Conclusion: Amrubicin was active for extensive-disease SCLC with acceptable toxicity. Further studies in combination with other agents for SCLC are warranted.

Keywords Amrubicin · Small cell lung cancer · Anthracycline · Previously untreated patients · Phase II study

Introduction

Small cell lung cancer (SCLC) is a major cause of cancer deaths and accounts for 15 to 20% of all lung cancers [1]. Although this cancer is initially highly responsive to chemotherapy, the vast majority of patients will ultimately relapse and die of recurrent disease within 2 years [2]. Recently, combination chemotherapy with irinotecan and cisplatin for extensive-disease SCLC produced more survival benefit than etoposide and cisplatin, the worldwide standard regimen since 1981 [3, 4]. Median survival time and 2-year survival rate of the standard regimen is 12.8 months and 19.5%, respectively. Clearly, new and more effective agents against SCLC are needed.

Amrubicin is a totally synthetic 9-aminoanthracycline, (+)-(7S, 9S)-9-acetyl-9-amino-7-[(2-deoxy-β-D-*erythro*-pentopyranosyl)oxy]-7, 8, 9, 10-tetrahydro-6, 11-dihydroxy-5,12-naphthacenedione hydrochloride, with a chemical structure similar to that of doxorubicin (Fig. 1) [5]. Amrubicin showed more potent antitumor activity than doxorubicin in several human tumor xenografts implanted in nude mice [6]. Acute toxicity of amrubicin is qualitatively similar to that of doxorubicin [7], however, amrubicin shows almost no delayed toxicity (e.g. cardiotoxicity) [8, 9].

Amrubicin is converted to an active metabolite, amrubicinol, by reduction of its C-13 ketone group to a hydroxy group. *In vitro* cytotoxic activity of amrubicinol was almost equipotent to that of doxorubicin and 20 to 220 times more potent than that of its parent compound, amrubicin [10]. Amrubicinol is considered to be closely associated with the efficacy and toxicity of amrubicin [11].
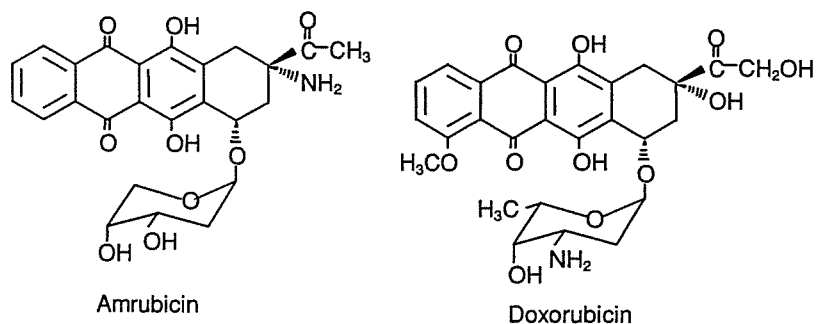
Despite their similarity in chemical structure, amrubicin has a different mode of action to doxorubicin [12]. Amrubicin and its active metabolite, amrubicinol, are inhibitors of DNA topoisomerase II. Amrubicin and amrubicinol exert cytotoxic effects by stabilizing topoisomerase II-mediated cleavable complexes, while doxorubicin does not inhibit this step of the catalytic cycle of topoisomerase II at concentrations for which it demonstrates cytotoxicity. Doxorubicin is a potent DNA intercalator, and its cytotoxicity is thought to be mainly due to this. Amrubicin and amrubicinol are about one-tenth weaker DNA intercalators than doxorubicin. Therefore, they are similar to etoposide in terms of inhibition of topoisomerase II by stabilizing the cleavable complexes, although etoposide does not show any DNA intercalating activity.

In a phase I–II study in patients with non-small cell lung cancer, amrubicin was administered as a 5-min intravenous infusion for 3 consecutive days [13]. The maximum tolerated dose (MTD) was 50 mg/m^2/day and the dose-limiting toxicities were leukopenia, neutropenia, thrombocytopenia, and gastrointestinal complications. The recommended dose for the phase II study was 45 mg/m^2/day for 3 consecutive days every 3 weeks.

Based on these experimental data and preliminary clinical reports indicating that amrubicin may be active against lung cancer, the West Japan Thoracic Oncology Group (WJ-TOG) evaluated it for use in SCLC. The WJTOG conducted a phase II study in previously untreated extensive-disease SCLC patients as a first-line therapy. Salvage chemotherapy with etoposide and cisplatin and an early cessation rule were set in place as precautionary measures.

Patients and methods

Eligibility criteria

Eligibility criteria included histologically or cytologically proven small cell lung cancer with extensive-disease defined as distant metastasis and/or disease involving the

Fig. 1 Chemical structures of amrubicin and doxorubicin

Amrubicin

Doxorubicin

contralateral hilar lymph nodes; no prior treatment; life expectancy of at least 2 months; the Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; at least one bidimensionally measurable lesion; age less than 80; adequate organ function, such as white blood cell (WBC) count of 4000×10^6/L or greater, hemoglobin level 10 g/dL or greater, platelet count 100×10^9/L or greater, AST and ALT less than 100 IU/L, bilirubin level 1.5 mg/dL or less, creatinine concentration 1.2 mg/dL or less, electrocardiogram (ECG) findings within normal range, and left ventricular ejection fraction (LVEF) of echocardiogram 60% or greater. All patients gave written informed consent. Ineligibility criteria were: brain or bone metastases requiring radiation; continuous long-term treatment with non-steroidal anti-inflammatory drugs and glucocorticoids; pulmonary fibrosis; serious complications and other active malignancy; or pregnant or nursing subjects.

This study was approved by the institutional review boards at each participating center.

Study design

Amrubicin (Sumitomo Pharmaceuticals Co., Ltd, Osaka, Japan) was dissolved in 20 mL normal saline and administered once intravenously as a 5-min infusion at a dose of 45 mg/m²/day on days 1 to 3, every 3 weeks.

Before treatment, all patients underwent a medical history, physical examination, hematology and serum biochemistry tests, urinalysis, ECG, LVEF, and baseline tumor measurements (chest radiography, CT scans, bone scintigraphy, and other measurements as appropriate). All measurable and assessable lesions were evaluated within 2 weeks before treatment. ECG and LVEF were undertaken within 1 month before treatment.

Complete and differential blood cell counts, platelet counts, hematocrit analysis, biochemical analysis including AST, ALT, alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, serum bilirubin, albumin, total protein, and electrolyte levels (Na, K, Cl, and Ca), and urinalysis (including protein, glucose, urobilinogen, and occult blood) were performed weekly as a rule. When severe myelosuppression was observed, complete and differential blood cell counts plus platelet counts were performed 2 times or more per week. ECG was undertaken every treatment cycle and LVEF every other cycle. Chest radiography and CT scans were carried out every cycle as a rule.

Subjective and objective symptoms were observed and recorded as appropriate.

Dose modifications were made according to WBC and platelet counts. If the WBC count nadir was lower than $1,000 \times 10^6$/L for 4 days or longer and/or the platelet count nadir was lower than 50×10^9/L, a dose reduction of 5 mg

was stipulated in the subsequent treatment course. Treatment was postponed until the WBC and platelet counts recovered to $\geq 3,000 \times 10^6$/L and $\geq 100 \times 10^9$/L, respectively.

In patients who demonstrated tumor regression of 25% or greater after the first course of chemotherapy, amrubicin treatment was continued. After the second course, patients had to have achieved tumor regression of 50% or greater to continue to receive the drug up to a maximum of 6 courses. Treatment of combination chemotherapy with etoposide (100 mg/m² on days 1, 2, and 3) and cisplatin (80 mg/m² on day 1) was recommended for patients who failed to fulfill any of the above criteria.

Evaluation of response and toxicity

Response was assessed according to the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14], which are virtually identical to those of the World Health Organization [15]. A complete response (CR) was defined as disappearance of all lesions for a minimum of 4 weeks. A partial response (PR) was defined as a 50% or greater decrease in the sum of the products of the diameters of measurable lesions for a minimum period of 4 weeks and no new lesions. No change (NC) was defined as a decrease in the tumor mass of less than 25% or any increase of less than 25%. Progressive disease (PD) was defined as an increase in the size of any measurable lesion by 25% or greater or the appearance of new lesions.

Toxicity grading was recorded based on the side effect record form in the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14].

Statistical analyses

The estimated sample size was 30 to guarantee that the lower limits of 95% confidence interval would be at least 20% at 40% of expected response rate. An early cessation rule was in place to terminate the study if at least 4 responses had not been seen among 15 patients evaluated. Median overall survival was estimated using the product-limit (Kaplan-Meier) method [16].

Results

Patient characteristics

Of 35 patients entered into this study between May 1995 and January 1997, 33 patients were eligible and assessable for efficacy and toxicity. There were 2 ineligible patients because of serious complications before treatment (cardiac

Table 1 Patient characteristics

Patient characteristics	No. of patients ($N = 33$)	%
Age (years)		
Median	66	
Range	42–78	
Sex		
Male	29	87.9
Female	4	12.1
Performance status (ECOG)		
0	5	15.2
1	26	78.8
2	2	6.1
Stage		
IIIB	1	3.0
IV	32	97.0
Prior therapy		
No	33	100

ECOG: Eastern Cooperative Oncology Group.

failure and aggravation of hepatitis, respectively), and they did not receive amrubicin. Characteristics of the 33 eligible patients are shown in Table 1. Of the 33 patients, 13 (39%) were 70 years of age or older, 88% were male, and 94% had an ECOG performance status of 0 or 1.

Efficacy

Response to amrubicin is shown in Table 2. The early cessation rule was not imposed to terminate the study, as 10 responses were seen after 15 patients were enrolled. Of 33

patients, 3 achieved a complete response, giving a CR rate of 9.1% (95% CI, 1.9–24.3%), and 22 a partial response, for an overall response rate of 75.8% (95% CI, 57.7–88.9%). Of 7 patients, 6 experiencing no change under amrubicin treatment were switched to salvage chemotherapy. Of these, 2 had partial responses and the others had no change.

The overall survival curve is shown in Fig. 2. Median survival time was 11.7 months (95% CI, 9.9–15.3 months), and 1-year and 2-year survival rates were 48.5% (95% CI, 31.4–65.5%) and 20.2% (95% CI, 6.4–34.4%), respectively.

