1, 8 and 15) was administered, whereas ACE (A: 50 mg/m^2 on day 1; C: 750 mg/m^2 on day 1; E 80 mg/m^2/day on days 1–3) was given in the second, fourth and sixth cycles. Each cycle was repeated every 4 weeks. At the end of six cycles, patients who had obtained a complete response were given prophylactic cranial irradiation.
Results In total, 28 patients were enrolled, of whom 27 were assessable for efficacy and safety. Objective responses, including 4 (15%) complete responses, were observed in 25 patients (93%). Median survival time was 12.9 months. The principal toxicity was myelosuppression; grade 4 neutropenia and thrombocytopenia were observed in 89 and 4%, respectively. Febrile neutropenia occurred in 30% of patients. Diarrhea was mild (grade 3–4; 4%). All toxicities were reversible and there were no treatment-related deaths. The mean percentage of the delivered doses, relative to the projected doses, of PI and ACE were 84.6 and 91.1%, respectively.
Conclusions These results indicate the PI–ACE regimen to have promising activity against ED-SCLC with moderate toxicities.

Keywords Small-cell lung cancer · Alternating chemotherapy · Irinotecan

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Introduction

Standard treatment for previously untreated extensive-disease small-cell lung cancer (ED-SCLC) is currently considered to be systemic chemotherapy consisting of cisplatin and etoposide (PE) [1]. However, the majority of responders relapse and the long-term survival rate is still quite low. To improve outcomes, several treatment strategies have been investigated. Alternating chemotherapy based on the Goldie–Coldman hypothesis was evaluated mainly in the late 1980s [2]. Fukuoka et al. [3] conducted a phase III trial comparing cyclophosphamide, doxorubicin, and vincristine

(CAV), PE with alternation of CAV and PE, in 300 SCLC patients to clarify whether rapid alternation of these two regimens produced superior therapeutic results as compared with either regimen alone. They showed a trend toward longer survival with alternating therapy as compared with the standard PE regimen.

Roth et al. [4] also conducted a phase III trial of the same three regimens in 437 patients with ED-SCLC. They found no significant differences in treatment outcomes among the regimens in terms of response rate and overall survival. Thus, alternating chemotherapy did not definitely improve the survival of SCLC patients as compared to standard treatments. One possible explanation for the negative results might be that these two regimens, CAV and PE, were partially cross-resistant [3, 4]. In addition, the outdated chemotherapy regimen, CAV, might have resulted in the failure of alternating chemotherapy to provide a survival advantage.

Recently, there have been several advances in chemotherapy for SCLC. First, a Japanese randomized phase III study comparing cisplatin and irinotecan (PI) with standard PE in previously untreated patients with ED-SCLC, demonstrated a significant survival benefit of PI with a median survival time of 12.8 versus 9.4 months [5]. Furthermore, Ando et al. [6] demonstrated that patients who relapsed after receiving a combination of platinum and etoposide responded well to subsequent PI chemotherapy with an overall response rate of 80%, which might suggest that irinotecan and etoposide are not cross-resistant. Second, Bunn et al. [7] demonstrated that a three-drug combination of doxorubicin, cyclophosphamide and etoposide (ACE) yielded a significant survival benefit in patients with ED-SCLC, as compared with the CAV regimen, in a randomized phase III trial.

There have been no investigations of alternating chemotherapy using new chemotherapeutic agents such as irinotecan. Given these background factors, we aimed to reappraise alternating chemotherapy in a prospective phase II trial using PI and ACE regimens. The primary endpoint of this trial was objective response rate, and secondary endpoints were toxicity and overall survival.

Patients and methods

Eligibility criteria

Patients were required to fulfill the following eligibility criteria: pathologically proven SCLC, extensive disease, no prior chemotherapy or thoracic irradiation, age ≤75 years, Eastern Cooperative Oncology Group performance status of 0 or 1, presence of measurable lesions, and adequate hematologic [white blood cell (WBC) count >4,000 per μl and platelet count >100,000 per μl], renal (creatinine clearance ≥60 ml per min), and hepatic (serum transaminases <1.5 × upper limit of normal range) functions. All participants provided written informed consent. Patients with massive pleural effusion, pericardial effusion or symptomatic brain metastases were excluded. The protocol was approved by the institutional review board of each participating institute. Baseline pretreatment evaluations included a complete history, physical examination, laboratory tests, chest radiograph and computed tomography (CT) scan of the chest. CT scan of the abdomen, magnetic resonance imaging of the brain and a radionuclide bone scan were also performed.

Assessments of antitumor activity and toxicity

Tumor response was assessed according to the World Health Organization criteria [8]. Complete response (CR) was defined as the disappearance of disease at all sites, partial response (PR) as a reduction of at least 50% in the sum of the products of the two largest perpendicular diameters of all measurable lesions, without progression in any other sites. No change was defined as a decrease of less than 50% or an increase of less than 25% in the sum of the products of the two largest perpendicular diameters of all measurable lesions for at least 4 weeks. Progressive disease was defined as an increase of 25% or more in the sum of the products of the two largest perpendicular diameters of all measurable lesions or the appearance of a new lesion. Tumor markers were not used to assess response. Response assessments were performed at the end of each cycle. A minimum duration of 4 weeks was required to document a response. The responses were finally confirmed by blinded extramural review.

All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC, Version 2.0).

