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Multi-institutional phase II trial of irinotecan, cisplatin, and etoposide for sensitive relapsed small-cell lung cancer

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Irinotecan (CPT-11) has been shown to exhibit excellent antitumour activity against small-cell lung cancer (SCLC). A multi-institutional phase II study was therefore conducted to evaluate the efficacy and toxicity of CPT-11 combined with cisplatin (CDDP) and etoposide (ETOP) (PEI regimen) for the treatment of sensitive relapsed SCLC. Patients who responded to first-line chemotherapy but relapsed more than 8 weeks after the completion of first-line therapy ($n = 40$) were treated using the PEI regimen, which consisted of CDDP (25 mg m^{-2}) weekly for 9 weeks, ETOP (60 mg m^{-2}) for 3 days on weeks 1, 3, 5, 7, and 9, and CPT-11 (90 mg m^{-2}) on weeks 2, 4, 6, and 8 with granulocyte colony-stimulating factor support. Five complete responses and 26 partial responses were observed, and the overall response rate was 78% (95% confidence interval 61.5–89.2%). The median survival time was 11.8 months, and the estimated 1-year survival rate was 49%. Grade 3/4 leucocytopenia, neutropenia, and thrombocytopenia were observed in 55, 73, and 33% of the patients, respectively. Nonhaematological toxicities were mild and transient in all patients. In conclusion, the PEI regimen is considered to be highly active and well tolerated for the treatment of sensitive relapsed SCLC.
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Small-cell lung cancer (SCLC) is one of the most chemosensitive solid tumours, and first-line combination chemotherapy improves survival. However, despite a high response rate to chemotherapy, the majority of SCLC patients relapse. At the time of recurrence, the tumour is broadly resistant to second-line chemotherapy and is lethal within a few to several months (Glisson, 2003). The further development of not only first-line chemotherapy but also of effective salvage chemotherapies is needed.

In predicting the efficacy of salvage chemotherapy, two major factors are important: the response to the initial chemotherapy and the duration of time between the last exposure to chemotherapy and the confirmation of recurrence (Postmus *et al*, 1987; Giaccone *et al*, 1988; Ardizzoni *et al*, 1997; Ebi *et al*, 1997). Based on these factors, relapsed SCLC is now commonly classified into two main groups. Patients who both respond to the initial chemotherapy and relapse more than 2 or 3 months after the completion of chemotherapy are considered to be 'sensitive relapse' patients, while patients whose tumour is stable or progresses during the initial chemotherapy or who have a recurrence within 2 or 3 months after the completion of chemotherapy are considered to be 'refractory relapse' patients (Giaccone *et al*, 1988). Since the outcomes of salvage chemotherapy for relapsed SCLC patients are different between these two groups, the ratios of sensitive and refractory cases must be carefully considered when evaluating the results of clinical trials for second-line chemotherapy.

The combination of cisplatin (CDDP) and etoposide (ETOP) (PE regimen) has been the standard chemotherapeutic regimen for SCLC (Fukuoka *et al*, 1991; Ihde, 1992; Roth *et al*, 1992; Aisner, 1996). Moreover, PE is a reasonable second-line chemotherapy for relapsed SCLC after combination chemotherapy consisting of cyclophosphamide, doxorubicin (ADM), and vincristine (VCR) (CAV regimen); the likelihood of a response to this regimen is 40–50% (Evans *et al*, 1984; Porter *et al*, 1985). Since PE has a relatively mild toxicity profile, other cytotoxic agent can be combined with PE.

Irinotecan (CPT-11), a camptothecin derivative topoisomerase I inhibitor, has been shown to exhibit excellent antitumour activity against SCLC in monotherapy and in combination with CDDP (Masuda *et al*, 1992; Kudoh *et al*, 1998). Based on these results, the Japan Clinical Oncology Group (JCOG) conducted a randomised phase III trial comparing CPT-11 and CDDP (IP regimen) with standard PE for previously untreated extensive stage (ED) SCLC (JCOG 9511) (Noda *et al*, 2002). The response rates were significantly higher for IP than for PE, and overall survival was also significantly better for IP than for PE. This was the first study to show the superiority of any one regimen over PE for the

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treatment of ED SCLC, and IP has become one of the standard regimens for ED SCLC in Japan. Thereafter, several clinical trials of CPT-11-containing regimens for patients with limited disease (LD), ED, and relapsed SCLC have been conducted by Japanese clinical study groups (Masuda *et al*, 1998; Mori *et al*, 2002; Sekine *et al*, 2002).

Consequently, a phase I trial of CPT-11 combined with weekly CDDP (25 mg m^{-2}) and biweekly ETOP (60 mg m^{-2}) (PEI regimen) was conducted, and the recommended dose of 90 mg m^{-2} of CPT-11 was repeated every 2 weeks (JCOG 9507) (Sekine *et al*, 2003). This regimen showed promising antitumour activity in patients with untreated ED SCLC (response rate, 91%, 1-year survival rate 46%). Moreover, since the drug dose and treatment schedule can be easily modified in a weekly regimen, this protocol is considered to be suitable for relapsed SCLC patients, who usually present with severe haematological toxicities during salvage chemotherapy because of poor bone marrow reserve (Masuda *et al*, 1990; Faylona *et al*, 1995).

Based on these results, we conducted two phase II trials to evaluate the efficacy and toxicities of PEI in patients with sensitive and refractory relapsed SCLC, separately. In this paper, the final results for the sensitive relapsed SCLC group are reported.

PATIENTS AND METHODS

Patient selection

Patients with histologically or cytologically confirmed SCLC who respond to first-line chemotherapy or chemoradiotherapy and relapsed more than 8 weeks after the completion of first-line treatment were candidates for the present study. Additional eligibility criteria were as follows: (1) age of 75 years or younger; (2) performance status of 0–2 on the Eastern Cooperative Oncology Group scale; (3) measurable disease; (4) adequate organ function as documented by a $4.0 \times 10^9 l^{-1} \leqslant$ WBC count $\leqslant 12.0 \times 10^9 l^{-1}$, haemoglobin level of $\geqslant 9.0$ g dl^{-1}, platelet count of $\geqslant 100 \times 10^9 l^{-1}$, total serum bilirubin level of $\leqslant 1.5$ mg dl^{-1}, a hepatic transaminase level of $\leqslant 2$ times the institutional upper limit of normal, a serum creatinine level of $\leqslant 1.5$ mg dl^{-1}; and (5) written informed consent. Patients were not eligible for the study if they had experienced any of the following events: (1) massive pleural effusion requiring drainage; (2) prior radiotherapy with an irradiated area larger than one-third of the bone marrow volume; (3) active infection; (4) contraindications for the use of CPT-11, including diarrhoea, ileus, interstitial pulmonary fibrosis, massive ascites, or hypersensitive reaction to CPT-11; (5) serious concomitant medical illness, including severe heart disease, uncontrollable diabetes mellitus or hypertension; or (7) pregnancy or lactation. This study was approved by the institutional review board at each participating institution.

Treatment schedule

Figure 1 shows the treatment schema of the PEI regimen. CDDP (25 mg m^{-2}) was administered intravenously (i.v.) over 60 min on day 1 and at 1-week intervals for 9 weeks; ETOP (60 mg m^{-2}) was administered i.v. over 60 min on days 1–3 of weeks 1, 3, 5, 7, and 9; and CPT-11 (90 mg m^{-2}) was administered i.v. over 90 min on day 1 on weeks 2, 4, 6, and 8. Hydration (2000 ml) and granisetron (40 μg kg^{-1}) were given on day 1. After day 1 on week 2, granulocyte colony-stimulating factor (G-CSF) (50 μg m^{-2}) was administered routinely according to JCOG 9507 on days when the cytotoxic drugs were not given, unless the WBC count exceeded $10.0 \times 10^9 l^{-1}$. Patients were expected to complete at least six cycles of this regimen; if the toxicities were acceptable and the tumour responded to the treatment, a maximum of nine cycles of chemotherapy were performed.

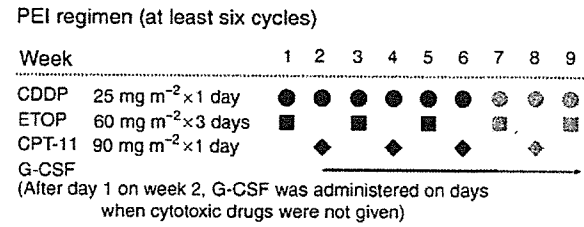

Figure 1 Treatment schedule.

Toxicity assessment and treatment

During the course of treatment, complete blood cell counts and differential counts were analysed twice a week, and routine chemistry measurements and a chest X-ray were performed once a week. Toxicity was graded according to the toxicity criteria of the JCOG (Tobinai *et al*, 1993), a modified version of the NCI Common Toxicity Criteria issued in 1991. Grade 4 neutropenia was defined as $<0.5 \times 10^9 l^{-1}$, and grade 3 neutropenia was defined as between (and including) $0.5-1.0 \times 10^9 l^{-1}$, according to the JCOG criteria. The second and subsequent cycles of chemotherapy were delayed for 1 week if one of the following toxicities was noted on day 1: a WBC count of $<2.0 \times 10^9 l^{-1}$, a platelet count of $<50 \times 10^9 l^{-1}$, a serum creatinine level of $\geqslant 2.0$ mg dl^{-1}, an elevated hepatic transaminase level or total serum bilirubin of grade 2 or higher, diarrhoea of grades 1–2, fever $\geqslant 38°C$, or a performance status of 3. The treatment was terminated if the above-mentioned criteria did not disappear in 3 weeks or if one of the following severe nonhaematological toxicities was noted: diarrhoea of grade 2 lasting for more than 1 week, diarrhoea of grade 3, neurotoxicity of grade 3, or drug-induced pneumonitis.