Toxicity

The major observed toxicity was hematologic, as shown in Table 3. All patients experienced leukopenia and neutropenia. Grade 3 or 4 leukopenia occurred in 51.5% of patients and grade 3 or 4 neutropenia in 84.8%. Anemia and thrombocytopenia were observed in 78.8% and 39.4% of patients, respectively, both with a frequency of grade 3 or 4 of 21.2%. Despite the severe hematologic toxicity of amrubicin, there was no febrile neutropenia or treatment-related death during the entire treatment of 33 patients. Granulocyte colony-stimulating factor (G-CSF) was used in 55 (40%) of a total of 136 cycles, in 13 patients (39%). Most hematologic toxicity in this trial was well-controlled without dose reduction: 88% of the total treatment cycles were delivered at the planned dosage of amrubicin, 45 mg/m^2/day.

Non-hematologic toxicities observed in more than 10% of patients were anorexia (54.5%), nausea and vomiting

Table 2 Response to amrubicin

No. of assessable patients	Response (No. of patients)				CR rate, % (95% CI)	Response rate, % (95% CI)
	CR	PR	NC	PD		
33	3	22	7	1	9.1 (1.9–24.3)	75.8 (57.7–88.9)

CR: complete response; PR: partial response; NC: no change; PD: progressive disease; 95% CI: 95% confidence interval.

Fig. 2 Overall survival of patients with extensive-disease small cell lung cancer treated with amrubicin. MST: median survival time; 95% CI: 95% confidence interval
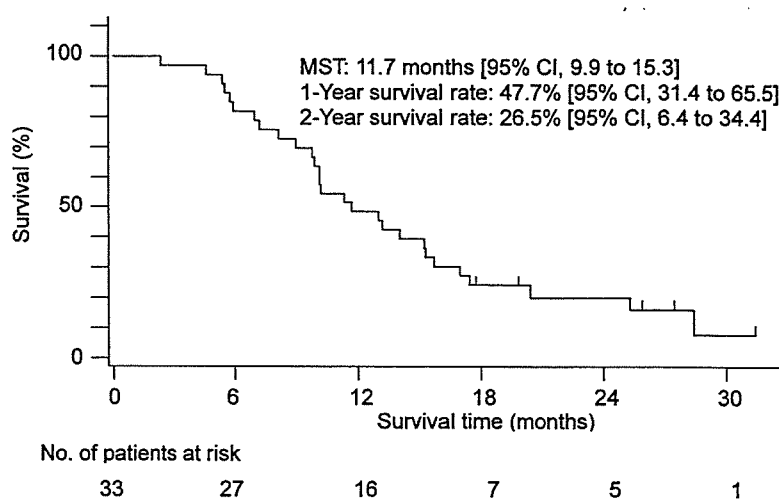
Table 3 Main treatment-related toxicity of amrubicin

Toxicity	No. of assessable patients	Toxicity grade others					
		1	2	3	4	≥1	≥3
		(No. of patients)				Frequency (%)	
Hematologic toxicity							
Anemia (hemoglobin)	33	12	7	6	1	78.8	21.2
Leukopenia	33	5	11	13	4	100	51.5
Neutropenia	33	1	4	14	14	100	84.8
Thrombocytopenia	33	3	3	1	6	39.4	21.2
Non-hematologic toxicity							
Stomatitis	33	2	1	0	0	9.1	0
Anorexia	33	12	3	3	-[a]	54.5	9.1
Nausea and vomiting	33	12	7	0	-[a]	57.6	0
Diarrhea	33	6	0	0	0	18.2	0
Fever	33	3	7	0	0	30.3	0
Phlebitis	33	1	1	0	0	6.1	0
Alopecia	33	11	8	1	-[a]	60.6	3.0
Total bilirubin elevation	33	1	1	0	0	6.1	0
AST elevation	33	5	0	0	0	15.2	0
ALT elevation	33	8	1	0	0	27.3	0
ALP elevation	33	1	0	0	0	3.0	0
BUN elevation	33	2	0	0	0	6.1	0
Others[b]	Headache, 1/33[c]; Rash, 1/33; Constipation, 1/33; Interstitial pneumonia, 1/33; Rhinorrhagia, 1/33; ECG abnormality, 3/32						

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; BUN: blood urine nitrogen; ECG: electrocardiogram.

[a]Toxicity grade was not defined for these toxicities.

[b]Toxicities which were not graded.

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

(57.6%), diarrhea (18.2%), fever (30.3%), alopecia (60.6%), AST increase (15.2%), and ALT increase (27.3%). Most of these were mild (≤grade 2), with only 3 patients (9.1%) experiencing grade 3 anorexia and 1 patient grade 3 alopecia (3.0%). A single patient developed interstitial pneumonia after the second cycle of treatment; however, it was reversibly recovered by steroid therapy and cessation of amrubicin treatment. ECG abnormality was observed in 3 patients (9.4%; supraventricular extrasystole, prolonged QT interval, and T wave flattening in 1 patient each), which did not need any treatment. No LVEF decrease was observed.

Discussion

Results of this phase II study demonstrate that amrubicin is an extremely active agent against extensive-disease SCLC. The complete response rate was 9.1% (95% CI, 1.9–24.3%), overall response rate 75.8% (95% CI, 57.7–88.9%), and median survival time 11.7 months (95% CI, 9.9–15.3 months). These results are comparable or even superior to those of the standard combination regimen of cisplatin and etoposide, used as the gold standard of extensive-disease SCLC

therapy since 1981 and remaining unchanged over the last 2 decades [4].

SCLC is sensitive to cytotoxic anticancer agents. Of anticancer drugs developed before 1990, a number of agents with response rates of 20% or greater for SCLC were listed as active drugs [17]. Of these drugs, etoposide, cisplatin, carboplatin, doxorubicin, cyclophosphamide, and vincristine, are still currently used as important constituents of combination regimens in the treatment of SCLC. In addition, several drugs with significant activity for SCLC have been developed since 1990. Irinotecan showed a response rate of 33% to 47% even in previously treated patients who are generally less sensitive to chemotherapy [18, 19]. Recently a new combination regimen of irinotecan plus cisplatin was demonstrated to be significantly superior to standard regimen of etoposide plus cisplatin in median survival time (12.8 months vs. 9.4 months, $P = 0.002$) [3]. In addition, topotecan, paclitaxel, docetaxel, and gemcitabine are reported to have response rates of 26% to 41% for extensive-disease SCLC patients without previous treatment [20–24]. Compared to these agents, amrubicin demonstrated a much higher response rate (75.8%) in this study, indicating it is a promising novel agent with potential to overcome the therapeutic plateau of SCLC.

The major toxicity of amrubicin was hematologic. Grade 3 or 4 leukopenia was frequently observed in 51.5% of patients and grade 3 or 4 neutropenia in 84.8% of patients. Despite such severe hematologic toxicity, 88% of the total treatment cycles could be delivered without dose reduction and non-hematologic toxicities were mild. Although anorexia (54.5%) and nausea and vomiting (57.6%) were frequently observed, there were no episodes of grade 3 or 4 toxicity, except for 3 patients (9.1%) with grade 3 anorexia and 1 patient (3.0%) with grade 3 alopecia. A single patient developed interstitial pneumonia; however, this was reversible with steroid therapy. ECG abnormalities were observed in 3 patients, but they were each reviewed by a medical cardiologist and judged not to be clinically significant. No LVEF decrease was observed. Results show that the toxic profiles of amrubicin are acceptable and favorable in the treatment of extensive-disease SCLC, although due to its hematologic toxicity, in particular neutropenia, G-CSF support is needed.

In conclusion, amrubicin is a very active and promising agent with acceptable toxicity for patients with SCLC. Further studies are warranted in combination with other agents for this disease.

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Expert Opinion

Irinotecan in the treatment of small cell lung cancer: a review of patient safety considerations

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A water soluble derivative of camptothecin, irinotecan (CPT-11) is effective against small-cell lung cancer (SCLC), as well as non-SCLC and gastrointestinal cancers. This extended review of recently concluded and ongoing studies focuses on irinotecan in the treatment of limited (LD) and extensive (ED) SCLC specifically considering the safety of patients. Irinotecan-induced diarrhoea is pervasive, and can be severe and life-threatening especially in combination with neutropenia. It can have a significant impact on patient quality of life, negatively influencing compliance with therapy and dose-intensity. For LD SCLC, irinotecan can be administered with radiotherapy concurrently or sequentially. In a Phase III study for ED SCLC comparing etoposide and cisplatin (EP) and irinotecan and cisplatin (IP) regimens, severe myelosuppression was more frequent in the EP arm than in the IP arm, and conversely severe or life-threatening diarrhoea was more frequent in the IP arm than in the EP arm. IP resulted in significantly higher response rates and overall survival in Japan, and confirmatory Phase III studies are ongoing. Irinotecan should not be administered to patients with any degree of ongoing diarrhoea above their baseline. Irinotecan can be administered with relative safety for patients with SCLC only through careful patient monitoring, especially regarding diarrhoea and myelosuppression.

1. Introduction

Lung cancer is the leading cause of cancer deaths worldwide, with > 900,000 deaths per year attributed to the disease [1]. About 15 – 20% of lung cancers are small-cell lung cancer (SCLC), although the frequency has been decreasing relative to other lung cancer over the last two decades [2]. SCLC is considered distinct from other non-small cell lung cancers (NSCLC) because of its clinical and biological characteristics [3]. The clinical characteristics of SCLC tend to be aggressive behaviour with rapid growth, early spread to distant sites, but more sensitive to chemotherapy and radiation. SCLC is usually staged as either limited disease (LD), in which the tumour is confined to the hemithorax of origin, the mediastinum, or the supraclavicular lymph nodes, or extensive disease (ED), in which tumours have spread beyond the supraclavicular areas. About 30% of patients with SCLC have LD. Management of most cases of LD SCLC involves combination chemotherapy, usually with a platinum-containing regimen, and thoracic radiation therapy (TRT). If a complete response is obtained, the patient may be offered prophylactic cranial irradiation. The median survival time (MST) of LD SCLC is 16 – 24 months with current forms of treatment, such as chemoradiotherapy with or without surgery. ED SCLC patients are treated with combination

chemotherapy, but the disease remains incurable. Usually a platinum-containing regimen is chosen. For ED SCLC, the MST is less than one year with currently available chemotherapy, and long-term survivors are still rare [4,5].

