exhibit a response, and skin rash, diarrhea and elevation in GOT/GPT levels were significant prognostic factors of survival.

4. Discussion

Gefitinib is a promising agent for the treatment of advanced NSCLC, but risk assessment is of critical importance to using it properly. Gefitinib was thought to be a relatively safe agent at first, and physicians in Japan tended to prescribe it without careful consideration of risks. In the first 4 months after its approval, 17,000 patients began taking gefitinib, the most rapid adoption of any antitumor agent in Japan. The Ministry of Health, Labour and Welfare has estimated that the incidence of ILD was 2.2%. However, since a follow-up survey of all of the cases has not been conducted and only limited data from sporadic reports by physicians were available, many ILD cases may not have been reported, and the actual incidence may have been higher than 2.2%. Although the sample size in the present study was small, the incidence of ILD was

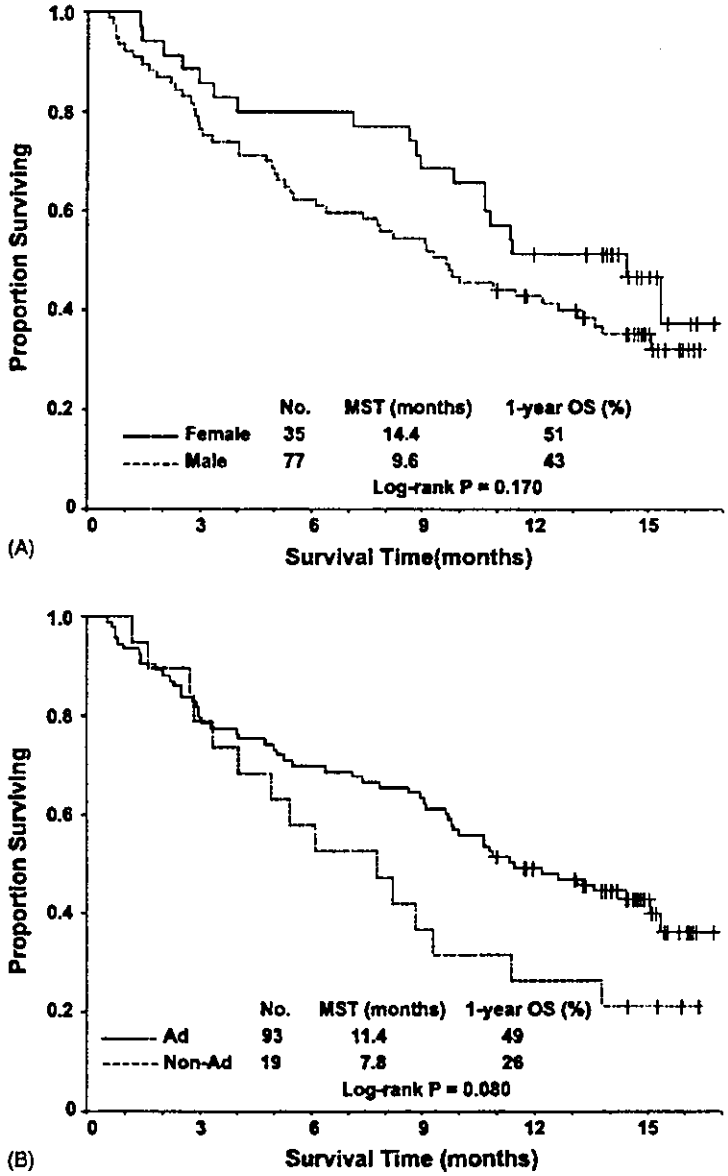

Fig. 2 Kaplan–Meier plot of overall survival according to subgroups: (A) female versus male; (B) adenocarcinoma versus non-adenocarcinoma; (C) never-smokers versus moderate/heavy smokers. MST: median survival time, OS: overall survival, Ad: adenocarcinoma.

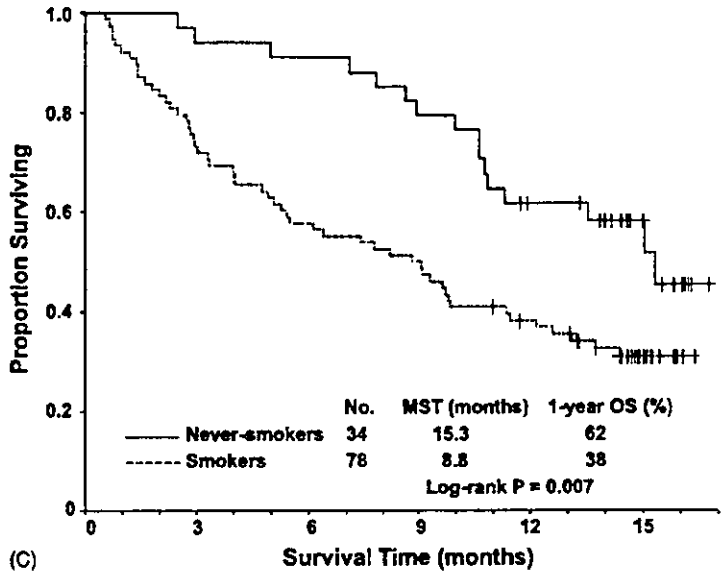

Fig. 2 (*Continued*).

as high as 5.4%. The risk of ILD appears to be around 2–5% if gefitinib is given to patients without careful risk assessment. We think that the incidence can be reduced by patient selection after a thorough risk assessment and that the proper use of gefitinib may enable great benefit, far exceeding its potential risks.

Our analysis of the risk factors for the development of ILD revealed pre-existing PF as a strong risk factor. Of the 112 patients in this study, 12 had PF at the start of gefitinib administration. Four (33%) of these patients subsequently developed ILD, 3 (25%) died as a result, and no response was seen in any of these 12 patients. A panel of experts convened by AstraZeneca Japan retrospectively analyzed 104 patients with NSCLC who developed ILD during gefitinib therapy in Japan and reported that 30 (29%) of them were diagnosed as pre-existing PF by chest X-rays or computed tomography scans taken before gefitinib administration [8]. The panel also noted that the patients with PF had a significantly higher mortality rate after the onset of ILD: it was 77% (23/30) among the patients with PF and 34% (25/74) among the patients without PF ($P < 0.001$) [8]. We conclude that gefitinib treatment may be harmful to patients with PF and recommend that gefitinib not be used if PF is apparent on the chest X-rays.

