Table II. Response to treatment, time to progression and overall survival of patients.

Response	Patients, n (%)		
	Relapse-sensitive (n=12)	Relapse-refractory (n=14)	Total (n=26)
Best response to treatment			
Complete	0 (0)	0 (0)	0 (0)
Partial	1 (8.3)	0 (0)	1 (3.8)
Stable disease	4 (33.3)	6 (42.8)	10 (38)
Progressive disease	7 (58.3)	8 (57.1)	15 (58)
Objective response rate	1 (8.3)	0 (0)	1 (3.8)
Disease control rate	5 (41.6)	6 (42.8)	11 (42.3)
Median time to progression (days)	34	32	33
Median overall survival (months)	8.4	4.0	5.3

Table III. Haematological and non-haematological toxicities.

Toxicity	Grade				3/4 (%)
	1	2	3	4	
Haematological					
Leukopenia	9	6	2	0	7.7
Neutropenia	9	2	1	1	7.7
Febrile neutropenia	0	0	0	0	0
Anaemia	13	5	1	1	7.7
Thrombopenia	2	0	2	0	7.7
Non-haematological					
Aspartate aminotransferase	8	0	1	0	3.8
Alanine aminotransferase	3	2	1	0	3.8
Hyponatremia	16	-	0	2	7.7
Hypokalemia	0	0	2	0	7.7
Anorexia	20	6	0	0	0
Nausea	6	4	0	0	0
Diarrhoea	5	3	1	0	3.8
Rash	4	3	2	0	7.7
Malaise	15	2	0	0	0
Infection without neutropenia	0	2	2	0	7.7

TS expression in neuroendocrine tumors has been examined, and higher TS expression was observed in SCLC and large cell neuroendocrine carcinoma compared to other types of lung cancer (17,18).

However, in contrast with pemetrexed, findings of phase II and III trials of S-1 against NSCLC did not demonstrate any obvious differences in the efficacy of S-1 against squamous and non-squamous NSCLC (7).

The reason for this discrepancy between pemetrexed and S-1 is unclear. S-1 may be able to inhibit higher levels of TS compared to pemetrexed. However, TS activity in SCLC may be considerably higher than S-1 can inhibit, since expression of TS in SCLC was shown to be markedly higher compared to TS expression in squamous cell carcinoma (17).

In addition, DPD inhibition may play an important role in NSCLC compared to SCLC. Several studies have demonstrated that 5-FU sensitivity is affected by DPD expression, which is an enzyme in NSCLC affecting 5-FU catabolism (19-22).

In conclusion, S-1 monotherapy is well-tolerated but has low activity in patients with relapsed previously treated SCLC patients, including those with a previous chemotherapy-sensitive disease. Findings of this study have shown that S-1 has minimal single-agent activity in relapsed SCLC.

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Etoposide and cisplatin versus irinotecan and cisplatin in patients with limited-stage small-cell lung cancer treated with etoposide and cisplatin plus concurrent accelerated hyperfractionated thoracic radiotherapy (JCOG0202): a randomised phase 3 study

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Summary

Background Four cycles of etoposide plus cisplatin and accelerated hyperfractionated thoracic radiotherapy (AHTRT) is the standard of care for limited-stage small-cell lung cancer (SCLC). Irinotecan plus cisplatin significantly improved overall survival compared with etoposide plus cisplatin for extensive-stage SCLC. We compared these regimens for overall survival of patients with limited-stage SCLC.

Methods We did this phase 3 study in 36 institutions in Japan. Eligibility criteria included age 20–70 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, and adequate organ functions. Eligible patients with previously untreated limited-stage SCLC received one cycle of etoposide plus cisplatin (intravenous etoposide 100 mg/m² on days 1–3; intravenous cisplatin 80 mg/m² on day 1) plus AHTRT (1·5 Gy twice daily, 5 days a week, total 45 Gy over 3 weeks). Patients without progressive disease following induction therapy were randomised (1:1 ratio, using a minimisation method with biased-coin assignment balancing on ECOG performance status [0 *vs* 1], response to induction chemoradiotherapy [complete response plus near complete response *vs* partial response and stable disease], and institution) to receive either three further cycles of consolidation etoposide plus cisplatin or irinotecan plus cisplatin (intravenous irinotecan 60 mg/m² on days 1, 8, 15; intravenous cisplatin 60 mg/m² on day 1). Patients, physicians, and investigators were aware of allocation. The primary endpoint was overall survival after randomisation; primary analysis was by intention to treat. This trial is registered with ClinicalTrials.gov, number NCT00144989, and the UMIN Clinical Trials Registry, number C000000095.

Findings 281 patients were enrolled between Sept 1, 2002, and Oct 2, 2006. After induction etoposide plus cisplatin and AHTRT, 258 patients were randomised to consolidation etoposide plus cisplatin (n=129) or irinotecan plus cisplatin (n=129). In the etoposide plus cisplatin group, median overall survival was 3·2 years (95% CI 2·4–4·1). In the irinotecan and cisplatin group, median overall survival was 2·8 years (95% CI 2·4–3·6); overall survival did not differ between the two groups (hazard ratio 1·09 [95% CI 0·80–1·46], one-sided stratified log-rank p=0·70). The most common adverse events of grade 3 or 4 were neutropenia (120 [95%] in the etoposide plus cisplatin group *vs* 101 [78%] in the irinotecan plus cisplatin group), anaemia (44 [35%] *vs* 50 [39%]), thrombocytopenia (26 [21%] *vs* six [5%]), febrile neutropenia (21 [17%] *vs* 18 [14%]), and diarrhoea (two [2%] *vs* 13 [10%]). There was one treatment-related adverse event leading to death in each group (radiation pneumonitis in the etoposide plus cisplatin group; brain infarction in the irinotecan plus cisplatin group).

Interpretation Four cycles of etoposide plus cisplatin and AHTRT should continue to be the standard of care for limited-stage SCLC.

Funding National Cancer Center and the Ministry of Health, Labour, and Welfare of Japan.

Introduction

The shift from non-filter to filter tobacco has resulted in a decrease in small-cell and squamous-cell lung cancer, and an increase in adenocarcinoma of the lung.[1] Currently, small-cell lung cancer (SCLC) accounts for 13% of all lung cancer, and about a third of patients with SCLC have limited-stage disease—ie, disease confined to the hemithorax.[2]

Combination chemotherapy is the cornerstone of SCLC treatment, and meta-analyses[3,4] have shown that addition of thoracic radiotherapy to combination chemotherapy significantly improves the survival of patients with limited-stage SCLC. Several randomised trials[5–7] have shown that early use of concurrent thoracic radiotherapy results in improved overall survival compared with sequential or late use when etoposide and cisplatin are used as combination

chemotherapy. The US intergroup phase 3 study[8] showed that accelerated hyperfractionated thoracic radiotherapy (AHTRT) with etoposide plus cisplatin for limited-stage SCLC resulted in significantly improved overall survival compared with standard fractionation, once-daily irradiation, with 5-year survival of 26% and 16%, respectively. Thus, etoposide plus cisplatin and AHTRT is now the standard of care in patients with limited-stage SCLC. However, many patients with limited-stage SCLC experience tumour recurrence and die from the disease, showing the need for improved therapy.

The Japan Clinical Oncology Group (JCOG) previously undertook a randomised phase 3 trial[9] (JCOG9511) comparing irinotecan plus cisplatin with etoposide plus cisplatin in patients with extensive-stage SCLC. Response and overall survival were significantly better for patients treated with irinotecan than those treated with etoposide. The result prompted us to explore the use of irinotecan and cisplatin in limited-stage SCLC. A phase 2 study[10] showed that irinotecan and cisplatin after concurrent etoposide plus cisplatin plus AHTRT for limited-stage SCLC was safe with acceptable side-effects, and the 3-year survival of 38% of patients was encouraging.

Therefore, we did a randomised phase 3 trial to compare overall survival of patients with limited-stage SCLC given three cycles of irinotecan plus cisplatin or etoposide plus cisplatin after one cycle of induction etoposide plus cisplatin and concurrent AHTRT.

Methods

Study design and participants

We did this randomised, open-label, phase 3 study in 36 institutions in Japan (appendix). We enrolled patients with histologically or cytologically confirmed limited-stage SCLC—defined as disease confined to one hemithorax, including ipsilateral hilar, bilateral mediastinal, and bilateral supraclavicular lymph node metastases. Pleural effusion of less than 1 cm width by chest CT was defined as limited-stage disease; malignant pleural effusion was defined as extensive-stage disease and excluded from the study. Additional eligibility criteria consisted of measurable disease, age 20–70 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, no previous treatment for SCLC, no history of anticancer chemotherapy, 4000 leucocytes per μL or greater, 10^5 platelets per μL or greater, haemoglobin of 90 g/L or greater, serum creatinine of 132·60 μmol/L or less, serum bilirubin of 34·21 μmol/L or less, serum aspartate aminotransferase of 100 IU/L or less, serum alanine aminotransferase of 100 IU/L or less, and partial pressure of oxygen of 9·33 kPa or greater. Consultation with a radiation oncologist was mandated before enrolment. We included patients aged between 20 years and 70 years because the previous JCOG trial[9] (JCOG9511) comparing irinotecan and cisplatin with etoposide plus cisplatin for extensive-stage SCLC included only patients aged 70 years or younger.

Exclusion criteria were active concomitant malignancy, active infection, uncontrolled heart disease or a history of myocardial infarction within the previous 6 months, unstable angina, uncontrollable hypertension or diabetes mellitus, interstitial pneumonia or active lung fibrosis on chest radiograph, psychiatric disease, malignant pericardial effusion, diarrhoea, intestinal obstruction or paralysis, and concurrent administration of any oral or intravenous steroid. We excluded pregnant or lactating women.

All patients enrolled in the study underwent an induction therapy of one cycle of etoposide plus cisplatin with concurrent AHTRT, eligible patients were registered again and randomised to consolidation chemotherapy consisting of three cycles of etoposide plus cisplatin or irinotecan plus cisplatin. The second registration eligibility criteria were: within 49 days from the first registration, ECOG performance status of 0–1, 3000 leucocytes per μL or greater, 10^5 platelets per μL or greater, serum creatinine of 132·60 μmol/L or less, serum bilirubin of 34·21 μmol/L or less, serum aminotransferase of 100 IU/L or less, no fever or diarrhoea within 24 h, no pulmonary infiltration beyond the radiation portal, no active infection, radiation dermatitis or oesophagitis of grade 2 or less, completion of induction chemoradiotherapy, no progressive disease, and tumour response to induction chemoradiotherapy as assessed by chest CT (complete response, near complete response, partial response, or stable disease). Because almost all patients with limited-stage SCLC are admitted to hospital during induction chemoradiotherapy in Japan, chest CT assessment within the specified timeframe was not problematic. The assessment of response to chemoradiation was done after day 23, counted from the start of induction chemoradiotherapy.