Treatment schedules and modifications

In the first cycle, PI was administered intravenously, and ACE was given intravenously in the second cycle (day 29 of the first cycle). Subsequently, PI and ACE were alternately administered and repeated every 4 weeks up to 6 cycles. PI consisted of cisplatin 60 mg/m^2 given on day 1 and irinotecan 60 mg/m^2/day on days 1, 8 and 15, same schedule as those in previous phase II and III trials [5, 9]. ACE was administered as cyclophosphamide 750 mg/m^2 on day 1, doxorubicin 50 mg/m^2 on day 1 and etoposide 80 mg/m^2/day on days 1–3. After completion of chemotherapy, prophylactic cranial irradiation was delivered at a dose of 30 Gy in 15 fractions to patients who had obtained CR. Each patient was pre-medicated with intravenous

dexamethasone (16 mg) and granisetron (3 mg). If grade 4 leucopenia, grade 4 neutropenia, or febrile neutropenia was noted, the use of granulocyte colony-stimulating factor (G-CSF) was permitted.

With the PI regimen, administration of irinotecan on days 8 or 15 was cancelled if the WBC count <3,000 per μl and/or the platelet count <100,000 per μl on the day of administration. For both the PI and the ACE regimens, initiation of the next cycle was delayed until recovery of the WBC count ≥ 4,000 per μl or the platelet count ≥ 100,000 per μl, and resolution of non-hematologic toxicities to ≤ grade 1. Patients were treated with at least two cycles of chemotherapy unless there was disease progression, unacceptable toxicity in the first cycle, or withdrawal of consent to participate in this study. Dose modification for the next cycle was defined as follows. If grade 4 leucopenia, neutropenia or thrombocytopenia was observed with the PI regimen, the dose of irinotecan in the next PI cycle was decreased by 10 mg/m^2. If the same toxicity occurred in the ACE regimen, doses of cyclophosphamide, doxorubicin and etoposide in the next ACE cycle were decreased by 100, 10 and 10 mg/m^2, respectively. For grade 3 diarrhea, the irinotecan dose in the next PI cycle was decreased by 10 mg/m^2. Irinotecan was discontinued for grade 4 diarrhea. In addition, the cisplatin dose in the next PI cycle was reduced to 40 mg/m^2 when creatinine clearance dropped to between 30 and 60 ml/min. A decrease to less than 30 ml/min required discontinuation of cisplatin.

The dose intensity of each drug was calculated, for each patient who received at least two cycles of chemotherapy, using the following formula: Dose intensity (mg/m^2/week) = Total milligrams of a drug in all cycles per body surface area/[(Total days of therapy)/7], where total days of therapy is the number of days from day 1 of cycle 1 to day 1 of the last cycle plus 28 days [10]. The mean dose intensity was then calculated.

Statistical considerations

A Minimax two-stage design was used to test whether there was sufficient evidence to determine a response rate of at least 85% (i.e. clinically promising) versus at most 70% (i.e. clinically inactive), accepting a false-positive rate (α) ≤ 10% and a false-negative rate (β) ≤ 10%. In this two-stage design, accrual was stopped at the first stage for 22 patients if 16 or more patients did not respond to the treatment, otherwise it was continued to a total of 52 patients. This alternating chemotherapy was judged to be effective if more than 41 patients responded to the treatment.

Statistical analyses were performed using the StatView® 5.0 program (BrainPower Inc., Calabasas, CA, USA). The correlations between dose intensity and response or survival were assessed with Kruskal–Wallis test or Spearman's rank correlation coefficient. Overall survival curve was constructed using the Kaplan–Meier product-limit method. *P*-values less than 0.05 were considered statistically significant.

Results

Patient characteristics

Twenty-eight patients with previously untreated ED-SCLC were enrolled between November 1999 and November 2002 at 11 institutions in Japan. Initially, we aimed to accrue a total of 50 patients in this trial. However, a low accrual rate prompted termination of patient registration in 2003. Twenty-seven (96%) of the 28 patients were assessable for efficacy and toxicity analysis. The one remaining patient did not satisfy the eligibility criteria because of limited-disease. Characteristics of the 27 patients are listed in Table 1. The majority were male and had metastatic disease, but no weight loss, prior to the registration.

Objective response and survival

Of the 27 patients, 4 achieved CR (14.8%), 21 PR (77.8%), resulting in a total response rate of 92.6% (95% confidence interval; 75.7–99.1%), whereas disease stabilization was obtained in 2 patients (7.4%). Survival analysis was performed for all 27 patients. Twenty-five (93%) patients had died at the time of this analysis. Mean follow-up time for surviving patients was 14.7 months, and the median survival time of all patients was 12.9 months ranging from 3.5 to 34.5 months (Fig. 1).

Hematological toxicity

Myelosuppression was the principal toxicity experienced with this regimen. Among 27 patients, grades 3 and 4 neutropenia were seen in 2 (7%) and 24 (89%), respectively (Table 2). Of these, eight patients (30%) developed febrile episodes. However, these conditions were reversible with appropriate supportive care. Anemia and thrombocytopenia were relatively mild with grade 4 toxicities in 7 and 4% of patients, respectively. The PI regimen had less severe hematological toxicity than the ACE regimen; grade 3 and

Table 1 Demographics of the 27 patients

Age [median (range)]	67 (47–75)
Gender (male/female)	22 (81%)/5 (19%)
Performance status (0/1)	9 (33%)/18 (67%)
Body weight loss (<5/≥5%)	25 (93%)/2 (7%)
Stage (IIIB/IV)	4 (15%)/23 (85%)