In our study, all patients were Japanese and a 33% response rate was observed. In the IDEAL 1 trial, 102 Japanese and 106 non-Japanese patients received gefitinib, and the response rate was 27.5% in the Japanese and 10.4% in the non-Japanese [5]. Whether this difference was attributable to ethnicity or an imbalance in other characteristics is unknown, but a high response rate in Japanese patients has been consistently observed in clinical practice.

Both the IDEAL 1 and 2 trials suggested "female gender" and "adenocarcinoma" as predictive factors for tumor response to gefitinib [5,6], and a retrospective analysis of gefitinib monotherapy for advanced NSCLC showed that "adenocarcinoma" (especially with bronchioloalveolar features) and "no history of smoking" were significantly correlated with response to gefitinib [9]. We observed the same tendency with a response rate of 53% in women, 38% in patients with adenocarcinoma, and 63% in never-smokers. "No history of smoking" was a significant predictive factor for response in multivariate analysis, and it was also a significant predictor of longer TTF and longer survival. Since both female gender and adenocarcinoma were significantly associated with no history of smoking, which of these characteristics are true predictive factors remains uncertain. It was also suggested that heavier smokers and male smokers specifically had a lower response rate among the patients with smoking history. Since heavier smokers tended to have a higher risk of ILD, we should carefully assess their risk-benefit ratio of gefitinib therapy before selecting therapeutic strategies.

There are some biological explanations for these clinical characteristics associated with response to gefitinib [10]. Although gefitinib inhibits the intracellular tyrosine kinase domain of EGFR, no correlation between expression of EGFR and response

has been demonstrated [11]. When EGFR and human epidermal growth factor receptor 2 (HER2) are coexpressed, HER2 is the preferred dimerization partner of EGFR, and EGFR-HER2 heterodimers have more signaling potency than EGFR homodimers [12]. Preclinical studies have indicated that tumor cell lines overexpressing HER2 or coexpressing EGFR and HER2 are sensitive to gefitinib [13–16]. Since EGFR/HER2-coexpression is more common in adenocarcinoma of the lung than in squamous cell carcinoma [13,17], the high response rate in adenocarcinoma may be attributable to it. In women, estrogens and estrogen receptors are involved in the development of NSCLC [18], and estrogens binding to its receptors upregulates EGFR and EGFR ligands [19]. The presence of estrogens and its receptors may impact EGFR signaling and the response of NSCLC to gefitinib in women. NSCLC in never-smokers may also have a different biology. Since several studies have indicated fewer mutations of the p53 and K-ras genes in never-smokers than in smokers [20,21], the relation between such tobacco-related mutations and gefitinib response should be investigated. Subgroups of patients who obtain a clinical benefit from gefitinib administration are needed to be identified more precisely, and molecular markers predictive of tumor response should be sought by using DNA microarrays and a proteomics-based approach.

Our analysis suggests that patients who suffer from skin toxicity, diarrhea, or liver toxicity have a greater clinical benefit from gefitinib treatment. A correlation between skin toxicity and survival has also been shown in a study of gefitinib for head and neck cancer [22] and in studies of erlotinib, another EGFR tyrosine kinase inhibitor [23]. Because these findings may be attributable to the responders having taken gefitinib for longer periods and the toxicities in these patients being evaluated more carefully, further studies are needed to confirm them. If the early onset of toxicities has predictive value for survival, it can be used for clinical decision making regarding continuation of gefitinib treatment.

5. Conclusion

When gefitinib is used to treat advanced NSCLC, it confers a higher risk of ILD on patients with PF and a greater clinical benefit on never-smokers, women, patients with adenocarcinoma, and patients with no history of thoracic radiotherapy. Gefitinib therapy is an important treatment option for patients with advanced NSCLC, but the proper use of it based on individual risk-benefit assessments is crucial.

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Dose Escalation Study of Paclitaxel in Combination with Fixed-Dose Irinotecan in Patients with Advanced Non-Small Cell Lung Cancer (JCOG 9807)

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Key Words
Paclitaxel · Irinotecan · Non-small cell lung cancer · G-CSF

Abstract
Background: Both irinotecan (CPT) and paclitaxel (Pac) are effective against non-small cell lung cancer (NSCLC), and besides, preclinical studies have demonstrated an additive or synergistic interaction between camptothecin and taxane. ***Methods:*** We conducted a phase I/II study of combination chemotherapy consisting of Pac and CPT to determine qualitative and quantitative toxicities and efficacy of the combination against advanced NSCLC. We fixed the dose of CPT at 60 mg/m^2 and escalated the Pac dose in 10 or 20 mg/m^2 increments from a starting dose of 80 mg/m^2, and repeated the cycle every 2 weeks. Prophylactic G-CSF was also administered. ***Results:*** Between February 1999 and April 2001, 24 patients were registered in the study. None of the patients had a history of prior chemotherapy, but surgical resection had been performed in 3 of them. None of the patients experienced dose-limiting toxicity (DLT) up to and including level 6. At dose level 7 of Pac, 180 mg/m^2, 2 patients experienced DLT, that is grades 2 and 3 dyspnea due to pneumonitis. Another patient experienced grade 1 dyspnea due to pneumonitis. Neutropenia, diarrhea, and other toxicities were mild; however, we concluded that dose level 7 of Pac was the maximum-tolerated dose. An objective response was observed in 58.3%. The median survival time was 370 days, and the 1-year survival rate was 54.2%. ***Conclusion:*** Pneumonitis was the DLT in this study, and Pac 160 mg/m^2 and CPT 60 mg/m^2 every 2 weeks are recommended for the phase II study. This combination shows appreciable activity against NSCLC.