The study protocol was approved by the Clinical Trial Review Committee of JCOG and the institutional review boards of the participating institutions. All patients provided written informed consent.

Procedures

Induction chemotherapy consisted of intravenous cisplatin 80 mg/m² on day 1 and intravenous etoposide 100 mg/m² on days 1–3. AHTRT was begun on day 2 of induction chemotherapy and administered twice daily, 5 days a week, (1·5 Gy per fraction, with 6 h or more between fractions) to a total dose of 45 Gy in 3 weeks. 30 Gy was delivered with 6–10 MV photons using anterior–posterior opposed fields that included the primary tumour; metastatic lymph nodes; and regional nodes, excluding the contralateral hilar nodes. Supraclavicular lymph nodes were also included when involved. A booster dose of 15 Gy was delivered to the primary tumour and metastatic lymph nodes. Conventional two-dimensional radiograph simulation and three-dimensional CT simulation were allowed for treatment planning; PET scanning was not required. The clinical target volume was equal to the gross tumour volume, including the primary tumour and metastatic nodes (1 cm or greater in shortest dimension).

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See Online for appendix

The planned target volumes for the primary tumour, metastatic lymph nodes, and regional nodes were defined as clinical target volume plus adequate margins (typically 0·5–1·0 cm laterally and 1·0–2·0 cm craniocaudally). The volume of the lung unaffected by cancer to receive 20 Gy or more was kept to 35% or less when three-dimensional CT simulation was used. Lung heterogeneity corrections were not used. If grade 3 non-haematological side-effects (excluding hyponatraemia, nausea, vomiting, and appetite loss), performance status of 3, grade 2 pneumonitis or pulmonary infiltrates, or a fever of 38·0°C or more developed, radiotherapy was withheld until recovery. Quality assurance reviews were done and the results are reported elsewhere.[11]

In the consolidation chemotherapy stage, patients assigned to etoposide plus cisplatin received intravenous cisplatin 80 mg/m² on day 1 and intravenous etoposide 100 mg/m² on days 1–3, repeated every 3 weeks for three cycles. Patients assigned to irinotecan plus cisplatin were treated every 3–4 weeks for three cycles; this regimen consisted of intravenous irinotecan 60 mg/m² on days 1, 8, and 15 and intravenous cisplatin 60 mg/m² on day 1. The doses of cisplatin were the same as in the previous JCOG trial (JCOG9511) in extensive-stage SCLC.[9]

If the leucocyte count decreased to less than 3000 leucocytes per μL or the platelet count fell below 10^5 platelets per μL on the first day of etoposide plus cisplatin or irinotecan plus cisplatin, chemotherapy was withheld until the counts recovered to above these cutoffs. Administration of irinotecan was skipped on day 8 or 15, or on both days, if the leucocyte count was less than 2000 leucocytes per μL, the platelet count was below 10^5 platelets per μL, or if there was any diarrhoea irrespective of grade, or a fever of 37·5°C or more. The dose of etoposide in subsequent cycles was reduced by 20 mg/m² from the planned dose if grade 4 leucopenia, grade 4 thrombocytopenia, or grade 3 non-haematological side-effects (excluding nausea, vomiting, appetite loss, hyponatraemia, and creatinine) developed. The dose of irinotecan in subsequent cycles was reduced by 10 mg/m² from the planned dose if grade 4 leucopenia or grade 4 thrombocytopenia, grade 2 or 3 diarrhoea, or grade 3 non-haematological side-effects (excluding nausea, vomiting, hyponatraemia, and creatinine) developed. The dose of cisplatin was reduced by 10 mg/m² if serum creatinine was higher than 132·60 μmol/L but not exceeding 176·80 μmol/L. Cisplatin was not administered if creatinine was higher than 176·80 μmol/L. Treatment was stopped in patients with non-haematological side-effects of grade 4.

Administration of granulocyte colony stimulating factor (G-CSF) was prohibited on the same days as chemotherapy or radiotherapy. Primary prophylactic G-CSF was not administered. For patients who had developed grade 4 neutropenia or grade 3 febrile neutropenia during previous cycles of chemotherapy, secondary prophylactic G-CSF administration was allowed. Prophylactic antibiotics were not administered.

Prophylactic cranial irradiation (25 Gy in ten fractions) was undertaken for patients showing a complete response or near complete response, defined as a reduction of 70% or more in the sum of the longest diameters of the target lesions.

Before enrolment in the study, each patient provided a complete medical history and underwent physical examination, blood cell count determinations, arterial blood gas, biochemical laboratory examinations, chest radiograph, electrocardiogram, chest CT scan and whole-brain CT or MRI, abdominal ultrasound or CT, and isotope bone scans. Data regarding the time interval between diagnosis and start of concurrent chemoradiotherapy were not collected. Blood cell counts, differential white cell counts and other laboratory data were obtained weekly during induction chemoradiotherapy. All patients were reassessed at the end of consolidation chemotherapy with the same imaging assessments as at the time of enrolment. For efficacy assessments after the end of study treatment, patients were monitored once a month for 1 year and once every 3 months after 1 year. If progression was suspected on the basis of worsening symptoms or abnormal laboratory test values, the site of suspected progression was examined. If recurrence or progression was established, restaging including chest CT, brain MRI or CT, abdominal ultrasound or CT, and bone scintigraphy were done.

Responses were assessed according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0. Response was defined as the proportion of patients whose best overall response was complete reponse or partial response according to RECIST. Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria (NCI-CTC) version 2.0. Serious adverse events were defined as grade 4 non-haematological or grade 5 adverse events.

Randomisation and masking

After induction chemoradiotherapy, eligible patients were randomly assigned in a 1:1 ratio to receive either three cycles of consolidation etoposide plus cisplatin or irinotecan plus cisplatin at the JCOG Data Center. Randomisation was done using a minimisation method with biased-coin assignment balancing on ECOG performance status (0 *vs* 1), response to induction chemoradiotherapy (complete response plus near complete response *vs* partial response and stable disease) and institution. Patients, treating physicians, and individuals assessing outcomes and analysing data were not masked to treatment allocation.

Statistical analysis

The primary endpoint was overall survival after randomisation. The planned sample size for randomisation was 250 and the expected number of events was 223, with a one-sided α of 2·5% and at least 70% power to detect a difference between groups, assuming 30·0% 3-year survival with etoposide plus cisplatin versus 42·5% with

irinotecan plus cisplatin. Final analysis was planned 5 years after completion of accrual. Secondary endpoints were adverse events associated with induction chemoradiotherapy, adverse events associated with consolidation chemotherapy, late radiation morbidity after thoracic irradiation, adverse events during treatment with prophylactic cranial irradiation, incidence of serious adverse events, and progression-free survival after randomisation.

Progression-free survival was calculated from the date of randomisation until the date of documented progression or death (in the absence of progression). Overall survival was calculated from the date of randomisation until the date of death from any cause. Both intervals were estimated by the Kaplan-Meier method.

Three interim analyses were scheduled. The first interim analysis was to assess the futility of the trial after half the planned sample size was randomised. The second interim analysis was planned immediately after patient accrual was completed to decide whether the preplanned follow-up was necessary in terms of efficacy. The third interim analysis was planned 2 years after completion of accrual, with the same aim as the second interim analysis. Results of the interim analyses were reviewed by the JCOG Data and Safety Monitoring Committee and investigators were masked to the results. Multiplicity for analyses of the primary endpoint was adjusted with the O'Brien-Fleming type α-spending function.[12]

The primary endpoint, overall survival after randomisation, was analysed with the log-rank test, stratified by ECOG performance status (0 *vs* 1) and response to induction chemoradiotherapy (complete response plus near complete response *vs* partial response plus stable disease). Hazard ratios (HR) were estimated with a Cox regression model, stratified by the same factors as the log-rank test. Unstratified log-rank tests and unstratified Cox regression models were used for all other analyses. The efficacy analyses were by modified intention to treat, including all patients enrolled at the second registration who did not violate any inclusion criteria. Safety analyses included all patients enrolled at the second registration who received at least one dose of study drug. Analyses were done by the JCOG Data Center using SAS (version 9.2).

This trial was registered with ClinicalTrials.gov, number NCT00144989 and UMIN Clinical Trials Registry, number C000000095.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

281 patients were enrolled between Sept 1, 2002, and Oct 2, 2006. Four patients were shown to be ineligible after the first registration, three did not receive study treatment because of progressive disease, and two did not receive AHTRT, one because of an inappropriate radiation field and one because of an allergy to etoposide (figure 1). After the induction etoposide plus cisplatin plus AHTRT, 258 patients were enrolled at the second registration and

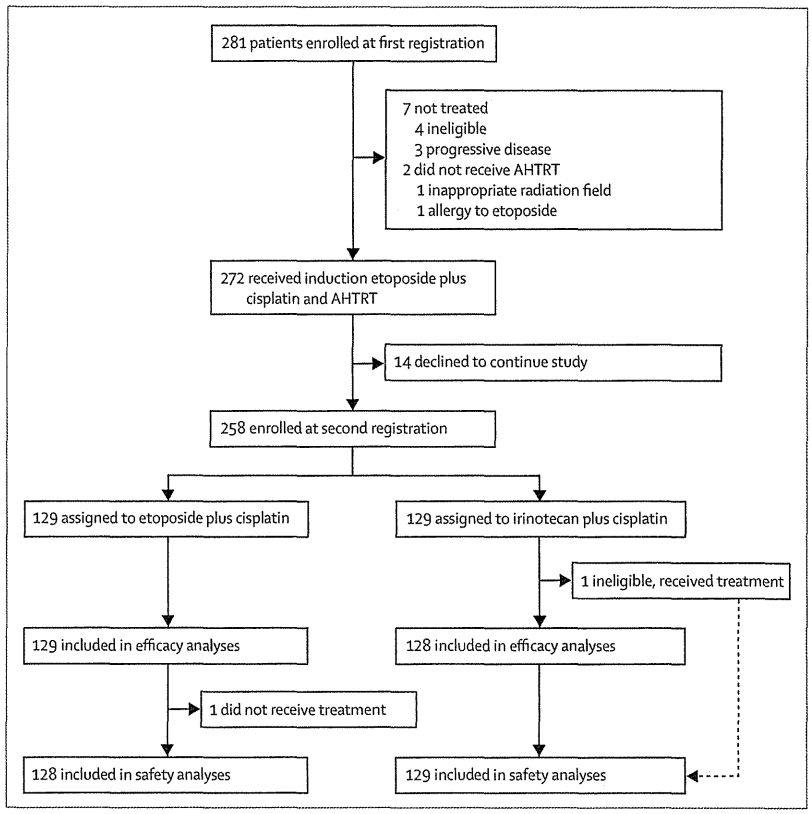

Figure 1: **Trial profile**
AHTRT=accelerated hyperfractionated thoracic radiotherapy.

