Table 1. Patient Characteristics

Characteristic	No. of Patients
Patients enrolled	42
Patients eligible	40
Sex	
Male	24
Female	16
Age, years	
Median	61
Range	44-74
Performance status	
0	14
1	26
Stage	
IIIB	3
IV	34
Recurrence after surgery	3
Histologic type	
Adenocarcinoma	30
Squamous cell carcinoma	3
Large cell carcinoma	7
Smoking history	
Current	27
Former	5
Never	8

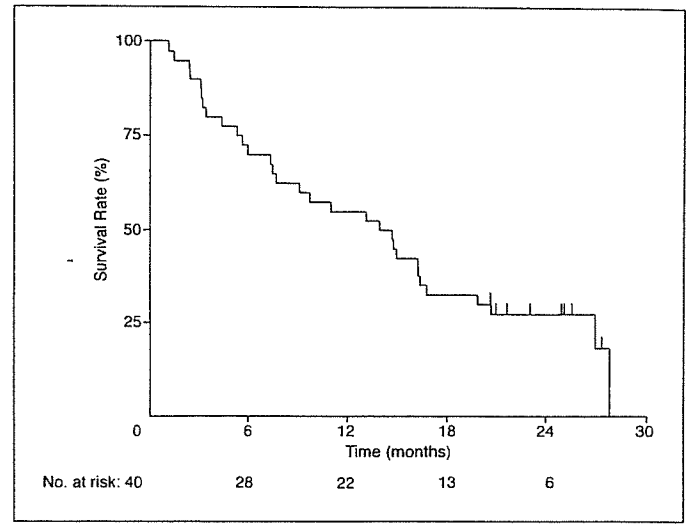

Fig 1. Overall survival of all eligible patients (n = 40) was calculated according to the Kaplan-Meier method. The median survival time was 13.9 months (95% CI, 9.1 to 18.7 months), and the 1-year survival rate was 55%.

patient from this subgroup achieved PR within 4 weeks, with the remaining patient achieving PR within 8 weeks. The background of the 12 responding patients was as follows: nine females, three males; 11 adenocarcinomas, one large-cell carcinoma; six individuals who never smoked, five current smokers, and one former smoker. Response rates based on patient characteristics were as follows: three of 24 (13%) males, nine of 16 (56%) females ($P = .0050$); 11 of 30 (37%) individuals with adenocarcinoma, one of 10 (10%) individuals with squamous or large-cell carcinoma ($P = .0048$); six of 32 (19%) current or former smokers, and six of eight (75%) individuals who never smoked ($P = .0048$).

The median follow-up time was 23 months, and nine patients were still alive at the most recent follow-up. The median survival time was 13.9 months (95% CI, 9.1 to 18.7 months), and the 1-year survival rate was 55% (Fig 1).

Safety and Toxicity

Toxicity was evaluated in all eligible patients. The most common toxicity was rash (Table 3). Thirty-eight percent and 13% of patients experienced grade 1 or 2 rash, respectively. One patient experienced grade 3 nausea and vomiting, leading to gefitinib treatment being terminated. Grade 3 hepatic toxicity was observed in one patient, also causing termination of gefitinib treatment.

The most problematic toxicity was ILD. We reviewed the medical records, chest x-rays, and CT films of all the cases, which were suspected as ILD by the physician in charge. ILD was diagnosed on the basis of standard or high-resolution CT findings of the chest (diffuse ground-glass opacity, consolidation, or infiltrate) and no response to antibiotics. We diagnosed that four patients experienced grade 5 ILD during or after first-line treatment with gefitinib. The first patient was a 61-year-old man. He developed dyspnea and fever elevation (38.1°C) on day 23 of the treatment with gefitinib and administration of gefitinib was terminated. Chest CT demonstrated bilateral diffuse ground-glass opacity, and PaO2 was 43.7mmHg in the room air. KL-6 antigen, a serum marker of interstitial pneumonia, was not elevated

Table 2. Efficacy of Single Agent Treatment With Gefitinib in Patients With Stage IIIb or IV Non–Small-Cell Lung Cancer

Type of Response	No. of Patients	% of Patients
Complete	0	0
Partial	12	30
CR + PR	12	30
95% CI	17 to 47	
Stable disease	16	40
Progression	12	30

Abbreviations: CR, complete response; PR, partial response.

Table 3. Maximum Toxicity Grades Associated With Single Agent Treatment With Gefitinib in 40 Patients With Non–Small-Cell Lung Cancer

	Toxicity Grade									
	1		2		3		4		5	
Toxicity	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Rash	15	38	5	13	0	0	0	0	0	0
Dry skin	4	10	0	0	0	0	0	0	0	0
Diarrhea	7	18	0	0	0	0	0	0	0	0
Nausea	3	8	0	0	1	3	0	0	0	0
Mucositis	6	15	0	0	0	0	0	0	0	0
Alopecia	4	10	0	0	0	0	0	0	0	0
Hyponatremia	24	60	0	0	3	8	0	0	0	0
Hypokalemia	12	30	0	0	0	0	0	0	0	0
Hepatic	11	28	2	5	1	3	0	0	0	0
Renal	4	10	1	3	0	0	0	0	0	0
ILD	0	0	0	0	0	0	0	0	4	10

Abbreviation: ILD; interstitial lung disease.

(351 U/mL) on day 24, but elevated on day 31 (1,400 U/mL). Beta-D-glucan, a serum marker of fungal infection and *Pneumocystis carinii* pneumonia, was also negative. Methylprednisolone and antibiotics were administered, with temporal improvement of ILD. However, subsequently, pulmonary function gradually deteriorated, leading to death. Autopsy revealed alveolar damage with organization around the bronchus and vessels in both neoplastic and non-neoplastic lesions, compatible with drug-induced ILD. The second patient was a 64-year-old man. Chest CT on day 27 showed stable disease, but administration of gefitinib was continued (protocol violation). Periodic chest x-ray film on day 45 showed abnormal shadow in the left lung field. High-resolution CT of the chest on the same day revealed reticular shadow on bilateral upper lobe. The treatment with gefitinib was terminated on day 45. KL-6 antigen was not elevated on day 49 (276 U/mL). Methylprednisolone and antibiotics were administered, but were not effective, leading to death. The third patient was a 67-year-old man. Chest CT on day 30 demonstrated enlargement of primary lesion and bilateral reticular shadow in subpleural lesions. Gefitinib was terminated on day 30. The patient developed dyspnea without fever elevation on day 37. Pao2 in the room air fell to 61.0 mmHg from 82.4 mmHg at pretreatment. Chest x-ray showed that the bilateral diffuse reticular shadow deteriorated. Methylprednisolone and antibiotics were administered, but were not effective, leading to death. Autopsy revealed severe fibrotic thickness of alveolar septum, compatible with severe interstitial pneumonia. There was no pathological evidence of carcinomatous lymphangiosis. The fourth patient was a 59-year-old woman. Chest x-ray showed consolidation in the left lung on day 21. Slight fever (37.9°C) developed on day 22. Blood culture was negative. Antibiotics were administered, but consolidation deteriorated and spread to both lungs on day 25. Gefitinib was terminated on day 25. KL-6 antigen was elevated to 3,590 U/mL. Methylprednisolone was administered, but was not effective, leading to death (Table 4). Four other patients experienced ILD after second-line or third-line chemotherapy. Two patients received second-line treatment with cisplatin plus vinorelbine (one and four courses), one patient received treatment with cisplatin plus gemcitabine (one course), and one patient received third-line treatment with docetaxel (four courses). Three of four patients received steroids, with temporal improvement of ILD being observed in two patients. However, ILD deteriorated during tapering of steroid treatment, with three patients subsequently dying. One patient stopped the third-line treatment with docetaxel, with the associated ILD showing improvement in this case without steroid treatment (Table 4).

We retrospectively reviewed the pretreatment chest x-rays and CT films of all patients. Interstitial shadow was not detected on pretreatment chest x-ray films in any patients. However, six patients showed evidence of interstitial shadow on pretreatment chest CT films. Three of the six patients with interstitial shadow, as determined by pretreatment chest CT, experienced ILD either during or following administration of gefitinib or second-line chemotherapy. None of the six patients responded to gefitinib treatment. On the other hand, four of 34 patients who showed no interstitial shadow on pretreatment chest CT films experienced ILD. Interstitial shadow as determined by pretreatment chest CT was not a statistically significant risk factor of ILD ($P = .0819$; Table 5).

Second-Line Chemotherapy

A total of 30 patients received second-line chemotherapy. Twenty-seven patients received platinum-based chemotherapy (cisplatin plus vinorelbine; n = 17), carboplatin plus paclitaxel (n = 5), cisplatin plus gemcitabine (n = 3), cisplatin plus docetaxel (n = 1), and cisplatin plus irinotecan (n = 1). The remaining three patients received vinorelbine plus gemcitabine or vinorelbine alone. Nine of 30 patients achieved PR with these second-line chemotherapies. The objective response rate of second-line chemotherapy was 30% (95% CI, 15% to 50%).