Introduction

Current chemotherapy regimens for metastatic non-small cell lung cancer (NSCLC) are not particularly effective, and the disease cannot be cured even with the most effective cisplatin-based combination chemotherapy. New agents and new combination chemotherapies have been investigated for metastatic NSCLC, and in the past decade, a number of new anticancer agents, including vinorelbine, gemcitabine, docetaxel, and paclitaxel (Pac), have been approved for the treatment of advanced NSCLC. Regimens based on combinations of these drugs with platinum compounds have presented interesting new possibilities for treating patients with NSCLC, and randomized studies comparing platinum-based combinations with single-agent treatment have demonstrated a

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small but significant survival benefit for combined treatment [1, 2]. A trial of nonplatinum combination chemotherapy has recently been instituted [3].

The camptothecin derivative irinotecan (CPT) is a topoisomerase I inhibitor and effective against NSCLC [4]. Pac preferentially binds to microtubles and impairs microtubular disassembly, and it is also effective against NSCLC [5]. The combination of a camptothecin and a taxane is attractive, because both have been shown to have a broad spectrum of clinical activity that is dependent on the schedule of administration, and the mechanisms of action and the nonhematologic toxicity profiles of the camptothecins and taxanes are different. Preclinical studies that have evaluated combinations of a camptothecin with a taxane have yielded promising results, and several studies have demonstrated an additive or synergistic interaction between camptothecin and taxane [6–10].

Accordingly, we expected the combination of Pac and CPT to display high activity against NSCLC. Pac was initially infused every 3–4 weeks; however, weekly infusions were shown to produce a higher dose intensity and to have promising activity [11]. CPT has been used in various schedules, including weekly, every 2 weeks, and every 3 weeks. Weekly or 2-week cycles are considerably better than 3-week cycles to increase the dose intensity of both drugs. An important criterion for the feasibility of the combination of Pac and CPT is a reduced incidence of neutropenia, which is dose-limiting toxicity (DLT) of both drugs, and prophylactic granulocyte colony-stimulating factor (G-CSF) has been used to prevent neutropenia. We previously reported that prophylactic administration of G-CSF when monocytopenia is first detected can lessen neutropenia caused by chemotherapy for lung cancer without increasing the total G-CSF dose beyond the standard dose [12]. Administration of G-CSF was started on day 6–8 in this study. A 2-week cycle is reasonable for combinations of Pac and CPT when G-CSF was prophylactically used after the detection of monocytopenia.

We designed a dose escalation study to determine the maximum dose of Pac that could be given with CPT when both drugs were delivered according to a 2-weekly schedule. We fixed the dose of CPT at 60 mg/m^2, which is the dose used in combination with cisplatin [13], and escalated the Pac dose form 80 mg/m^2 in 10 or 20 mg/^{2} increments. The aims of this study were: (1) to determine the qualitative and quantitative toxicities of the combination chemotherapy; (2) to determine the recommended dose of Pac with CPT, and (3) to determine the efficacy of combination chemotherapy against advanced NSCLC by evaluating the objective response rate and survival rate.

Table 1. Patient characteristics

	Patients
Total	24
Age, years	
Median	59
Range	44–69
Gender	
Male	18
Female	6
PS (ECOG)	
0	4
1	20
Clinical stage	
IIIA/B	5
IV	16
Postoperative recurrence	3
Histology	
Adenocarcinoma	16
Squamous cell carcinoma	7
Large cell carcinoma	1
Prior treatment (surgery)	3

Patients and Methods

Patients

Patients with histologically or cytologically proven unresectable NSCLC were registered for Pac and CPT combination chemotherapy. Eligibility criteria were an expected survival of at least 6 weeks, age <70 years, Eastern Cooperative Oncology Group performance score (PS) ≤1, leukocyte count ≥4,000/μl, hemoglobin ≥10 g/dl, platelet count ≥100,000/μl, total serum bilirubin ≤1.5 mg/dl, aspartate aminotransferase and alanine aminotransferase ≤90 IU/l, serum creatinine ≤1.5 mg/dl, and creatinine clearance ≥50 ml/min. Patients who had experienced postoperative recurrence were eligible for this study, but a period of rest of 4 or more weeks was required after surgery. Patients who had received chemotherapy or radiotherapy were excluded from this study. Written informed consent was obtained from every patient.

Chemotherapy

All patients without disease progression were treated every 2 weeks for a total of 4 courses of chemotherapy. CPT was dissolved in 250 ml of 5% glucose and administered as a 90-min infusion at a fixed dose of 60 mg/m^2 on day 1. Pac was dissolved in 500 ml of 5% glucose and administered as a 3-hour infusion at a starting dose of 80 mg/m^2 on day 1 and escalated in 10 or 20 mg/m^2 increments per dose level (table 1). Premedication consisted of a 20-mg dexamethasone infusion (14 and 7 h before Pac), and a 50-mg ranitidine and 50-mg oral dose of diphenhydramine (30 min before Pac). After completion of the 90-min CPT infusion, a 30-min ranitidine infusion was administered, and a 3-hour Pac infusion was then administered. G-CSF, 50 or 2 μg/kg/day, was administered subcutaneously when the leukocyte, neutrophil, or monocyte count fell below 2,000, 1,000 or 150/μl, respectively. G-CSF was stopped if the leukocyte or neutrophil count rose above 10,000 or 5,000/μl, respectively. Patients were

given 5-HT_3 antagonist intravenously before administration of CPT on day 1. Subsequent courses of chemotherapy were started when the patients satisfied the organ function criteria: leukocyte count ≥3,000/μl, neutrophil ≥1,500/μl, platelet count ≥75,000/μl, less than grade 1 nonhematologic toxicities, except alopecia. Grade 3 nausea and vomiting did not preclude subsequent courses of chemotherapy. If the DLT was reached the dose of Pac and CPT in the subsequent course was reduced by 10 mg/m^2. Chemotherapy was repeated for a maximum of 6 courses unless the disease progressed, but it was stopped if the tumor response was judged to be no change (NC) after 4 courses. Physical examination, a complete blood cell count, biochemical tests, and chest radiographs were obtained weekly. Tumor response was evaluated according to World Heath Organization criteria [14]. Complete response was defined as the complete disappearance of all evidence of tumor for at least 4 weeks. PR was defined as at least a 50% reduction in the sum of the product of the two greatest perpendicular diameters of all indicator lesions or a reduction of more than 50% in evaluable disease for at least 4 weeks with no appearance of new lesions or progression of any existing lesions. PD was defined as at least a 25% increase in the tumor area or the appearance of new lesions. All other outcomes were classified as NC. Toxicities were evaluated according to the JCOG criteria [15].