	First registration (n=281)	Second registration	
		Etoposide and cisplatin (n=129)	Irinotecan and cisplatin (n=129)
Age (years)	61 (32–70)	60 (32–70)	62 (39–70)
Sex			
Men	228 (81%)	103 (80%)	106 (82%)
Women	53 (19%)	26 (20%)	23 (18%)
ECOG performance status			
0	170 (60%)	86 (67%)	85 (66%)
1	111 (40%)	43 (33%)	44 (34%)
Response to induction chemoradiotherapy*			
Complete response	..	3 (2%)	4 (3%)
Near complete response	..	28 (22%)	26 (20%)
Partial response	..	92 (71%)	87 (67%)
Stable disease	..	6 (5%)	12 (9%)

Data are median (IQR) or n (%). ECOG=Eastern Cooperative Oncology Group. *According to Response Evaluation Criteria In Solid Tumors (version 1.0).

Table 1: **Characteristics of patients**

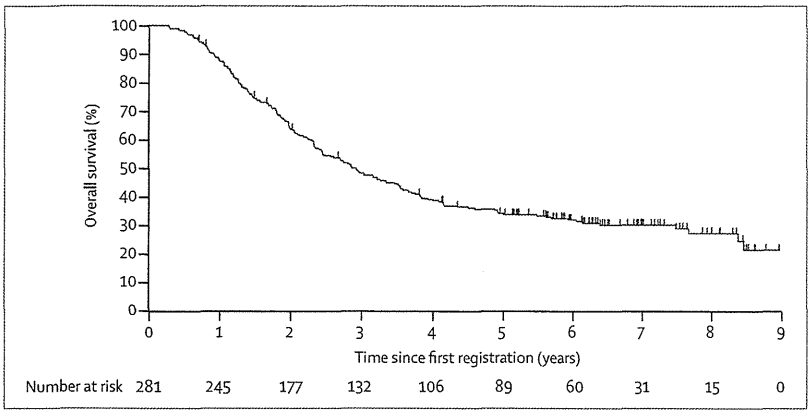

Figure 2: **Overall survival after first registration**
*One-sided p value from stratified log-rank test, with Eastern Cooperative Oncology Group performance status and response to induction chemoradiotherapy as strata.

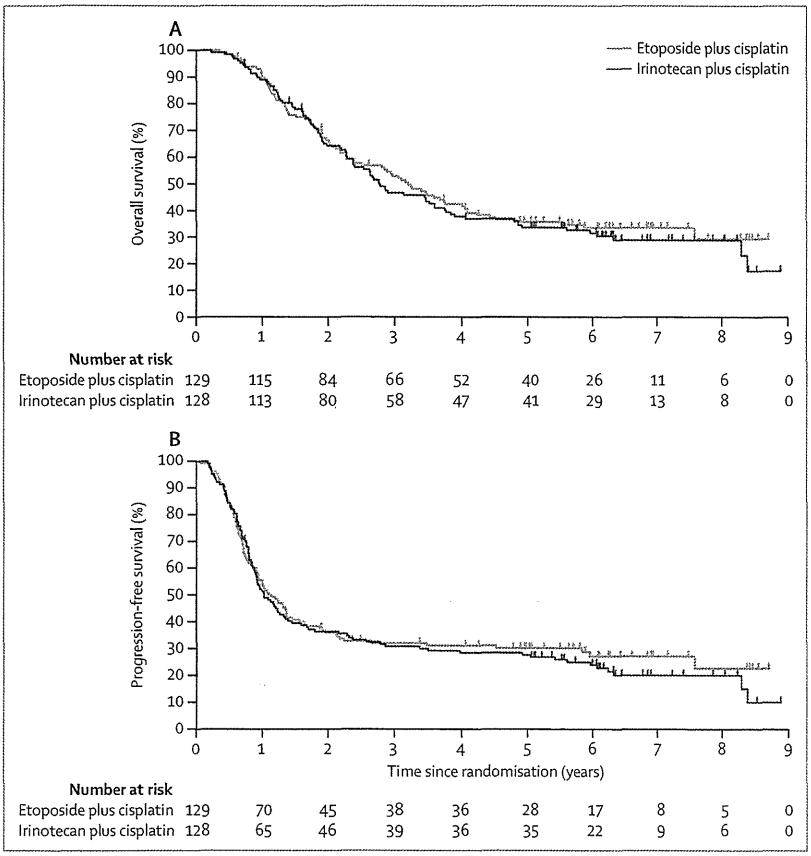

Figure 3: **Overall survival (A) and progression-free survival (B) after randomisation**
*p value from unstratified log-rank test.

randomised to consolidation etoposide plus cisplatin (n=129) or irinotecan plus cisplatin (n=129). One patient in the irinotecan plus cisplatin group was shown to be ineligible after the second registration because of contralateral hilar node metastasis, this patient was excluded from the efficacy analyses, but included in the safety analyses. Table 1 shows the characteristics of the patients.

Of 129 patients who were randomised to the etoposide plus cisplatin group, 116 patients (90%) received three cycles of consolidation chemotherapy, four (3%) received two cycles, eight (6%) received one cycle, and one (1%) had no consolidation therapy. In the irinotecan plus cisplatin group, 110 of 128 (86%) patients received three cycles of consolidation chemotherapy, six (5%) received two cycles, 12 (9%) received one cycle. The main reasons for non-completion of three cycles of consolidation chemotherapy in the both groups were adverse events (eight patients in the etoposide plus cisplatin group, 12 patients in the irinotecan plus cisplatin group) and patient refusal because of adverse events (nine patients in the etoposide plus cisplatin group, 14 patients in the irinotecan plus cisplatin group); one patient in each group did not complete consolidation chemotherapy because of progressive disease. In the etoposide plus cisplatin group, 115 (89%) of 129 patients received at least 70% of the planned of dose of etoposide, and 116 (90%) of 129 received at least 70% of the planned dose of cisplatin; in the irinotecan plus cisplatin group, 88 (69%) of 128 received at least 70% of the planned dose of irinotecan and 110 (86%) of 128 received at least 70% of the planned dose of cisplatin. Prophylactic cranial irradiation was administered to 76 patients in the etoposide plus cisplatin group and 73 in the irinotecan plus cisplatin group.

Of 281 patients who entered into the first registration, median follow-up for the 88 censored patients was 6·3 years (IQR 5·6–7·2); median overall survival was 2·9 years (95% CI 2·5–3·5), 3-year overall survival was 48·4% (95% CI 42·4–54·1), and 5-year overall survival was 34·3% (28·7–39·9; figure 2). Of 257 patients included in the final analysis of the primary outcome, median follow-up for the 84 censored patients was 6·2 years (IQR 5·4–7·0); there were 173 events. In the etoposide plus cisplatin group, median overall survival was 3·2 years (95% CI 2·4–4·1), 3-year overall survival was 52·9% (95% CI 43·9–61·1), and 5-year overall survival was 35·8% (27·4–44·1). In the irinotecan plus cisplatin group, median overall survival was 2·8 years (95% CI 2·4–3·6), 3-year overall survival was 46·6% (37·7–55·1) and 5-year overall survival was 33·7% (25·5–42·0; HR 1·09 [95% CI 0·80–1·46]; p=0·70 from one sided stratified log-rank test; figure 3A). The results of the unstratified analysis did not differ from those of the stratified analysis (data not shown).

Figure 3B shows the Kaplan-Meier curves for progression-free survival in the two groups. Median progression-free survival was 1·1 years (95% CI 0·9–1·4) in the etoposide plus cisplatin group and 1·0 years (0·9–1·4) in the irinotecan plus cisplatin group (HR 1·10; 95% CI 0·83–1·45; p=0·74 from one sided unstratified log-rank test). In the etoposide group, 3-year progression-free survival was 32·0% (95% CI 24·1–40·1) and 5-year progression-free survival was 30·2% (22·4–38·3). In the irinotecan plus cisplatin group, these were 30·8% (23·0–38·9) and 27·7% (20·2–35·6), respectively.

	Etoposide plus cisplatin plus AHTRT*				Consolidation chemotherapy							
	Grade 1–2	Grade 3	Grade 4	N	Etoposide plus cisplatin				Irinotecan plus cisplatin			
					Grade 1–2	Grade 3	Grade 4	N	Grade 1–2	Grade 3	Grade 4	N
Leucopenia	16 (6%)	148 (54%)	109 (40%)	273	12 (9%)	81 (63%)	34 (27%)	128	28 (22%)	76 (59%)	25 (19%)	129
Anaemia	86 (32%)	1 (<1%)	0	273	76 (59%)	33 (26%)	11 (9%)	128	72 (56%)	42 (33%)	8 (6%)	129
Thrombocytopenia	108 (40%)	20 (7%)	0	273	56 (44%)	22 (17%)	4 (3%)	128	28 (22%)	6 (5%)	0	129
Neutropenia	12 (4%)	57 (21%)	203 (74%)	273	6 (5%)	33 (26%)	87 (68%)	128	28 (22%)	62 (48%)	39 (30%)	129
Hypoalbuminaemia	194 (72%)	0	..	271	102 (80%)	0	..	127	109 (84%)	0	..	129
Bilirubin	72 (26%)	1 (<1%)	0	272	20 (16%)	0	0	128	21 (16%)	0	0	129
Aspartate aminotransferase	54 (20%)	1 (<1%)	0	273	19 (15%)	1 (1%)	0	128	29 (22%)	0	0	129
Alanine aminotransferase	91 (33%)	4 (1%)	0	273	38 (30%)	1 (1%)	0	128	47 (36%)	0	0	129
Creatinine	67 (25%)	0	0	273	55 (43%)	0	0	128	35 (27%)	0	0	129
Fever	75 (27%)	0	0	274	28 (22%)	1 (1%)	0	128	33 (26%)	0	0	129
Alopecia	207 (77%)	..	..	270	94 (76%)	..	..	123	93 (74%)	..	..	126
Weight loss	43 (16%)	0	..	274	17 (13%)	0	..	128	20 (16%)	1 (1%)	..	129
Anorexia	158 (58%)	22 (8%)	1 (<1%)	274	82 (64%)	12 (9%)	0	128	78 (60%)	16 (12%)	0	129
Diarrhoea	28 (10%)	3 (1%)	0	274	10 (8%)	2 (2%)	0	128	68 (53%)	13 (10%)	0	129
Dysphagia–oesophageal†	229 (84%)	5 (2%)	0	274	34 (27%)	0	0	128	34 (26%)	1 (1%)	0	129
Nausea	139 (51%)	17 (6%)	..	274	82 (64%)	7 (5%)	..	128	82 (64%)	7 (5%)	..	129
Stomatitis or pharyngitis	38 (14%)	1 (<1%)	0	274	16 (13%)	0	0	128	15 (12%)	0	0	129
Vomiting	53 (19%)	3 (1%)	0	274	26 (20%)	3 (2%)	0	128	21 (16%)	5 (4%)	0	129
Febrile neutropenia	..	67 (25%)	0	271	..	21 (16%)	0	128	..	18 (14%)	0	129
Infection with grade 3 or 4 neutropenia	0	37 (14%)	0	272	0	15 (12%)	0	128	0	8 (6%)	0	129
Infection without neutropenia	7 (3%)	11 (4%)	1 (<1%)	274	11 (9%)	4 (3%)	0	128	16 (12%)	8 (6%)	0	129
Pneumonitis or pulmonary infiltrates	2 (1%)	1 (<1%)	0	274	9 (7%)	1 (1%)	0	128	16 (12%)	0	0	129