Mutation Status of the EGFR Gene

Out of 42 enrolled patients, 16 patients were diagnosed pathologically, 22 were diagnosed cytologically, and four patients recurred after surgical resection. Biopsied specimens were available in nine patients. Therefore, tissue samples were available in a total of 13 patients. These 13 patients included four PRs, six with stable disease, and three PDs. *EGFR* mutations were detected in four tumor tissues, including the in-frame nucleotide deletions in exon 19 (n = 3) and an L858R mutation in exon 21 (n = 1). One tumor had an in-frame deletion and

Table 4. Four Patients Developed Interstitial Lung Disease During First-Line Chemotherapy With Gefitinib, With Another Four Patients Showing ILD During Either Second- or Third-Line Chemotherapy

Age (years)	Sex	Smoking Index	Pathology	Onset of ILD	Response to Gefitinib	Death From Chemotherapy
61	M	1,520	AD	Day 23*	PD	Day 74
64	M	880	AD	Day 45*	SD	Day 51
67	M	1,880	SQ	Day 37†	PD	Day 45
59	F	0	AD	Day 21*	PD	Day 35
61	M	820	AD	Day 131‡	SD	Day 154
68	M	2,000	LA	Day 37‡	PD	Day 106
68	M	705	AD	Day 225	PR	Day 87
59	M	1,170	AD	Day 108‖	SD	Alive

Abbreviations: ILD, interstitial lung disease; M, male; F, female; AD, adenocarcinoma; SQ, squamous cell carcinoma; LA, large-cell carcinoma; PD, progressive disease; SD, stable disease; PR, partial response.
*During gefitinib administration.
†One week after discontinuation of gefitinib.
‡ After 2nd-line chemotherapy of cisplatin and vinorelbine.
§ After 2nd-line chemotherapy of cisplatin and gemcitabine.
‖ After 3rd-line chemotherapy of docetaxel.

Table 5. Interstitial Shadow on Pretreatment Chest Computed Tomography Films and ILD

Interstitial Shadow on Pretreatment Chest Computed Tomography Scans	No ILD	ILD
No existence	29	5
Existence	3	3

NOTE. $P = .0819$.
Abbreviation: ILD interstitial lung disease.

an E746V mutation in exon 19. All four PR patients had *EGFR* mutations (Table 6).

DISCUSSION

This phase II study was designed to evaluate the efficacy and safety of first-line single agent treatment with gefitinib in patients with advanced NSCLC. There is no other paper that evaluates single agent treatment with gefitinib prospectively in patients with advanced NSCLC. The observed response rate of 30% (95% CI, 17% to 47%), median survival of 13.9 months and 1-year survival of 55% are promising. However, grade 5 ILD occurred in 10% (95% CI, 3% to 24%) of patients. This high rate of ILD was not acceptable. The incidence of ILD was seen to be less than 1% in two randomized controlled studies comparing gefitinib with placebo in combination with gemcitabine and cisplatin or paclitaxel and carboplatin.[12,13] The reason for the high incidence of ILD observed in our study is unknown. The West Japan Thoracic Oncology Group analyzed 1,976 patients receiving gefitinib retrospectively. In this case, the incidence of ILD was 3.2% (95% CI, 2.5% to 4.6%) and the death rate due to ILD was 1.3% (95% CI, 0.8% to1.9%). Multivariate analyses found that risk factors included being male, individuals who smoked, and complication of interstitial pneumonia.[14] Our retrospective analyses revealed that three of six patients with interstitial shadow on pretreatment chest CT films, but not detected on chest x-ray films developed ILD; on the other hand, five of 34 patients without interstitial shadow developed ILD. Interstitial shadow on pretreatment chest CT was a marginally significant risk factor of ILD ($P = .0819$). It might be suggested that patients with interstitial shadow on pretreatment chest CT films be excluded from administration of gefitinib; however, our analyses were biased because we analyzed retrospectively and did not blind patient clinical information. Prospective analysis is needed to evaluate interstitial shadow by chest CT before treatment with gefitinib.

The Southwest Oncology Group conducted a phase II trial to evaluate gefitinib in patients with advanced bronchioloalveolar carcinoma (SWOG 0126). Previously untreated (n = 102) and treated (n = 36) patients were entered and eligible in SWOG 0126. The response rate was 19% and the median survival time was 12 months in the untreated population.[19] These subset analyses were comparable to our results.

Recently, mutations in the tyrosine kinase domain of EGFR were found to be associated with gefitinib sensitivity in patients with NSCLC.[16,20,21] Our retrospective analyses demonstrated that *EGFR* mutations were detected in four of 13 patients, and those four patients achieved PR in the single agent treatment of gefitinib. These results were compatible with previous reports.[16,20,21]

Thirty patients received second-line chemotherapy, including platinum-based (n = 27) and nonplatinum-based (n = 3) regimens; the response rate was 30%. Pretreatment with gefitinib does not seem to adversely affect the response of second-line chemotherapy. However, our small-scale study does not suggest the best second-line regimen. Platinum combined with any third-generation agents including paclitaxel, docetaxel, vinorelbine,

Table 6. Mutation Status of the *EGFR* Gene

Sex	Age (years)	Pathologic Type	Smoking Status	Overall Survival (months)	*EGFR* Gene	Effect of Mutation	Response to Gefitinib	Response to Second Line Chemotherapy
M	68	AD	Current	14.9	Deletion of 15 nucleotides (2236-2250)	In-frame deletion (E746-A750)	PR	PD
F	67	AD	Current	16.2	Deletion of 15 nucleotides (2236-2250)	In-frame deletion (E746-A750)	PR	PD
F	54	AD	Current	5.6	Deletion of 18 nucleotides (2238-2255) and substitution of T for A at nucleotides 2237	In-frame deletion (L747-S752) and amino acid substitution (F746V)	PR	NR
F	57	AD	Never	25.4	Substitution of G for T at nucleotide 2573	Amino acid substitution (L858R)	PR	SD
M	61	AD	Current	7.5	Wild	—	SD	SD
M	54	AD	Current	9.7	Wild	—	SD	SD
M	45	AD	Current	16.2	Wild	—	SD	PR
M	59	AD	Current	14.7	Wild	—	SD	PR
M	67	SQ	Current	2.4	Wild	—	SD	NR
M	59	AD	Current	24.9	Wild	—	SD	PR
M	61	AD	Current	2.4	Wild	—	PD	NR
F	61	SQ	Current	3.4	Wild	—	PD	PD
F	61	AD	Current	16.3	Wild	—	PD	PR

Abbreviations: EGFR, epidermal growth factor receptor; M, male; F, female; AD, adenocarcinoma; SQ, squamous cell carcinoma; PR, partial response; SD, stable disease; PD, progressive disease; NR, not received.

gemcitabine, or irinotecan is probably acceptable as the current standard first-line chemotherapy.

First-line single agent with gefitinib is active, but produces unacceptably frequent ILD in the Japanese population. Being female, as well as adenocarcinoma, those who never smoked, and *EGFR* mutation were associated with response to gefitinib. Patients who responded to gefitinib did not experience ILD during gefitinib chemotherapy. Further research via genetics and image analysis is needed to avoid ILD and identify a subgroup of patients that benefit from gefitinib treatment. If this is realized, single agent treatment with gefitinib could be an option as first-line chemotherapy in selected patients with advanced NSCLC. Furthermore, randomized trials are warranted to compare first-line single agent treatment with gefitinib followed by second-line platinum-based chemotherapy with first-line platinum-based chemotherapy followed by second- or third-line gefitinib treatment.

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Small-Cell Lung Cancer: Current Therapy and Novel Agents

ABSTRACT

Among patients with lung cancer, approximately 15% have small-cell lung cancer (SCLC). Although, without therapy, untreated SCLC is a rapidly proliferating tumor with a poor prognosis, response rates to chemotherapy and radiotherapy are high. SCLC is usually staged as either limited disease or extensive disease. Extensive disease is treated primarily with chemotherapy. A recent Japanese randomized trial compared IP (irinotecan [Camptosar]/cisplatin [Platinol]) with EP (etoposide/cisplatin). Patients in the IP arm had significantly better outcomes than patients in the EP arm. In the IP arm, the response rate was 84%, and the median overall survival period was 12.8 months. Limited disease is usually treated with concurrent chemotherapy and accelerated radiation therapy, and approximately 20% of patients are cured. Further investigations to improve local control and inhibit distant metastasis are clearly warranted. The dose-rate escalation in radiotherapy (administered concurrently with chemotherapy) is important in improving local control, and the introduction of molecular-targeting agents is necessary to inhibit distant metastasis.

An estimated 75,000 new cases of lung cancer were diagnosed in Japan in the year 2002, and approximately 15% of these cases were small-cell lung cancer (SCLC). Like non–small-cell lung cancer, SCLC is strongly associated with tobacco use. Left untreated, SCLC is a rapidly proliferating tumor with a poor prognosis, but response rates to chemotherapy and radiotherapy are high. SCLC is usually staged as either limited or extensive disease. The standard treatment for extensive disease is chemotherapy, and for limited disease is chemotherapy plus radiotherapy.