DLT was defined as toxicity during the first 4 courses consisting of grade 4 neutropenia lasting 4 days or more, or grade 4 neutropenia with a fever of 38°C or higher, grade 4 thrombocytopenia, ≥grade 2 depression of PaO_2, grade 2 dyspnea, grade 3 or 4 other nonhematologic toxicity, except alopecia, nausea, and vomiting, or failure to complete the 4 courses within 9 weeks because of toxicity. Patient refusal was also defined as a DLT.

At least 3 patients assessable for toxicity were treated at each dose level. If none of the first 3 patients experienced a DLT, then escalation to the next dose level proceeded. If 1 patient developed a DLT, the cohort was expanded to 6 patients. We planned a 20 mg/m^2 increase by dose level after level 3 when none of the patients at levels 1 and 2 experienced DLT. The maximum tolerated dose (MTD) was defined as the dose level at which at least 2 of 3 patients or 3 of 6 patients experienced a DLT. The recommended dose of Pac for the phase II study was defined as the most dose-intensive level below the MTD. If the MTD was not defined by the Pac dose level at 210 mg/m^2, which is the recommended dose for Pac alone in Japan, the most dose-intensive level including 210 mg/m^2 of Pac would be recommended as the dose for phase II study. The Committee of JCOG and the Institutional Review Board of the Kanagawa Cancer Center reviewed and approved the protocol prior to commencement.

Results

Patient Characteristics

Between February 1999 and April 2001, 24 patients were registered in the study. The patients' characteristics are summarized in table 1. Eighteen patients were male, and 6 were female; their median age was 59 years (range 44–69 years). Four patients had a PS of 0, and the other 20 patients had a PS of 1. Sixteen patients had adenocarcinoma, 7 had squamous cell carcinoma, and 1 had large cell carcinoma. None of the patients had a prior history of chemotherapy, but surgical resection had been performed in 3 of them.

Table 2. Planned doses and administered doses of Pac and CPT

Dose level	Pac mg/m^2	CPT mg/m^2	Number of patients	Number of cycles
1	80	60	3	12
2	90	60	3	16
3	100	60	3	12
4	120	60	3	9
5	140	60	3	14
6	160	60	3	13
7	180	60	6	26

Dose Escalation and Determination of MTD

The numbers of patients and cycles at each dose level are listed in table 2. All patients were assessable for toxicity. Three patients each were registered at dose levels 1 and 2. None of the patients experienced DLT during 4 courses. At dose levels 3 and 4, 2 and 1 of the 3 patients registered developed PD during the third cycle and the first cycle, respectively, but none of them experienced DLT. None of the 3 patients registered at level 5 experienced DLT. One of the 3 patients registered at dose level 6 developed PD during the first cycle, but none of them experienced DLT. At dose level 7, 1 of the first 3 patients experienced DLT, grade 2 dyspnea, because of pneumonitis during the third cycle, and another 3 patients were registered. One of the additional 3 patients experienced a DLT, grade 3 dyspnea, because of pneumonitis in the fourth cycle, and another additional patient experienced grade 1 dyspnea because of pneumonitis in the fifth cycle. Two patients experienced DLTs at level 7, but these were both cases of pneumonitis; since another patient experienced pneumonitis, which is a very serious toxicity, we concluded that the dose level 7 of Pac, 180 mg/m^2, was the MTD. As a result, Pac 160 mg/m^2 and CPT 60 mg/m^2 every 2 weeks were recommended for the phase II study.

Treatment Administration and Toxicities during All Cycles of Treatment

The 24 patients received a total of 102 cycles of treatment, and the mean number of cycles was 4 (range 1–6). There were no treatment-related deaths. Five patients were excluded from treatment, 4 because of disease progression and 1 because of an adverse event, that is pneumonitis. Tables 3 and 4 list the overall incidence of hematologic and nonhematologic adverse events among all

Table 3. Hematologic toxicities by number of cycles

Dose level	Number of patients	Number of cycles	Incidence of adverse events (JCOG grade)											
			neutrophils				infection	hemoglobin			platelets			
			1	2	3	4		1	2	3	1	2	3	4
1	3	12	0	0	0	0	0	0	2	0	0	0	0	0
2	3	16	1	1	0	0	0	2	1	0	0	0	0	0
3	3	12	1	0	1	0	0	0	1	0	0	0	0	0
4	3	9	1	1	0	0	3	2	0	0	0	0	0	0
5	3	14	1	1	1	0	1	1	2	0	0	0	0	0
6	3	13	0	1	1	0	0	2	0	0	0	0	0	0
7	6	26	1	2	2	0	1	4	1	0	0	0	0	0

JCOG = Japan Clinical Oncology.

patients in this study. G-CSF was used for a mean of 5.2 days in 97 of the 102 courses in the phase I study, depending on the presence of monocytopenia. One third of the patients experienced grade 3 neutropenia at dose levels 3, 5, 6 and 7, but there was no grade 4 neutropenia. Infectious episodes were observed in 3, 1 and 1 patients at dose levels 4, 5 and 7, respectively, but all of the episodes were mild and improved with response to antibiotics. Anemia was mild, and there was no thrombocytopenia. None of the patients received transfusions. All acute nonhematologic adverse events, i.e. liver, renal, gastrointestinal, cardiac, and circulatory toxicities, were grade 1 or 2, and the patients recovered before the next cycle. Diarrhea was observed in 50% of the patients and nausea or vomiting in 62.5%, both high incidences, but they were mild and improved with medication. The incidences of hypotension and arrhythmia were low, and they were mild. Mild myalgia or arthralgia was observed at all dose levels. All events were observed during the 1st week of administration; the patients recovered before the next cycle. Neuropathy was observed in two thirds of the patients at dose levels 5–7 and persisted for several months. Pneumonitis developed in half of the patients at level 7, even though none of the patients below level 7 experienced it. The episodes were manifested by a high fever between chemotherapy cycles 3–5. All of the patients were treated with methylpredonisolone, and their dyspnea resolved. Every course could be performed in 2-week cycles except 1 course of the second cycle at level 5.