Dose modifications for toxicity

The CPT-11 dosage was reduced to 67.5 mg m^{-2} (25% reduction) in subsequent cycles if one of the following toxicities was noted: a WBC count of $<1.0 \times 10^9 l^{-1}$, or a platelet count of $<25 \times 10^9 l^{-1}$. If the above-mentioned toxicities reappeared after a 25% reduction in the dosage, the CPT-11 dosage was further reduced to 50 mg m^{-2} (44% reduction). Since CDDP (25 mg m^{-2}) and ETOP (60 mg m^{-2}) in this regimen were relatively low dose, no dose modifications for these drugs were permitted.

Pretreatment evaluation

Pretreatment assessment included a complete blood cell count, differential counts, routine chemistry measurements, creatinine clearance, blood gas analysis, electrocardiogram, chest X-rays, computed tomography (CT) scan of the chest, brain CT scan or magnetic resonance imaging (MRI), abdominal CT scan or ultrasound sonography, radionuclide bone scan, and bone X-rays, if indicated.

Response evaluation

Objective tumour responses were evaluated in all enrolled patients according to the WHO criteria issued in 1979 (WHO, 1979). A complete response (CR) was defined as the disappearance of all known disease for at least 4 weeks with no new lesions appearing. A partial response (PR) referred to a decrease in the total tumour size of at least 50% for at least 4 weeks without the appearance of new lesions. No change (NC) was defined as the absence of a partial or complete response and the appearance of no progressive or new lesions for at least 4 weeks. Progressive disease (PD) was

defined as a 25% or greater increase in the size of any measurable lesion or the appearance of new lesions. Patients whose responses were not evaluated were included in the analysis as not evaluable (NE).

Statistical methods

The primary end point of this study was the response rate, defined as the proportion of patients whose best response was CR or PR among all eligible patients, and its confidence interval was based on an exact binomial distribution. Simon's two-stage minimax design was used to determine the sample size and decision criteria. Assuming that a response rate of 40% in eligible patients would indicate a potential usefulness of the regimen while a rate of 20% would be the lower limit of interest and that alpha = 0.05 and beta = 0.20, the estimated number of required patients was 33 (Simon, 1989). Finally, this regimen would be considered worthy of further testing if 11 (33%) or more eligible patients showed an objective response. At the first stage decision, this regimen would be rejected if four (22%) or fewer of 18 eligible patients had an objective response. Thus, we determined that the sample size would be 35 registered patients. The planned accrual period was 2 years, and the follow-up period was set as 1 year after the completion of accrual. Secondary end points were toxicity and overall survival. The duration of overall survival was measured from the date of registration to the date of death from any cause or the last follow-up examination. Progression-free survival was calculated from the date of registration until evidence of PD. All patients started the treatment within 1 week of registration. The survival distribution was estimated by the method of Kaplan and Meier (1958).

RESULTS

Patient characteristics

From October 1998 to March 2001, 40 patients were enrolled in this study. The first-stage decision was made in October 1999, when 22 patients were registered. Three CRs and 13 PRs were observed in 18 analysed patients, resulting in a response rate of 89% (95% confidence interval (CI), 65.3–98.6%). This result did not meet the criteria for stopping the study as defined in the protocol, and the study was continued. At the time of the final analysis, there were three censored cases (8%). The median follow-up period for these cases was 25.5 months (range, 4.4–46.1 months).

The clinical characteristics of the enrolled patients are listed in Table 1. Of the 40 patients in the total, 29 (73%) were male and 11 (27%) were female; the median age was 67 years. A total of 39 patients (97%) had a good performance status of 0 or 1. The extent of the disease at the time of recurrence was LD in five patients (12%) and ED in 35 (88%). All 40 patients had been previously treated using platinum-based chemotherapy, such as PE in 11 patients, carboplatin plus ETOP in 11, PE plus weekly CDDP/VCR/ADM/ETOP (CODE) in six, CDDP plus CPT-11 in six, PEI in two, and other regimens in four. Eight (20%) of these patients received thoracic radiotherapy. All patients were eligible, and the toxicity and efficacy of the regimen was evaluated in all 40 patients.

Compliance with treatment

A total of 251 treatment cycles were administered, with a median of six cycles per patient (range, 1–9 cycles). A total of 32 patients (80%) completed six or more cycles of chemotherapy, and the median number of weeks for completing six cycles of chemotherapy was 7 weeks (range 6–10 weeks). Eight patients could not complete the planned six or more cycles for the following reasons: toxicities in four cases (grades 4 and 3 diarrhoea, grade 3 liver dysfunction, and grade 3 erythema); patient refusal in three cases; and PD in one case. Six patients (15%) had their dosage of CPT-11 reduced because of leucocytopenia in three, thrombocytopenia in two, and both in one.

Clinical response and survival

All the patients were included in the analyses of tumour response and survival. Five CRs (13%) and 26 PRs (65%) were observed, for an overall response rate of 78% (31 out of 40 patients; 95% CI, 61.5–89.2%). Four NC, four PD, and one NE were also observed. One patient was lost to follow-up and only two patients were still alive as of April 16, 2003. The median survival time (MST) was 11.8 months (95% CI, 10.1–13.5 months), and the estimated 1-year survival rate was 49% (Figure 2).

Table 1 Patient characteristics

Total no. of patients	40
Age, median (range)	67 (41–74)
Sex	
Male	29
Female	11
ECOG performance status	
0	9
1	30
2	1
Disease extent at relapse	
Limited disease	5
Extensive disease	35
Prior chemotherapy	
CDDP/ETOP	11
CBDCA/ETOP	11
CDDP/ETOP/CODE	6
CDDP/CPT-11	6
PEI	2
Others	4
Prior thoracic radiotherapy	8

ECOG = Eastern Cooperative Oncology Group; CDDP = cisplatin; ETOP = etoposide; CBDCA = carboplatin; CODE = cisplatin/vincristine/doxorubicin/etoposide; CPT-11 = irinotecan; PEI = cisplatin/etoposide/irinotecan.

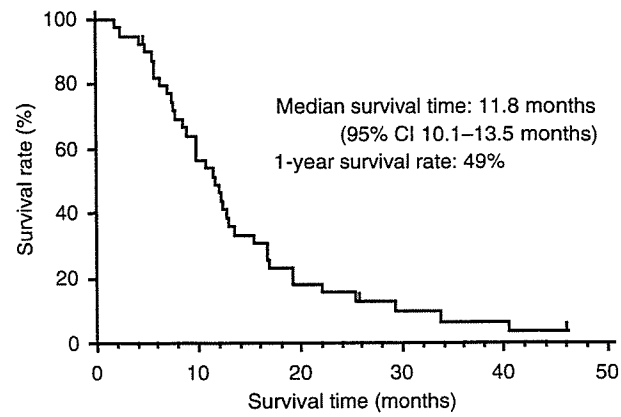

Figure 2 Overall survival ($n = 40$).

Clinical

Site of first relapse and progression-free survival

The majority of patients ($n=30$, 75%) experienced a systemic relapse after completing PEI, including 17 patients (43%) with central nerve metastases. Six patients (15%) developed only a locoregional recurrence, and one had no recurrence and died of acute myocardial infarction. No data on recurrence patterns were available in three patients because these patients were followed up at other hospitals. In all, 13 patients received additional chemotherapy treatment after recurrence (no data on response to third-line chemotherapy were available), while four patients underwent palliative chest radiotherapy and 18 underwent whole-brain irradiation for cerebral metastases. One patient, who achieved a CR by this regimen, developed a locoregional recurrence and underwent a right upper lobectomy. He has not experienced any further relapse and is still alive. The median progression-free survival period was 5.0 months (95% CI, 4.1–5.9 months) (Figure 3).

Toxicities

All the patients were included in the toxicity analysis. Severe toxicities were mainly haematological. Grades 3–4 leucopenia, neutropenia, and thrombocytopenia were observed in 22 (55%), 29 (73%), and 13 (33%) patients, respectively (Table 2). Nonhaematological toxicities were mild and transient in all patients. Grades 3–4 diarrhoea was noted in only three patients (8%) (Table 3). No treatment-related deaths occurred.

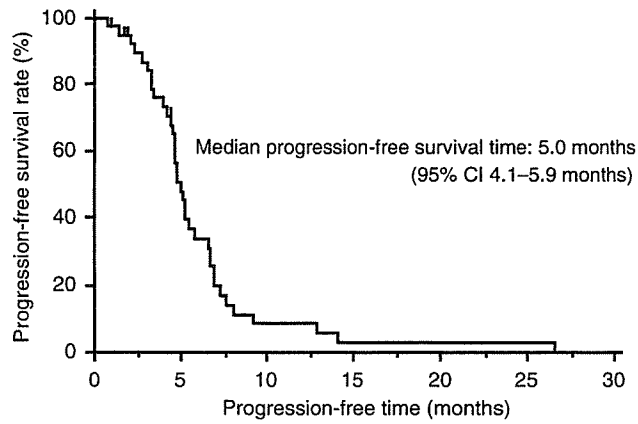

Figure 3 Progression-free survival ($n=40$).