Furthermore, the prognosis is exceedingly poor for patients who receive second-line therapy after relapse. Response is influenced by the time to progression after cessation of first-line therapy. Patients who relapse less than three months after the completion of first-line therapy are termed refractory; they have response rates that are lower than for those patients who relapse more than three months after therapy, who are termed sensitive. The objective for these patients is palliation and increased quality of life, and therefore salvage therapy should be limited to patients with a good performance status (PS) and without significant comorbidities [3].

A water soluble derivative of camptothecin, irinotecan hydrochloride (CPT-11), a topoisomerase I inhibitor, has been synthesised for use in chemotherapy. The chemical structures of irinotecan and its major metabolites found in plasma are shown in Figure 1. Irinotecan is converted by hepatic and peripheral carboxylesterase to its active metabolite 7-ethyl-10-hydroxycamptothecin (SN38). This is subsequently glucuronidated by hepatic uridine diphosphate glucuronosyl transferase-1A1 (UGT 1A1), the enzyme responsible for bilirubin glucuronidation with multi-genetic variants, to SN38-glucuronide (SN38G) [6]. The patient with UGT1A1*28 has an impaired capacity for glucuronidation of SN-38, increased exposure to SN-38, and there is increased clinical toxicity when treated with irinotecan. To measure UGT1A1*28, in August 2005, FDA in the US cleared the Invader Molecular Assay for irinotecan dosing. However, irinotecan activity is not determined by the product of one gene [7]. Irinotecan, SN-38 and SN-38 glucuronide (SN-38G) may be shunted out of the cell via members of the ATP-binding cassette transporters [8]. The metabolism and pharmacogenetics of irinotecan is beyond the scope of this review, but there are some excellent reviews on this subject [9-11].

It should be cautioned that there are drug–drug interactions [12] with irinotecan. Exposure to irinotecan and its active metabolite SN-38 is substantially reduced in patients receiving the CYP3A4 enzyme-inducing anticonvulsants phenyntoin, phenobarbital or carbamazepine [13]. Rifampin, rifabutin and St. John's Wort are also CYP 3A4 inducers [14,15]. St. John's Wort is contraindicated during irinotecan therapy. Ketoconazole, a strong inhibitor of CYP3A4 [16], and contraindicated during irinotecan therapy, should be discontinued in patients at least one week prior to starting irinotecan therapy.

In Japan, 1245 cancer patients received irinotecan as a single agent in Phase I or Phase II trials that were conducted to obtain approval for commercial use from the Ministry of Health, Labour and Welfare. Of the 1245 patients, 55 (4.4%) died from toxicities of irinotecan, mainly myelosuppression and/or diarrhoea [17].

The onset of diarrhoea can occur early or be delayed beyond 24 h after injection of irinotecan. Early-onset diarrhoea is a cholinergic effect. Anticholinergic drugs, such as atropine, seem to easily reverse this side effect. Late-onset diarrhoea represents the dose-limiting toxicity (DLT) of irinotecan; it can be severe and life-threatening, especially in combination with neutropenia. Late-onset diarrhoea is treated with loperamide, and identification of high-dose loperamide as an effective remedy for this toxic effect greatly facilitated development of irinotecan [18,19]. These studies established the usefulness of high-dose loperamide. Patients should be instructed to take high-dose loperamide at the first onset of any irinotecan-associated late-onset diarrhoea that has occurred at least 12 h after drug administration. This therapy has been widely used for the management of diarrhoea caused by irinotecan.

For the treatment of SCLC, initial irinotecan is usually administered on days 1 and 8 every 3 weeks or on days 1, 8 and 15 every 4 weeks. The dose ranges from 50 to 70 mg/m² when administered weekly. As an example of the dose modification of irinotecan, Kudoh et al. [20] used the following dose modification: irinotecan is not given on days 8 or 15 if the leukocyte or platelet counts were < 3000/μl or < 75,000/μl, respectively. It is also withheld if the patient develops diarrhoea of grade 2 (increase of 4 – 6 stools/day, or nocturnal stools) or worse (grade 3: increase of > 6 stools/day or incontinence; grade 4: physiological consequences requiring intensive care). The next course of treatment can only be initiated if the leukocyte count is ≥ 4000/μl, the platelet count is ≥ 10,000/μl, serum creatinine is less than the upper limit of normal, and diarrhoea has been resolved. There is no dose modification for the leukocyte count, platelet count or diarrhoea during the same course. The dose of irinotecan in the next course was reduced by 10 mg/m² if the leukocyte count was < 2000/μl, the platelet count was < 50,000/μl, or diarrhoea was grade 3 to 4. This dose modification was applied in most studies with minor variation. For example, in some studies, the delay in the irinotecan doses was applied when the leukocyte count was < 2000/μl [21] instead of 3000/μl.

Another available topoisomerase-I inhibitor, topotecan, has achieved response rates of up to 22% in previously treated patients with SCLC and survival almost double that achieved with other single agents. Compared with cyclophosphamide/doxorubicin/vincristine (CAV), single-agent topotecan achieved a higher response rate, longer survival and statistically significant improvements in dyspnoea, hoarseness, fatigue, anorexia and interference with daily activities [22,23]. The incidence of grade 3 – 4 diarrhoea was extremely low (1%). The clinical comparison of these two topoisomerase-I inhibitors has not been tried. This review focuses mainly on the recent results of irinotecan in the treatment of SCLC in connection with patient safety considerations.
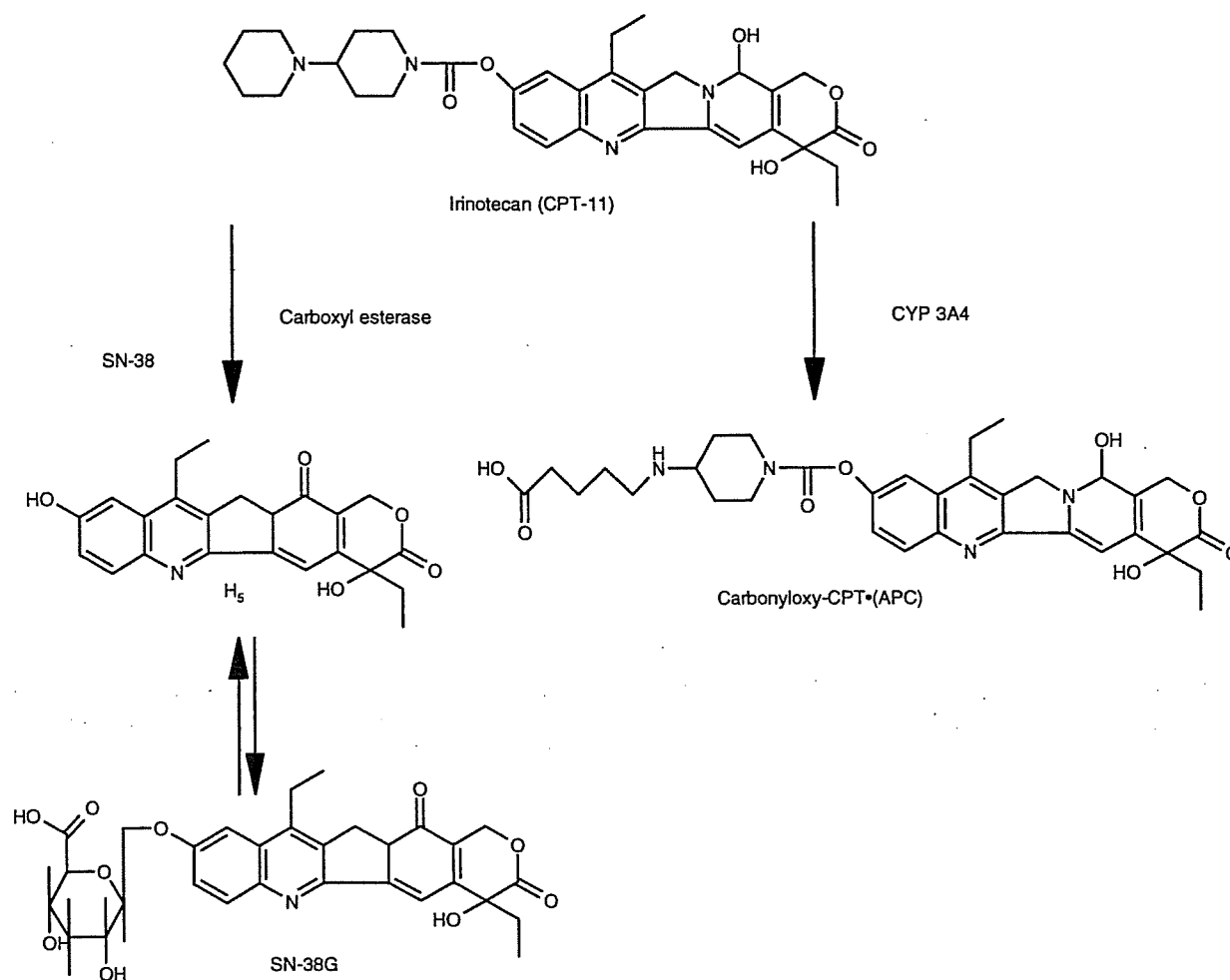
Figure 1. Metabolism of irinotecan. Chemical structures of CPT-11 and its major metabolites.

2. Irinotecan containing regimens as front-line treatment

2.1 Irinotecan plus cisplatin for ED SCLC

Clinically, irinotecan was proved to be effective against SCLC [24]. Negoro et al. have demonstrated that 13 (37%) out of 35 patients responded, including 33% of previously treated patients and 50% of chemotherapy-naive patients. In a Phase II trial of irinotecan for previously treated SCLC, the response rate was 47% out of 16 patients [25].

IP was tested in a Phase II trial for patients with previously untreated SCLC [20]. A total of 40 patients (53%) had LD and 35 patients (47%) had ED. Initially, irinotecan 80 mg/m² over 90-minutes infusion was given on days 1, 8 and 15, and cisplatin 60 mg/m² was given every 4 weeks. After 3 of the initial 10 patients experienced severe haematological toxicity, diarrhoea and hepatic toxicity, and one patient died of diarrhoea and neutropenia, the irinotecan dose was reduced to 60 mg/m². The response rate was 84%, with a complete response rate of 29%. The MST was 14.3 months for LD

patients and 13.0 months for ED patients, an encouraging result. Although the survival of LD was not increased significantly, this may be due to the small number of LD SCLC patients accrued. This study prompted a Phase III study of the Japan Clinical Oncology Group (JCOG 9511).