Data were missing for some patients. AHTRT=accelerated hyperfractionated thoracic radiotherapy. *Including two patients who did not undergo radiotherapy. †Related to radiation.

Table 2: **Adverse events**

The two groups did not differ in terms of sites of primary failure. Of 175 patients who had disease progression, in the etoposide plus cisplatin group, 30 had local progression within the radiation field, seven had local progression outside of the radiation field, 26 had progression to the brain, and 35 had systemic progression to other sites; in the irinotecan plus cisplatin group, 27 had local progression within the radiation field, six had local progression outside of the radiation field, 33 had progression to the brain, and 38 had systemic progression to other sites (some patients had progression to more than one site).

In a planned subgroup analysis, women in the etoposide plus cisplatin group had improved overall survival compared with those in the irinotecan plus cisplatin group (median overall survival not reached, 5-year overall survival 55·3% [95% CI 33·8–72·3] *vs* median overall survival 2·4 years [1·6–3·4], 5-year overall survival 26·1% [10·6–44·7] in the irinotecan group; unstratified HR 2·56; 95% CI 1·20–5·44, one-sided p=0·99) whereas outcomes for men did not differ between the groups (0·90; 0·65–1·24, one-sided p=0·25). Other prespecified subgroup analyses, including age (≤60 years old *vs* >60 years old), stage by UICC-TNM 7th edition (≤IIIA *vs* ≥IIIB), ECOG performance status (0 *vs* 1), response to induction chemoradiotherapy (complete response plus near complete response *vs* partial repsonse plus stable disease), bodyweight loss during 6 months (≤5% *vs* >5%), and smoking history (<20 packs per year *vs* ≥20 packs per year) did not differ between the two groups (data not shown).

Of 129 eligible patients randomised to the etoposide plus cisplatin group, 128 (99·2%) had an overall response (24 complete response; 54 near complete response; 50 partial response); of 128 patients in the irinotecan plus cisplatin group, 123 (96·1%) had an overall response (30 complete response; 57 near complete response; 36 partial response).

Table 2 shows side-effects associated with concurrent chemoradiotherapy and consolidation chemotherapy. During consolidation chemotherapy, the most common adverse events of grade 1 or 2 were hypoalbuminaemia (102 [80%] in the etoposide plus cisplatin group *vs* 109 [84%] in the irinotecan plus cisplatin group) and alopecia (94 [76%] *vs* 93 [74%]). The most common adverse events of grade 3 or 4 were neutropenia (120 [95%] in the etoposide plus cisplatin group *vs* 101 [78%] in the irinotecan plus cisplatin group), anaemia (44 [35%] *vs* 50 [39%]), thrombocytopenia (26 [21%] *vs* six [5%]), febrile neutropenia (21 [17%] *vs* 18 [14%]), and diarrhoea (two [2%] *vs* 13 [10%]). 12% of patients in the in the etoposide plus cisplantin group and 6% in the irinotecan plus cisplatin group had infection with grade 3 or 4 neutropenia. However, grade 3 febrile neutropenia did not differ between the two groups. Grade 3 or 4 leucopenia was less frequent in the

Panel: Research in context

Systematic review

Combination chemotherapy is the cornerstone of treatment of small-cell lung cancer (SCLC). We searched PubMed for reports of randomised clinical trials published in English up to Sept 30, 2013, using the terms "lung neoplasms", "small-cell lung cancer", "radiotherapy", and "not non-small-cell lung cancer". We also searched the reference lists of retrieved articles. The quality of evidence was assessed mainly on the basis of whether the standard chemotherapy regimen, etoposide plus cisplatin, was used as the reference group. Meta-analyses[3,4] have shown that addition of thoracic radiotherapy to combination chemotherapy significantly improves the survival of patients with limited-stage SCLC. Several randomised trials[5–7] have shown that early use of concurrent thoracic radiotherapy is better than sequential or late use, when etoposide and cisplatin are used as combination chemotherapy. The US intergroup phase 3 study[8] showed that accelerated hyperfractionated thoracic radiotherapy (AHTRT) with etoposide plus cisplatin for limited-stage SCLC was better than standard fractionation, once-daily irradiation.

Interpretation

At present, standard treatment for patients with limited-stage SCLC is etoposide plus cisplatin with thoracic radiotherapy. AHTRT is recommended when logistically acceptable. As far as we are aware, JCOG0202 is the first randomised trial investigating the efficacy of irinotecan plus cisplatin in patients with limited-stage disease. The hypothesis that irinotecan plus cisplatin could improve overall survial for these patients compared with etoposide plus cisplatin was refuted. Four cycles of etoposide plus cisplatin and concurrent AHTRT should be the standard of care in patients with limited-stage SCLC, and discouragement and cessation of tobacco use is still the most effective strategy to reduce deaths from SCLC.

irinotecan plus cisplatin group than in the etoposide plus cisplatin group; grade 3 or 4 diarrhoea was more frequent in the irinotecan plus cisplatin group than in the etoposide plus cisplatin group (table 2).

Late radiation morbidity after thoracic irradiation did not differ between the two groups (two [1·6%] grade 3 and two [1·6%] grade 4 events in the etoposide plus cisplatin group *vs* two [1·6%] grade 3 events in the irinotecan plus cisplatin group). Only one event [1·3%] of nausea of grade 3 due to prophylactic cranial irradiation was reported in the etoposide and cisplatin group.

Study treatment was terminated because of side-effects in 17 patients (13%) in the etoposide plus cisplantin group and in 26 patients (20%) in the irinotecan plus cisplatin group. There were three treatment-related deaths. One treatment-related death from pneumonitis occurred 86 days after induction chemoradiotherapy (induction etoposide plus cisplantin plus AHTRT). The patient was not randomised because a diffuse interstitial shadow occurred after 28·5 Gy of AHTRT. One patient in the etoposide plus cisplantin group died of radiation pneumonitis 116 days after completion of study treatment. One patient in the irinotecan plus cisplatin group died of brain infarction during the third course of consolidation chemotherapy.

Discussion

In this study of 258 patients with limited-stage SCLC, three cycles of irinotecan plus cisplatin did not improve overall survival compared with three cycles of etoposide plus cisplatin, after one cycle of etoposide plus cisplatin with concurrent AHTRT (panel). Randomisation was done after completion of induction chemoradiotherapy, thus the findings are unlikely to be biased by induction chemoradiotherapy.

JCOG previously reported the results of a randomised phase 3 trial[9] (JCOG9511) comparing irinotecan plus cisplatin versus etoposide plus cisplatin for extensive-stage SCLC. Median overall survival was 12·8 months and 19·5% patients were alive at 2 years in the irinotecan plus cisplatin group, whereas in the etoposide plus cisplatin group, median overall survival was 9·4 months only 5·2% of patients were alive after 2 years (p=0·002 from unadjusted log-rank test). Similar trials[13–15] done mainly in white patients with extensive-stage SCLC, including the Southwest Oncology Group trial[13] (S0124) using almost the same eligibility criteria and identical treatment regimens as JCOG9511, did not confirm the JCOG results. These results suggest pharmacogenomic differences between Japanese and non-Japanese patients.[16] Despite several negative trials, two meta-analyses[17,18] using non-individual-patient data showed a significant survival improvement with irinotecan compared with etoposide in patients with extensive-stage SCLC. However, the efficacy of irinotecan plus cisplatin shown in extensive-stage SCLC was not observed in the Japanese patients with limited-stage SCLC in our current study.

Side-effects were as expected. Severe non-haematological adverse events were much the same between the two groups, except for grade 3 or 4 diarrhoea which occurred in 10% of patients in the irinotecan plus cisplatin group and only 2% of patients in the etoposide plus cisplatin group. Late radiation reactions were not increased in the irinotecan plus cisplatin group. 86% of patients in the irinotecan plus cisplatin group received the planned three cycles of consolidation chemotherapy, and 90% received three cycles in the etoposide plus cisplatin group. Thus, compliance does not explain the negative results in the present study.

5-year overall survival in patients who received standard etoposide plus cisplatin plus concurrent AHTRT has been reported to be 24–26% in two phase 3 studies[7,8] in limited-stage SCLC. Although we failed to show an improvement in survival with our investigational regimen, the 5-year overall survival of 34·3% for all patients in the present study would be the best outcome reported so far. The 5-year overall survival of 55·3% in women who received standard etoposide plus cisplatin consolidation therapy is encouraging. This favourable result might be attributable to selection of patients, such as inclusion of patients with ECOG performance status of 0 or 1, and aged 70 years or younger. However, this selection bias does not fully explain the difference because the proportion of patients with ECOG performance status of 2 in other trials was only about 5%.[7,8] Radiotherapy quality control undertaken in the present study might have contributed to the improved

outcome, because radiotherapy protocol deviations are associated with overall mortality.[11,19] Optimum care of patients, including full disclosure of prognosis in the consent form for the study, might be another factor related to the favourable outcome.[20,21]

Full dose irinotecan cannot be combined with radiotherapy.[22] Thus, it is unlikely that the addition of irinotecan to radiotherapy improves the outcome of patients with limited-stage SCLC who receive combined chemotherapy and radiotherapy treatment. In future trials, new active agents with radiosensitising potential are needed. Testing of different radiotherapy regimens would be another option to improve outcomes in limited-stage SCLC. A randomised trial to establish whether administration of high-dose thoracic radiotherapy, 70 Gy (2 Gy once daily over 7 weeks) or 61·2 Gy (1·8 Gy once daily for 16 days followed by 1·8 Gy twice daily for 9 days), will improve survival compared with 45 Gy (1·5 Gy twice daily over 3 weeks) is underway in the USA (NCT00632853).