Table 2 Haematological toxicities (JCOG toxicity criteria)

	0	1	2	3	4	% of Grs 3 and 4
Leucocytopenia	2	3	13	17	5	55
Neutropenia	3	4	4	12	17	73
Anemia	2	4	16	18	—	45
Thrombocytopenia	10	7	10	7	6	33
Elevated total bilirubin	33	—	6	1	0	3
Elevated GOT	32	7	0	1	0	3
Elevated GPT	30	7	2	1	0	3
Elevated creatinine	37	3	0	0	0	0
Hyponatremia	28	4	6	0	2	5
Hypokalemia	32	5	3	0	0	0

Grs = grades; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase.

Table 3 Nonhaematological toxicities (JCOG toxicity criteria)

	0	1	2	3	4	% of Grs 3 and 4
PS	1	30	4	5	0	13
Infection	28	4	7	1	0	3
Fever	29	7	4	0	0	0
Nausea/vomiting	11	15	11	3	—	8
Diarrhoea	15	16	6	2	1	8
Mucositis	36	4	0	0	0	0
Arrythmia	36	2	0	1	1	5
Eruption	37	1	1	1	0	3
Alopecia	16	17	7	—	—	—
Allergy	39	0	1	0	0	0

Grs = grades; PS = performance status.

DISCUSSION

Despite a high response rate to first-line chemotherapy, most patients with SCLC experience a relapse within a year of the completion of therapy (Hansen, 1992). Although many relapsed patients in good physical condition undergo second-line chemotherapy, the results are disappointing. The obtained response is usually brief, and the median survival period is generally less than 4 months (Albain *et al*, 1993; Glisson, 2003).

Although one phase III trial for patients with relapse SCLC comparing the use of topotecan with CAV has been reported (von Pawel *et al*, 1999), a standard treatment for relapsed SCLC has not been agreed upon. However, the repeated use of the original induction regimen is the most popular treatment for sensitive relapsed patients. Reinduction chemotherapy has been reported to produce a response rate of 50%, and patients who relapsed more than 3 months after the end of their previous chemotherapy regimen were sensitive to reinduction chemotherapy (Giaccone *et al*, 1987; Postmus *et al*, 1987). Giaccone *et al* (1988) suggested that sensitive tumour cells, which were not completely eradicated by the induction chemotherapy, regrow spontaneously after the suspension of chemotherapy, eventually constituting a clinically significant part of the tumour burden. In the present study, two patients received the PEI regimen as a reinduction chemotherapy, and both patients showed PRs.

Many clinical trials of salvage chemotherapy for relapsed SCLC have been reported. In these studies, the single administration of CPT-11 or ETOP produced good results, with response rates of 16–47% and an MST of 3.5–6.2 months (Einhorn *et al*, 1990; Johnson *et al*, 1990; Masuda *et al*, 1992; Le Chevalier *et al*, 1997). Moreover, CPT-11 or ETOP-containing combined chemotherapy regimens showed favourable results, with response rates of 20–88% and an MST of 4.7–8.7 months (Table 4) (Evans *et al*, 1985; Masuda *et al*, 1990; Sculier *et al*, 1990; Gridelli *et al*, 1991; Roth *et al*, 1992; Faylona *et al*, 1995; Kubota *et al*, 1997; Masuda *et al*, 1998; Groen *et al*, 1999; Nakanishi *et al*, 1999; von Pawel *et al*, 1999; Domine *et al*, 2001; Kosmas *et al*, 2001). Therefore, these two drugs are considered to be key drugs for the treatment of relapsed SCLC. In particular, the combination of CPT-11 and ETOP (a combination of topoisomerase I and II inhibitors) produced a high response rate (71%) and the best survival results (MST, 8.7 months) (Masuda *et al*, 1998). In addition, a weekly chemotherapy regimen containing ETOP (CODE) was highly active in patients with relapsed SCLC, with a favourable response rate (88%) and survival duration (MST, 8.2 months) (Kubota *et al*, 1997). In the two studies mentioned above, four patients (16%) with refractory relapsed SCLC were included in the CPT-11 and ETOP study, and six patients (35%) with refractory relapsed SCLC were included in the CODE study. Three and five of these patients achieved PR, respectively.

Table 4 Combination chemotherapy studies for relapsed small-cell lung cancer

Author	Regimen	No. of pts	% of ref pts (%)	RR (%)	RR in ref pts (%)	MST (month)
Sculier	CAV	61	75	21	5	6.2–7.5
von Pawel	CAV	104	20	18	5	6.2
Roth	CAV	41	32	12	8	NM
Roth	PE	59	46	22	15	NM
Evans	PE	78	50	55	28	NM
Masuda	PE	20	NM	50	NM	4.7
Gridelli	CCNU/MTX	33	100	21	21	4.0
Faylona	PE/IFO	46	41	55	50	6.8
Kubota	CODE	17	35	88	83	8.2
Masuda	CPT-11/ETOP	25	16	71	75	8.7
Nakanishi	CPT-11/CDDP	5	100	20	20	NM
Domine	GEM/PTX	31	58	50	40	NM
Groen	CBDCA/PTX	35	100	74	74	7.2
Kosmas	CDDP/IFO/PTX	33	61	73	70	6.5

Pts = patients; ref = refractory; RR = response rate; MST = median survival time; CAV = cyclophosphamide/doxorubicin/vincristine; PE = cisplatin/etoposide; CCNU = lomustine; MTX = methotrexate; IFO = ifosfamide; CODE = cisplatin/vincristine/doxorubicin/etoposide; CPT-11 = irinotecan; ETOP = etoposide; CDDP = cisplatin; GEM = gemcitabine; PTX = paclitaxel; CBDCA = carboplatin; NM = not mentioned.

The response and survival data from Japanese clinical trials for relapsed SCLC were generally better than those obtained in western countries. We have no proof that this difference depends on either drug metabolism or tumour sensitivity. It is possibly related to the difference in patient follow-up interval between Japan and western countries. Since intensive follow up after completion of first-line treatment is common in Japan, relapses can be detected in the early stage by CT or MRI before becoming symptomatic. Therefore, relapsed patients had a relatively good performance status, and showed good responses to second-line chemotherapy as well as better survival results.

The weekly regimen was designed to increase the overall relative dose intensity of the chemotherapeutic drugs (Murray *et al*, 1991). However, several phase III trials have made it clear that intensive weekly chemotherapy does not improve the survival of patients with SCLC (Furuse *et al*, 1998; Murray *et al*, 1999). On the other hand, drug dosages and treatment schedules are easy to modify in weekly chemotherapy regimens. Since patients with relapsed SCLC may have lower bone marrow reserve, a high-dose regimen or intensified dosage can lead to treatment-related death (Masuda *et al*, 1990; Faylona *et al*, 1995). In the PEI regimen, the individual dosage of each drug is within the commonly used range and the dose given at one time is lower than that of a standard 3-week cycle regimen. The PEI regimen therefore permits greater flexibility in dosage adjustment and treatment delays based on laboratory data or the physical condition of patients. Thus, this regimen is considered to be suitable for the treatment of patients with relapse SCLC. In addition, this weekly schedule may be of great advantage for enabling the synergistic effects of ETOP (a topoisomerase II inhibitor) and CPT-11 to be realised because the development of resistance to topoisomerase II inhibitors has been reported to increase tumour sensitivity to subsequent treatment with topoisomerase I inhibitors (Vasey and Kaye, 1997).

Three cytotoxic drugs were used in this PEI regimen. However, three-drug combination chemotherapy was reportedly associated with more severe toxicity and showed no survival benefit as compared with the two-dug combination (Mavroudis *et al*, 2001; Niell *et al*, 2002). The main reason for mild toxicities was that the PEI regimen consists of a weekly schedule. With a weekly chemotherapy regimen, drug dosages and treatment schedules can easily be adjusted according to haematological data and the patient's physical condition. These careful modifications resulted in a mild toxicity profile with the PEI regimen. Moreover, the PEI regimen did not consist of concomitant administration of three drugs but rather weekly alternative administration of a two-drug combination chemotherapy, that is, PE and IP. As a result, the toxicity profile was similar with that of two-drug combination chemotherapy.

Although all the patients in this study were sensitive relapsed cases, the overall response rate of 78% is one of the best results reported for relapsed SCLC. Moreover, although only selected patients with a good performance status were included in this study, it is notable that the median survival time was 11.8 months and the 1-year survival rate was 49%. In JCOG- 9511, the MST was 12.8 months in the IP arm and 9.4 months in the PE arm for chemotherapy naive ED SCLC patients (Noda *et al*, 2002). Our survival data for PEI is almost equivalent to that of first-line treatment. Salvage chemotherapy may be possible to prolong the survival of sensitive relapsed SCLC patients who are in good physical condition.

Since second-line chemotherapy for relapsed SCLC patients is a palliative treatment, a reasonable toxicity profile is essential. The main toxicities of the PEI regimen were haematological. Although G-CSF was routinely administered, Grades 3–4 leucopenia and neutropenia were observed in 55 and 73% of patients, respectively. Grades 3–4 thrombocytopenia was observed in 33% of patients. However, the frequencies of these haematological toxicities were approximately equal to that of first-line PE treatment (Noda *et al*, 2002). Nonhaematological toxicities were mild and transient in all patients. Grades 3–4 diarrhoea was noted in only three patients (8%). Irinotecan dose modifications as a result of haematological toxicities were only performed in six patients (15%). All toxicities were easily manageable, and no treatment-related deaths occurred.