Efficacy

Although patients were treated at different starting dose levels, it was possible to assess the response rate and other efficacy variables for the 24 patients assessable for response. The outcome of chemotherapy in the 24 patients is shown in table 5. There was a complete response in 1, a PR in 13 patients, NC in 4 patients, and PD in 6 patients, and the overall response rate was 58.3%. Median time to progression was 177 days (range 79–413 days). Six patients were alive and the other 18 patients died during the follow-up period. The median survival time was 370 days, and the 1-year survival rate was 54.2%.

Discussion

Combined analysis of the two randomized phase III studies demonstrated that CPT combined with cisplatin significantly improved survival compared to vindesine and cisplatin in patients with advanced NSCLC [13, 16, 17]. CPT is believed to be a key drug against NSCLC in Japan. Over the past decade, a number of new anticancer agents including CPT, vinorelbine, gemcitabine, docetaxel, and Pac have been approved for the treatment of metastatic NSCLC. The combination of one or more of these agents with a platinum compound has resulted in high response rates and prolonged survival [1, 2]. However, a randomized study of a comparison of new anticancer-containing chemotherapy regimens such as cisplatin and Pac, cisplatin and gemcitabine, cisplatin and docetaxel, or carboplatin and paclitaxel conducted in the Eastern Cooperative Oncology Group did not show the differences among them [18]. Therefore, more active new combination chemotherapy is required to improve the treatment of NSCLC, and we conducted the combination chemotherapy with nonplatinum agents.

neurologic		dyspnea[1]				alopecia		stomatitis		myalgia[2]	arthralgia[2]
G1/2	G3/4	G1	G2	G3	G4	G1/2	G3/4	G1/2	G3/4		
0	0	0	0	0	0	3	0	1	0	2	2
0	0	0	0	0	0	3	0	0	0	1	1
0	0	0	0	0	0	3	0	0	0	1	1
0	0	0	0	0	0	2	0	0	0	1	1
2	0	0	0	0	0	3	0	1	0	0	1
2	0	0	0	0	0	2	0	0	0	1	1
4	0	1	1	1	0	3	0	0	0	4	2

Table 5. Antitumor activity by dose level

Dose level	Number of patients	Complete response	Partial response	Stable disease	Progressive disease
1	3	0	0	2	1
2	3	0	2	1	0
3	3	0	1	0	2
4	3	0	2	0	1
5	3	0	2	0	1
6	3	0	2	0	1
7	6	1	4	1	0
Total	24	1	13	4	6

Moreover, if pneumonitis had not occurred as a DLT, a higher dose of Pac might have been possible in this combination.

Both dose-dependent and dose-independent nonhematologic toxicities were observed. The dose-independent toxicities, hypotension, arrhythmia, liver damage, diarrhea, nausea, and vomiting, were mild. The delayed toxicities, pneumonitis and neuropathy, were considered the DLT in this study. Two thirds of the patients at PAC levels 5–7 experienced neuropathy, but none of the patients at levels 1–4 did. Some of them refused to continue chemotherapy despite being responders, and some did not recover from the neuropathy before death. Thus, neuropathy was considered to be a cumulative toxicity. Pneumonitis causing a high fever and depressed PaO_2 was observed in 3 patients only at PAC level 7. No abnormal shadows were detected on plain chest X-P films, but a CT scan showed a reticular shadow in each of the patients. They were all treated with methylpredonisolone, 1 g per day for 3 days, and recovered from respiratory failure. The pneumonitis occurred during the latter half of chemotherapy. The analysis of the relationship between the total doses of Pac and occurrence of pneumonitis revealed a total PAC dose of 540, 720 and 900 mg/m^2, respectively, in the 3 patients who developed pneumonitis. The total dose in the patients who did not experience pneumonitis at levels 6 and 7 was 720 mg/^{2} in 2 patients, 960 mg/m^2 in 2 patients, and 1,080 mg/m^2 in 1 patient. Based on these data, the risk of pneumonitis is unlikely to be dependent on the total dose of Pac during all courses. The pneumonitis is thought to be attributable to a booster effect of an allergic reaction when 180 mg/m^2 or higher of Pac was combined with CPT, and thus it is not expected to occur at doses of PAC below 180 mg/m^2.

In conclusion, this phase I study demonstrated that the DLT of Pac and CPT combination chemotherapy is pneumonitis, but this regimen is feasible for 2-week infusion cycles and has a high activity against NSCLC. A large phase II study will be required to confirm the feasibility and activity of this combination as a treatment for NSCLC.

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High Body Mass Index Correlates with Increased Risk of Venous Irritation by Vinorelbine Infusion

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Background: Vinorelbine is currently one of the most active chemotherapeutic agents. However, it is also a moderate vesicant that is well known to cause venous irritation and phlebitis. We conducted this study to identify clinical risk factors related to the incidence of venous irritation caused by peripheral vinorelbine infusion.
Methods: Medical records were used to investigate retrospectively a total of 201 cases of non-small cell lung cancer treated with a chemotherapeutic regimen containing vinorelbine. Venous irritation was evaluated in every course and graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. Gender, age, body mass index (BMI), chemotherapeutic regimen, dose of vinorelbine and prior chemotherapy were used as clinical variables.
Results: A total of 928 vinorelbine infusions were administered to the 201 patients, among whom venous irritation occurred in 63 (31%). The incidence of venous irritation was 28% in the normal BMI (<25) group and 45% in the high BMI (25 or more) group and the difference between the two groups was statistically significant (P = 0.037). There were no significant correlations between the incidence of venous irritation and the clinical variables except BMI. In the multivariate analysis BMI was also a significant independent variable that correlated with increased risk of venous irritation (P = 0.017).
Conclusions: Care is required when using vinorelbine to treat patients with a high BMI, especially with regard to the development of venous irritation.