Nearly one-third of patients with SCLC present with limited disease, which is defined as disease confined to one hemithorax, without pericardial or pleural effusion, that can be encompassed by a single radiotherapy port.[1] Limited disease is a potentially more curable form of SCLC than extensive disease, and yet, before the use of chemotherapy, patients diagnosed with limited-stage SCLC survived an average of only 3 months.

History of Chemotherapy for SCLC

In the 1960s and 1970s, when cyclophosphamide (Cytoxan, Neosar) therapy was shown to be superior to best supportive care, the CAV regimen (cyclophosphamide/doxorubicin [Adriamycin]/vincristine) became standard treatment for SCLC. In the late 1970s, the effectiveness of EP chemotherapy (etoposide/cisplatin [Platinol]) was demonstrated in patients resistant to cyclophosphamide, and in the 1980s, EP became the standard rather than cyclophosphamide-based therapy. Given its mild side effects, the combination of radiotherapy and EP (without the need for drug dose reductions) subsequently became the standard approach.

In the late 1990s, the antitumor activities of new drugs such as paclitaxel, irinotecan (Camptosar), topotecan (Hycamtin), and amrubicin (investigational in the United States) were demonstrated in SCLC. Ongoing investigations are exploring the use of these agents, and the possibility that they might be more effective than EP.

Table 1

New Agents for the Treatment of Small-Cell Lung Cancer

Authors	Drug	Number of Patients	Prior Therapy	Response Rate
Negoro et al, 1991[5]	Irinotecan	27	Yes	33%
	Irinotecan	8	No	50%
Masuda et al, 1992[6]	Irinotecan	15	Yes	47%
Smyth et al, 1994[7]	Docetaxel	28	Yes	25%
Cormier et al, 1994[8]	Gemcitabine	26	No	27%
Ettinger et al, 1992[9]	Ifosfamide	43	No	49%
Ettinger et al, 1995[10]	Paclitaxel	32	No	34%
Kirschling et al, 1994[11]	Paclitaxel	37	No	41%
Ardizzoni et al, 1997[12]	Topotecan	47	Yes	6.4%
Schiller et al, 1996[13]	Topotecan	48	No	39%
Jassem et al, 1993[14]	Vinorelbine	25	Yes	16%
Furuse et al, 1996[15]	Vinorelbine	24	Yes	12.5%

In comparing state-of-the-art therapies for extensive and limited SCLC from 1981 and 2003, both median survival and 3-year survival rates have shown improvement. In this dataset, patient selection bias would presumably be a significant factor, but progress in therapy is critical to such improvement. The incorporation of new agents has been crucial in the establishment of more effective therapy.

Treatment of Extensive-Stage SCLC

Cisplatin and Etoposide

Platinum-based chemotherapy is the mainstay of treatment regimens for extensive disease. In a meta-analysis of 19 randomized trials comparing a cisplatin-based regimen with a non–cisplatin-based regimen, patients randomized to the regimen containing cisplatin had a significantly higher probability of response and survival, with no significant increase in toxicity.[2] Berghmans et al presented a detailed analysis of the roles of etoposide and cisplatin in the treatment of SCLC.[3] Thirty-six eligible trials were performed between 1980 and 1998; 1 trial compared cisplatin with no cisplatin, 17 compared etoposide with no etoposide, 9 compared cisplatin plus etoposide with no cisplatin plus etoposide, and 9 compared cisplatin plus etoposide with etoposide alone. These trials concluded that the use of cisplatin and/or etoposide offered a significant survival advantage to patients with SCLC.

In another meta-analysis, Chute et al evaluated all 21 cooperative group phase III trials performed in North America between 1972 and 1993.[4] Patients with extensive disease who were treated during a similar time interval and were listed in the Surveillance, Epidemiology, and End Results (SEER) database were also included in the analysis. The median survival time of patients in the control arms of the phase III trials initiated between 1972 and 1981 was 7.0 months; for those enrolled in control arms between 1982 and 1990, the median survival was 8.9 months ($P = .001$). Trends i the number of trials and the survive periods of patients over time wer examined. A modest 2-month prolor gation in median survival was dem onstrated in patients with extensiv disease. This improvement in survi al was independently associated wit both a cisplatin-based regimen an improvement in best supportive car measures.

Several other agents with signif cant activity in SCLC were studied i the 1990s (Table 1).[5-15]

Irinotecan

Irinotecan is a derivative of camp tothecin, an inhibitor of nuclea enzyme topoisomerase I. Topoisom erase I creates single-strand breaks i DNA during DNA replication. Tw trials have evaluated the use of irinc tecan in patients with SCLC.[5,6] Ne goro et al evaluated 35 patients, 27 c whom had received prior treatment.[Responses were seen in 9 of the 2 previously treated patients and 4 c the 8 previously untreated patient The principal toxicities were neutr penia and diarrhea. Masuda et al stu ied 16 previously treated patients wi SCLC.[6] Irinotecan (100 mg/m^2) w administered weekly, with dosag adjusted for toxicity. Responses we seen in 7 of the 15 evaluable patient producing an overall response rate 47%. The principal toxicities were d arrhea and neutropenia. Two patien suffered from grade 3 or 4 pulmona toxicity, and one of these patients su sequently died.

Irinotecan's mechanism of actic is complementary to that of cisplati Studies in preclinical models ha shown that these two agents exhit synergistic activities, and their toxic ty profiles also show minimal ove lap. For these reasons, irinotecan w an ideal drug for clinical trials wi cisplatin as a first-line combin therapy.[16-18]

A phase II trial of cisplatin pl irinotecan as first-line combined the apy in patients with SCLC (includi 35 patients with extensive diseas was conducted by the West Japan Th racic Oncology Group. In this tri both agents were administered at dose of 60 mg/m^2; irinotecan was a

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ministered on days 1, 8, and 15 of each 28-day cycle, and cisplatin was administered on day 1.[19] For patients with extensive disease, the overall response rate was 86%, with 29% of patients achieving complete responses. The median survival was 13.0 months, with a 2-year survival rate of 17.5%.

Based on the results of this phase II trial, the Japan Clinical Oncology Group (JCOG) conducted a multi-institutional randomized phase III trial (JCOG-9511), comparing IP with EP in patients with previously untreated extensive disease.[20] The patient characteristics in this trial included an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2 and an age of 70 years or less. Patients with symptomatic central nervous system metastases requiring radiation or corticosteroid treatment were excluded from the study. The experimental arm consisted of irinotecan (60 mg/m^2) administered on days 1, 8, and 15 of each 4-week cycle, with cisplatin (60 mg/m^2) administered on day 1 for a total of four 4-week cycles (IP). This treatment regimen was compared with a regimen of etoposide (100 mg/m^2) administered on the first 3 days of each 3-week cycle, with cisplatin (80 mg/m^2) administered on day 1 for a total of four 3-week cycles (EP). The principal end point was overall survival.

The projected accrual for this trial was 230 patients (115 patients per arm). An interim analysis conducted after 77 patients had been accrued in each arm showed a significant survival advantage for the IP arm. Therefore, further enrollment in the trial was discontinued. The response rate was significantly higher in the IP arm than in the EP arm (84% v 68%; P = .02). Additionally, the IP arm showed a statistically significant improvement in progression-free survival (6.9 vs 4.8 mo; P = .003) and median overall survival (12.8 vs 9.4 mo; P = .002; Figure 1).

The results of JCOG 9511 were the most provocative ever seen in patients with previously untreated SCLC. The IP regimen is thus another platinum-based combination that should be considered for the treatment of extensive disease. Appropriately, the combination of cisplatin and irinotecan has become the new standard treatment for patients with extensive disease in Japan.

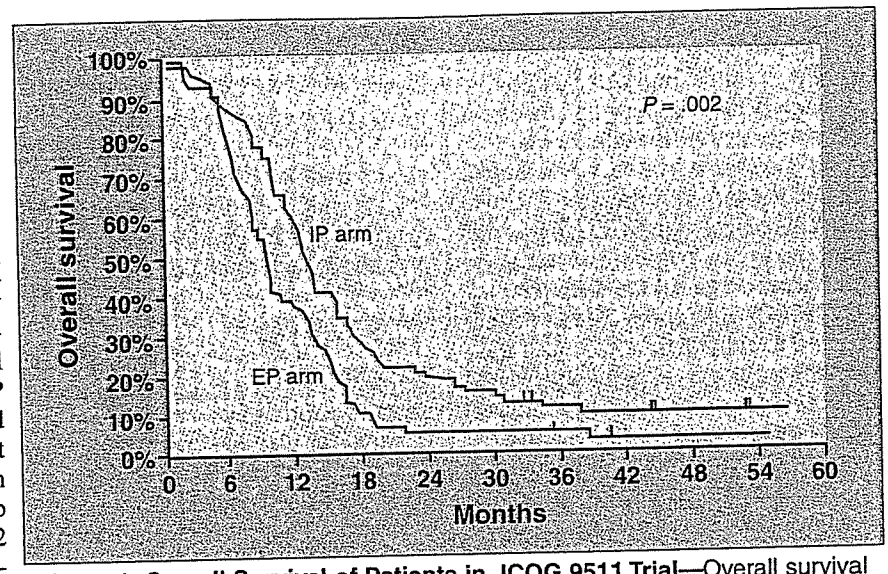

Figure 1: Overall Survival of Patients in JCOG 9511 Trial—Overall survival of patients with extensive small-cell lung cancer assigned to treatment with irinotecan plus cisplatin (IP) or etoposide plus cisplatin (EP). JCOG = Japan Clinical Oncology Group.