In conclusion, PEI is a highly active and well-tolerated treatment for sensitive relapsed SCLC. Another phase II trial restricted to refractory relapsed SCLC patients is presently being performed by our clinical group. Further phase III studies comparing PEI regimen with rechallenges of the same drugs used in the first-line chemotherapy regimen should clarify the role of second-line chemotherapy for sensitive relapsed SCLC and are now being planned.

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Topographical distribution of allelic loss in individual lung adenocarcinomas with lymph node metastases

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Adenocarcinomas of the lung are characterized by morphological heterogeneity, and since carcinogenesis has been suggested to be a multistep process involving sequential accumulation of multiple genetic alterations, the morphological heterogeneity may represent a cross-sectional view of genetic alterations within individual tumors. Therefore, to elucidate whether, and which, genetic alterations accumulated in relation to morphological cancer progression, we examined 56 microdissected sites for topographical distribution of loss of heterozygosity (LOH) in 12 adenocarcinomas of the lung with bronchioloalveolar (BA) and invasive components in their primary tumors and metastases to lymph nodes. The morphological changes from noninvasive BA lesions to invasive and metastatic components were characterized by a significant rise in the prevalence of allelic losses ($P<0.05$). Individually, eight cases (67%) showed accumulation of genetic alterations from BA lesions to metastases. LOHs in multiple foci in one case were compared to determine whether they were shared at all tumor sites as an early event or localized in metastases as an additional event. LOHs at 5q and 17p may be crucial steps in the early phase of development to metastasis, while 18q loss may be an additional step. These findings suggested that the cancer cells in some pulmonary adenocarcinomas evolved from the BA lesions to the invasive and metastatic lesions.
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Carcinoma of the lung is one of the most common human cancers and is the predominant cause of cancer-related death throughout the world. Of the four major histological subtypes of lung cancer, the incidence of adenocarcinoma has been gradually increasing, but the morphological and genetic progression schemes of adenocarcinoma of the lung have not been elucidated as clearly as those of colon cancer,[1] which is the human cancer that has been most intensively investigated in this regard. Elucidation of the genetic sequence responsible for the progression of adenocarcinoma of the lung from *in situ* to invasive and metastatic carcinoma is an important goal in understanding the biological basis of this malignancy.

One of the most characteristic features of adenocarcinoma of the lung is its high degree of morphological heterogeneity. We have often observed morphologically noninvasive bronchioloalveolar carcinoma (BAC) components replacing pre-existing alveolar epithelium (the replacing-growth-type) at the periphery of invasive adenocarcinomas. According to the WHO classification,[2] they were diagnosed as 'adenocarcinoma mixed bronchioloalveolar and other subtypes (acinar, solid, papillary).' This morphological heterogeneity may represent a cross-sectional view of clonal evolution within individual tumors. That is, some noninvasive BACs may evolve into invasive adenocarcinoma as they acquire invasiveness during carcinogenesis, as observed in the adenoma–carcinoma sequence of colon carcinogenesis.[1,3–5] Adenocarcinomas mixed bronchioloalveolar (BA) and other subtypes are supposed to contain bronchioloalveolar (BA) components of BACs and

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invasive components evolving from the BAC in the individual tumor. They contain noninvasive components and invasive components that we usually observe in each tumor as part of the adenoma–carcinoma sequence. In addition, it has never been determined whether adenocarcinomas containing a morphologically noninvasive BA component (adenocarcinoma mixed BA and other subtypes) evolve from BAC. We recently examined 66 cases of replacing-growth-type small adenocarcinoma of the lung less than 2 cm in size to investigate allelic losses at eight loci on the eight chromosomes carrying the most important cancer-associated genes by the laser capture microdissection method, in which cancer cells are selectively collected.[6] The 66 cases were divided into three groups according to the Noguchi's classification of small adenocarcinomas of the lung:[7] 12 localized bronchioloalveolar carcinomas (LBACs), 28 LBACs with alveolar collapse, and 26 LBACs with active fibroblastic proliferation. We confirmed that the prevalence of loss of heterozygosity (LOH) increased as adenocarcinoma of the lung underwent histological progression from LBAC (16.7%) to small but advanced LBAC with fibroblastic proliferation (96.2%). Deletions of four of the markers, 3p, 17p, 18q, and 22q, increased significantly during the carcinogenic steps from noninvasive to invasive carcinoma, and these molecular genetic data were consistent with the morphological progression of the adenocarcinoma.

It has been suggested that certain types of genetic alterations may be involved in the early phase of tumorigenesis, whereas others may play a role in late events during tumor progression. However, such inferences have essentially been based on statistical arguments after analysis of a set of different tumors[6,8,9] and whether they are indeed applicable to the progression scheme in individual tumors has not been specifically addressed in regard to adenocarcinoma of the lung. An alternative approach therefore seemed to be necessary, for those relying on the statistical correlation between the frequency of each genetic alteration and histological and/or disease progression, but such an approach has rarely been taken in studies of the genetic changes in adenocarcinoma of the lung.

The pathologic staging of malignant tumors, including of non-small cell lung carcinoma (NSCLC), is based on the concept that primary tumor growth and local invasion precede lymphatic dissemination, which is regarded as a later event in lung cancer progression. From the standpoint of molecular genetics as well, the metastatic event is considered to be a relatively late event that follows multiple sequential and selective steps of clonal evolution. Metastases are thought to be established through selected clonal tumor cells that carry all genetic alterations involved in the genesis and progression of carcinoma.[10,11] However, no studies have carefully compared the accumulation of genetic changes and histopathological progression from a noninvasive lesion to an invasive or metastatic lesion in an individual pulmonary adenocarcinoma mixed BA and other subtypes.

In the present study, we examined the topographical distribution of LOH events occurring in the process of neoplastic progression within individual tumors and assessed whether previous inferences are indeed applicable to the progression scheme within individual tumors. To do so, we examined multiple pathologically well-defined specimens from individual tumors with lymph node metastases in order to investigate whether, and which genetic alterations are accumulated in relation to histopathological progression in individual tumors.

Materials and methods

Patients and Tissue Samples

We selected 12 cases of adenocarcinoma of the lung in which the primary tumor measured 3 cm or less in greatest dimension and contained BA components and invasive components (adenocarcinoma mixed BA and other subtypes) and there were metastases in the resected lymph nodes. All tumor samples were obtained from surgical resections at the Department of Thoracic Oncology of the National Cancer Center Hospital East (Chiba, Japan) between 1998 and 2000. All patients underwent lobectomies and lymph node dissections curative intent with no preoperative adjuvant therapy.

A 3-μm-thick section from each block was stained with hematoxylin–eosin (HE) and used as a guide to localize regions of interest for microdissection. We examined all identifiable components that appeared histotopographically different and contained sufficient cells (more than 100 cells). To investigate the clear relationship between pathology and genetic alteration, we collected the lesions whose subtypes we could define clearly and which consisted of the histologically uniform cells. In the microdissection analysis, two or three 10-μm-thick sections from each specimen were deparaffinized with xylene and stained with hematoxylin alone. The stained sections were dried, and multiple lesions in each tumor were microdissected separately with a Pixcell Laser Capture Microdissection System (Arcturus Engineering Inc., Mountainview, CA, USA).[12] Finally, 100–200 tumor cells and the same number of normal cells were microdissected from each specimen, and their genomic DNAs were extracted as described previously. Separated tumor areas (3–6 areas per case) that were histotopographically dissimilar were selected microscopically on HE-stained sections. We identified a total of 56 foci, which included samples from the 12 BA component sites, 30 invasive regions of primary tumors (17 acinar, eight solid, five papillary), and 14 metastases (13 lymph node metastases, one intrapulmonary metastasis). Normal control DNAs were extracted from the corresponding lymph nodes without metastasis.

Multiplex Polymerase Chain Reaction–Loss of Heterozygosity Analysis

To evaluate LOH, we used 24 polymorphic microsatellite markers located at the following sites in the 10 genes reported to play a major role in human carcinogenesis:[13–22] *FHIT* (3p) (D3S1300, D3S1312, and D3S1313), *APC* (5q) (D5S346 and D5S82), *p16* (9p) (D9S171 and D9S162), *TSC-1* (9q) (D9S149, D9S150 and DBH), *Int-2* (11q) (INT-2), *Rb* (13q) (D13S270, D13S273, and D13S176), *TSC-2* (16p) (D16S291 and D16S292), *p53* (17p) (TP53 and D17S520), *Smad 4* (18q) (D18S46, D18S363, and D18S474), and *Band M* (22q) (D22S1140, D22S1170, and D22S1161). The use of more than one microsatellite marker ensured a higher yield of information for each genomic locus. Polymerase chain reaction (PCR) reactions for the fluorescent-labeled markers were carried out in a volume of 20 μl that included 2 or 3 μl of 10 × PCR buffer and 5–25 pmol of each primer, 1 μl of template DNA, 200 μM of each deoxynucleotide triphosphate (dNTP), and 1.0 or 1.5 U of *Taq* DNA polymerase. To detect the amplified fragments, the samples were run on a Model 377 Genetic Analysis System (Applied Biosystems, Foster City, CA, USA) using Gene Scan 377 software (Applied Biosystems, Foster City, CA, USA). Markers that identified two distinguishable alleles of different sizes but similar intensity in normal DNA were termed 'informative' (heterozygous). Markers that yielded a single major peak in normal DNA were termed 'noninformative' (homozygous). LOH was defined as loss of one allele that corresponded to an allele present in informative cases. A result was scored as LOH if there was a greater than 50% reduction in allele ratio in the tumor relative to the normal control DNA.