At the present time, the results of our study indicate that four cycles of etoposide plus cisplatin plus concurrent AHTRT should continue to be the standard of care in patients with limited-stage SCLC. Because SCLC is strongly smoking-related, discouragement and cessation of tobacco use is still the most effective strategy to reduce deaths from SCLC.[23]

Contributors

TT was the chief investigator of the trial. KK, TH, SI, MN, MK, AY, FI, KT, SN, MH, HO, NY, TShin, HS, KM, KN, NS, and TT designed the trial and wrote the protocol. KK, TH, MN, MK, AY, FI, KT, SN, MH, HO, NY, TShin, HS, KM, KN, and TT enrolled patients. JM and TShib were responsible for data management, statistical analysis, and data interpretation. KK drafted the report. All authors were involved in writing the report and approved the final version.

Conflicts of interest

KK has received honoraria and a research grant from Daiichi-Sankyo. TT has received honoraria from Daiichi-Sankyo and Bristol-Myers Squibb. KN has received honoraria from Bristol-Myers Squib, Nippon Kayaku, and Daiichi-Sankyo. All other authors declare that they have no conflicts of interest.

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Microcavity Array System for Size-Based Enrichment of Circulating Tumor Cells from the Blood of Patients with Small-Cell Lung Cancer

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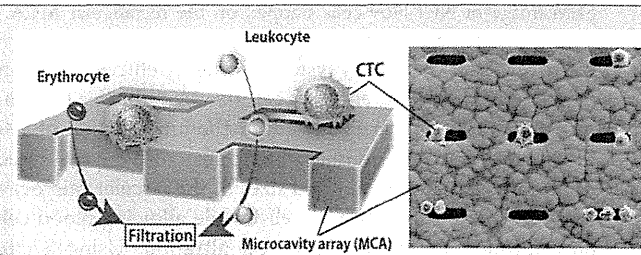
Supporting Information

ABSTRACT: In this study, we present a method for efficient enrichment of small-sized circulating tumor cells (CTCs) such as those found in the blood of small-cell lung cancer (SCLC) patients using a microcavity array (MCA) system. To enrich CTCs from whole blood, a microfabricated nickel filter with a rectangular MCA (10^4 cavities/filter) was integrated with a miniaturized device, allowing for the isolation of tumor cells based on differences in size and deformability between tumor and blood cells. The shape and porosity of the MCA were optimized to efficiently capture small tumor cells on the microcavities under low flow resistance conditions, while allowing other blood cells to effectively pass through. Under optimized conditions, approximately 80% of SCLC (NCI-H69 and NCI-H82) cells spiked in 1 mL of whole blood were successfully recovered. In clinical samples, CTCs were detectable in 16 of 16 SCLC patients. In addition, the number of leukocytes captured on the rectangular MCA was significantly lower than that on the circular MCA ($p < 0.001$), suggesting that the use of the rectangular MCA diminishes a considerable number of carryover leukocytes. Therefore, our system has potential as a tool for the detection of CTCs in small cell-type tumors and detailed molecular analyses of CTCs.



Circulating tumor cells (CTCs) are defined as tumor cells circulating in the peripheral blood of patients with metastatic cancer. The number of CTCs in peripheral blood has prognostic value in patients and can be used to evaluate therapeutic effects.[1,2] Furthermore, molecular analysis of CTCs provides valuable information for the characterization of CTCs and understanding cancer metastasis.[3,4] Detection of abnormal genes in CTCs can also contribute to the planning of personalized medicine using molecular-targeted drugs.[5,6] However, as CTCs are extremely rare (1 in 10^9 blood cells), enrichment is required to increase detection sensitivity to an acceptable level. The most often used CTC enrichment technique is immunomagnetic separation, which uses magnetic beads coated with monoclonal antibodies that target an epithelial cell marker such as EpCAM to enrich CTCs.[7,8] However, several studies have shown that the presence of EpCAM on tumor cells varies with tumor type.[9,10] The expression of various epithelial cell markers such as EpCAM is down-regulated to increase the invasiveness and metastatic potential via the epithelial-to-mesenchymal transition.[11−14] It has been suggested that the lower detection of CTCs with the CellSearch system (Veridex, Raritan, NJ), which is a semi-automated immunomagnetic separation system, in patients with advanced nonsmall-cell lung cancer (NSCLC) may be due to the loss of EpCAM expression.[15] Therefore, CTC enrichment methods based on an antigen−antibody reaction cannot isolate CTCs stably or reproducibly from all tumor types.

Other groups have reported CTC separation methods based on differences in size and deformability between CTCs and hematologic cells. Isolation by size of epithelial tumor cells (ISET) can be achieved by filtration because tumor cells (>8 μm) are larger than leukocytes.[16−19] ISET using a polycarbonate filter is an inexpensive, user-friendly method for enriching CTCs, as it enables the enrichment of EpCAM negative CTCs on the basis of size. CTC detection sensitivity with ISET in clinical tests of patients with metastatic lung cancer has been previously reported as higher than that with a conventional EpCAM expression-based CellSearch System.[20−22]

Recently, microfabricated devices for size-based separation of tumor cells have been developed to enable efficient enrichment of CTCs.[23−28] We have also developed a miniaturized

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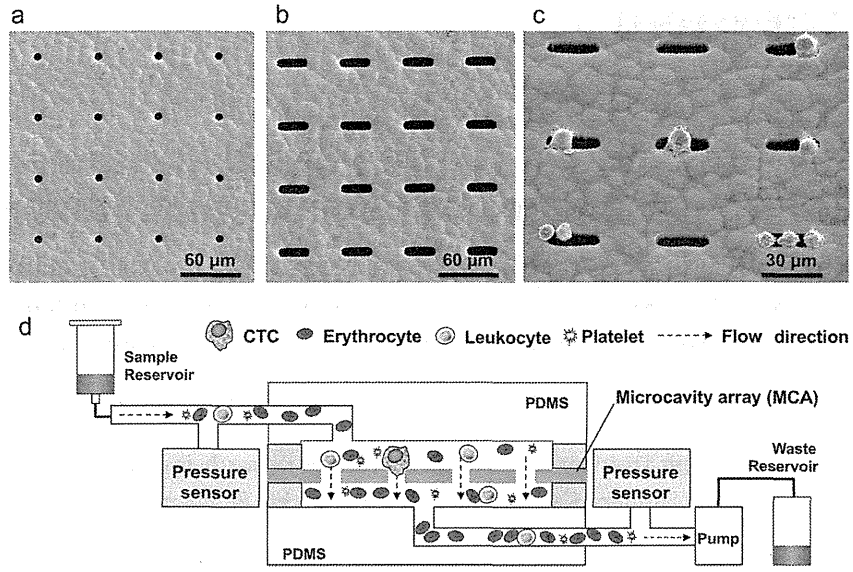

Figure 1. MCA for size-based isolation of circulating tumor cells. (a) SEM image of the circular MCA. (b) SEM image of the rectangular MCA. (c) SEM image of NCI-H69 cells trapped on the rectangular MCA. (d) Schematic image of the CTC enrichment device.

microcavity array (MCA) system for highly efficient enrichment of cells based on differences in cell size.[29−31] In this system, use of a miniaturized device allowed for the introduction of a series of reagents through the microfluidic structure for the detection of tumor cells. The shapes and sizes of the circular microcavities were designed to trap tumor cells, while allowing blood cells to flow through during whole blood filtration. Using a circular microcavity with an 8 μm diameter, the detection efficiency of NSCLC cells in a 1 mL blood sample spiked with 10−100 cells was approximately 96%. Our recent clinical study showed that the circular MCA system might be superior to the CellSearch System for detecting CTCs in patients with NSCLC and small-cell lung cancer.[32]

Although some studies have described morphological variations in tumor cells, little is known about the morphological features of CTCs.[33] It was reported that a portion of the CTCs detected in the blood of a patient with colorectal cancer were the same size as or smaller than leukocytes, whereas the majority of CTCs were larger than benign leukocytes.[34] In addition, the size of the CTCs detected by the CellSearch System and ISET differed significantly. In a study of lung cancer patients, Krebs et al. reported that the size range of cells estimated by CellSearch was 4−18 μm and that measured by ISET was 12−30 μm.[15] Moreover, CTC clusters, defined as contiguous clusters of cells containing 3 or more nuclei, were observed by ISET in 38% of the patients examined, whereas CellSearch detected no CTC clusters. These results indicate that the immunomagnetic separation techniques cannot isolate large clusters (20−130 μm), and ISET lost small CTCs, which include cells with features suggestive of apoptosis, such as irregular nuclear or cytoplasmic condensation or frank fragmentation into dense rounded structures.

In this study, we further optimized the structure of MCA for enrichment of small-sized tumor cells such as those found in small-cell lung cancer (SCLC). We developed a rectangular MCA by electroforming to improve the number and purity of small tumor cells recovered from whole blood. Using this MCA, we conducted a clinical test for the enrichment and detection of CTCs from the blood of patients with metastatic SCLC. Our results highlight the potential of our MCA system for the detection of CTCs in patients with solid tumors such as lung cancer tumors and for further detailed molecular analyses of CTCs.

EXPERIMENTAL SECTION

Fabrication of CTC Enrichment Device. MCAs were made of nickel by electroforming as follows: a stainless steel plate was coated with SU-8 photoresists and exposed to UV light through a photomask to form the MCA pattern. By electroformation, nickel was built up in the bare areas of the stainless steel plate between the photoresists. Finally, the electroformed MCA was separated from the stainless steel plate. Each circular microcavity was fabricated with a diameter of 8−9 μm, and the rectangular microcavity was fabricated with a width of 5−9 μm and a length of 30 μm. The distance between each microcavity was 60 μm, and 10 000 (100 × 100) cavities were arranged in an 18 × 18 mm sheet (Figure 1a,b). A poly(dimethylsiloxane) (PDMS) structure equipped with a vacuum microchannel (i.d. 500 μm) was fitted directly beneath the MCA to apply negative pressure for CTC isolation, while a chamber for blood sample introduction was constructed on the upper side (Figure 1d). Master mold substrates comprising poly(methyl methacrylate) (PMMA) were prepared by a computer-aided modeling machine (PNC-300; Roland DG Corp., Shizuoka, Japan). PDMS layers were then fabricated by pouring a mixture of Sylgard 184 silicone elastomer (Dow Corning Asia Ltd., Tokyo, Japan) and curing agent (10:1) onto either the master molds or a blank wafer, followed by curing for at least 20 min at 85 °C. Upon curing, the PDMS substrates were carefully peeled off the molds. The CTC enrichment device was constructed by assembling the MCA and the PDMS layers using spacer tape in the same manner as previously described.[31] The sample inlet was connected to a reservoir, while the vacuum microchannel was connected to a peristaltic pump.