Key words: vinorelbine – venous irritation – phlebitis – body mass index – lung cancer

INTRODUCTION

Vinorelbine is a semi-synthetic *Vinca* alkaloid that differs chemically from vinblastine in a modification in the catharantine moiety of the molecule (1). Vinorelbine has been shown to have low neurotoxicity and clearly higher activity than other *Vinca* alkaloids. Vinorelbine is currently one of the most active agents for the treatment of a variety of solid tumors and it is especially used for the treatment of metastatic non-small-cell lung cancer (NSCLC) (2), breast cancer (3) and Hodgkin's disease (4). The highly selective affinity of vinorelbine for mitotic tubulin-associated protein may account for this pattern of toxicity. In clinical studies, toxic side-effects frequently reported for vinorelbine included myelosuppression, constipation and peripheral neuropathy, all at mild to moderate levels.

Vinorelbine is also a moderate vesicant that is known to cause venous irritation and the incidences of venous irritation of ~10–50% have been reported in patients who received vinorelbine as a 6–30 min peripheral infusion (3,5–10). Venous irritation is generally characterized by injection site reactions, local reactions or superficial phlebitis. Symptoms include erythema, pain at the injection site, vein discoloration and tenderness along the vein (7).

Several investigators have tried to reduce the incidence of venous irritation by various methods (11–13). However, the exact mechanism responsible for this phenomenon remains unknown and the risk factors related to incidence of venous irritation caused by peripheral infusion of vinorelbine have never been reported. Since cure of patients with metastatic solid tumors is rare, an important approach for them is to decrease toxicity and to increase the effectiveness of treatment. We conducted this study to identify clinical risk factors related to the incidence of venous irritation caused by peripheral infusion of vinorelbine.

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Table 1. Patients' characteristics

Variable	No. of patients
Total No. of patients	201
Gender	
Male	155
Female	46
Age (years)	
Median	64
Range	31–81
BMI	
Normal: <25	161
High: ≥25	40
Chemotherapeutic regimen	
VNR + CDDP	123
VNR + GEM	58
VNR + CDDP + MMC	8
VNR + CDDP + GEM	5
VNR alone	7
VNR (dose, mg/m^2)	
25	198
20	3
Prior chemotherapy	
Negative	175
Positive	26

VNR, vinorelbine; CDDP, cisplatin; GEM, gemcitabine; MMC, mitomycin-C.

SUBJECTS AND METHODS

PATIENTS

We retrospectively reviewed the medical records of 201 NSCLC patients treated with a chemotherapeutic regimen containing vinorelbine between July 1999 and August 2002 at the National Cancer Center Hospital East. The chemotherapeutic regimens consisted of vinorelbine (VNR) 20–25 mg/m^2 weekly, alone or in combination with cisplatin (CDDP), gemcitabine (GEM) or mitomycin-C (MMC). VNR was diluted in 50 ml of normal saline and all infusions were administered through a peripheral vein over a period between 6 and 10 min, followed by flushing the vein with 200 ml of fluid to minimize the risk of venous irritation. All patients who received at least one dose of VNR were considered assessable for this study. The characteristics of all patients are listed in Table 1. Body mass index (BMI) (body weight in kilograms divided by the square of body height in meters) was used as the criterion for obesity. In accordance with the standard of the Japan Society for the Study of Obesity, a BMI of below 25 was defined as normal and 25 or more as high (14).

EVALUATION OF VENOUS IRRITATION

The medical records were used to evaluate venous irritation for every course and it was graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 for injection site reaction: grade 0, none; grade 1, pain, itching or erythema; grade 2, pain or swelling, with inflammation or phlebitis; and grade 3, ulceration or necrosis that is severe or prolonged or requires surgery. Venous irritation was categorized as positive or negative, with positive being defined as experience of grade 1 or more venous irritation at least once during treatment.

STATISTICAL ANALYSIS

The correlations between the incidence of venous irritation and the clinical variables were evaluated by the chi-squared test or Fisher's exact test, as appropriate. We used gender (male, female), age (lower, <70 years; higher, ≥70 years), BMI (normal, <25, high, ≥25), chemotherapeutic regimen (VNR alone, VNR in combination: VNR + CDDP, VNR + GEM, VNR + CDDP + MMC or VNR + CDDP + GEM), dose of VNR per body (<40, ≥40 mg/body) and prior chemotherapy (positive, negative) as clinical variables. Multivariate analysis was performed by the logistic regression procedure to determine the relationship between the incidence of venous irritation and the clinical variables. P values <0.05 were considered significant. A two-sided statistical test was used in all analyses. Statistical analysis software (StatView-J Version 5.0, Macintosh) was used for the analyses.

RESULTS

INCIDENCE OF VENOUS IRRITATION

A total of 928 infusions of VNR were administered to the 201 patients. The median number of infusions per patient was four (range, 1–14). Venous irritation occurred in 63 of the 201 patients (31%) infused with VNR and after 74 of the 928 infusions (8%), with 17% of the venous irritation events (11/63) occurring after the first VNR infusion. Five of 18 high BMI patients who developed venous irritation experienced two or more episodes of venous irritation (27%). In contrast, three of 45 normal BMI patients who developed venous irritation experienced two or more episodes of venous irritation (7%). A significant difference was observed between the two groups (P = 0.036). Grade 1 venous irritation was observed in 15% (n = 11), grade 2 in 81% (n = 60) and grade 3 in 4% (n = 3). The relationship between venous irritation and the clinical variables is summarized in Table 2. The incidence of venous irritation was 28% in the normal BMI (<25) group and 45% in the high BMI (≥25) group and the difference between the two groups was statistically significant (P = 0.037). On the other hand, there were no significant correlations between the incidence of venous irritation and the clinical variables except BMI.

Table 2. Relationship between clinical variables and venous irritation

Variable	No. of patients (n = 201)	Incidence of venous irritation (%)	P-value
Gender			
Male	155	33 (51/155)	0.38
Female	46	26 (12/46)	
Age (years)			
Lower age: <70	150	34 (51/150)	0.16
Higher age: ≥70	51	24 (12/51)	
BMI			
Normal: <25	161	28 (45/161)	0.037
High: ≥25	40	45 (18/40)	
Chemotherapeutic regimen			
VNR alone	7	29 (2/7)	>0.99
VNR in combination	194	31 (61/194)	
VNR dose per body (mg)			
<40	83	31 (26/83)	0.99
≥40	118	31 (37/118)	
Prior chemotherapy			
Negative	175	32 (56/175)	0.6
Positive	26	27 (7/26)	

VNR, vinorelbine.