However, several points must be examined before the IP regimen is established as standard therapy worldwide. Clinical studies have been initiated to confirm these promising results by three groups: Pfizer, the Southwest Oncology Group (SWOG), and Aventis. The Pfizer group reported the interim results of this study as a North American study at ASCO 2003.[21] These investigators observed superior compliance and lower toxicity, as compared to the findings of JCOG 9511.

Amrubicin

Another excellent antitumor agent developed in Japan is amrubicin. In single-agent therapy, this drug produced a high response rate of 75.8% in extensive disease (median survival: 11.7 mo).[22] A phase II study of amrubicin plus cisplatin demonstrated a response rate of 86.8%.[23]

Etoposide/Cisplatin–Based Combinations

Another approach to improving efficacy is the addition of new agents to EP therapy. Phase II/III studies of a regimen known as PET (cisplatin, etoposide, paclitaxel [Taxol]) have been performed in the United States. In a phase II study with 80 patients, the response rate was 57%, median survival was 11 months, and treatment-related death was 14%.[24] The results of a controlled study comparing EP with PET in 572 patients showed a slightly superior complete response rate in the PET arm, but there was no difference in median survival or the 1-year survival rate between the two therapy arms, Moreover, grade 5 toxicity was higher in the PET arm.[25].

A phase II trial of irinotecan/cisplatin/etoposide (IPE) administered weekly (arm A) or every 4 weeks (arm B) for extensive disease (JCOG 9902-DI) was also performed. In arm B, the response rate was 77%, and median survival was 12.9 months.[26] A randomized trial comparing IP with IPE administered every 3 weeks in patients with previously untreated extensive disease is currently being conducted in Japan.

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Molecular-Targeted Therapy

Although there are many controlled studies comparing dose-intensive weekly chemotherapy with non–cross-resistant alternative chemotherapy, solid data have yet to be obtained. Molecular-targeted therapy has also been investigated in SCLC, but at this time, no agents in this class have proven effective against the disease.

There are no reported cases of a response to either a farnesyl transferase inhibitor among previously treated SCLC patients, or to imatinib mesylate (Gleevec) among untreated SCLC patients. A controlled study comparing marimastat (investigational in the United States) after four cycles of EP with a placebo showed no difference in survival and significantly greater toxicity in the marimastat arm.[27] At ASCO 2003, the optimal dose of oblimersen sodium (Genasense), an investigational Bcl-2 inhibitor, was evaluated in combination therapy with etoposide and carboplatin (Paraplatin). A randomized trial to assess the combination of oblimersen, etoposide, and carboplatin in SCLC will be conducted by the Cancer and Leukemia Group B (CALGB).[28] Furthermore, neither vascular endothelial growth factor (VEGF) nor cyclooxygenase (COX)-2 expression is reportedly a significant prognostic factor in SCLC.[29]

Reference Guide

Therapeutic Agents Mentioned in This Article

Amrubicin
Carboplatin (Paraplatin)
Cisplatin
Cyclophosphamide (Cytoxan, Neosar)
Doxorubicin
Etoposide
Imatinib mesylate (Gleevec)
Irinotecan (Camptosar)
Marimastat
Oblimersen sodium (Genasense)
Paclitaxel
Topotecan (Hycamtin)
Vincristine

Brand names are listed in parentheses only if a drug is not available generically and is marketed as no more than two trademarked or registered products. More familiar alternative generic designations may also be included parenthetically.

Recurrent Disease

Most SCLC patients are destined to relapse, and the prognosis of these relapsed patients is poor. No standard therapy for patients with recurrent SCLC after a complete response has been established. Patients who relapse less than 3 months after first-line therapy are commonly called refractory, and those who relapse at least 3 months after therapy are called sensitive. Sensitive cases may be re-treated with the same induction regimen used initially. Refractory patients who are in satisfactory clinical condition should be offered a second-line regimen. Such patients are usually registered in a phase I or phase II study of new antitumor agents, such as topotecan, irinotecan, or paclitaxel.[30-32]

Elderly Patients

The proportion of elderly patients among all lung cancer cases is rapidly increasing with the aging of society. In many of these cases, general chemotherapy is not feasible due to unsatisfactory performance status, and treatment of such patients should be a focus of future research. Although there is no difference in survival between elderly and other populations, the death rate due to toxicity is high in the elderly population. A trial of oral etoposide among patients who did not respond to CAV was reported (and its use in elderly patients was anticipated), but a later controlled study revealed higher toxicity and less efficacy with the oral agent, compared to standard chemotherapy.[33,34] Recently, oral etoposide has not been used as a single agent.

Treatment of Limited-Stage SCLC

SCLC has long been recognized to be clinically responsive to radiation therapy, and in vitro irradiation of SCLC cell lines has shown that they often have a greater intrinsic radiosensitivity than adenocarcinomas or squamous cell lung cancer cell lines. Consequently, many early trials combining radiation therapy with chemotherapy in patients with SCLC used low total radiation dosages.

A number of trials conducted in the 1970s and 1980s compared chemotherapy alone with chemotherapy and thoracic radiation therapy in patients with limited disease. These trials varied with regard to the radiation dosage, timing, and choice of chemotherapeutic agents used. Warde and Payne analyzed these trials and found that the addition of thoracic radiation therapy improved local control and survival rates.[35] Pignon et al obtained individual patient data from these trials and updated the analyses after their original publication.[36] They found that the addition of thoracic radiation therapy increased the 3-year survival rate from 8.9% to 14.3%—an absolute improvement of 5% and a relative improvement of nearly 50%.

In the 1990s, several trials examined whether radiation therapy and chemotherapy should be administered concurrently or sequentially and whether radiation therapy should be administered early or late in the overall course of treatment. Murray and Coldman performed a meta-analysis of trials that combined chemotherapy and thoracic radiation therapy, using 3-year progression-free survival as a surrogate end point for long-term survival.[37] The best results were seen when thoracic radiation therapy was administered 3 to 5 weeks after the start of chemotherapy. When radiation therapy was further delayed, the survival benefit decreased and approached that seen with chemotherapy alone.

The rapid growth of many SCLC cell lines encouraged the exploration of accelerated radiation treatment schedules, with two fractions administered per day and a modest reduction in fraction size from the usual 1.8–2.0 Gy to 1.5 Gy. Two prospective trials compared this approach with conventional daily fractionation. Tur-

risi et al compared 45-Gy doses administered in 25 fractions for more than 5 weeks with 45-Gy doses administered in 30 fractions for more than 3 weeks. The chemotherapy regimen in this study consisted of four cycles of cisplatin plus etoposide. The accelerated regimen resulted in an improved local control rate (intrathoracic failure in the accelerated therapy arm was 36%; in the standard therapy arm, 52%) and 5-year survival rate (twice-daily regimen: 26%; standard regimen: 16%). Although an increased incidence of grade 3 esophagitis (26% vs 11%, respectively) was observed, no other significant differences in toxicity were seen.[38]

Conclusions

The incidence of SCLC has been decreasing. In 1998, SCLC reportedly accounted for only 13.8% of all lung cancers. A two-tiered staging system is generally utilized for diagnosis. Platinum-based chemotherapy is the standard treatment for extensive disease. The cisplatin/irinotecan combination has become the new standard treatment for patients with extensive disease in Japan. Limited disease is treated with concurrent chemotherapy and accelerated radiation therapy, enabling approximately 20% of all patients to be cured.

Future investigation of strategies to improve local control and inhibit distant metastasis is warranted. To improve local control, researchers need to explore dose-rate escalation of radiotherapy administered concurrently with chemotherapy, and for inhibiting distant metastasis, molecular-targeting agents are an important approach.

This article is reviewed on pages 52, 55, and 57.

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The Horiike/Saijo Article Reviewed

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As described by Horiike and Saijo, small-cell lung cancer (SCLC) is commonly divided into limited and extensive disease. Patients with limited disease are generally treated with combined-modality therapy, whereas those with extensive disease are treated with chemotherapy alone. However, different definitions of limited and extensive disease exist. The widely used Veterans Administration Lung Study Group (VALSG) staging system defines limited disease as disease confined to the ipsilateral hemithorax, excluding contralateral supraclavicular and hilar adenopathy. Alternatively, the International Association for the Study of Lung Cancer (IASCL) includes all patients without distant metastatic disease in this category.

This is a clinically relevant distinction, as treatment options and prognostication may differ. One report comparing the outcome of patients with limited disease by VALSG or IASCL criteria suggests that the IASCL classification may provide more accurate prognostic stratification.[1] Of note, patients with limited disease and contralateral nodal involvement are often excluded from enrollment in clinical trials.