DNA Analysis

We used two approaches to data analysis:[9] (1) to correlate morphologic changes with allelic losses, we calculated the mean Fractional Regional Loss (FRL) index, defined as: FRL index = total number of chromosomal regions with LOH/total number of informative regions, and (2) to determine whether the deletions in the individual chromosomal regions were progressive in individual foci, we determined the frequencies of loss of individual markers on the basis of a Fractional Allelic Loss (FAL) index defined as: FAL index = total number of markers with LOH/total number of informative markers. Fisher's exact test was used for the statistical analysis in these approaches, and probability values of $P<0.05$ were regarded as statistically significant.

Results

Accumulation of Genetic Alterations in Individual Tumors

Microsatellite markers on 10 chromosome arms were used to perform a PCR-based multifocal LOH assay on a total 56 tumor sites microdissected from 12 adenocarcinomas mixed BA and other subtypes of the lung. The results for each locus are summarized in Table 1. At least one allelic loss was detected in all 56 sites examined. To gain a greater understanding of the relationship between the morphologic and genetic anatomy of each specimen, two representative cases are shown in Figures 1 and 2, respectively. Normal components are designated 'N,' bronchioloalveolar components 'BA,' acinar components 'A,' solid components 'S,' papillary components 'P,' intrapulmonary metastases 'PM,' and lymph node metastases 'LNM.' Multiple foci representing the same histology are distinguished by a numerical designation. We used the changes (including the presence of allelic loss and the specific allele lost) to determine whether the foci in individual cases were clonally related. A diagram shows the least complex pathway of tumor progression compatible with the LOH data in each case. The diagrams were drawn based on the assumption that, whenever compatible with the data, an observed LOH event represents a single occurrence in the evolution of the tumor, so that tumor components with LOH at the same locus are postulated to share a common precursor.

In case 2 (Figure 1), genetic alterations accumulated in the evolution of the tumor from the BA component to the invasive and metastatic tumor components. Case 3 (Figure 2), on the other hand, is characterized by alterations in the primary tumor that were not detectable in the metastasis (TP53 (smaller allele) in A1, D22S1140 in BA). Although genetic alterations accumulated in the evolution of the tumor to metastasis, two subclonal pathways were demonstrated in the primary tumor.

As shown in these representative cases, genetic alterations accumulated during the evolution of the tumors. However, as demonstrated by case 3, tumor evolution along a single clonal pathway from BA to invasive and metastatic components was not detected in every case. Four of the BA components (4/12: 33.3%) and five of the invasive components (5/30: 16.7%) appeared to have evolved through independent subclonal pathways. In regard to the relationship among histological subtypes in the invasive lesions, in seven cases there were multiple morphological subtypes in the primary tumors, even though two of them displayed an identical LOH pattern. The remaining five cases did not show any distinct relationship between morphology and LOH status.

Allelic Loss in Histopathological Progression (Figure 3)

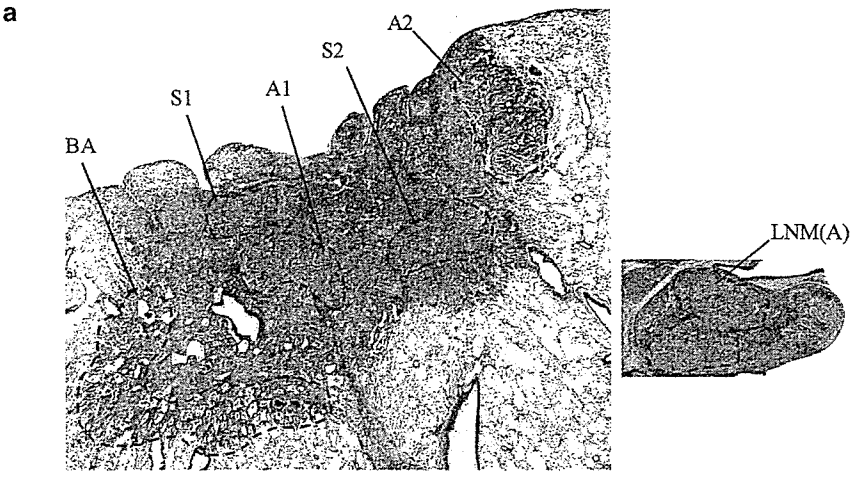
To elucidate whether accumulation of genetic alterations is related to histopathological progression, we compared mean FRL indices among BA-type lesions, invasive lesions, and metastases. The mean FRL indices for invasive lesions of the primary

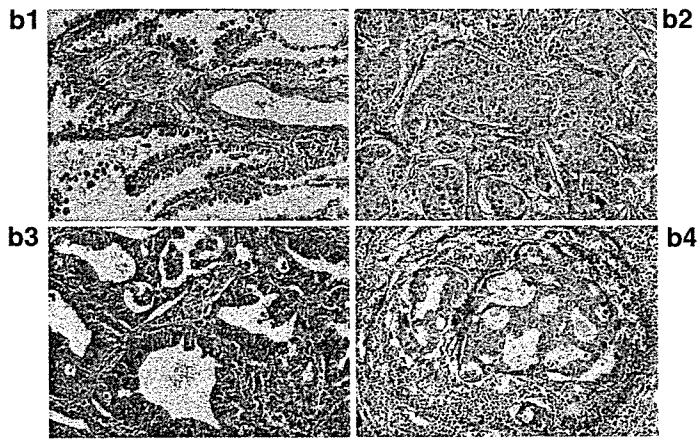
Table 1 Genetic analysis of samples from different portions of individual tumors

Case	*Region*	*Histological subtype*	*LOH*									
			3p	*5q*	*9p*	*9q*	*11q*	*13q*	*16p*	*17p*	*18q*	*22q*
1	BA	BA	+	NI	+	+	NI	+	−	+	−	−
	Invasive 1	A1	+	NI	+	+	NI	+	−	+	−	−
	Invasive 2	A2	+	NI	+	+	NI	+	−	+	−	+
	Metastasis 1	PM(A)	+	NI	+	+	NI	+	+	+	−	+
	Metastasis 2	LNM(A)	+	NI	+	+	NI	+	−	+	+	+
2	BA	BA	−	−	−	−	NI	−	NI	−	−	+
	Invasive 1	S1	−	−	−	−	NI	−	NI	+	−	+
	Invasive 2	A1	−	−	−	−	NI	+	NI	+	+	+
	Invasive 3	S2, A2	+	−	−	−	NI	+	NI	+	+	+
	Metastasis	LNM(A)	+	−	−	−	NI	+	NI	+	+	+
3	BA	BA	−	+	NI	+	−	NI	+	−	+	+
	Invasive 1	A1	−	+	NI	−	−	NI	+	−/+	−	−
	Invasive 2	A2	−	+	NI	+	−	NI	+	+/−	+	−
	Invasive 3	A3	−	+	NI	+	+	NI	+	+/−	+	−
	Metastasis	LNM(A)	−	+	NI	+	+	NI	+	+/−	+	−
4	BA	BA	+	+	−/+	−	NI	+	+	+	+	+
	Invasive 1	A1, S	+	+	+/−	−	NI	+	+	+	+	+
	Invasive 2	A2, A3	+	+	+/−	+	NI	+	+	+	+	+
	Metastasis	LNM(A)	+	+	+/−	−	NI	+	+	+	+	+
5	BA	BA	+	+	NI	−/+	−	+	NI	+	+	+
	Invasive 1	A, P	+	+	NI	−/+	−	+	NI	+	+	+
	Invasive 2	S	+	+	NI	+/−	−	+	NI	+	+	+
	Metastasis	LNM(P)	+	+	NI	−/+	−	+	NI	+	+	+
6	BA	BA	+	+	NI	−	−	+	NI	NI	+	−
	Invasive	P	−	+	NI	−	−	+	NI	NI	+	−
	Metastasis	LNM(P)	−	+	NI	−	−	+	NI	NI	+	−
7	BA	BA	+	NI	NI	+	−	+	−	+	−	−
	Invasive	A, S	+	NI	NI	+	−	+	−	+	+	−
	Metastasis	LNM(A, S)	+	NI	NI	+	−	+	−	+	+	−
8	BA	BA	+	+	−	+	−	−	−	+	NI	−
	Invasive	S1, S2	+	+	+	+	+	+	+	+	NI	−
	Metastasis	LNM(S)	+	+	+	+	+	+	+	+	NI	−
9	BA	BA	−	NI	NI	+	−	−	−	+	+	NI
	Invasive	A, P	−	NI	NI	+	−	−	−	+	+	NI
	Metastasis	LNM(A)	−	NI	NI	+	−	−	−	+	+	NI
10	BA	BA	+	+	+	+	−	NI	−	−	+	−
	Invasive	A1, A2	+	+	+	+	+	NI	−	−	+	−
	Metastasis	LNM(A)	+	+	+	+	+	NI	−	−	+	−
11	BA	BA	−	+	NI	−	−	+	−	+	−	−
	Invasive	A, S, P	−	+	NI	+	−	+	−	+	−	−
	Metastasis	LNM(A)	−	+	NI	+	−	+	−	+	−	−
12	BA	BA	+	−	+	+	NI	−	−	+	+	−
	Invasive	A, P	+	−	+	+	NI	−	−	+	+	−
	Metastasis	LNM(A)	+	−	+	+	NI	−	−	+	+	−