The JCOG conducted a multi-centre, randomised, Phase III study which compared irinotecan plus cisplatin with etoposide plus cisplatin (EP) in patients with ED SCLC (JCOG 9511) (Figure 2) [26]. IP consisted of four 4-week cycles of 60 mg/m² of irinotecan on days 1, 8 and 15, and 60 mg/m² of cisplatin on day 1. The regimen of etoposide and cisplatin consisted of four 3-week cycles of 100 mg/m² of etoposide on days 1, 2 and 3, and 80 mg/m² of cisplatin on day 1. The delivered dose intensity for irinotecan was 80%. The results are listed in Table 1. This study was terminated early because an interim analysis found a statistically significant difference in survival between the two arms. The MST was 12.8 months in the IP arm and 9.4 months in the EP arm (p = 0.002). At two years, the proportion of patients surviving was 19.5% in the IP group and 5.2% in the EP

Table 1. IP versus EP in phase III studies.

	IP	EP	p-value	IP	EP	p-value
	JCOG9511 study [26]			*Hanna's study* [27]		
	(n = 75)	(n = 77)		(n = 210)	(n = 104)	
Irinotecan: delivered dose intensity	80%			90%		
Survival						
Median survival time (months)	12.8	9.4	0.002	9.3	10.2	0.6226
1-year survival (%)	58.4	37.7		35.4	36.7	
2-year survival (%)	19.5	5.2		8.0	7.9	
Haematological						
Neutropenia	65.3	92.2	< 0.001	36.2	86.5	< 0.0001
Anaemia	26.7	29.9	0.72	4.8	11.5	< 0.0268
Thrombocytopenia	5.3	18.2	0.002	4.3	19.2	< 0.0001
Nonhaematological				(n = 216)	(n = 106)	
Diarrhoea	16	0	< 0.001	21.3	0	0.0001
Response				(n = 221)	(n = 110)	
Complete response	2.6	9.1		3.6	2.7	
Partial response	81.8	58.4		44.3	40.9	
Overall response	84.4	67.5	0.02	48.0	43.6	
Stable disease	2.6	20.8		4.1	7.3	
Progressive disease	3.9	11.7		20.0	20.0	
Not evaluable	6.5	0		28.1	29.1	

EP: Etoposide and cisplatin; IP: Irinotecan and cisplatin.
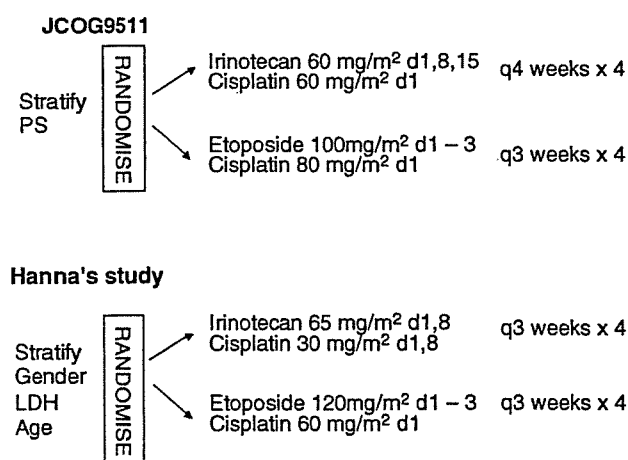
Figure 2. Two Phase III randomised trials.
LDH: Lactate dehydrogenase.

group. This was the first study to show the superiority of any one regimen over etoposide plus cisplatin for the front-line treatment of ED SCLC, and IP has become one of the standard regimens for ED SCLC in Japan. Severe myelosuppression was more frequent in the EP group than in the IP group. On the other hand, severe diarrhoea was more frequent in the IP arm than in the EP arm. Despite the dose modifications, major deviations from the protocol resulted in failure to reduce the dose of chemotherapy (in 6 patients); administration of irinotecan despite the presence of grade 1 (increase of < 4 stools/day) or 2 diarrhoea (in 9 patients); continuation of the study treatment despite grade 2 to 3 pulmonary toxicity (in 3 patients); and continuation of the treatment despite grade 3 hepatic toxicity (in 1 patient). There were 3 treatment-related deaths in the IP arm; one patient died of bleeding from a metastatic site in the lung, another patient died of sepsis associated with neutropenia and diarrhoea, and the third patient died of pneumonia associated with neutropenia. These three treatment-related deaths in the IP arm occurred during the first or second cycle of treatment and were attributed to haematological toxicities of the first cycle. This may indicate that severe haematological toxicities, as well as diarrhoea, during the first cycles of chemotherapy should be managed carefully. All cases of grade 1 to 4 diarrhoea occurred during the first and second cycles of the IP arm but early suspension of treatment may have prevented death associated with diarrhoea in all but one patient, which

involved a protocol violation because the patient was given irinotecan on day 8 of the first cycle despite the presence of grade 1 diarrhoea. This suggests that irinotecan should not be administered to patients with any degree of ongoing diarrhoea above their baseline.

Confirmatory studies are underway; currently, there is only one concluded study showing IP superiority, but it had a small sample size. Additionally, pharmacogenomic differences may exist between Japanese and Western populations.

Hanna et al. presented a Phase III trial comparing IP with EP in patients with previously untreated ED SCLC at the ASCO meeting in 2005 (Figure 2, Table 1) [27]. This was designed to confirm the JCOG9511 trial. However, the dose and schedule were modified to increase dose intensity.

The IP arm consisted of cisplatin 30 mg/m^2 and irinotecan 65 mg/m^2 on days 1 and 8 every 3 weeks. The EP arm was cisplatin 60 mg/m^2 on day 1, and etoposide 120 mg/m^2 on days 1 – 3 every 3 weeks for 4 cycles, or disease progression, or intolerable toxicity. This was planned to improve tolerability, achieve greater dose intensity and maintain or improve efficacy. The 336 patients were stratified by gender, lactate dehydrogenase level and age, and were randomised in a 2:1 fashion, with 221 treated with IP (median age, 63 years; range, 37 – 82 years; male, 57.5%) and 109 to EP (median age, 62 years; range, 38 – 83 years; male, 57.3%). Baseline characteristics were well balanced across the 2 arms, with a high representation of PS of 0 or 1 (IP, 92.3%; EP, 88.2%). After 30 patients with PS 2 were enrolled, study amendment excluded PS 2 patients. Delivered dose intensity of irinotecan was 39 mg (94%), higher than that of the JCOG9511 trial (80%). In both arms, 65% of patients received 4 or more cycles. Selected grade 3 or 4 toxicities in IP versus EP arm were: diarrhoea (21 versus 0%), neutropenia (35 versus 84%), febrile neutropenia (4 versus 11%). Grade 3 or 4 haematological toxicities were significantly more common with EP than IP. There was a trend towards more febrile neutropenia in the EP arm (10 versus 4%), and significant differences were seen in rates of dehydration (13 versus 3%; p = 0.15), vomiting (13 versus 4%; p = 0.0445), and diarrhoea (21 versus 0%; p < 0.0001). The survival of EP in both trials was similar (MST: 10.2 months in this study and 9.4 months in the JCOG9511 trial). However, the MST of IP was 9.3 months in this trial and 12.8 months in the JCOG9511 trial. Differences in outcome of this study from the JCOG trial may be due to pharmacogenomic or patient characteristic differences, or a change in the dose/schedule of IP. Pharmacogenomic studies among ethnic populations are needed to address this issue. It is likely that IP will prove to be at least as effective as other treatments for patients with ED SCLC.

Other Phase III trials will clarify these issues, including a SWOG S0124-randomised Phase III trial with the dose and schedule of each arm the same as the JCOG9511 trial, and a Phase III study started in June 2002 – (NCT00143455) sponsored by Pfizer. In this second study, IP consists of irinotecan

65 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 on day 1. EP consists of etoposide 100 mg/m^2 on days 1 – 3 and cisplatin 80 mg/m^2 on day 1 every 3 weeks. The results of these studies are awaited.

The debate continues regarding the optimal dose of combination chemotherapy as related to improvement of the outcome of SCLC. However, the author can state that too low a dose intensity may lead to poor results. Takigawa et al. used fractionated administration of IP in 15 patients with ED SCLC [28]. Both irinotecan at a dose of 50 mg/m^2 and cisplatin at a dose of 60 mg/m^2 were given on days 1 and 8, and repeated every 4 weeks up to 4 cycles. Although objective response rates were 80%, no complete response (CR) were obtained. The MST was 9.4 months and one-year survival was 40.0%. They stopped enrollment because of no CR and poor survival compared to Kudoh's data [20]. The dose intensity may be low because this regimen had a lower dose of irinotecan (50 mg/m^2) and a two-week rest period.

Han et al. reported a Phase II study of dose-intensified weekly IP in chemo-naive patients with ED SCLC [29]. The initial six patients received cisplatin 50 mg/m^2 followed by irinotecan 90 mg/m^2 on day 1 and 8 of a 21-day cycle (level I), with one treatment death and three febrile neutropenias. The doses of cisplatin and irinotecan were then reduced to 40 mg/m^2 and 80 mg/m^2, respectively (level II). The overall response rate was 97%, with a complete response rate of 26%. The MST was 11.1 months and 1- and 2-year survival rates were 44.1% and 11.8%, respectively. Major grade 3 or 4 toxicities included neutropenia (89%), anaemia (59%) and diarrhoea (27%). There were three treatment-related deaths, occurring in elderly patients aged > 60 years and/or relative poor baseline PS 2 or 3. Although they adopted the oral alkalisation and control of defecation to prevent irinotecan-induced side effects, especially delayed diarrhoea, they are uncertain whether or not this preventive treatment reduced the observed incidence of severe delayed diarrhoea.

2.2 Irinotecan plus carboplatin for ED SCLC

Schmittel et al. studied the DLT and maximum tolerated dose (MTD) of a dose escalation of carboplatin to a fixed dose of irinotecan (IC) in Caucasian patients [30]. They demonstrated that the maximum tolerated dose is irinotecan 50 mg/m^2 administered on day 1, 8 and 15, and carboplatin at an area under the concentration–time curve (AUC) of 5 mg/ml x min, on day 1 of a 4-week cycle. DLT (neutropenia, thrombocytopenia and diarrhoea) was comparable to the results of the Japanese trial at a dose of 60 mg/m^2 of irinotecan and AUC = 5 of carboplatin [31].