Cell Culture and Labeling. The SCLC cell lines NCI-H69 and NCI-H82 and the NSCLC cell lines NCI-H358 and NCI-H441 were cultured in RPMI 1640 medium containing 2 mM L-glutamine (Sigma-Aldrich, Irvine, UK), 10% (v/v) FBS (Invitrogen Corp., Carlsbad, CA), and 1% (v/v) penicillin/

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streptomycin solution (Invitrogen Corp.) for 3–4 days at 37 °C in a humidified atmosphere containing 5% CO_2. Immediately before each experiment, confluent cells were trypsinized and resuspended in PBS.

To measure tumor cell size, the cell size distribution was measured using a CASY cell counter and Analyzer System (Model TTC; Schärfe System GmbH, Reutlingen, Germany). In the performance test, the tumor cells were labeled with CellTracker Red CMTPX (Molecular Probes, Eugene, OR), and labeling was achieved by incubating the cells with a tracking dye (5 μM) for 30 min. The cells were then pelleted by centrifugation (200*g* for 5 min); the supernatant was decanted, and the cells were washed twice with PBS to remove any excess dye. Finally, the cells were resuspended in PBS containing 2 mM EDTA and 0.5% BSA.

Blood Sample Preparation. Normal human blood samples were collected from healthy donors at the Tokyo University of Agriculture and Technology in accordance with Institutional Review Board procedures. Samples were collected in a collection tube with EDTA to prevent coagulation and used within 24 h. The clinical study was conducted at Shizuoka Cancer Center (UMIN clinical trial registry, number UMIN000005189), and patients with pathologically proven SCLC with radiological evident metastatic lesions were eligible for the study. The institutional review board at Shizuoka Cancer Center approved the study protocol, and all patients provided written informed consent. Peripheral blood samples were collected from 16 patients with histologically or cytologically confirmed metastatic SCLC. For each patient, 10–15 mL of blood was collected in EDTA tubes for CTC enumeration by the MCA system and processed within 2 h.

Tumor Cell Entrapment Operation. Blood samples or cell suspensions (1–5 mL) were added to the reservoir. Subsequently, negative pressure was applied to the cell suspension with a peristaltic pump connected to the vacuum line. The sample was passed through the microcavities at a flow rate of 200 μL/min for 0.5–10 min. To remove blood cells that remained on the array, PBS containing 2 mM EDTA and 0.5% BSA (2 mL) was then added to the reservoir and passed through the microcavities at a flow rate of 200 μL/min for 10 min.

Staining of Trapped Cells for the Identification of CTCs. Cell fixation solution and cell staining solution were introduced into the reservoir and passed through the microcavities with a peristaltic pump after washing. To stain the CTCs with an anticytokeratin antibody, the trapped cells were fixed by passing 400 μL of 1% PFA in PBS through the MCA at a flow rate of 20 μL/min for 20 min. After washing with 100 μL of PBS, the cells were subsequently treated with 200 μL of 0.2% Triton X-100 in PBS at a flow rate of 20 μL/min for 10 min. After permeabilization, the cells were treated with 3% BSA in PBS at a flow rate of 20 μL/min for 30 min. To identify CTCs and leukocytes, 600 μL of cell staining solution containing 1 μg/mL Hoechst 33342 (Molecular Probes, Invitrogen Corp.), a cocktail of antipan-cytokeratin antibodies (Alexa488-AE1/AE3 [1:100 dilution; eBioscience, San Diego, CA] and FITC-CK3-6H5 [1:60 dilution; Miltenyi Biotec, Auburn, CA]) and a PE-labeled anti-CD45 antibody [1:120 dilution; BD Biosciences, San Jose, CA]) was passed through the microcavities at a flow rate of 20 μL/min for 30 min. Finally, the array was washed with 1 mL of PBS containing 2 mM EDTA and 0.5% BSA to remove excess dye.

Identification and Enumeration of CTCs by Fluorescence Microscopy. After the tumor cells were recovered, an image of the entire cell array area was obtained using a fluorescence microscope (BX61; Olympus Corporation, Tokyo, Japan) integrated with a 10× objective lens and a computer-operated motorized stage, WU, NIBA, and WIG filter sets, a cooled digital camera (DP-70; Olympus Corporation), and Lumina Vision acquisition software (Mitani Corporation, Tokyo, Japan). In clinical trials, an entire image of the cell array area was obtained using a fluorescence microscope (Axio Imager Z1; Carl Zeiss, Oberkochen, Germany) integrated with a 10× or 20× objective lens and a computer-operated motorized stage, WU, FITC, and Texas Red filter sets, a digital camera (AxioCam HRc; Carl Zeiss), and AxioVision acquisition software (Carl Zeiss). Image analysis was then performed, and objects satisfying the predetermined criteria were counted. Fluorescence intensities and morphometric characteristics such as cell size, shape, and nuclear size were considered when performing CTC identification and nontumor cell exclusion; cells were characterized by a round to oval morphology and a visible nucleus (i.e., Hoechst-33342 positive), and those that were positive for cytokeratin and negative for CD45 were identified as CTCs (Supporting Information Figure S-1).

Measurement of Pressure Drop across the MCA. To measure the pressure drop across the MCA, disposable blood pressure transducers (ADInstruments, Colorado Springs, CO) were connected to the upstream and downstream lines of the CTC recovery device. A multichannel data-recording unit (PowerLab System; ADInstruments) was used for continuous pressure monitoring, and data was analyzed using LabChart software (ADInstruments).

Statistical Analysis. For comparison of the recovery rate of rectangular MCA (ϕ 8 μm) with the circular MCA (8 × 30 μm), 1000 cells of each of NCI-H69, NCI-H82, and NCI-H358 were spiked into 1 mL of whole blood of a healthy donor and then processed by the MCA assay in triplicate. Comparison of the average number of tumor cells and leukocytes recovered were carried out using unpaired, two-tailed Student's *t*-test. In the clinical test, the same blood samples were processed through the circular and rectangular MCAs side-by-side. Comparison of the number of CTCs and leukocytes recovered was carried out using a two-tailed Wilcoxon test. In addition, we tested the correlations between variables by calculating the Spearman's rank correlation coefficients. All statistical analyses were performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). *P* values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Pressure Drop via the MCA. In a previous study, we optimized the size of the circular MCA system to enable efficient recovery of tumor cells from human whole blood without clogging.[31] Our device successfully recovered more than 80% of the tumor cells spiked in 1 mL of whole human blood without pretreatment (e.g., density gradient centrifugation or erythrocyte lysis) when 8–9 μm diameter microcavities were used. However, 1000–3000 leukocytes were trapped on the MCA when 1 mL of whole blood was passed through the MCA. Because the diameter of the circular microcavity was smaller than the tumor cells and leukocytes, a number of microcavities were occupied by single cells after whole blood filtration. Therefore, the pressure gradient across the circular

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MCA increased during blood filtration, and this increase might have caused the loss of small tumor cells through the cavities and damage to trapped cells.

In this study, a rectangular MCA was developed to improve the recovery of small tumor cells. The width of this rectangular microcavity was designed to be 5–9 μm for effective capture of CTCs based on typical tumor cell size. The length of the rectangular microcavity was designed to be 30 μm to prevent the occupation of microcavities by single cells and increase the porosity of the substrate; the lengths of rectangular microcavities were measured by microscopic observation. The widths were measured to be 4.7 ± 0.1, 6.0 ± 0.2, 7.2 ± 0.1, 8.3 ± 0.1, and 9.2 ± 0.4 μm.

First, the pressure drop during filtration was compared between the circular and rectangular MCAs. During PBS filtration using either MCA, the pressure drop was similar (0.3–0.4 kPa). In contrast, during filtration of an NCI-H441 cell suspension containing an excess number of cells compared to the number of microcavities, the pressure drop across the MCA increased as a function of cell number (Figure 2). The

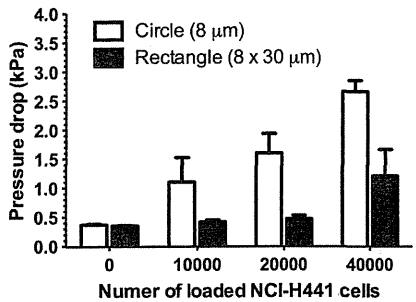

Figure 2. Pressure drop across the MCA during filtration of NCI-H441 cell suspensions.

pressure drop across the circular MCA was higher than that across the rectangular MCA due to occupation of microcavities by single cells. As shown in Figure 1c, although multiple cells were simultaneously trapped on single rectangular microcavities, the microcavities were not completely clogged.

The change in the pressure during filtration of a 3 mL whole blood sample and subsequent washing was then monitored (Figure 3). The pressure immediately increased after introduction of the blood sample and then gradually increased further as a function of filtration time. After washing solution was introduced, the pressure across the MCA immediately

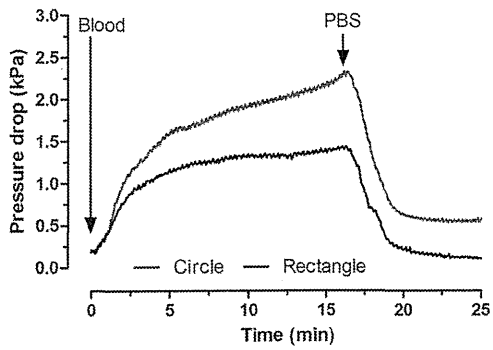

Figure 3. Monitoring the increase in pressure drop across the MCA during filtration of whole blood. Three milliliters of blood were introduced into the MCAs and then washed with PBS.

decreased. However, the maximum pressure drop across the circular MCA (2.3 kPa) was higher than that across the rectangular MCA (<1.5 kPa). In addition, with the rectangular MCA, the pressure returned to the original value after introduction of the washing buffer, whereas that of the circular MCA was slightly higher than the original value. This result suggests that some microcavities were occupied by blood cells with the circular MCA; the pressure was maintained at a higher level than the original value even after washing the MCA. These results suggest that the rectangular MCA enabled a reduction in the flow resistance and pressure drop across the microcavities in agreement with Kuo et al.[35]

Optimization of the Rectangular MCA. To optimize the design of the rectangular MCA, microcavities of various sizes were tested using tumor cell-spiked whole blood (Figure 4).