Limited-Stage SCLC

The standard treatment of limited disease in the United States consists of four cycles of EP (etoposide, cisplatin [Platinol]) every 3 weeks with concurrent thoracic radiotherapy. Clinical trials investigating sequential non–cross-resistant chemotherapy, dose intensity, dose-dense administration, and "maintenance" chemotherapy beyond four cycles have not consistently demonstrated benefit. At this time, the routine use of cytokines to maintain or increase dose intensit is not evidence-based and may be det rimental when used with thoraci radiotherapy.[2]

Thoracic radiotherapy is initiate during the first or second cycle o chemotherapy, as controlled clinica trial data indicate early irradiation of fers a survival advantage. If radio therapy is initiated after the first cycle limited radiation fields to postchemo therapy tumor volume appear to b adequate. Horiike and Saijo sugges that hyperfractionated administratio of thoracic radiation may be superio to a conventional daily schedule, base on an Eastern Cooperative Grou study comparing the two approaches which found improved local contro and median survival with the acceler ated regimen.[3] However, the tota dose of radiation used (45 Gy) wa the same in both arms; higher dose of radiation are generally recommend with once-daily schedules. Hyper fractionated thoracic radiation t 45 Gy or once-daily treatment to 50 70 Gy with concurrent chemotherap are both acceptable approaches in th United States.[4] Ongoing clinical tr

Continued on page 5

als are investigating escalating doses of radiation, the role of radioprotectants such as amifostine (Ethyol), and alternating regimens of chemotherapy and thoracic radiation.

Prophylactic cranial irradiation (PCI) is usually offered to patients with limited disease after a complete or near-complete response. Numerous clinical trials support PCI in this setting, with a meta-analysis of seven randomized trials showing an absolute decrease of 25% (from 58.6% to 33.3%) in the cumulative incidence of brain metastases at 3 years and an absolute increase in overall survival of 5%.[5] Limited data from these trials suggest that PCI up to 36 Gy in small fractions is well tolerated with few neuropsychological sequelae if given sequentially rather than concurrently with chemotherapy. Controlled data for the delayed effects of sequential PCI on cognitive function are awaited.

Extensive-Stage SCLC

Presently, the standard first-line treatment of extensive disease in the United States is four to six cycles of etoposide and a platinum-containing compound. Many oncologists substitute carboplatin (Paraplatin) for cisplatin, as it is less toxic and appears to have similar efficacy. The only randomized study comparing EP to etoposide/carboplatin found no significant difference in response or survival and less toxicity with carboplatin, but the trial was underpowered to show equivalence in either limited or extensive disease.[6,7].

An alternative to EP is IP (irinotecan [Camptosar], cisplatin). Horiike and Saijo summarize the Japan Clinical Oncology Group trial comparing EP to IP, which was terminated after an interim analysis of 154 patients found significant improvement in response rate, progression-free survival, and median survival in the IP group. The results of an ongoing confirmatory Southwest Oncology Group phase III trial may change the standard of care in the United States. The use of carboplatin with irinotecan is also being investigated.

Salvage Therapy

Although most patients respond to chemotherapy, the median duration of response is only 6 to 8 months. Salvage therapy can provide palliation with improved quality of life and possibly longer survival, depending on performance status, tumor extent, and interval between first-line therapy and relapse/progression. However, the toxicities of chemotherapy can be substantial and responses short. An open discussion with shared medical decision-making, including consideration of a patient's realistic assessment and physician recommendation, is essential.

Based on data from a phase III trial involving 211 patients, topotecan (Hycamtin) has largely replaced the CAV regimen (cyclophosphamide [Cytoxan, Neosar], doxorubicin, vincristine) in the second-line treatment of progressive SCLC.[8] Although both regimens had similar response rates and median survivals, topotecan resulted in better control of disease-related symptoms. Topotecan has also led to regression of brain metastases in some patients with extensive disease.[9] Other regimens for progressive disease incorporate paclitaxel/docetaxel (Taxotere), gemcitabine (Gemzar), vinorelbine (Navelbine), ifosfamide (Ifex), and most recently, premetrexed (Alimta). Although some of these regimens have resulted in minimal improvements in survival, associated toxicities do not support their routine use. Horiike and Saijo describe the promising activity of amrubicin, a new anthracycline developed in Japan. A phase III trial comparing amrubicin and cisplatin to IP is planned.[10]

Targeted Therapy

Novel targeted therapies for SCLC are actively being explored. Numerous targets have been identified, including the c-kit receptor, vascular endothelial growth factor receptor, neural cell adhesion molecule (NCAM), gastrin releasing peptide (GRP), matrix metalloproteinases (MMPs), retinoid signaling pathway, bcl-2, and various gangliosides. Initial approaches using the c-kit inhibitor imatinib (Gleevec); the antisense oligonucleotide against bcl-2, oblimersen (Genasense); the MMP inhibitor marimastat; the synthetic retinoid fenretinide; and the BEC2/BCG vaccine against the G3 ganglioside have yet to show benefit in phase II and III clinical trials. Other agents being developed include toxin-conjugated antibodies against the neural cell adhesion molecule (NCAM), gastin-releasing peptide (GRP) antibodies, and replacement of lost retinoblastoma protein or p53 function with gene therapy.

Conclusions

Although there has been progress in treating SCLC over the past 25 years, overall prognosis is still poor. Current trials are exploring innovative chemoradiation schedules with dose escalation of both chemotherapy and radiation in limited disease. Irinotecan and other newer agents are actively being evaluated in extensive disease. Targeted therapy to date has been unsuccessful but holds promise for the future.

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The Horiike/Saijo Article Reviewed

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Lung cancer is the most common cause of cancer death in the United States, with a projected 160,000 or more Americans dying of the disease in 2004. Small-cell lung cancer (SCLC) comprises approximately 14% of lung cancer cases. In the past 5 years, as outlined by Horiike and Saijo, significant advances have been made both in defining new combination chemotherapy options for extensive-stage SCLC and in optimizing radiotherapy for limited-stage SCLC. Despite these advances, and despite the exquisite sensitivity of SCLC to radiation and chemotherapy, the majority of patients with SCLC still die within 2 years of diagnosis. Clearly new approaches are needed.

Characteristic molecular alterations are present in the large majority of SCLC cases. These include upregulation of bcl-2, overexpression of IGF-1R, activating mutation of Rb, and inactivation of p53. These oncogenic alterations in key regulators of cell survival and proliferation are each present in 80% to 95% of patients with SCLC and together offer opportunities for selective targeted anticancer therapy. To date, only a small subset of these possible targets have been explored therapeutically.

Apoptotic Pathways

Overexpression of bcl-2, a central regulator of apoptotic induction, is a key molecular alteration associated with chemotherapeutic resistance and poor outcome in SCLC. Preclinical models suggest that suppression of bcl-2 in SCLC augments the sensitivity of SCLC to standard chemotherapeutic agents. We have conducted phase I studies in SCLC patients evaluating the combination of oblimersen (Genasense)—an antisense oligonucleotide directed against bcl-2 mRNA—either with paclitaxel or with etoposide and carboplatin (Paraplatin).[1] These studies have supported an ongoing randomized trial by the Cancer and Leukemia Group B assessing carboplatin and etoposide with or without oblimersen in untreated extensive-stage SCLC.

Inhibition of nuclear factor (NF)-kappaB, another key survival factor, also promotes tumor cell apoptosis. Bortezomib (Velcade) is a novel proteasome inhibitor that, among other effects, suppresses NF-kappaB activation by stabilizing the inhibitory regulator of NF-kappaB, I-kappaB kinase.[2] A phase II trial of bortezomib in previously treated SCLC patients is currently being conducted by the Southwest Oncology Group.

Failure to proceed through the G1 cell-cycle checkpoint triggers an apoptotic response in cancer cells. CCI-779 is a novel agent that inhibits the translation of multiple proteins, including critical factors that regulate progression through the G1 checkpoint.[3] A phase II trial of CCI-779 as maintenance therapy for patients with extensive SCLC after response to initial therapy has completed accrual. The results of this study should be available in the next year.

Signal Transduction

Activation of the receptor tyrosine kinase c-kit has been found in multiple malignancies including SCLC. Trials of imatinib (Gleevec)—a high-affinity inhibitor of c-kit—in SCLC

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have shown no benefit either as a single agent or in combination with chemotherapy.[4] Recent data suggesting that c-kit exon 9 and 11 mutations (as opposed to overexpression) are present in about 10% of SCLC cases suggest that reevaluation of imatinib specifically in this subset of patients may be warranted.[5]

Other receptor tyrosine kinases that have been strongly implicated in SCLC survival and proliferative signaling include c-MET and IGF-1R. Agents specifically targeting these alternative receptor tyrosine kinases are in preclinical and very early-phase clinical evaluation.

Angiogenesis

Serum levels of the critical angiogenic regulator vascular endothelial growth factor (VEGF) have been correlated with vessel density, advanced disease stage, and poor outcome in SCLC.[6-8] The Eastern Cooperative Oncology Group is currently conducting a phase II study of the anti-VEGF monoclonal antibody bevacizumab (Avastin) with cisplatin and etoposide. Analysis of an oral small molecule inhibitor of VEGF (ZD6474) in SCLC patients after response to first-line therapy has been initiated by the National Cancer Institute of Canada.