Subsequently, Schmittel et al. presented a randomised Phase II trial comparing IC and etoposide plus carboplatin (EC) in ED SCLC [32]. Chemotherapy-naive ED SCLC patients were randomly assigned to receive carboplatin AUC = 5 either in combination with 50 mg/m^2 of irinotecan on days 1, 8 and 15 or with etoposide 140 mg/m^2 on days 1 – 3. In the IC arm, treatment was repeated every four weeks; in the EC arm, every

three weeks. IC improved response rate (10% CR and 61% partial response (PR) in IC, 0% CR and 50% PR in EC) and progression free survival (9 months, p = 0.03) over standard EC (6 months). The MST was 12 months in the IC arm and 10 months in the EC arm, but with no significant difference. Patients with EC had significantly higher incidence of grade 3 to 4 leucopenia, neutropenia and thrombocytopenia. Grade 3 – 4 diarrhoea developed more frequently in the IC arm (11 versus 6%), but with no significant difference. Haematotoxicity was favourable in the IC arm. They extended into a randomised Phase III trial to assess impact on overall survival, and concluded this study showed that even when carboplatin is used instead of cisplatin, the survival of IC and EC was not significantly different, and that myelosuppression was more frequent in EC than IC.

2.3 Irinotecan plus etoposide for ED SCLC

A Phase II study of irinotecan and etoposide (IE) for chemotherapy-naive ED SCLC was recently conducted without platinum by the West Japan Thoracic Oncology Group (WJTOG) [33]. A total of 50 patients were enrolled. This regimen consisted of irinotecan 60 mg/m² on days 1, 8 and 15, and etoposide 80 mg/m² on days 2 – 4. The overall response rate was 66% with a complete response rate of 10%. The MST was 11.5 months and the 1-year survival rate was 43.2%. Grade 3 – 4 neutropenia, thrombocytopenia and diarrhoea were 62.9, 4 and 2%, respectively. There was no treatment-related death. This regimen seems to be equal to the EP regimen. The dose intensity of irinotecan and etoposide achieved with this regimen was not adequate. This may be the reason for the low incidence of diarrhoea (2%). A schedule of irinotecan administered on days 1 and 8 at 3-week intervals may be preferred.

2.4 Triplets including irinotecan for ED SCLC

JCOG9902-DI was a randomised Phase II trial to compare two kinds of three-drug combinations of cisplatin, etoposide and irinotecan (PEI regimens) for the treatment of ED SCLC [34]. A total of 60 patients were randomised to receive either arm A (cisplatin 25 mg/m² on day 1, on weeks 1, 3, 5, 7 and 9 and etoposide 60 mg/m² on days 1 – 3, on weeks 2, 4, 6, 8), or arm B (cisplatin 60 mg/m² on day1, irinotecan 60 mg/m² on days 1, 8, 15 and etoposide 50 mg/m² on days 1 – 3, every week for 4 cycles). Prophylactic G-CSF support was provided in both arms. This study suggested that the PEI combinations in both schedules have significant activity against ED SCLC with acceptable toxicity. The CR rate of 17% and MST of 12.9 months in arm B were much more promising compared with the CR rate of 7% and MST of 8.9 months in arm A. They concluded that arm B should be selected for future Phase III studies. However, because irinotecan administration often needed to be skipped, especially on day 15, they suggested a 3-week schedule in which irinotecan is administered only on days 1 and 8.

Briasoulis *et al* showed that irinotecan can be safely combined with cisplatin and etoposide in a convenient and simple

schedule of administration over three days [35]. They treated 36 patients with irinotecan on day 1 in combination with fixed doses of cisplatin (20 mg/m²) and etoposide (75 mg/m²), both for 3 consecutive days. Irinotecan dose was escalated from 60 mg/m² by increments of 40 mg/m² in this Phase I trial. The MTD of irinotecan was 140 mg/m² and the recommended optimal dose 120 mg/m². DLTs were febrile neutropenia and grade 3 diarrhoea. This same regimen is being studied with concurrent TRT in a total dose of 54 Gy in 30 fractions (1.8 Gy once daily) [36].

Thompson *et al* reported a Phase II trial of the Minnie Pearl Cancer Research Network at the 2005 ASCO meeting [37]. They added a molecular targeted agent, imatinib (60 mg/day, per os) to chemotherapy of irinotecan (60 mg/m² on days 1, 8 and 15) and carboplatin (AUC = 4) every 4 weeks. Imatinib targets c-kit expression. Grade 3/4 haematological toxicity included: neutropenia (29%/16%), anaemia (13%/1%) and thrombocytopenia (7%/0%). The response rate was 66% with 10% CR. Grade 3 diarrhoea was observed in 21%. There were no treatment-related deaths. The MST was 8.5 months. This suggests that C-kit expression did not correlate with survival and that imatinib offers no efficacy at a cost of increased toxicity when combined with irinotecan and carboplatin in the treatment of ED SCLC.

3. Irinotecan-containing regimens for relapsed or refractory SCLC

Huisman *et al* have summarised 21 Phase II studies and 3 randomised trials of second-line chemotherapy in patients with SCLC reported from 1989 to 1999 [38]. They found a cumulative response rate of 21% for multi-drug regimens and 19% for single agents. As yet there is no standard second-line treatment established for patients with SCLC who fail or relapse after front-line treatment.

Irinotecan was combined with various anticancer drugs in doublet or triplet. As doublets, these include cisplatin [39], weekly or every three weeks carboplatin [40,41], etoposide [42], gemcitabine [43,44], ifosfamide [45] and paclitaxel [46]. The responses vary from 10 to 94%, and the MST ranges from 5.8 to 8.9 months. As described earlier on triplet including irinotecan [34,47], a three-drug combination Phase II study of irinotecan, cisplatin and etoposide (PEI regimen) was conducted only for sensitive relapsed SCLC (40 patients) [48]. This Phase II regimen consisted of cisplatin 25 mg/m² weekly for 9 weeks, etoposide 60 mg/m² for 3 days on weeks 1, 3, 5, 7 and 9, and irinotecan 90 mg/m² on weeks 2, 4, 6 and 8 with G-CSF support after day 1 on week 2. The results showed a response rate of 78% (CR rate of 13%) and the MST of 11.8 months. A total of 39 patients (98%) had a good PS of 0 or 1. Grade 3 – 4 neutropenia, thrombocytopenia, and diarrhoea were observed in 73, 33, and 8%, respectively. Nonhaematological toxicities were mild and transient.

Another three-drug combination of cisplatin, ifosfamide and irinotecan with G-CSF was conducted by Fujita *et al* [49].

The response rate was 94.4% and the MST was 11.1 months, encouraging result. Because of patient selections, it is difficult to make wholly valid conclusions about the most effective regimen based only on Phase II results. However, three-drug combinations containing irinotecan with G-CSF support may have better survival and feasibility than the doublets. The disadvantage is that triplet regimens require G-CSF support, which may make out-patient treatment difficult.

4. Irinotecan containing regimen for LD SCLC

Two meta-analyses showed that the addition of TRT to chemotherapy in patients with LD SCLC improves survival at two and three years by 5.4% [50,51]. In these meta-analyses, non-platinum-based combination chemotherapies were commonly used, with only a few trials using platinum-based chemotherapy. Cisplatin and etoposide plus TRT is now widely regarded as the standard regimen for LD SCLC, and presents acceptable toxicity [52]. Turrisi et al. reported results of once-daily versus twice-daily (b.i.d) TRT with four cycles of cisplatin and etoposide. Results showed that the MST was significantly superior in the b.i.d arm (23 versus 19 months) [53].

Irinotecan showed potent radiosensitising effects in human lung tumour xenografts which were related to the cell cycle [54]. Kubota et al. reported a pilot study of concurrent etoposide and cisplatin plus accelerated hyperfractionated TRT followed by irinotecan and cisplatin for LD SCLC (JCOG9903) [21]. Treatment consisted of etoposide 100 mg/m² on days 1 – 3, cisplatin 80 mg/m² on day 1, and concurrent b.i.d TRT of 45 Gy beginning on day 2. The IP regimen started on day 29 and consisted of irinotecan 60 mg/m², days 1, 8, 15 and cisplatin 60 mg/m² on day 1, with three 28-day cycles. A total of 31 patients were accrued. Although a pilot study, the MST was 20.2 months and 1-, 2- and 3-year survival rates were 76%, 41%, and 38%, respectively. This encouraging regimen proved safe with acceptable toxicities. A randomised Phase III trial comparing EP with IP following EP plus concurrent TRT for LD SCLC is now underway (JCOG0202).

The WJTOG also conducted a similar regimen [55]. Treatment included cisplatin 80 mg/m² on day 1 and etoposide 100 mg/m² on days 1 – 3 with concurrent TRT (1.5 Gy/b.i.d, a total dose of 45 Gy) followed by 3 cycles irinotecan 60 mg/m² on days 1, 8 and 15 and cisplatin 60 mg/m². The results of 51 patients were almost identical to JCOG9903; overall response and CR rate was 87.8% and 40.8%, respectively; Grade 4 toxicity included neutropenia (83.7%), anaemia (10.2%), thrombocytopenia (0%), diarrhoea (2%) and infection (2%); the MST was 21.5 months and 2-year survival rate was 45.7%.

A Phase II study of IP induction followed by concurrent b.i.d TRT with EP chemotherapy for LD SCLC was conducted [56] and also showed encouraging results. Treatment consisted of two cycles of cisplatin 40 mg/m² and irinotecan 80 mg/m² on days 1 and 8 of a 3-week cycle. This was followed by two 3-week cycles of cisplatin 60 mg/m² on days 43

and 64, and etoposide 100 mg/m² on days 43 – 45 and 64 – 66, with concurrent b.i.d TRT total of 45 Gy beginning on day 43. Thirty-five patients were accrued. The MST was 25 months (but it should be noted that this is a single institution Phase II study).

In these studies, irinotecan was used on an induction or adjuvant setting, and both regimens were encouraging. However, randomised study in which both modalities are compared has not been conducted.