Multivariate Analysis

The results of the multivariate analysis of six variables (gender, age, BMI, chemotherapeutic regimen, dose of VNR and prior chemotherapy) are shown in Table 3. BMI (normal versus high) turned out to be a significant independent variable correlated with increased risk of venous irritation (P = 0.017).

Table 3. Multivariate analysis: relationship between clinical variables and venous irritation

Variable	Odds ratio	95% CI	P-value
Gender			
(male, female)	0.563	0.246–1.292	0.17
Age (years)			
(lower <70, higher ≥70)	0.518	0.242–1.108	0.09
BMI			
(normal <25, high ≥25)	2.522	1.176–5.411	0.017
Chemotherapeutic regimen			
(VNR alone, VNR in combination)	0.976	0.177–5.383	0.97
VNR dose per body (mg)			
(<40, ≥40)	0.645	0.317–1.315	0.22
Prior chemotherapy			
(negative, positive)	0.763	0.295–1.971	0.57

VNR, vinorelbine; CI, confidence interval.

DISCUSSION

We examined the clinical risk factors related to the incidence of venous irritation caused by peripheral infusion of vinorelbine. The results showed that high BMI was associated with a significantly increased risk of venous irritation over normal BMI (P = 0.017). The reasons for this are considered to be as follows. One is the relationship between obesity and venous thrombotic disease. Obesity, as indicated by an elevated BMI, is clearly associated with cardiovascular disease and diabetes worldwide and is also a detectable risk marker of venous thrombotic disease including superficial vein thrombosis and phlebitis (15). In our study, the incidences of history of cardiovascular diseases and venous thrombosis were 55% (22/40) in the high BMI group and 20% (32/161) in low BMI group, the difference being statistically highly significant (P < 0.0001). Patients with high BMI would therefore be expected to have impaired venous valve functions and be prone to superficial vein thrombosis and thus tend to have stagnant venous return as a result. Because of this, vinorelbine may adhere to the peripheral vein in the injection site and cause venous irritation and phlebitis. Another is that for patients with high BMI there may be technical difficulties with injection. The peripheral vein of patients with high BMI is often difficult to locate compared with that of patients with normal BMI and therefore there may be practically no reasonable venous access for peripheral infusion. Consequently, patients with high BMI may tend to develop minor leakage that might be a cause of venous irritation owing to failure of peripheral infusion. Moreover, as another possible risk factor related to the incidence of venous irritation, the infusion site of VNR such as the difference in the diameter of the vein may also be considered to be a risk factor. However, unfortunately, we could not clarify the relationship between infusion site of VNR and venous irritation, because this study was a retrospective analysis.

Vinorelbine is generally well tolerated and can be administered safely in outpatient settings. However, it is a moderate vesicant with the potential to cause venous irritation and phlebitis (16). Our results suggest that care is required, especially with regard to the development of venous irritation, if vinorelbine is administered through a peripheral vein to patients with a high BMI.

The use of drugs with anti-thrombotic and protective endothelial cell activity, such as heparin and defibrotide, has been investigated in an attempt to reduce the incidence of venous irritation by vinorelbine. Lozano et al. (13) administered heparin with vinorelbine. In their study, a population of 23 patients was randomized to arm A, in which vinorelbine plus 5000 U of heparin was diluted in 500 ml of normal saline and infused over 2 h, or arm B, in which vinorelbine was diluted in 50 ml of normal saline and infused over 10 min. However, arm A, with heparin, was found to be inferior to arm B in terms of pain control at the injection site (13). In another study, defibrotide was used to prevent venous irritation. A total

of 360 infusions were delivered and the incidence of venous irritation was 5%. Maisano et al. reported that defibrotide could be used to prevent venous irritation by vinorelbine (12). Incidentally, vinorelbine has been shown to be a mast cell activator and to induce histamine release in rats (17,18). On the basis of these findings, cimetidine, which inhibits histamine actions in endothelial cells, was administered prior to vinorelbine infusion and an incidence of phlebitis of only 6% among a total of 127 vinorelbine infusions was reported (11). Recently, a retrospective study reported the incidence of phlebitis with administration of vinorelbine by intravenous bolus injection (19). The results indicated that the incidence of phlebitis by bolus injection was lower than that with drip infusion but other toxicities were equivalent. Although these methods of preventing venous irritation may show promise, there have been no randomized controlled trials to verify the benefit of these methods, hence a randomized controlled study is needed to draw definite conclusions about their efficacy.

In conclusion, our study is the first to statistically investigate clinical risk factors related to the incidence of venous irritation caused by peripheral infusion of vinorelbine. Our findings indicated that high BMI is associated with a significantly increased risk of venous irritation by vinorelbine. Care is required especially in regard to the development of venous irritation when vinorelbine is administered through a peripheral vein to patients with a high BMI. We suggest that BMI (high or normal) should be considered as a stratification factor in randomized controlled trials to compare the incidence of venous irritation caused by peripheral infusion of vinorelbine. Currently in our department, a randomized controlled study of 1 min bolus injection versus 6 min drip infusion is being conducted in order to investigate the best intravenous administration of vinorelbine.

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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.