Thalidomide (Thalomid) functions in part as an angiogenesis inhibitor and appears to be well tolerated in combination with carboplatin and etoposide in patients with SCLC.[9] A phase III trial of this combination is also under way.

Immunotherapy

The consistency of molecular alterations associated with SCLC, serving as potential tumor-specific antigens, has made SCLC an attractive target for immunotherapy. Results to date however have been disappointing. Two recent large randomized phase III trials, including evaluation of the anti-idiotype antibody BEC2, have had clearly negative results.[10]

Conclusions

Small-cell lung cancer is an aggressive disease despite initial sensitivity to chemotherapy and radiation. The recent elucidation of key biologic determinants of SCLC carcinogenesis has suggested targets for novel therapeutic development that may translate into improved outcome for patients with this disease. Further efforts should focus on exploitation of these key oncogenic determinants.

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—Charles M. Rudin, MD, PhD

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A third review, by Drs. Dubey and Schiller, appears on the following page.

The Horiike/Saijo Article Reviewed

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In this article, Drs. Horiike and Saijo provide a comprehensive review of the treatment of small-cell lung cancer (SCLC). We concur with the authors and would like to highlight certain areas of interest.

The incidence of SCLC has declined over the past several years, the etiology for which is unclear. Nevertheless, it remains a lethal malignancy: More than half of those diagnosed will have distant disease with a 5-year survival rate of approximately 2%.

Extensive Disease

Small-cell lung cancer is an exquisitely chemosensitive disease. Clinical trials have demonstrated the survival benefit of combination chemotherapy over single-agent therapy.[1,2] The authors have addressed the benefit of cisplatin-based chemotherapy in comparison to other regimens. Even though higher doses of etoposide are associated with improved response rates, they have had no impact on survival, and increasing doses of cisplatin have affected neither response rates nor survival.[3,4] Further evidence for the lack of benefit of high-dose chemotherapy in SCLC can be drawn from the negative results obtained after dose-intense chemotherapy using granulocyte colony-stimulating factor (G-CSF, Neupogen) and autologous bone marrow transplant.[5,6] The implication is that higher doses offer no added benefit, at the expense of increased toxicity.

The most compelling evidence of advances in chemotherapeutic management in recent years has been the combination of cisplatin and irinotecan (Camptosar), to which the authors allude. In the study by the Japanese Clinical Oncology Group (JCOG), the cisplatin/irinotecan combination had a median survival advantage of approximately 3 months and a 2-year survival benefit of approximately 15% over the cisplatin/etoposide regimen. An ongoing North American trial using the same drug combination will attempt to confirm these results in the American population. Interim analyses indicate an improved toxicity profile in the North American study. However, the North American study excluded patients with a performance status of 2, whereas 16% of patients in the JCOG study had a performance status of 2. In addition, the North American regimen differed from the JCOG regimen, with both drugs being administered on days 1 and 8 of a 21-day cycle.

The elderly remain a special population. Surveillance, Epidemiology, and End Results (SEER) data indicate that the incidence of SCLC peaks at age 60 to 80 years. The medical comorbidities that frequently accompany patients in this age range necessitate special considerations in treatment. Moderately aggressive regimens using carboplatin (Paraplatin) and etoposide have been well tolerated and have resulted in efficacy comparable to that in younger cohorts. Functional status, and not age alone, should guide the choice of chemotherapy in this subgroup.

Even in the general SCLC population, direct comparisons of cisplatin/etoposide and carboplatin/etoposide have demonstrated similar efficacy and an improved toxicity profile in the carboplatin arm.[7] Thus, while the cisplatin vs carboplatin debate in non–small-cell lung cancer is ongoing, carboplatin-based regimens have become an attractive option in small-cell lung cancer, particularly for those with poor performance status and medical comorbidities.

In the United States, topotecan (Hycamtin) is the Food and Drug Administration (FDA)-approved drug for the treatment of relapsed SCLC. Other regimens that are effective in this setting include combinations of cyclophosphamide (Cytoxan, Neosar), doxorubicin (Adriamycin), and vincristine (the CAV regimen), as well as etoposide, gemcitabine (Gemzar), and paclitaxel. The prognosis of sensitive disease is better than that of refractory disease but continues to be poor.

Brain metastases from SCLC are distinct from those associated with other malignancies. The underlying chemosensitivity coupled with disruption of the blood-brain barrier due to the presence of malignant deposits in the brain make chemotherapy without radiation therapy an appealing therapeutic option. In fact, the response rate of initial brain metastases to chemotherapy is approximately 70%. However, the response rates to chemotherapy alone at relapse decrease to 40%.[8]

Limited Disease

Cure can be achieved in a small number of patients with aggressive combined-modality chemoradiation. Concurrent chemoradiation offers improved benefit over sequential treatment. As discussed by the authors, twice-daily hyperfractionated radiation concurrent with chemotherapy offers the greatest survival benefit.[9] However, patients must be cautiously selected for concurrent and accelerated treatment regimens, given the higher toxicities accompanying these modalities.

Since it appears that, in SCLC, "more is better," trials have been designed to incorporate consolidation chemotherapy after concurrent chemoradiation.[10-13] Consolidation therapies in these trials have included older and newer agents, such as carboplatin/paclitaxel, single-agent cy-

clophosphamide, vincristine/methotrexate/etoposide alternating with doxorubicin/cyclophosphamide, and interferon. Median survival has ranged from 11 to 18 months without evidence of survival benefit. Even the addition of BEC2/BCG vaccination offered no benefit after response to chemotherapy and radiation.[14]

In SCLC patients, the brain is a sanctuary for relapse. The 2- to 3-year cumulative risk of brain metastases after treatment of limited disease is approximately 50%.[15,16] A meta-analysis demonstrated that prophylactic cranial irradiation (PCI) not only decreased the incidence of brain metastases, but also improved survival. On careful examination, neurocognitive deficits have been found in patients prior to PCI, and no significant deterioration was found after PCI.[17,18] In randomized trials, patients who underwent PCI experienced the same neurocognitive difficulties as those who did not, and these difficulties did not interfere with day-to-day functioning. Thus, cognitive decline may be more a reflection of the actual disease process than of the treatment. That said, PCI should be offered to those who have achieved a complete response to chemoradiation.

Conclusions

Although SCLC is chemosensitive, with higher response rates than most other solid tumors, it remains an elusive disease with a propensity to recur and develop resistance. A ceiling has been reached in terms of combination and intensification of chemotherapeutic regimens. Nevertheless, there has been some progress against SCLC.[19]

Future approaches include exploitation of novel targeted agents such as Bcl-2 antisense oligonucleotide, tyrosine-kinase inhibitors, vascular endothelial growth factor (VEGF) inhibitors, and proteasome inhibitors. We agree with the authors that more patients need to be enrolled in clinical trials. The major challenge with targeted agents is that their cytostatic rather than cytotoxic nature makes it difficult to use conventional criteria to assess response. Surrogate markers are needed to evaluate efficacy. The fact that patients with SCLC represent a minority of the lung cancer cohort may provide an additional challenge in accrual to larger trials.

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—Joan H. Schiller, MD

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SNP Communication

Genetic Polymorphisms of UGT1A6 in a Japanese Population

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Summary: Thirteen single nucleotide polymorphisms (SNPs), including 6 novel ones, were found in exon 1 and its flanking region of UDP-glucuronosyltransferase (UGT) *1A6* from 195 Japanese subjects. Several novel SNPs were identified, including 269G>A (R90H), 279A>G (S93S), and 308C>A (S103X) in exon 1, and IVS1+109C>T, IVS1+120A>G, and IVS1+142C>T in the intron downstream of exon 1. Among these SNPs, 308C>A confers termination of translation at codon 103, resulting in the production of an immature protein that probably lacks enzymatic activity. The allele frequencies were 0.003 for all the 6 SNPs. In addition, the 3 known nonsynonymous SNPs were detected: 19T>G (S7A), 541A>G (T181A), and 552A>C (R184S) with frequencies of 0.226, 0.218, and 0.226, respectively. High linkage disequilibrium was observed among 19T>G (S7A), 315A>G (L105L), 541A>G (T181A), 552A>C (R184S), and IVS1+130G>T, as reported in Caucasian and African-American populations. Then, 11 haplotypes in *UGT1A6* were estimated. The novel nonsynonymous variant, 269A or 308A, was shown to be located on the same DNA strand together with 19G, 315G, 541G, 552C, and IVS1+130T. Our results provide fundamental and useful information for genotyping *UGT1A6* in the Japanese, and probably Asian populations.

Key words: *UGT1A6*; single nucleotide polymorphisms; amino acid alteration; nonsense alteration

On October 25, 2004, these variations were not found on the UDP Glucuronosyltransferase home page (http://som.flinders.edu.au/FUSA/ClinPharm/UGT/), the Japanese Single Nucleotide Polymorphisms (JSNP) (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB (http://www.pharmgkb.org/do/) databases.