There have been a few trials of concurrent chemoradiotherapy including irinotecan for patients with SCLC as well as NSCLC. Recently, a combined modality treatment of IC and TRT followed by bevacizumab (antiangiogenic anti-VEGF antibody) in the treatment of LD SCLC was conducted in a Phase II trial by the Minnie Pearl Cancer Research Network [57]. Induction therapy consisted of irinotecan 50 mg/m² on days 1 and 8, carboplatin AUC = 5 on day1, TRT 1.8 Gy single daily dose to total dose of 61.2 Gy (34 fractions), beginning with the 3rd cycle. Chemotherapy was repeated every three weeks for four cycles. As a maintenance therapy, bevacizumab 10 mg/kg i.v. every 2 weeks was given until disease progression, or a maximun of 10 doses (20 weeks) were administered. The response rate was 81% with 28% CR. This regimen was well tolerated with rare grade 4 toxicity and no treatment-related deaths. One-year progression-free and overall survival were 68% and 71%, respectively. These results suggest that irinotecan can be safely administered with TRT concurrently.

Sohn et al. also reported a Phase II study of IP with concurrent TRT in LD SCLC [58]. Chemotherapy of irinotecan 60 mg/m² on days 1, 8 and 15 and cisplatin 40 mg/m² on days 1 and 8 were repeated every 4 weeks until a maximum of 6 cycles. TRT of 2 Gy/day was commenced on day 1 of the second chemotherapy cycle up to a total of 54 Gy. The results are not concluded at this time.

Langer et al. reported a Phase I study of IP and either b.i.d TRT (45 Gy) or once daily RT (70 Gy) to determine if irinotecan can be safely integrated with concurrent TRT and cisplatin in LD SCLC [59]. Acute DLT was defined as grade 4 oesophagitis, pneumonitis, or diarrhoea; grade 4 neutropenic fever; or any attributable grade 5 fatal toxicity (≤ 90 days after RT). Although preliminary, there has been no attributable DLT in the 26 patients that have been enrolled. In combination with cisplatin 60 mg/m² every 3 weeks x 4 and either b.i.d TRT or once daily TRT, irinotecan 40 mg/m² on days 1 and 8 was safe and feasible. Irinotecan at 50 mg/m² on days 1 and 8 every 3 weeks x4 was also feasible in combination with cisplatin and b.i.d TRT. These reports allow us to conclude that irinotecan can be administered with radiotherapy sequentially or concurrently.

5. Expert opinion and conclusion

Irinotecan is effective against SCLC. For the treatment of ED SCLC, IP regimen is at least comparable to EP regimen.

The degree of myelosuppression in the IP regimens was less than that of the EP regimens. However, diarrhoea was more often observed in the IP than the EP regimens, and can lead to severe side effects when the IP regimen is used incautiously. Pharmacogenetic study of irinotecan may prompt one to use the drug in a safer way to avoid severe toxicities. McLeod suggests that at the least, irinotecan $300 - 350$ mg/m^2 every 3 weeks should not be given to patients with a known UGT1A1*28 genotype until more definitive guidelines are established [60]. However, the use of UGT1A1*28 genotyping to predict toxicity is controversial and its clinical implications are unclear. Furthermore, whether or not these recommendations are also applicable to patients with SCLC should be elucidated upon because a lower dose of irinotecan is usually administered weekly for the treatment of SCLC, rather than the every 3 or 4 weeks for colorectal cancers. In the coming decade, we must confront the metabolic and pharmacogenomic differences in various populations for the treatment of cancer. For this, international cooperative studies are warranted and indeed of immense importance.

Considering patient safety, irinotecan can indeed be administered relatively safety in patients with SCLC, provided there is careful monitoring of patients, especially regarding diarrhoea and myelosuppresion. Further studies to avoid severe toxicities are needed to advance the safe use of this otherwise promising drug.

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EGFR and ErbB2 mutation status in Japanese lung cancer patients

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Much evidence has accumulated that the epidermal growth factor receptor (EGFR) and its family members are strongly implicated in the development and progression of lung cancers. Somatic mutations of the EGFR gene were found in about 25–40% of Japanese lung cancer patients. More recently, erbB2 mutations are found in about 4% of European-derived lung cancer patients. We have investigated EGFR and erbB2 mutation status in 95 surgically treated nonsmall cell lung cancer (NSCLC) cases from Nagoya City University Hospital. Seventy-five adenocarcinoma cases were included. The presence or absence of EGFR and ernB2 mutations of kinase domains were analyzed by reverse transcription polymerase chain reaction (RT-PCR) amplifications and direct sequences. We have also investigated erbB2 mutation status in 27 surgically treated NSCLC cases followed by treatment with gefitinib from Kinki-chuo Chest Medical Center. EGFR mutations (CTG→CGG; L858R) were found from 14 of 95 lung cancer patients. We also detected the deletion 1a-type mutations from 9 patients and deletion 4-type mutations from 6 patients in exon 19. In exon 20, 4 mutations including 2 novel mutations were found. Total EGFR mutations were present in 35 patients (36.8%). These mutation statuses were significantly correlated with gender (women 73.3% vs. men 20%, p < 0.0001), smoking status (never smoker 69.4% vs. smoker 16.9%, p < 0.0001), pathologic subtypes (adenocarcinoma 45.1% vs. nonadenocarcinoma 12.5%, p = 0.0089) and differentiation status of the lung cancers (well 51% vs. moderately or poorly 18.4%, p = 0.0021). On the other hand, erbB2 mutation was only found from 1 of 95 patients, at exon 20. This patient was female and a never smoker with adenocarcinoma. This 12 nucleotide insertion mutation (2324–2325 ins ATACGTGATGGC) was located in the exon 20 at kinase domain (775–776 ins YVMA). There was no erbB2 mutation in 27 gefitinib-treated NSCLC patients. In total, we have found only 1 erbB2 mutation from 122 (0.8%) Japanese NSCLC patients. There was a significantly higher erbB2 positive (2+/3+) ratio in EGFR mutant patients (13/25, 52.0%) compared to EGFR wild-type patients (10/62, 16.1%; p = 0.0247). The NSCLC specimen with erbB2 mutation showed 1+ immunoreactivity. The EGFR mutation status might correlate with the clinicopathologic features related to good response to gefitinib, such as gender, smoking history and pathologic subtypes of lung cancers. However, erbB2 mutation is rare from Japanese lung cancer and is of limited value for molecular target therapy.
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Key words: EGFR; lung cancer; mutations; erbB2; Japanese

Lung cancer is a major cause of death from malignant diseases, due to its high incidence, malignant behavior and lack of major advancements in treatment strategy.[1] Lung cancer was the leading indication for respiratory surgery (42.2%) in 1998 in Japan.[2] More than 15,000 patients underwent surgical operations at Japanese institutions in 1998.[2] The clinical behavior of the lung cancer is largely associated with its stage. The cure of the disease by surgery is only achieved in cases representing an early stage of lung cancer.[3]

There is much accumulated evidence that epidermal growth factor receptor (EGFR) and its family members are strongly implicated in the development and progression of numerous human tumors, including lung cancer.[4,5] The erbB family comprises 4 structurally related receptors: ErbB1 (EGFR), ErbB2 (HER2-neu), ErbB3 and ErbB4. On ligand stimulation, the receptor forms either homodimers or heterodimers, which activate their cytoplasmic domain. This tyrosine-auto-phosphorylated region functions as a docking site for messenger proteins, which initiate cascades of cytoplasmic and nuclear mitogenic pathways.[6] Inhibition of this pathway is facilitated by several newly developed compounds that have shown promising results in preclinical and clinical trials.[7] The EGFR tyrosine kinase inhibitor, gefitinib, was approved in Japan for the treatment of nonsmall cell lung cancer (NSCLC) since 2002. Trastuzumab is a recombinant DNA-derived monoclonal antibody that selectively binds to p185 HER2, the protein product of erbB2. Trastuzumab was approved for breast cancer[8] and clinical trials for NSCLC is underway.[9,10]

Recently, we have found that novel EGFR mutations' status at ATP binding pockets in Japanese NSCLC patients were correlated with the clinicopathologic features related to good response to gefitinib.[11] These EGFR mutations are predominantly found in Japanese lung cancer patients (about 25%) when compared to USA patients (about 8%[12–14] to 10%[15]). Kasaoka et al. have reported that the EGFR mutation ratio is 40% of Japanese lung cancer patients.[16] Actually, EGFR mutations in lung cancer have been correlated with clinical response to gefitinib therapy in vivo and in vitro.[11–13] More recently, it has been reported that novel erbB2 mutations at kinase domain were found in 4% of European-derived NSCLC patients.[17]

To determine the EGFR and erbB2 mutation status in Japanese lung carcinoma for screening purposes, we investigated EGFR and erbB2 mutation status by the RT-PCR amplifications and direct sequences. The findings were compared to the clinicopathologic features of lung cancer.

Material and methods

Study subjects

The study group included 95 lung cancer patients who had undergone surgery (but did not receive gefitinib) at the Department of Surgery II, Nagoya City University Medical School between 1997 and 2002. We have also investigated erbB2 mutation status for 27 NSCLC patients who had undergone surgery followed by treatment with gefitinib at the National Hospital Organization, Kinki-chuo Chest Medical Center. The lung tumors were classified according to the general rule for clinical and pathologic record of lung cancer in Japan.[18] All tumor samples were immediately frozen and stored at −80°C until assayed.

The clinical and pathologic characteristics of the 95 lung cancer patients are as follows: 52 cases at stage I, 9 at stage II and 34 at stage III–IV. The mean age was 64.9 years (range, 42–82). Among the 95 lung cancer patients, 71 (74.7%) were diagnosed as adeno-

carcinoma, 17 (17.9%) were squamous cell carcinoma and 4 (4.2%) were adenosquamous cell carcinoma. The samples from these patients had never been sequenced for *EGFR* before.

PCR assays for EGFR and erbB2

Total RNA was extracted from lung cancer tissues and adjacent nonmalignant lung tissues using Isogen kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. RNA concentration was determined by spectrophotometer and adjusted to a concentration of 200 ng/ml. About 10 cases were excluded because tumor cells were too few to sufficiently extract tumor RNA. RNA (1 μg) was reverse transcribed by Superscript II enzyme (Gibco BRL, Gaithersburg, MD) with 0.5 μg oligo $(dT)_{12-16}$ (Amersham Pharmacia Biotech, Piscataway, NJ). The reaction mixture was incubated at 42°C for 50 min and then at 72°C for 15 min. We then used 1 μl of each DNA for PCR analyses. The PCR reactions were performed using LA-Taq kit (Takara Bio, Shiga, Japan) in a 25 μl reaction volume. The primer sequences for *EGFR* gene for kinase domain (exons 18–21) were as follows: the forward primer, 5-CTCTTACACCCAGTGGAGAA-3 and the reverse primer, 5-CATCCACTTGATAGGCACTT-3 (572 bp). The cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 40 sec, 60°C for 40 sec, 72°C for 45 sec The primer sequences for *erbB2* gene for kinase domain (exons 19–22) were as follows: the forward primer, 5-CGCTTTTGGCACAGTCTACA-3 and the reverse primer, 5-GGGATCCCATCGTAAGGTTT-3 (594bp). The cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 40 sec, 60°C for 40 sec, 72°C for 45 sec. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA). Amplified cDNAs were separated on 1% agarose gels, and the bands were visualized by ethidium bromide and photographed under ultraviolet transillumination. These samples were sequenced by ABI prism 3100 analyzer (Applied Biosystems Japan, Tokyo, Japan) and analyzed by BLAST and chromatograms by manual review.