BA = bronchioloalveolar component; A = acinar component; S = solid component; P = papillary component; PM = intrapulmonary metastasis; LNM = lymph node metastasis; NI = non-informative; + = LOH; − = no LOH; −/+ or +/− = deletion of smaller or larger allele. (These designations are used only for the case in which heterogenous LOH detected in restricted tumor sites.)
All sites collected were classified into three regions: BA, invasive, and metastasis. 'BA' means only BA lesions. 'Invasive' means invasive lesions in the primary tumor (acinar, solid, and papillary). 'Metastasis' means intrapulmonary metastasis and lymph node metastasis. The sites in each region that had the same LOH pattern were summarized in the same group and numbered. For example, in case 2 the tumor contained S1, A1, S2, and A2 as invasive lesions. They were investigated separately and demonstrated three LOH patterns: invasive 1, 2, and 3. S2 and A2 showed the same LOH patterns and they were classified as invasive 3.

tumor and metastasis (0.66 and 0.69) were significantly higher than that for BA-type lesion (0.55); ($P<0.05$), however, the difference between the mean FRL index of the invasive components and metastatic components was not significant. The histological change from BA to invasive and to metastatic components was characterized by a significant rise in the mean FRL indices. The 12 cases could be divided into three groups according to the patterns of allelic losses. (1) Alleles that were retained in the BA lesion were deleted in either metastatic lesions or invasive and metastatic lesions. Eight cases (67%) showed accumulation of genetic alterations from BA lesions to metastases. (2) All foci had the same allelic losses. Two cases (17%) showed the same LOH patterns in all foci examined. (3) The markers used in this study could not be used to analyze the LOH patterns. Two cases, cases 5 and 6, had allelic loss in only one region in only one component (invasive and BA lesion), and the other components showed the same LOH patterns.





c

	BA	A1	A2	S1	S2	LNM (A)
D3S1300	–	–	■	–	■	■
D3S1312	–	–	■	–	■	■
D3S1313						
D5S346						
D5S82	–	–	–	–	–	–
D9S171						
D9S162	–	–	–	–	–	–
D9S149	–	–	–	–	–	–
D9S150	–	–	–	–	–	–
D8H	–	–	–	–	–	–
INT-2						
D13S270	–	□	□	–	□	□
D13S273	–	□	□	–	□	□
D13S176	–	■	■	–	■	■
D16S291						
D16S292						
TP53	–	□	□	□	□	□
D17S520						
D18S46	–	■	■	–	■	■
D18S363	–	■	■	–	■	■
D18S474	–	■	■	–	■	■
D22S1140	■	■	■	■	■	■
D22S1170						
D22S1161						

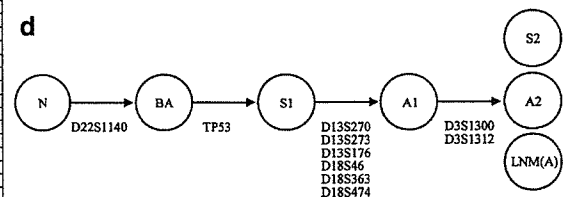


Figure 1 Analysis of LOH in an adenocarcinoma mixed BA, acinar, and solid (case 2). (a), Histology: low-power view showing regions chosen for laser capture microdissection (hematoxylin and eosin staining). BA: bronchioloalveolar component; S1 and S2: solid component dissected from different regions of the specimen; A1 and A2: acinar component dissected from different regions of the specimen; LNM (A): lymph node metastasis (acinar component). Primary tumor, which included a focus of BA-type carcinoma (BA) and two invasive acinar carcinomas (A1 and A2) and two invasive solid carcinomas (S1 and S2), and a metastatic tumor (lymph node metastasis; LNM) were analyzed. The metastatic tumor was acinar carcinoma. (b) Microscopic appearance of the four tumor areas (HE staining; original magnification, × 200). b1: BA component; b2: solid component; b3: acinar component; b4: acinar component in the lymph node metastasis. (c) LOH data obtained from microdissected tumor components. □: LOH of top band, ■: LOH of bottom band, -: no LOH, blank space: noninformative (d) Clonal relationship between microdissected tumor components suggested by LOH data. Open circles indicate a putative intermediate or precursor cell. LOH is indicated on the diagrams by microsatellite markers that showed allelic loss. BA, which contained one allelic loss (marker D22S1140), may be the precursor lesion of other invasive lesions as well as metastatic lesion. In the same manner, S1, which contained one allelic loss (marker TP53) in addition to the loss (marker D22S1140), may be the precursor lesion of other invasive and metastatic lesions. A1, which contained six additional allelic losses, may be the precursor lesion of the remaining lesions. Three lesions (S2, A2, and LNM (A)) had lost alleles at all 10 markers.

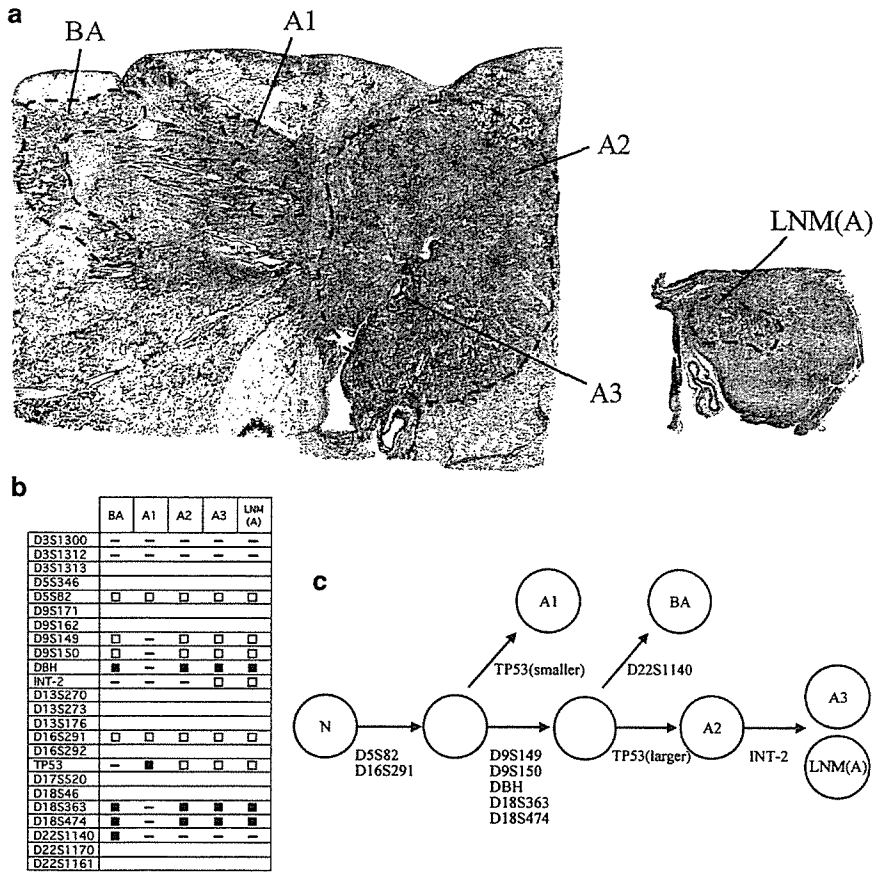


Figure 2 Analysis of LOH in an adenocarcinoma mixed BA and acinar (case 3). (a) Histology: BA: BA component; A1, A2, and A3: acinar component dissected from different regions of the specimen; LNM (A): lymph node metastasis (acinar component). Tumor components included BA-type carcinoma (BA) and three foci of invasive acinar components (A1–3) in the primary tumor and another acinar focus in the lymph node metastasis (LNM (A)). (b) LOH data obtained from microdissected tumor components. □: LOH of top band, ■: LOH of bottom band, -: no LOH, blank space: noninformative. (c) Clonal relationship between microdissected tumor components suggested by LOH data. Designation of LOH results is as in Figure 1. All components contained two allelic losses (marker D5S82 and D16S291). A1 showed LOH at the smaller allele of TP53 that was not detected in other components. In the same manner, only the BA component contained one allelic loss (marker D22S1140). These results suggest that the A1 and BA components must have evolved along subclonal pathways and that they shared common precursors, indicated by the open circle in the diagram. A2 showed LOH at the larger allele of TP53 in addition to the common precursor, and may be the precursor lesion of A3 and LNM (A).