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Table 1. Primers utilized for *UGT1A6* amplification and sequencing

	Direction	Primer Name	Sequences	Position[a]
1st Amplification	forward	UGT1A6ZF	TTCTGTAGGGACTTCTGGGACTA	107550
	reverse	UGT1A6ZR	TCAGGAGGGCATCTGTAAACACT	111117
2nd Amplification	forward	UGT1A6-1stF	TAACTTTTCAGAGAGGGAGAAGC	109328
	reverse	UGT1A6-1stR	ACTTCAGCCTCAGGTCTCCTAT	110642
Sequencing	forward	UGT1A6-1stF	TAACTTTTCAGAGAGGGAGAAGC	109328
	forward	UGT1A6seqF2	TGACAAGCTGCTGGTGGTC	109687
	forward	UGT1A6seqF3-2	CCCAGACCCTGTGTCCTACAT	110164
	reverse	UGT1A6seqR1	AAAGCAAATTAACTTCAGGCA	109808
	reverse	UGT1A6seqR2	ACAAATTAACAAGGAAGTTGGC	110258
	reverse	UGT1A6-1stR	ACTTCAGCCTCAGGTCTCCTAT	110642

[a] The position of the 5′ end of each primer on AF297093.1

Introduction

The UDP-glucuronosyltransferase enzymes (UGTs) are members of a superfamily of membrane-anchored enzymes located in the endoplasmic reticulum that catalyze the conjugation of glucuronic acid to a nucleophilic substrate.[1] Glucuronidation is important for the detoxification and elimination of a large number of endogenous and exogenous compounds. So far, two *UGT* gene subfamilies have been identified in humans, *UGT1* and *UGT2*.[2] The human *UGT1A* gene cluster is located on chromosome 2q37 and consists of at least 13 different exon 1's, including four inactive exon 1's (1A2P, 1A11P, 1A12P, and 1A13P), and common exons 2 to 5. One of the exon 1's can be spliced onto the other common exons.[3] The *N*-terminal domains (encoded by the exon 1's) of the *UGT1A* proteins determine their substrate-binding specificity, and the *C*-terminal domain (encoded by the exons 2 to 5) is important for binding to UDP-glucuronic acid.[4]

UGT1A6 is expressed in liver, bile ducts, stomach, colon, kidney, and brain.[1,5,6] *UGT1A6* plays important roles in the elimination of phenols and amines, including 1-naphthol, irinotecan, acetaminophen, and β-blockers.[1,7-10] In addition, 5-hydroxytryptamine (serotonin) was reported as an endogenous substrate of *UGT1A6*.[6] Several genetic polymorphisms with functional changes were reported for *UGT1A6*. Firstly, Ciotti *et al.* reported two nonsynonymous single nucleotide polymorphisms (SNPs) 541A>G (T181A) and 552A>C (R184S), both of which are in high (but not complete) linkage disequilibrium with each other.[10] These variations are located in the putative endoplasmic reticulum-localization signal.[11] The variant enzyme with the two amino acid alterations had reduced activities *in vitro* for 4-nitrophenol, methylsalicylate, 3-*O*-methyl-dopa, and β-blockers, such as propranolol, whereas it had almost comparable activities for 3-iodophenol compared to the wild-type enzyme. On the other hand, a recent study showed that the other SNP, 19T>G (S7A), located in the N-terminal signal sequence, was also highly linked with 541 A>G (T181A) and 552A>C (R184S).[12] The homozygous variant enzyme with all 3 SNPs (corresponding to the homozygote) glucuronidated 4-nitrophenol approximately two-fold higher than the wild-type *in vitro*. Interestingly, concomitant expression of both the wild-type and the variant with these 3 SNPs (corresponding to the heterozygote) showed decreased activity *in vitro*.

Though UGT1A6 is important for detoxification of many compounds, comprehensive sequence analysis for the genetic polymorphisms of *UGT1A6* in Asian populations, which includes the Japanese, is currently lacking. In this study, exon 1 of *UGT1A6* was sequenced from 195 Japanese subjects. Six novel SNPs, including two nonsynonymous ones, were identified from this sequence analysis.

Materials and Methods

Human genomic DNA samples: All 195 subjects in this study were Japanese patients with various solid cancers (88 subjects), who were administered irinotecan, or patients with arrhythmia (107 subjects), who were administered anti-arrhythmic drugs and β-blockers. Genomic DNA was extracted from blood leukocytes and used as a template for the polymerase chain reaction (PCR). All of the ethics committees of the National Cancer Institute, the National Cardiovascular Center, and the National Institute of Health Sciences approved this study. Written informed consent was obtained from all participating subjects.

PCR conditions for DNA sequencing: First, exon 1 of *UGT1A6* was amplified from genomic DNA (150 ng) using 2.5 units of *Z*-Taq (Takara Bio. Inc., Shiga, Japan) with 0.2 μM of the 1st amplification primers (see **Table 1**). The first PCR conditions consisted of 30 cycles of 98°C for 5 sec, 55°C for 5 sec, and 72°C for

Table 2. Summary of *UGT1A6* SNPs detected in a Japanese population

SNP ID				Position			Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Number of subjects			
This Study	dbSNP (NCBI)	Pharm GKB[c]	Reference	Location	AF 297093.1	From the translational initiation site or from the end of exon 1 (IVS1+)			Wild-type	Hetero-zygote	Homo-zygote	Frequency
MPJ6_U1A068	rs6759892	O	12)	Exon 1	109628	19	TGCCTCCTTCGCT/GCATTTCAGAGAA	S7A	117	68	10	0.226
MPJ6_U1A069		O		Exon 1	109714	105	GGTCCCTCAGGAC/TGGAAGCCACTGG	D35D	178	17	0	0.044
MPJ6_U1A070[a]				Exon 1	109878	269	AGCTGAAGAACCG/ATTACCAATCATT	R90H	194	1	0	0.003
MPJ6_U1A071[a]				Exon 1	109888	279	CCGTTACCAATCA/GTTTGGAAACAAT	S93S	194	1	0	0.003
MPJ6_U1A072[a]				Exon 1	109917	308	TTGCTGAGCGATC/AATTCCTAACTGC	S103X	194	1	0	0.003
MPJ6_U1A073	rs4365456	O	12)	Exon 1	109924	315	GCGATCATTCCTA/GACTGCTCCTCAG	L105L	117	68	10	0.226
MPJ6_U1A074	rs2070959[b]	O	10)	Exon 1	110150	541	TCCCTGGAGCATA/GCATTCAGCAGAA	T181A	120	65	10	0.218
MPJ6_U1A075	rs4365457	O	10)	Exon 1	110161	552	TACATTCAGCAGA/CAGCCCAGACCCT	R184S	117	68	10	0.226
MPJ6_U1A076		O		Exon 1	110236	627	TTCCCAACGAGTG/TGCCAACTTCCTT	V209V	192	3	0	0.008
MPJ6_U1A077[a]				Intron 1	110579	IVS1+109	TTCCTGGAGAAAC/TGGTGGGGGGAAG		194	1	0	0.003
MPJ6_U1A078[a]				Intron 1	110590	IVS1+120	ACGGTGGGGGGAA/GGTGATACCCGGC		194	1	0	0.003
MPJ6_U1A079	rs7592281	O		Intron 1	110600	IVS1+130	GAAGTGATACCCG/TGCTCGGAGCAGC		124	61	10	0.208
MPJ6_U1A080[a]				Intron 1	110612	IVS1+142	GGCTCGGAGCAGC/TGGGAACACATAG		194	1	0	0.003

[a] Novel variations detected in this study.
[b] Also included in the JSNP database as IMS-JST006083.
[c] SNPs publicized in the PharmGKB database were shown as "o".

190 sec. Then, the PCR products were amplified by *Ex*-Taq (0.625 units) (Takara Bio. Inc.) with the 2nd amplification primers (0.2 μM) designed in the introns (see **Table 1**, 2nd Amplification). The second round of PCR was 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min. These PCR products were then treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and were directly sequenced on both strands using an ABI Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) (see **Table 1**, Sequencing). The excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany). The eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All the SNPs were confirmed by repeating the PCR on genomic DNA and sequencing the newly generated PCR products.

Linkage disequilibrium (LD) and haplotype analysis: LD analysis was performed by SNPAlyze software (Dynacom Co., Yokohama, Japan), and a pairwise LD between SNPs was obtained for the rho square (r^2) values. Some of the haplotypes were unambiguous from subjects with homozygous SNPs at all sites or a heterozygous SNP at only one site. Separately, the diplotype configurations (a combination of haplotypes) were inferred by LDSUPPORT software, which determines the posterior probability distribution of the diplotype configuration for each subject based on the estimated haplotype frequencies.[13] The diplotype configurations of all subjects had a probability (certainty) of 1. The haplotypes inferred in only one chromosome are described with the haplotype name and a question mark in **Table 3**, since the predictability for these very rare haplotypes is known to be low in some cases.