Immunohistochemistry

Tissue blocks were cut into 4 mm sections and mounted on silane-coated slides. The slides were then deparaffinized in xylene, dehydrated in a grade alcohol series and blocked for endogenous peroxidase with 3% H_2O_2 in absolute methanol. After microwave pretreatment in Blockace solution, immunostaining was done at 4°C overnight with a rabbit polyclonal c-erbB2 oncoprotein antibody (A04085, DakoCytomation, Glostrup, Denmark) at a 1:200 dilution. The expression of erbB2 was scored as follows: −, no discernible staining or <10% of cell stained; 1+, >10% of cytoplasmic staining or plasma membrane staining with weak intensity; 2+, >10% of plasma membrane staining with moderate intensity; and 3+, >10% of plasma membrane staining with strong intensity.

Statistical methods

Statistical analyses were done using the Mann-Whitney U-test for unpaired samples and Wilcoxon's signed rank test for paired samples. Linear relationships between variables were determined by means of simple linear regression. Correlation coefficients were determined by rank correlation using Spearman's test and χ^2test. The overall survival of lung cancer patients was examined by the Kaplan-Meier methods, and differences were examined by the log-rank test. All analysis was done using the Stat-View software package (Abacus Concepts, Berkeley, CA) and was considered significant when the p-value was less than 0.05.

Results

EGFR gene mutation status in Japanese lung cancer patients

Using the primer sets for *EGFR* kinase domain, a PCR product of 572 bp was obtained. When we visualized the PCR products
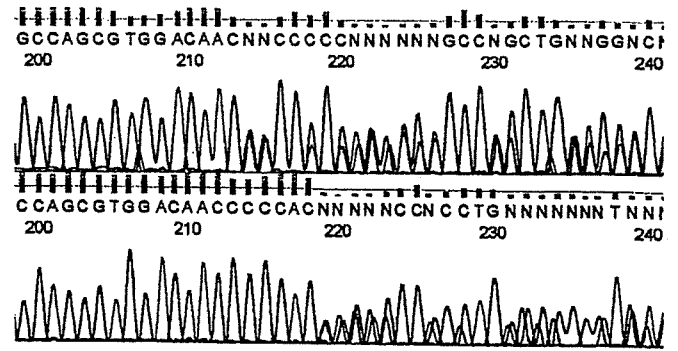

FIGURE 1 – Novel EGFR mutation at exon 20. Top: a male well-differentiated adenocarcinoma patient had the novel 2312–2313 insertion CAA. Bottom: a female, well-differentiated adenocarcinoma patient had the novel 2319–2320 insertion AACCCCCAC.

with 1% agarose gel, these samples were further studied. In exon 18, there was no G719S mutation found from this study. In exon 19, 9 patients had the del 1a type mutation, 6 patients had the deletion 4 type mutation and 1 patient had the del 1b type mutation. Seven were male and 10 were female. Thirteen were nonsmokers and 4 were smokers. Fifteen patients had adenocarcinoma, 1 had squamous cell carcinoma and 1 had adenosquamous cell carcinoma. Three of the tumors were moderately differentiated, 2 were poorly differentiated and 11 were well differentiated. Five of 15 adenocarcinomas showed bronchioloalveolar carcinoma (BAC) pattern at the edge of tumor. Thus *EGFR* mutation status at exon 19 was significantly correlated with gender ($p = 0.0172$) and tobacco-smoking ($p = 0.0008$) but not with pathologic stages (stage I vs. II–IV, $p = 0.9144$), subtypes (adenocarcinoma vs. nonadenocarcinoma, $p = 0.2675$) and differentiation of lung cancer (well vs. moderately or poorly differentiated, $p = 0.3812$).

In exon 20, 3 patients had theheterozygous in-frame insertion mutations. Two were male and 1 was female. All 3 were smokers. A female, well-differentiated adenocarcinoma patient had the novel 2319-2320 insertion AACCCCCAC. A male well-differentiated adenocarcinoma patient had the novel 2312-2313 insertion CAA (Fig. 1). We have found one point mutation, C2369T (T790M). This patient also has the predominant L858R mutation (Fig. 2).

For exon 21, 14 patients had the L858R mutation and 1 patient had the L861Q mutation. Four were male and 11 were female. Twelve were nonsmokers and 3 were smokers. All 15 patients had adenocarcinoma, 1 was moderately differentiated and 14 were well differentiated. Six of 15 adenocarcinomas exhibited the BAC pattern at the edge of the tumor. Thus, exon 21 mutation status was significantly correlated with gender ($p = 0.0005$), smoking status ($p = 0.0007$), pathologic stages ($p = 0.0152$), the pathologic subtypes ($p = 0.0329$) and differentiation of lung cancer ($p = 0.0033$).

The mutations detected in lung cancer specimens from 95 lung cancer patients are summarized in Table I. Taken together, 36 mutations were found from 35 lung cancer samples in our analysis. Total *EGFR* mutations were present in 35 patients (36.8%). These mutation statuses were significantly correlated with gender (women 73.3% vs. men 20%, $p < 0.0001$), smoking status (never smoker 69.4% vs. smoker 16.9%, $p < 0.0001$), pathologic subtypes (adenocarcinoma 45.1% vs. nonadenocarcinoma 12.5%, $p = 0.0089$) and differentiation status of the lung cancers (well 51% vs. moderately or poorly 18.4%, $p = 0.0021$).

The overall survival of 95 lung cancer patients from Nagoya City University, with follow-up through December 30, 2003, was studied in reference to the *EGFR* mutation status. The patient with the mutation in the *EGFR* gene ($n = 35$, 4 were dead) had a significantly better prognosis than the patient with wild-type *EGFR* ($n = 60$, 20 were dead; log-rank test $p = 0.0143$, Breslow-Gehan-Wilcoxon test $p = 0.0220$), although the observation period was short (Fig. 3). But a multivariate analysis revealed that pathologic
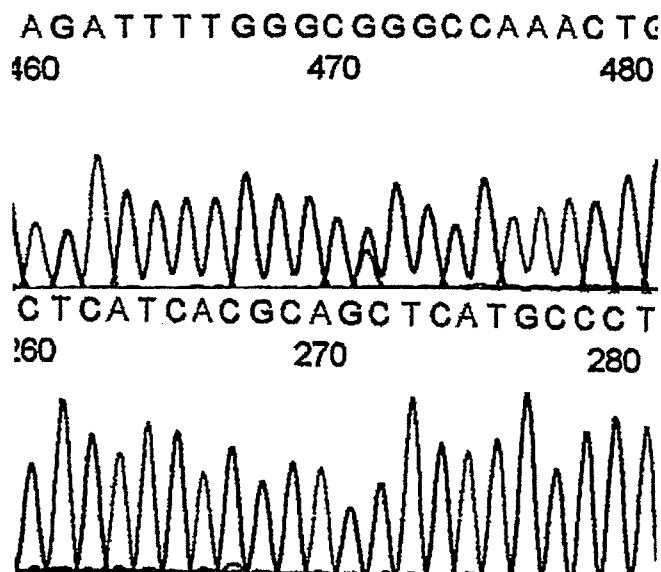
A G A T T T T G G G C G G G C C A A A C T C
460   470   480

C T C A T C A C G C A G C T C A T G C C C T
260   270   280

FIGURE 2 – The premoninant L858R (2573 T to G) mutation in exon 21 (top) and T790M (2369 C to T) mutation at exon 20 (bottom) within the EGFR kinase domain.

TABLE I – CLINICOPATHOLOGIC DATA OF 95 LUNG CANCER PATIENTS

Factors	EGFR gene status		
	Mutation patients	Wild-type patients	p-value
Mean age (years)			
64.9 ± 9.0	35	60	
Stage			
I	25 (72.4%)	27 (45.8%)	0.0274
II–IV	10 (28.6%)	32 (54.2%)	
Lymph node metastasis			
N0	8 (22.9%)	21 (35.0%)	0.3119
N+	27 (77.1%)	39 (65.0%)	
BI			
Never smoker	25 (71.4%)	11 (34.0%)	0.001
Smoker	10 (28.6%)	49 (66.0%)	
Differentiation			
Well	26 (78.8%)	23 (42.6%)	0.0021
Moderately or poorly	7 (21.2%)	31 (57.4%)	
Pathologic subtypes			
Adeno	32 (91.4%)	39 (74.7%)	0.0089
Nonadeno	3 (8.6%)	21 (25.3%)	
Age			
≤ 65	19 (54.3%)	29 (48.3%)	0.7269
> 65	16 (45.7%)	31 (51.7%)	
Gender			
Male	13 (37.1%)	52 (86.7%)	< 0.0001
Female	22 (62.9%)	8 (13.3%)	

N+, lymph node metastasis positive; Adeno, adenocarcinoma.

stage (p = 0.0006) was the only significant factor but not EGFR mutation (p = 0.1824).