Prevalence and Intratumor Topography of LOH Events

LOH was observed in 75.0% (126/168) of the informative markers on the 10 chromosome arms, including 3p (77.3%), 5q (80.0%), 9p (88.9%), 9q (85.2%), 11q (37.5%), 13q (83.3%), 16p (28.6%), 17p (93.8%), 18q (91.7%), and 22q (46.7%). In order to investigate the sequence of molecular genetic changes involved in the development of metastasizing adenocarcinoma mixed BA and other subtypes of the lung, we analyzed the topographical distribution of allelic losses in the tumors. Multiple foci from the same case were compared to determine whether the LOH events were shared at all tumor sites as a relatively early event derived from a progenitor cell, or localized in either metastatic lesions or invasive and metastatic lesions as an additional event directing toward invasion and metastasis. Losses on 5q were detected uniformly in the various regions of cases that tested positive for LOH (two cases were uniformly negative and three were noninformative), while homogeneous distribution of LOH was observed on 3p (70.6%), 9p (25.0%), 9q (43.5%), 11q (0.0%), 13q (66.7%), 16p (50.0%), 17p (80.0%), 18q (59.1%), and 22q (71.4%; and Figure 4). Losses on 11q were not detected uniformly at all tumor sites tested, but localized in either metastatic lesions or invasive and metastatic lesions, while heterogeneous distribution of LOH was observed on 3p (11.8%), 9p (37.5%), 9q (39.1%), 13q (33.3%), 16p (50.0%), 17p (13.3%), 18q (40.9%), and 22q (14.3%; and Figure 4). As shown in Figure 4, 5q loss was observed frequently (80.0%) and most uniformly (100%) in multiple sites, and was therefore interpreted as an obligatory early event in the progression of metastasizing pulmonary adenocarcinoma mixed BA and other subtypes. As 17p loss was most frequent (93.3%) and relatively uniform (80.0%), it was also interpreted as an early event. The loss of 18q was

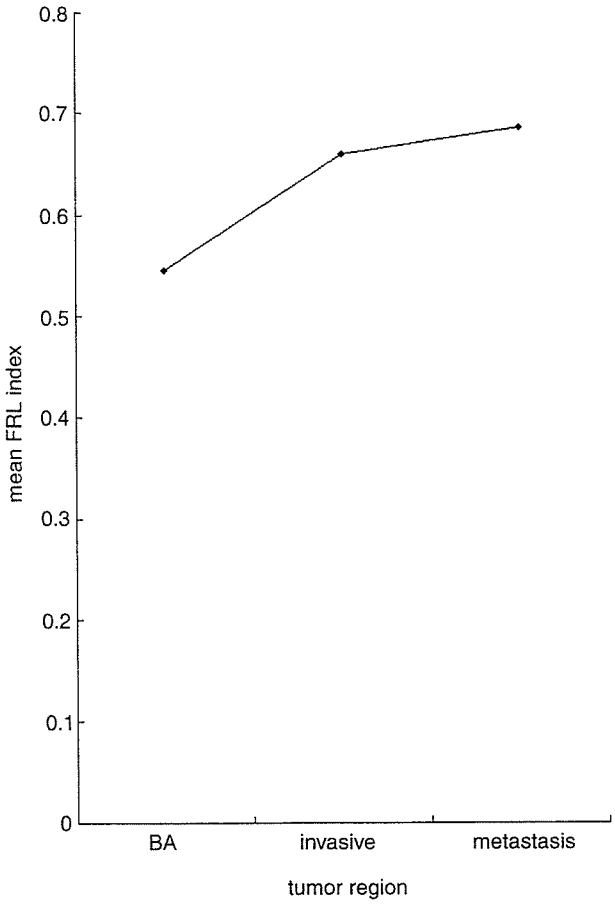


Figure 3 Mean fractional regional loss indices for the BA, invasive, and metastatic components in 10 chromosomal regions.

relatively frequent (91.7%) and heterogeneous (40.9%), and therefore indicative of late alterations required for malignant progression.

Discussion

Invasive and/or metastatic components contained more additional allelic losses than BA components. It has already been postulated that tumor cells evolved from BA to invasive and metastatic components because of the difference in histology between the lepidic pattern along the alveolar walls in BA components and invasiveness in other components.[7] Almost all metastases contained the same or more allelic losses when compared with invasive lesions in each individual tumor, although no significant difference was found in the mean FRL indices of the invasive and metastatic lesions. This suggests that tumor cells that showed noninvasive BA-type morphology evolved into invasive lesions and then to metastases, acquiring the invasive and metastatic phenotype through the process of clonal evolution occurring during multistep tumor progression. Eight cases (67%) actually showed accumulation of genetic alterations during morphological progression, but the tumor components examined thus far in the remaining four cases have shown genetic homogeneity or no obvious accumulation of genetic alterations despite great morphological divergence. This might be explained by the genetic alterations being present on loci other than those examined in this study. Although the additional allelic losses found in the metastases can be explained by the accumulation of genetic aberrations during the

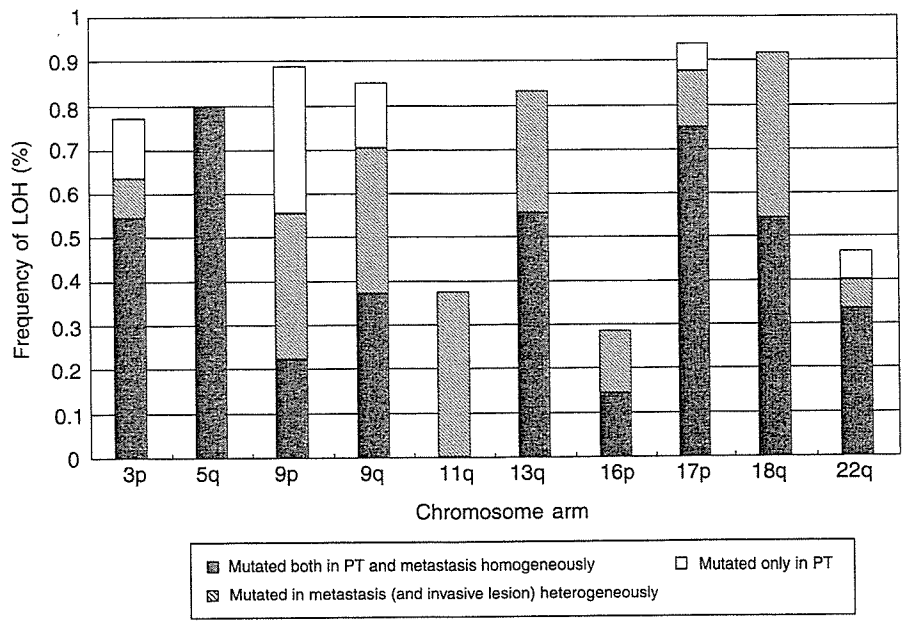


Figure 4 Prevalence and intratumor heterogeneity of allelic loss in primary tumors and lymph node metastases. PT, primary tumor.

course of tumor progression, genetic alterations that were not detectable in the metastases were present in the primary tumor. These findings indicate that the primary tumor progresses genetically even after the metastasis has occurred, that is, the predominant clone of some primary tumor components no longer represents the metastatic clones that we investigated.

Using multiple microdissected specimens within individual cases that included primary and metastatic tumors, we have shown highly frequent LOH at 5q and 17p even in the lowest-grade portions (ie, BA lesions) and found identical alleles to be deleted in all portions examined in each affected primary tumor and metastatic tumor, even though quite divergent histopathologically. These results suggest that LOH at 5q and 17p may be crucial steps in the early phase of the development to lymph node metastasis and that they are retained throughout successive clonal evolutions. A similar phenomenon was previously reported by Boland *et al*[23] based on an analysis of tumor progression schemes in colon cancers. They used multiple microdissected samples of colonic tumors showing cross-sections of the 'adenoma–carcinoma sequence' and detected a clear, abrupt occurrence of LOH at 5q at the transition phase from normal epithelium to adenoma. We observed LOH on at least one of the two loci on 5q and/or 17p uniformly in almost all cases examined (11/12; 92%). However, it is uncertain why such presumably early lesions are not present in all tumors. A number of cell types are thought to be potential precursors of lung cancers, and different initiation events may be involved depending on the differentiation pathway to which they are committed. Thus, certain cell types may not require inactivation of all putative tumor suppressor genes on 5q and 17p.

Previous studies have shown allelic loss at the 17p loci to be involved at a relatively early stage of NSCLC,[8,24,25] and its loss may be associated with the genesis of NSCLC. *p53* is believed to play a role as a 'guardian' that maintains the integrity of the genome by participating in the DNA damage checkpoints in the cell cycle. Inactivation of *p53* has been reported to lead to increased frequency of mutations, chromosomal rearrangements, and abnormal chromosomal segregations.[26–29] Recent studies have suggested that the LOH at specific chromosomal loci, 1p, 3p, 5q, 9p, 17q, and 22q, is associated with a worse prognosis of NSCLC, although studies of patients from different populations have yielded conflicting results.[14,30–39] LOH at the *APC/MCC* gene cluster at chromosome 5q has been reported to correlate with poorer survival of patients with NSCLC.[38] In the present study, the tumors were small but were associated with lymph node metastasis, and a worse prognosis was assumed. Seven of nine informative cases (78%) showed allelic losses at 5q in all foci, including BA lesions. Our previous study also concluded that this deletion is a relatively early event in the progression of adenocarcinoma of the lung.[6] The high prevalence of 5q deletion in this study might indicate that 5q loss plays an important role in the progression of metastatic tumors and that it was determined in the early stage.