Results and Discussion

UGT1A6 exon 1 and its flanking region were sequenced from 195 Japanese subjects. Genbank accession number AF297093.1 was utilized for the reference sequence. Thirteen SNPs, including 6 novel ones [3 were in the exon 1 and 3 in the following intron], were detected (see **Table 2**). All of the detected SNPs were found in Hardy-Weinberg equilibrium. Since we did not find any significant differences in the SNP frequencies between the two disease types (by χ^2 test or Fisher's exact test, $p>0.31$), the data for all subjects were analyzed as one group.

Two novel nonsynonymous SNPs, 269G>A (R90H) and 308C>A (S103X), were found as individual heterozygotes at a 0.003 frequency (**Fig. 1**). Among them, 308C>A confers the termination of translation at codon 103, resulting in the production of an immature protein most probably with null activity, since it lacks 81% of the structure, including the C-terminal domain important for UDP-glucuronic acid binding.

Table 3. *UGT1A6* haplotypes in a Japanese population

Nucleotide change[a]		19 T>G	105 C>T	269 G>A	279 A>G	308 C>A	315 A>G	541 A>G	552 A>C	627 G>T	IVS1 +109 C>T	IVS1 +120 A>G	IVS1 +130 G>T	IVS1 +142 C>T	Frequency		Haplotypes in ref 12)
Amino acid change		S7A	D35D	R90H	S93S	S103X	L105L	T181A	R184S	V209V							
Haplotypes[b]	*1	*1a													0.726	0.774	*1
		*1b													0.044		
		*1c													0.003		
		*1d?[c]													0.003		
	*2	*2a													0.197	0.213	*2
		*2b													0.010		
		*2c													0.003		
		*2d?[c]													0.003		
	*4	*4a													0.008	0.008	*4
	*5	*5a													0.003	0.003	
	*6	*6a													0.003	0.003	

[a] A of the translational start codon of *UGT1A6* is numbered 1. AF297093.1 was used as the reference sequence.
[b] The haplotypes were described as a number plus a small alphabetical letter.
[c] The haplotypes inferred in only one chromosome are described with the haplotype name and a question mark.

The functional significance of the other SNP, 269G>A (R90H), is currently unknown. Further functional analysis using a heterologous expression system should be pursued. Moreover, further study is necessary to evaluate the real frequencies of the very rare SNPs found in only one chromosome.

Also, the 3 known nonsynonymous SNPs, 19T>G (S7A), 541A>G (T181A), and 552A>C (R184S), were detected at 0.226, 0.218, and 0.226 frequencies, respectively. The frequencies for 541A>G (T181A) and 552A>C (R184S) were comparable to those of Asians in the previous report.[14] High linkage disequilibrium ($r^2 \geqq 0.90$) was observed among 19T>G (S7A), 315A>G (L105L), 541A>G (T181A), 552A>C (R184S), and IVS1+130G>T, as reported in Caucasian and African-American populations.[12] The r^2 values were below 0.035 for the other combinations of SNPs.

Using the detected SNPs, haplotype analysis was then performed (**Table 3**). We basically followed Nagar *et al.*[12] for the haplotype numbering of *UGT1A6*, since they included the highly linked four SNPs, 19T>G (S7A), 315A>G (L105L), 541A>G (T181A), and 552A>C (R184S). The subtype numbering followed their frequencies (from high to low frequencies). Several haplotypes were first unambiguously assigned by homozygous SNPs at all sites (**1a* and **2a*) or a heterozygous SNP at only one site (**1b*, **1c*, **2c*, and **5a*). Note that the subject with novel heterozygous 308C>A (S103X) had homozygous 19T>G (S7A), 315A>G (L105L), 541A>G (T181A), 552A>C (R184S), and IVS1+130G>T (**2a* haplotype), indicating that 308C>A (S103X) is linked with these SNPs (forming the **5a* haplotype). Separately, the diplotype configuration (a combination of haplotypes) for each subject was estimated by LDSUPPORT software. The additionally inferred haplotypes were one **1* subtype (**1d*), two **2* subtypes (**2b* and **2d*), **4a*, and **6a*. As for **6a*, cloning and sequencing of DNA fragments obtained from the subject with heterozygous 269G>A (R90H) revealed that 269A is located on the same DNA strand together with 19G, 315G, 541G, 552C, and IVS1+130T. The determined/inferred haplotypes were summarized in **Table 3**. We did not detect **3* haplotype, which consists of only SNP 19T>G (S7A). The most frequent haplotype was **1a* (frequency: 0.726), followed by **2a* (0.197), **1b* (0.044), and **2b* (0.010). The frequencies of the other haplotypes were less than 0.01. The total frequency of the **2* haplotypes (0.213) was almost comparable to those in Caucasians (0.274) and African-Americans (0.243).[12] On the other hand, the **4* frequency (0.008) was significantly lower ($p<0.01$ by Fisher's exact test) than those in Caucasians (0.052) and African-Americans (0.047).[12]

In conclusion, 13 SNPs were detected, including 6 novel ones, in *UGT1A6* from the Japanese population.

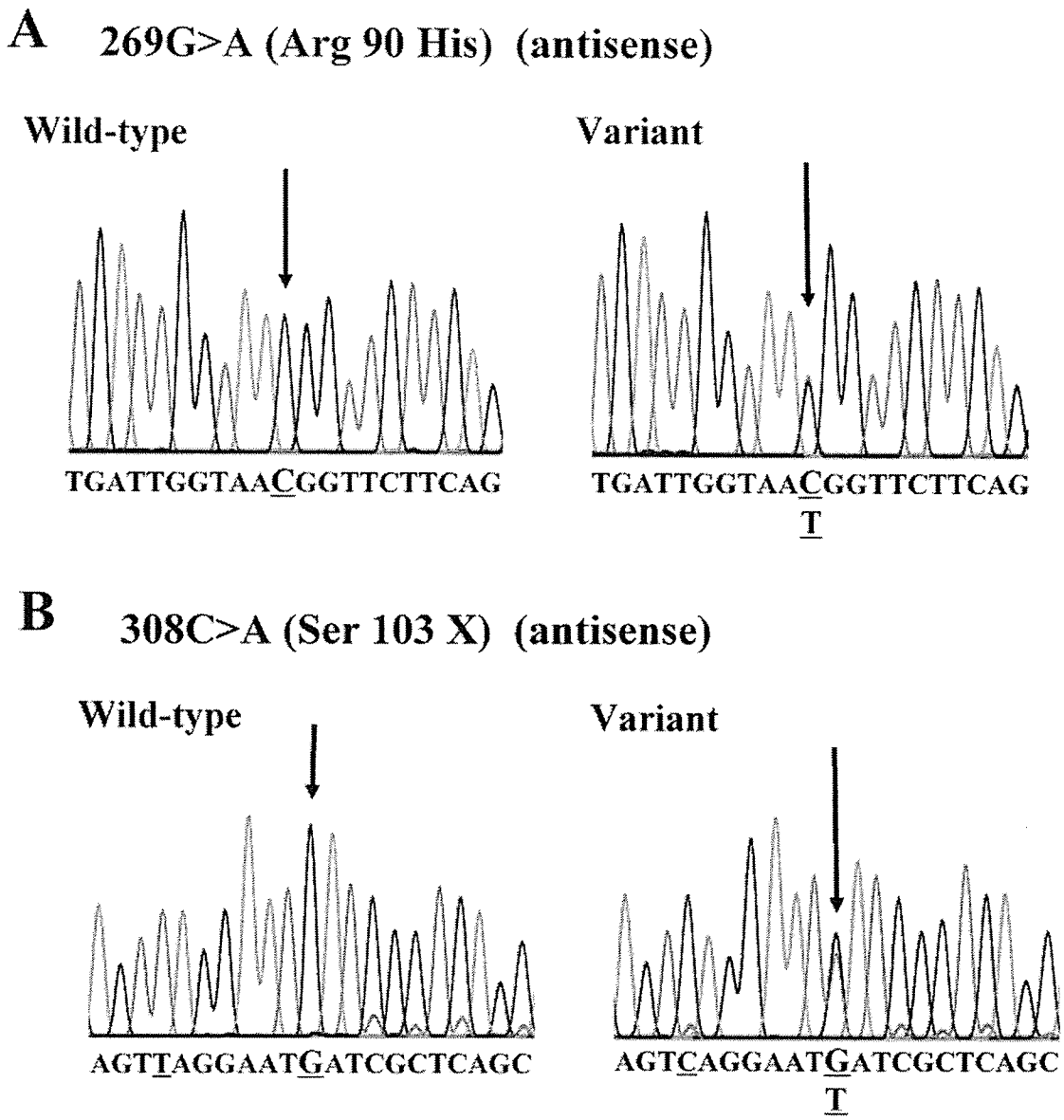

Fig. 1. The novel nonsynonymous SNPs of human *UGT1A6*. (A) MPJ6_U1A070 (wild-type 269G/G; variant 269G/A). (B) MPJ6_U1A072 (wild-type 308C/C; variant 308C/A). Arrows indicate the positions of the nucleotide changes. Note that the patient with heterozygous 308C>A also had homozygous 315A>G (L105L) alterations (shown in blue).

Using the detected SNPs, 11 haplotypes were determined and/or inferred. Our results provide fundamental and useful information for genotyping *UGT1A6* in the Japanese, and probably Asian populations.

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