ErbB2 gene mutation status in Japanese lung cancer patients

We identified only one erbB2 mutation from 95 NSCLC patients. This 12-nucleotide insertion mutation (2324-2325 ins ATACGTGATGGC) was located in the exon 20 at kinase domain (775-776 ins YVMA) (Fig. 4). This patient was a female non-smoker with well-differentiated adenocarcinoma, without EGFR mutation. Adjacent normal lung tissue exhibited a wild-type sequence for the erbB2 gene, suggesting that this mutation was somatic. We have also done sequencing for 27 gefitinib-treated NSCLC patients. Among 27 patients, 9 patients had EGFR mutations (data not shown). However, no erbB2 mutation was found
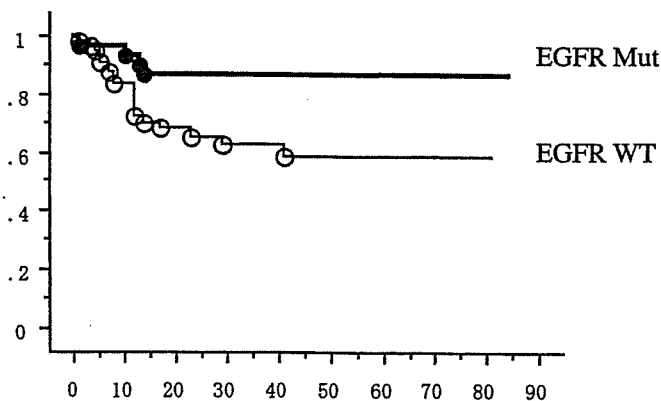

FIGURE 3 – The patient with a mutation in the EGFR gene (n = 35, 4 were dead) had a significantly better prognosis than the patient with wild-type EGFR (n = 60, 20 were dead) (log-rank test, p = 0.0143; Breslow-Gehan-Wilcoxon test, p = 0.0220), although the observation period was short.

: G A A G C A T A C G T G A T G G C A T A N N T G A T G G C T G G T G T G G G C T C C C C A T A T G
a   100   110   120   130   140

G C C A T C A C G T A T G C C A T C A C G T A T G C T T C G T C T A A G A T T T C T T T G T T G G
b   430   440   450   460   470

: G A A G C A T A C G T G A T G G C T G G T G T G G G C T C C C C A T A T G T C T C C C G C C T T C
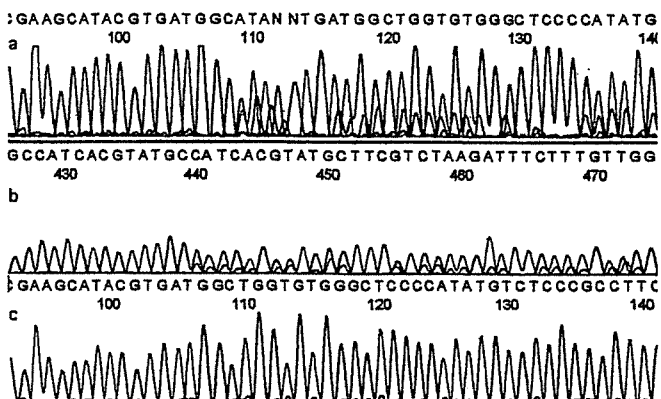c   100   110   120   130   140

FIGURE 4 – Detection of the insertion mutation in the erbB2 gene in genomic DNA extracted from lung cancer. (a) The 12 nucleotide insertion mutation (2324–2325 ins ATACGTGATGGC) was located in the exon 20 at kinase domain (775–776 ins YVMA). (b) Reverse sequence was performed and confirmed. (c) Adjacent normal lung tissue showed a wild-type sequence for the erbB2 gene.

within the kinase domain. Totally, we have found only 1 erbB2 mutation from 122 (0.8%) Japanese NSCLC patients.

Immunohistochemistry

The immunohistochemical evaluation was done according to the scoring system described in Material and Methods. Immunohistochemistry was done only for 87 patients because the tissue blocks were not available for other patients. The erbB2-positive (2+/3+) ratio was 26.4% (23/87). There was a significantly higher erbB2-positive ratio in EGFR-mutant patients (13/25, 52.0%) compared to EGFR wild-type patients (10/62, 16.1%) (p = 0.0247). The patient with erbB2 mutation exhibited 1+ immunoreactivity (Fig. 5).

Discussion

We obtained findings that EGFR mutation status was significantly correlated with gender and smoking history of lung cancers. This was in agreement with the recent reports that EGFR gene mutations are common in lung cancers from never smokers[13,14] and females with adenocarcinoma.[11,14] However, our analysis also suggested that erbB2 mutation might be less common in Japanese NSCLC patients.

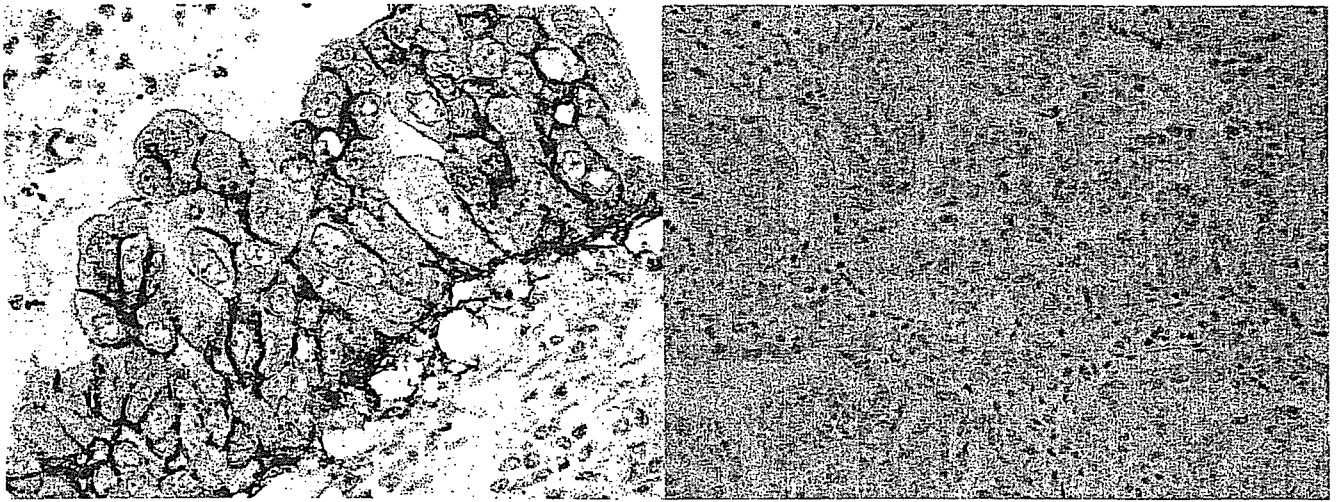FIGURE 5 – Immunohistochemistry for ErbB2. Left: erbB2-positive (3+) section. Right: the NSCLC specimen with *erbB2* mutation exhibited 1+ immunoreactivity.

Overexpression of EGFR/ErbB2 and ErbB ligands is correlated with advanced diseases and poor patient prognosis.[19] Although *EGFR* is more abundantly expressed in lung carcinoma,[20,21] *erbB2* overexpression is less common; it is found in <35% of patients with nonsmall cell lung cancers, mainly in those with adenocarcinoma.[21] Amplification of *EGFR* and *erbB2* mRNA[22] or overexpression of their proteins[23] has been found to relate to survival in patients with NSCLC, although contradictory results have also been reported.[24,25] The drug trastuzumab, a humanized antibody against the extracellular domain of erbB2, has been approved for treatment of metastatic breast cancer and is most effective in breast cancer with *erbB2* amplification. Preliminary results suggested that the combination of chemotherapy and trastuzumab is well tolerated for NSCLC.[21] However, results from phase II trials of trastuzumab as a treatment for NSCLC have not shown any advantage for most patients[22] and have provided insufficient evidence to proceed to phase III trials.[23] Because the presence of a mutation appears to be a determination of response to therapy, as is the case with gefitinib and *EGFR* mutations, we therefore investigated the *erbB2* and *EGFR* gene mutation status. However, we have found only 1 *erbB2* mutation from 122 Japanese lung cancer patients. More recently, Shigematsu et al. reported that *erbB2* mutations were found in 3% (8/269) of Japanese NSCLC.[26] The single *erbB2* mutation we have found was the same as the one repeatedly found by Shigematsu et al.[26] Because very few NSCLC patients have gene amplification of *erbB2*, trastuzumab in the treatment of NSCLC might have a limited role.[9] Lung cancers that coexpress both EGFR and erbB2 appear to have more virulent behavior.[27] In addition, EGFR-erbB2 heterodimers are associated with a stronger and more sustained proliferative signal than EGFR homodimers.[22,28] Blockade of a signaling pathway may in theory be overcome by compensatory activation of a separate pathway in the same tumor cell. Because there was a significantly higher erbB2-positive ratio in *EGFR*-mutant patients, blockade of both may ultimately yield superior results.

Because so many *EGFR* mutation phenotypes were discovered, it would be of interest to determine whether resistance to *EGFR* inhibition emerges through secondary mutation as is the case in imatinib-treated chronic myelogenous leukemia.[29] In our analysis, a female never smoker adenocarcinoma patient had the predominant L858R mutation as well as T790M mutation. Actually, this case was untreated with EGFR kinase inhibitors. Threonine 315 to isoleucine substitution in the Abl kinase domain was a critical structural determinant controlling inhibitor sensitivity of STI571.[29] Introduction of bulkier hydrophobic side chains at the Thr-790 position fully preserved the cellular kinase activity of the *EGFR* in the presence of selective kinase inhibitors, indicating potential mechanisms of molecular resistance formation as previously found for BCR-Abl at T315I. Previous *in vitro* study showed that mutation of T790M in the *EGFR* revealed a hotspot for resistance formation against gefitinib,[30] also *in vivo*.[31]

Over the decades, the incidence of lung adenocarcinoma has increased worldwide. Most individuals with lung adenocarcinoma (especially women) are nonsmokers,[32] who are corresponding with the sensitive population to gefitinib. In Taiwan, *EGFR* mutation ratio from adenocarcinoma was also high (55%, 38 of 69), and all of the adenocarcinomas with *EGFR* mutation were well to moderately differentiated.[33] These data were compatible for our results. Because well-differentiated adenocarcinoma patients had a better prognosis,[34] *EGFR* mutant patients showed better prognosis in our univariant analysis. The reason why many mutations were especially found in Asian, female nonsmoker adenocarcinoma remains unknown. Human papilloma virus type 16/18 infections,[35] cooking oil fume,[36] nutritional status, genetic susceptibility, immunologic infection, tuberculosis and asthma[32] have been investigated as causes of lung cancer occurring in nonsmoking women.

The findings of the breakdown of *EGFR* mutations among the 3 exons were interesting. The exon 21 mutations correlated with pathologic stage and subtype, unlike mutations in exon19. Since exon 21 mutations are more closed to the activation loop of *EGFR*, these may be more correlated with gefitinib sensitivity. Especially since 3 patients with exon 20 mutations were smokers, all of the mutations might not be equally correlated with sensitivity for gefitinib.

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