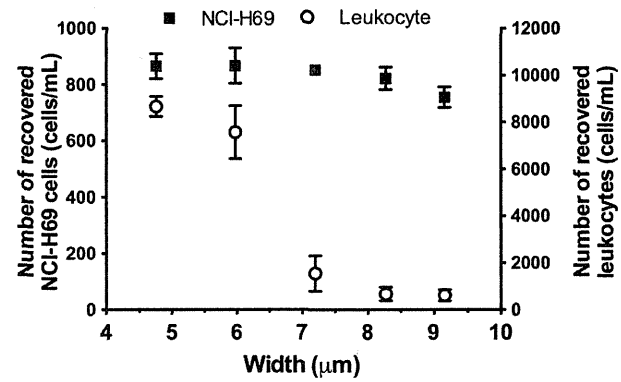

Figure 4. Relationship between the size of the rectangular microcavities and the number of recovered cells. Whole blood samples were spiked with CellTracker Red-stained NCI-H69 cells at 1000 cells/mL and recovered using various MCAs. The average number of tumor cells and leukocytes recovered is given.

The widths of the rectangular microcavities fabricated in this study were considerably smaller than the average diameter of the SCLC cell lines NCI-H69 (12.5 μm) and NCI-H82 (13.5 μm). Although the number of NCI-H69 cells recovered decreased slightly as the width of the rectangular microcavity increased, the recovery rates of all rectangular MCAs were higher than those of the circular MCAs. In addition, the number of trapped leukocytes varied according to the width of the rectangular microcavity. When the average width of the rectangular microcavity was greater than 7 μm, a number of leukocytes passed through the microcavities, resulting in a decrease in the number of captured leukocytes. Our previous study showed that the number of leukocytes captured depended on the effects of size, i.e., the size difference between leukocytes and microcavities, and retention, i.e., cellular deformability and adhesiveness.[30] Since leukocytes include cells that differ in size, deformability, and adhesiveness, the number of captured cells increased as the width of the MCA decreased. Therefore, to obtain a high recovery rate and high tumor cell purity, subsequent tumor cell-recovery experiments were conducted using rectangular MCAs containing microcavities with an average width of 8 μm.

To compare the recovery rates of the rectangular and circular MCAs, an NSCLC cell line (NCI-H358) and 2 SCLC cell lines (NCI-H69 and NCI-H82) were evaluated. When NCI-H358 cells were used in the recovery test, no obvious difference was observed between the 2 MCAs (Table 1). In contrast, when NCI-H69 and NCI-H82 cells were used, the recovery rates

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Table 1. Comparison of the Cell Recovery Rates between the Circular and Rectangular MCAs[a]

	number of recovered cells			
microcavity type	NCI-H358	NCI-H69	NCI-H82	leukocytes
circular	897 ± 40	672 ± 75	646 ± 58	1515 ± 334
rectangular	898 ± 34	802 ± 48	829 ± 49	251 ± 30

[a]Whole blood samples were spiked with 3 different lung cancer cell lines, NCI-H358, NCI-H69, and NCI-H82, at 1000 cells/mL and were recovered using the circular MCA (ϕ 8 μm) and the rectangular MCA (8 × 30 μm). The average number of tumor cells and leukocytes recovered is shown.

using the optimized rectangular MCA (80% ± 5% and 83% ± 5%, respectively) were significantly higher than those using the circular MCA (67% ± 7%; $p = 0.01$ by t-test and 65 ± 6%; $p < 0.01$ by t-test, respectively). In addition, the number of leukocytes captured on the rectangular MCA was 7-fold lower than that captured on the circular MCA. As a result, tumor cells were enriched more than 7000-fold on the rectangular MCA from whole blood. As described above, the occupation of microcavities by blood cells increased the pressure. We observed that, once cells occupied the circular microcavities, other cells were preferentially driven toward the unoccupied microcavities and passed through them at a higher pressure. Therefore, we believe that the excessive flow resistance and pressure increase caused cell deformation and allowed the cells to pass through the circular microcavities, resulting in increased loss of small tumor cells such as SCLC cells. The fluctuation in pressure during filtration using the rectangular MCA was smaller than that using the circular MCA. The rectangular MCA enabled recovery of small tumor cells with high efficiency.

We previously reported that approximately 98% of cells recovered with the circular MCA were viable.[31] In this study, the viability of the recovered tumor cells was further validated by expanding the cells in culture. NCI-H358 cells recovered on the MCA were retrieved with a micropipet and then reseeded in a culture dish. Adherence and growth of the NCI-H358 cells captured from whole blood was observed (Supporting Information Figure S-2).

Study of Blood Sample Preparation. This study was aimed at understanding the conditions that enable efficient direct recovery of small tumor cells from whole blood. Small-sized tumor cells and cellular aggregates can be lost as a result of blood sample pretreatments before filtration, such as density gradient centrifugation and erythrocyte lysis, because they require a cycle of decantation and pipetting, resulting in the loss of rare cells. In addition, due to either the migration of cells to the plasma layer or the presence of aggregates, CTCs can be easily lost during density gradient centrifugation.[36] In addition, we avoided cell fixation before filtration to recover intact CTCs. To evaluate the effect of anticoagulants on blood filtration, heparin tubes were also used for blood collection and spiked tumor cell recoveries were compared. During filtration of 1 mL of whole blood collected in a heparin tube, the pressure across the circular and rectangular MCAs increased by 40 and 9.5 kPa, respectively, suggesting that the microcavities were clogged by blood cells. Indeed, fluorescent imaging of the array after filtration of whole blood collected in a heparin tube showed blood clotting (data not shown). Therefore, all experiments were performed using blood collected in an EDTA tube, and the effect of the time that elapsed before mixing the collected blood with anticoagulant on the recovery of spiked tumor cells was determined. Blood was collected without using an anticoagulant and then dispensed into EDTA tubes after a certain period; as a result, the recovery rates of spiked tumor cells varied according to the time that elapsed before mixing the collected blood with an anticoagulant (Supporting Information Figure S-3). The cell recovery of a sample prepared 1 min after blood collection was approximately 10% lower than that of a sample prepared immediately after blood collection. This was confirmed by clogging of the array in the fluorescence images after filtration of these samples. Furthermore, the number of macroscopic blood clots on the array was increased as a function of elapsed time. Therefore, we collected blood samples

Table 2. Numbers of CTCs and Leukocytes Recovered from SCLC Patients Using the Circular and the Rectangular MCAs

	number of recovered CTCs (cells/mL)		number of recovered leukocytes (cells/mL)		percentage of CTCs in total captured cells (%)	
patient no.	circular MCA	rectangular MCA	circular MCA	rectangular MCA	circular MCA	rectangular MCA
1	1.7	2.3	2677	456	0.06	0.51
2	1.3	11.3	3741	2569	0.03	0.44
3	5.3	51.3	4354	4652	0.12	1.09
4	0.9	3.0	1556	545	0.06	0.55
5	3.0	2.0	1831	881	0.16	0.23
6	1.8	4.5	2843	1516	0.06	0.29
7	16.3	12.6	2096	1096	0.77	1.13
8	0.3	14.7	2613	3188	0.01	0.46
9	1.7	1.4	2781	457	0.06	0.31
10	0.6	0.9	3611	660	0.02	0.14
11	5.3	2.1	2058	598	0.26	0.34
12	4.9	0.3	2197	855	0.22	0.03
13	14.7	0.6	2014	852	0.72	0.07
14	0.3	0.7	1460	630	0.02	0.11
15	3.0	0.4	1063	659	0.28	0.06
16	19.3	72.7	2341	2605	0.82	2.71
median ± SEM	2.4 ± 1.5	2.2 ± 5.2	2269 ± 220	854 ± 306	0.09 ± 0.07	0.33 ± 0.17

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using evacuated blood collection tubes to prevent blood coagulation.

Clinical Test. For the clinical evaluation, 16 patients with extensive SCLC were enrolled in the study. The same blood samples were processed through the circular and rectangular MCAs side-by-side. Joosse et al. reported that broadening the spectrum of keratin detection increased CTC detection, thereby reducing the number of false-negatives.[37] Therefore, in this study, to stain a broad range of keratins, an anticytokeratin antibody cocktail of Alexa488-AE1/AE3 and FITC-CK3-6H5 was used for CTC detection. In addition, to evaluate the detection sensitivity and capacity, 7.5 mL of healthy donor blood, spiked with 1 tumor cell, was assessed with the MCA used in the recent report.[32] Therefore, we believe that the capacity of this method is limited to 7.5 mL of whole blood. In this study, we performed the clinical tests with 2–4 mL of whole blood considering the heterogeneity of patients' blood samples.

With the rectangular MCA, CTCs were detectable in 16 of 16 SCLC patients (Table 2). The number of CTCs isolated with the rectangular (range, 0.3–72.7 cells/mL; median, 2.2 cells/mL) and circular MCAs (range, 0.3–19.3 cells/mL; median, 2.4 cells/mL) did not differ significantly (p = 0.60, Wilcoxon test).[32] There was no statistically significant correlation between them (Spearman's rho was 0.14, p = 0.60). We speculated that the reason for this was the tendency of circulating CTCs in clinical samples to form aggregates, including CTC clusters that are large enough to be captured on single microcavities. In contrast, the number of leukocytes captured with the rectangular (range, 456–4652 cells/mL; median, 854 cells/mL) and circular MCAs (range, 1063–4354 cells/mL; median, 2269 cells/mL) differed significantly (p < 0.001, Wilcoxon test). There was no statistically significant correlation between them (Spearman's rho was 0.41, p = 0.12). The percentage of CTCs in total captured cells on the rectangular MCA (0.33% ± 0.17%) was significantly higher that on the circular MCA (0.09% ± 0.07%) (p = 0.03, Wilcoxon test). There was no statistically significant correlation between them (Spearman's rho was 0.09, p = 0.73). On average, the number of leukocytes captured with the rectangular MCA was approximately 2-fold lower than that with the circular MCA. However, the number of leukocytes captured varied widely among patients compared to the number of leukocytes from normal blood samples. We attributed this to the differences between cancer patients and normal volunteers in blood properties, such as viscosity and aggregability, which depend on plasma protein concentration or hematocrit. Although we have improved the number and purity of small tumor cells recovered from whole blood in this study (which might be lost with the conventional size-based enrichment methods), further clinical studies should be performed with larger cohorts of patients with various types of cancers to improve the number and purity of CTCs isolated from patient blood. In previous reports,[31,32] we performed the CTC enumeration assays using negative control samples (healthy donor blood). In these samples, no CTCs (false-positive) were observed by fluorescent imaging. However, leukocytes clogged the microcavities, increasing the background fluorescent noise of the MCA substrate or trapped cells. Therefore, we believe that the reduction in the number of leukocytes captured on the MCA is a significant factor for the prevention of false-positive CTCs and further gene analysis of CTCs.