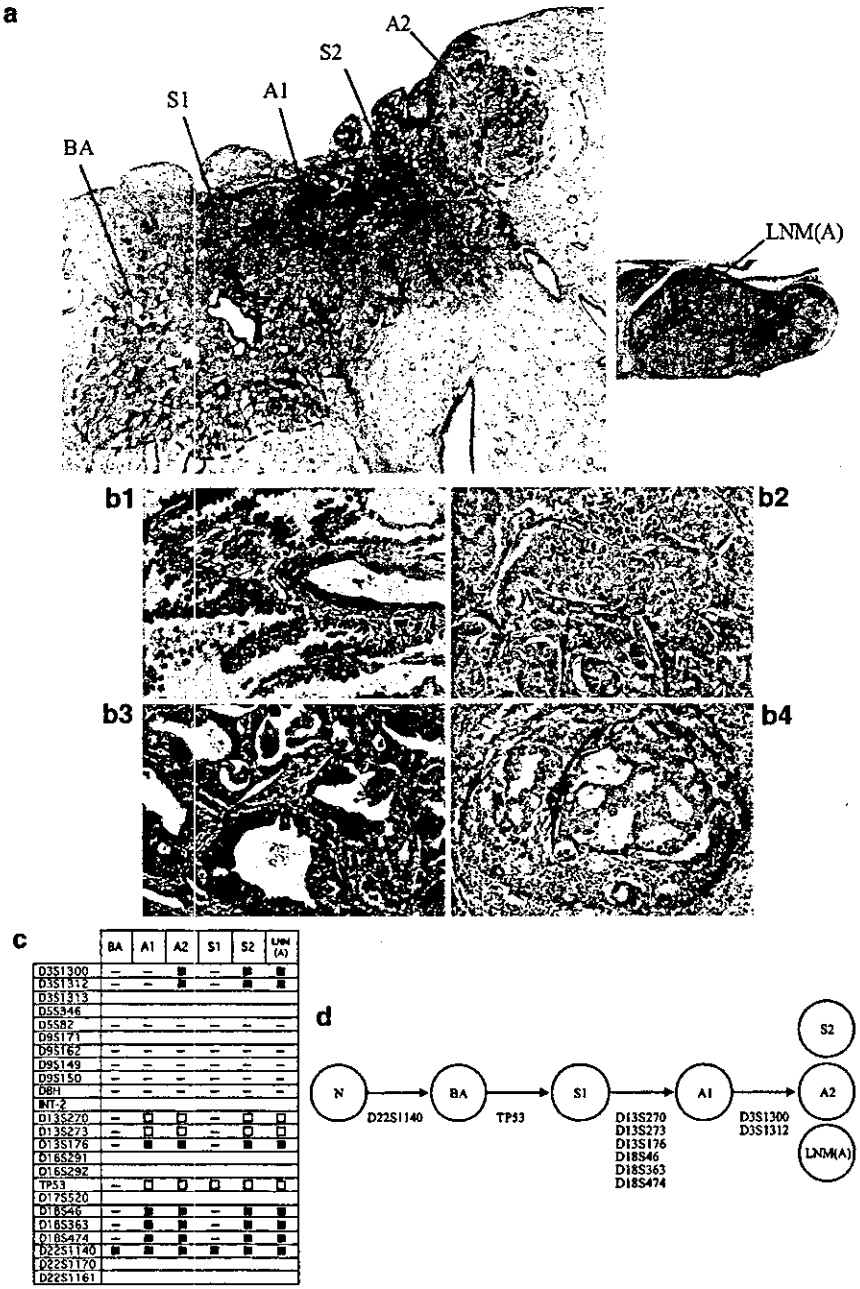

Figure 1 Analysis of LOH in an adenocarcinoma mixed BA, acinar, and solid (case 2). (a), Histology: low-power view showing regions chosen for laser capture microdissection (hematoxylin and eosin staining). BA: bronchioloalveolar component; S1 and S2: solid component dissected from different regions of 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.

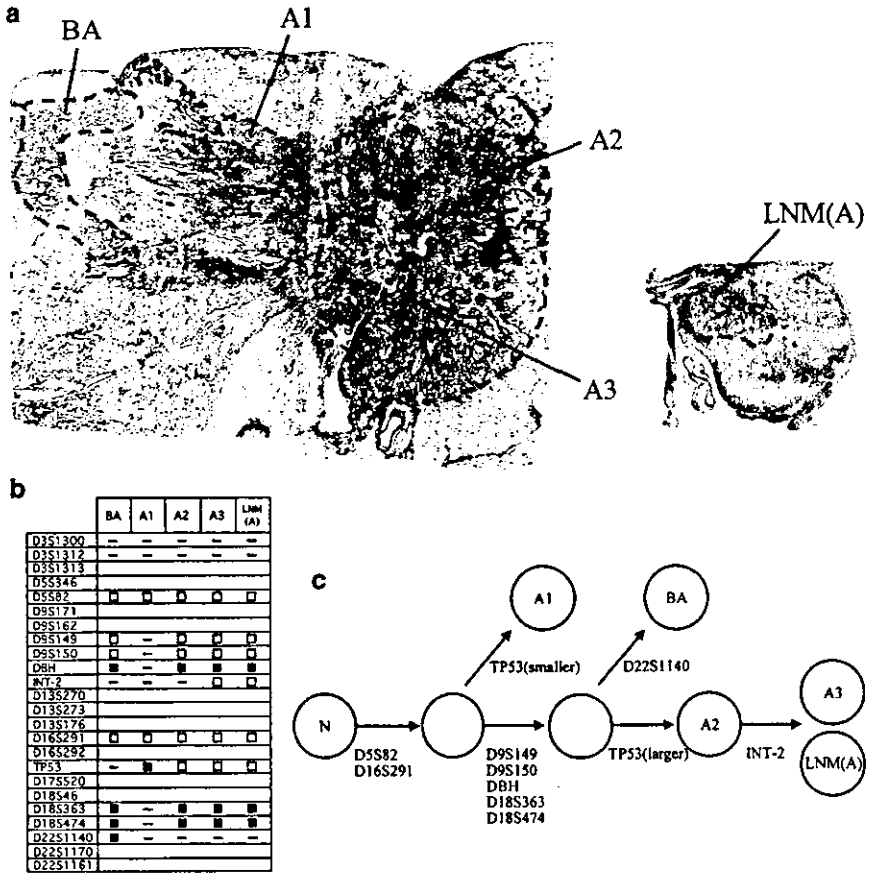

Figure 2 Analysis of LOH in an adenocarcinoma mixed BA and acinar (case 3). (a) Histology: BA: BA component; A1, A2, and A3: acinar component dissected from different regions of the specimen; LNM (A): lymph node metastasis (acinar component). Tumor components included BA-type carcinoma (BA) and three 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