Although we showed frequent LOH at 18q in the tumors examined, approximately 40% of allelic losses were found in either metastatic lesions or invasive and metastatic lesions, not in all portions of each affected primary tumor and metastatic tumor examined. Therefore, LOH at 18q appears to have a role as late event in the metastatic progression of adenocarcinomas mixed BA and other subtypes of the lung. Shiseki *et al*[8] reported that loss at 18q plays an important role in the progression of NSCLC based on a comparison of stage I NSCLC and brain metastases. Lymph node metastases, most malignant portions, were shown to carry 18q deletions at even higher frequency than 5q or 17p deletions in the present study. However, it should be noted that accumulation of LOH at 18q occurred at various stages of tumor progression within individual tumors toward lymph node metastasis, that is, some LOH at 18q occurred in BA lesions, and some in metastatic lesions. This indicated a clear distinction from LOH at 5q and 17p, especially at 5q. Since approximately 60% of 18q deletions are present in all portions of individual tumors, it remains unclear whether LOH at 18q has a role both as an early event and a late event or acts at various steps in tumors that progress to lymph node metastasis.

In summary, we examined the topographical distribution of LOH on 10 chromosome arms, and the results suggest that tumor cells accumulate genetic alterations as they evolve from the BA lesions to the invasive and metastatic lesions. Early occurrence of 5q and/or 17p deletions and successive clonal expansion during the progression of individual tumors was inferred. By contrast, LOH at 18q seemed to be acquired at various stages during tumor progression to metastasis. Similar studies analyzing more genetic loci in a larger number of cases are warranted. Furthermore, since the lung cancers resulted from various genetic and epigenetic alterations, it would also be interesting to examine the topographical differences in other genetic or epigenetic changes, such as DNA methylation.

Acknowledgements

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Randomized Trial of Oral Versus Intravenous Antibiotics in Low-risk Febrile Neutropenic Patients with Lung Cancer

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Background: Neutropenic fever is one of the most serious adverse effects of cancer chemotherapy. Neutropenia may cause a life-threatening bacterial infection. Therefore, febrile neutropenic inpatients are empirically treated with intravenous broad-spectrum antibiotics. Recently, several studies have suggested the presence of low-risk groups among febrile neutropenic patients.

Methods: A prospective randomized trial was conducted to compare treatment with oral ciprofloxacin (200 mg) and amoxicillin-clavulanate (375 mg) administered every 8 h against that with intravenous ceftazidime (1 g) administered every 12 h in low-risk febrile neutropenic patients with lung cancer. All patients received chemotherapy and antibiotic therapy while being hospitalized.

Results: A total of 177 patients with lung cancer agreed to participate in this study prior to undergoing chemotherapy. Among them, a total of 36 neutropenic patients with 42 febrile episodes were enrolled in the study. Treatment was successful without the need for modification in 91% of the episodes in patients receiving the oral regimen and 79% of the episodes in patients receiving the intravenous regimen. No treatment-related deaths occurred. One patient developed nausea while receiving the oral regimen, so the oral regimen was changed to the intravenous regimen in this patient.

Conclusions: This prospective study suggested that treatment with oral antibiotics ciplofloxacin plus amoxicillin-clavulanate was effective for low-risk febrile neutropenic patients after chemotherapy.

Key words: oral antibiotics – low-risk – febrile neutropenia

INTRODUCTION

Neutropenic fever is one of the most serious adverse effects in cancer chemotherapy. Neutropenia may cause a life-threatening bacterial infection. The risk of infection increases in patients with a neutrophil count of <1000/mm^3 (1). As a result, most cancer patients remain in hospital after undergoing chemotherapy in Japan, and empirical broad-spectrum intravenous antibiotics are administered to febrile neutropenic patients. This approach is effective in reducing morbidity and mortality but is associated with toxicity related to intravenous antibiotics, as well as physical and psychological discomfort for the patient. In addition, parenteral antibiotic administration requires insertion of an intravenous catheter, which carries a risk of infection. Prolonged hospitalization may cause infection to drug-resistant organisms, is expensive, and has a detrimental effect on quality of life.

Recently, several studies have suggested the presence of low-risk groups among febrile neutropenic patients (2–4). Medical complications were less frequent overall for patients whose neutropenia (<500/mm^3) resolved in 7 days or less, compared to other patients (4). A study demonstrated that neutropenia lasted for 1 week or less in 85% of the patients selected using the following exclusion criteria: hepatic insufficiency (alanine aminotransferase activity > four times normal), a history of recurrent pyrexia of undetermined origin (PUO), shock (systolic blood pressure <80 mmHg or peripheral circulatory failure), any other comorbid conditions requiring hospitalization (except for anemia or thrombocytopenia) and the expectation of prolonged neutropenia (>7 days) based on the presence of aplastic anemia, myelodysplasia, leukemia or other causes (5). Patients who did not meet any of these exclusion criteria were considered to belong to a low-risk group. A randomized trial comparing oral ciplofloxacin and amoxicillin-clavulanate with

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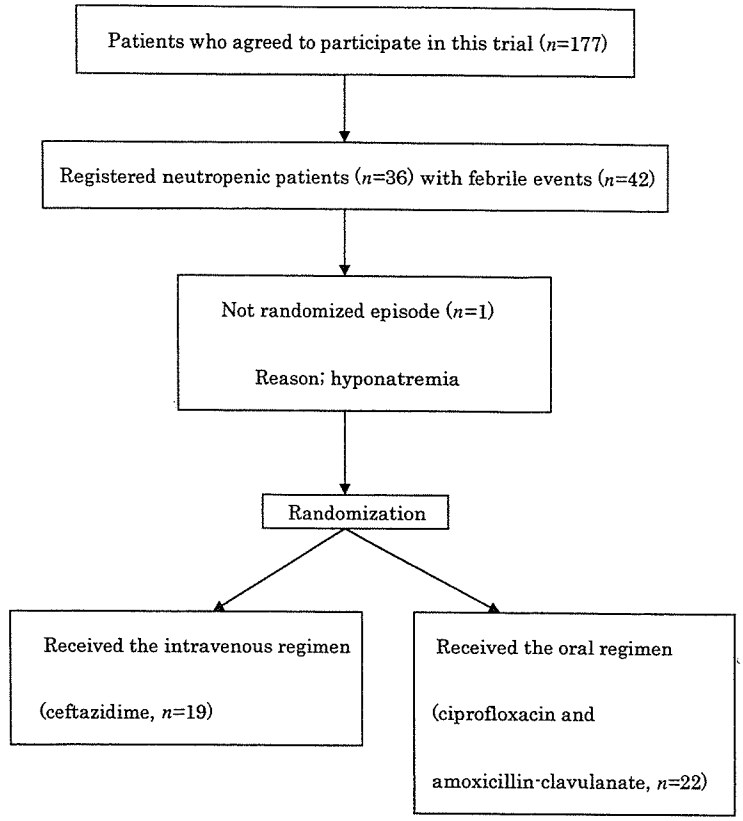

Figure 1. Study flow diagram.

intravenous aztreonam and clindamycin was conducted in these low-risk febrile neutropenic patients (6). This trial demonstrated that oral antibiotics were as effective as intravenous ones.

We conducted a randomized trial to compare oral ciplofloxacin and amoxicillin-clavulanate with intravenous ceftazidime, which was empirically used, in low-risk febrile neutropenic patients with lung cancer. The combination of ciplofloxacin and amoxicillin-clavulanate provides sufficient coverage against gram-negative enteric bacilli and gram-positive cocci. The aim of our trial was to determine whether an oral regimen was an acceptable alternative to an intravenous regimen in low-risk patients.

PATIENTS AND METHODS

Criteria for Eligibility

Eligible patients included those with lung cancer and neutropenia after having undergone platinum-based chemotherapy. Patients were required to have a single axillary temperature of 37.5°C or higher after platinum-based chemotherapy, an absolute leukocyte count $\leq 1000/mm^3$ or a neutrophil count $\leq 500/mm^3$. Other criteria included an age of 20 years or more and an ECOG performance status (PS) of between 0 and 2 (inclusive). The exclusion criteria included the following conditions: previous anaphylactic reactions or hypersensitivity to any of the antibiotics used or related products; antibiotic treatment within the preceding 96 h; prior administration of non-steroidal anti-inflammatory drugs (NSAIDs); recurrent PUO; renal insufficiency (serum creatinine ≥2.5 mg/dl or need for dialysis); hepatic insufficiency (aspartate aminotransferase/alanine aminotransferase levels > four times the normal value); systolic blood pressure ≤90 mmHg or peripheral circulatory failure; uncontrolled hypercalcemia; altered sensorium; respiratory rate ≥30 breaths/min; serum sodium ≤128 mg/dl; and the inability to take oral medications because of painful mouth ulcers, intestinal malabsorption or severe nausea and vomiting. All patients were required to provide their written informed consent prior to undergoing chemotherapy, and the institutional review board at the National Cancer Center approved the study's protocol.

Treatment Plan

All patients received chemotherapy and antibiotic therapy on an inpatient basis. The baseline evaluation included a physical examination (blood pressure, pulse and respiratory rate, temperature). Cultures were obtained of blood, sputum, throat, urine and feces (anal swabs). Patients were randomly assigned to one of two regimens using consecutive sealed envelopes. The oral regimen consisted of ciprofloxacin (200 mg) plus amoxicillin-clavulanate (375 mg) administered every 8 h, while the intravenous regimen consisted of ceftazidime (1 g) administered every 12 h. Granulocyte colony-stimulating