Recently, various microfluidic devices have been developed to improve the enrichment of CTCs from whole blood.[24,27,28,35,38] The advantage of the size-based enrichment techniques is that it enables simple and rapid processing of a large volume of whole blood (≥1 mL) compared to other techniques. In addition, our microcavity array was integrated with a microfluidic device so that enrichment, staining, and washing processes in the microfluidic assay could be performed within one integrated device. We have also developed a technique for single cell isolation by using microcapillaries and subsequent gene analysis using MCA.[29,39] We could obtain only CTCs from a patients' blood by integrating these techniques with the rectangular MCA. However, further clinical studies should be performed with larger cohorts of patients with various types of cancers to assess whether the MCA system is a more appropriate tool for CTC enumeration and characterization of metastatic tumors in patients with cancers other than lung cancer.

CONCLUSION

In this study, we improved the structure of the MCA to efficiently recover small-sized tumor cells by size-based isolation of tumor cells from whole blood. Using this system, CTCs were successfully recovered from the whole blood of patients with SCLC with higher purity than the previously developed system. Therefore, the MCA system has potential as a tool for the efficient recovery of CTCs with high purity in patients with small cell-type tumors, while offering additional advantages in cost, portability, and capacity for further detailed analyses of CTCs.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): MH, TYoshino, YKikuhara, HKanbara, and TM have applied for patents related to the MCA system.

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Chemoradiotherapy for Limited-disease Small-cell Lung Cancer in Elderly Patients Aged 75 Years or Older

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Background: As clinical trials for limited-disease small-cell lung cancer often exclude elderly patients due to comorbidities and a decline in organ function, the most suitable treatment for limited-disease small-cell lung cancer patients aged 75 years or older still remains unclear.
Methods: From July 2002 to June 2011, 20 consecutive patients aged 75 years or older, with Stage II to IIIB limited-disease small-cell lung cancer, were scheduled to be treated with concurrent or sequential chemoradiotherapy at the Shizuoka Cancer Center. We reviewed the medical charts of the patients and evaluated their characteristics, treatment compliance, toxicity and antitumor efficacy.
Results: Five patients were treated with concurrent chemoradiotherapy and the other 15 patients were scheduled to be treated with sequential chemoradiotherapy. Of these 15 patients, 12 were treated with four cycles of etoposide (80 mg/m^2, days 1–3, q3–4w) plus carboplatin (area under the curve 5, day 1, q3–4w), followed by thoracic radiotherapy. Of the five patients treated with concurrent chemoradiotherapy, discontinuation of chemotherapy/thoracic radiotherapy occurred in two patients due to toxicity and they suffered a prolonged decrease in performance status. Of the 12 patients treated with etoposide plus carboplatin followed by sequential thoracic radiotherapy, the response rate, median progression-free survival and median overall survival time were 91%, 244 and 601 days.
Conclusions: These results suggest that concurrent chemoradiotherapy is not feasible for all limited-disease small-cell lung cancer patients aged 75 years or older. The alternative of four cycles of etoposide plus carboplatin followed by thoracic radiotherapy is a candidate for the standard treatment of limited-disease small-cell lung cancer patients in this age group. A further trial is warranted to develop and evaluate the optimal treatment for elderly patients with limited-disease small-cell lung cancer.

Key words: small-cell lung cancer – limited-disease small-cell lung cancer – elderly – chemoradiotherapy – chemotherapy – radiotherapy – feasibility – efficacy

INTRODUCTION

Small-cell lung cancer (SCLC) accounts for 10–15% of all lung cancer cases, with individuals aged 70 years or older constituting 25–40% of SCLC patients (1,2). Limited-disease SCLC (LD-SCLC) is confined to one hemithorax and its regional lymph nodes, and can be treated using a

single radiation therapy port. Approximately 30–40% of all SCLC patients present with LD-SCLC (1,2). The proportion of elderly SCLC patients continues to increase with the growing geriatric population (1,3).

The combination of chemotherapy and radiotherapy, particularly etoposide plus cisplatin with early concurrent twice-daily thoracic radiotherapy (TRT), is regarded as the standard treatment for LD-SCLC, provided the patients are in a good general condition (4–6). However, many clinical trials for LD-SCLC have excluded elderly patients for reasons, such as comorbidities or a decline in organ function (7,8). Takada et al. (6) reported that etoposide plus cisplatin and concurrent TRT are more effective for the treatment of LD-SCLC than are etoposide plus cisplatin and sequential TRT, but patients aged 75 years or older were excluded from this trial.

Retrospective subset analyses of patients with LD-SCLC treated with etoposide plus cisplatin and concurrent early chemoradiotherapy (CRT) in Phase III trials have shown that severe hematological toxicity, pneumonitis of Grade 4 or more and treatment-related death occurred much more often among patients aged 70 years or older than among younger patients (9,10). Although the response rate and 5-year event-free survival rate did not significantly differ between these two subgroups, there was a trend for them to be worse in older patients, and significant difference in the 5-year overall survival rate favored patients younger than 70 years in one trial (9,10). These results suggest that this regimen is too toxic for elderly LD-SCLC patients and the most suitable method of treatment remains unclear.

The objective of our retrospective analysis was to discover the optimal treatment method for elderly patients with LD-SCLC aged 75 years or older. We compared the patient characteristics, treatment compliance, toxicity and antitumor efficacy between those undergoing concurrent and sequential CRT. Then, we focused on etoposide plus carboplatin and sequential TRT, as this is the most common method for treating elderly LD-SCLC patients in our institute, and evaluated their characteristics, treatment compliance, toxicity and antitumor efficacy of this regimen.

PATIENTS AND METHODS

Patient Selection

We reviewed 20 consecutive patients with Stage II–IIIB LD-SCLC, aged 75 years or older, whose treatment plan involved concurrent or sequential CRT at the Shizuoka Cancer Center between July 2002 and June 2011. The TNM stage was classified using TNM stage version 6 (11). Chest CT, abdominal CT, bone scintigram or FDG-PET, and brain magnetic resonance imaging (MRI)/CT were performed before treatment in all patients.

The inclusion criteria for concurrent or sequential CRT in our institution are generally as follows: a performance status (PS) of 0–2; white blood cell count, $\geq 3.0 \times 10^3/mm^3$; neutrophil count, $\geq 1.5 \times 10^3/mm^3$; platelet count, $\geq 1.0 \times 10^5/mm^3$; serum creatinine, ≤ 1.5 mg/dl; total bilirubin, ≤ 1.5 mg/dl and a transaminase level less than twice the upper limit of the normal value. The exclusion criteria were interstitial lung disease identified by a chest radiograph; the presence of malignant pleural or pericardial effusion prior to radiotherapy and serious complications, such as severe respiratory failure, active infectious diseases, serious heart diseases and poorly controlled hypertension/diabetes mellitus. The study protocol was approved by the institutional review board of Shizuoka Cancer Center.

Chemotherapy

The combination of etoposide (80 or 100 mg/m^2) on days 1–3 plus cisplatin (80 mg/m^2) on day 1, cisplatin (25 mg/m^2) on days 1–3, or carboplatin [area under the curve (AUC) 5] on day 1 were administered intravenously to elderly LD-SCLC patients every 3–4 weeks. The administered drug and its dose were determined by the physician in charge. The treatment cycles were repeated every 3–4 weeks for four cycles. The criteria for starting subsequent cycles of treatment in our institution are generally the same as the inclusion criteria for concurrent or sequential CRT mentioned in the 'Patient selection' section. If these criteria were not met, subsequent cycles were withheld until the noted abnormality had resolved. If there was no resolution of the abnormality after 7 weeks from the first day of the cycle, chemotherapy was stopped. Generally, the doses of etoposide and cisplatin or carboplatin were reduced or chemotherapeutic regimens were changed in the event of Grade 4 anemia, Grade 4 thrombocytopenia, prolonged Grade 4 leukopenia/neutropenia or Grade 3 or more severe non-hematological toxicity during the previous treatment cycle.

Radiotherapy

Generally, TRT was started concurrently in the first cycle of chemotherapy or sequentially after four cycles of chemotherapy in the elderly LD-SCLC patients. The timing and prescribed dose of TRT was determined by the physician in charge. All patients were required to undergo a chest CT to facilitate treatment planning. The primary tumor (gross tumor volume; GTV primary) was delineated in the pulmonary windows, and the nodal involvement (GTV node) was delineated in the mediastinal windows. The clinical target volume (CTV) included the GTV primary; GTV node; ipsilateral hilum and the elective mediastinum, for which the lower border was 3.0 cm below the carina up to 40 Gy in a once-daily fraction of 2 Gy per fraction or 30 Gy in twice-daily fractions of 1.5 Gy per fraction. Thereafter, CTV included the GTV primary and GTV node. The planning target volume was the CTV plus a margin to ensure that the planned dose was actually delivered to the CTV. The total planned dose was usually 50 Gy in a once-daily fraction or 45 Gy in twice-daily fractions. The initial field in the sequential arm was also based on the pretreatment tumor volume.

TRT was suspended if a patient experienced Grade 4 thrombocytopenia, radiation pneumonitis, fever caused by infection, a decrease in arterial oxygen pressure exceeding 10 mmHg or if a patient had difficulty swallowing a liquid diet. It was ensured that the normal lung volume receiving more than 20 Gy (V20) was $\leq$35% of the total lung volume. The maximum spinal cord dose was limited to 45 Gy in a once-daily fraction or 36 Gy in twice-daily fractions at any level.

After TRT, prophylactic cranial irradiation (PCI) was administered to patients with a complete or near-complete response represented by a scar-like shadow on a chest CT if the physician in charge judged the patient would benefit from PCI. The PCI consisted of 25 Gy/10 fr.

Evaluation of Efficacy and Toxicity

All the patients were evaluated for lesions approximately every 2 months by CT, MRI, bone scintigraphy or PET during the treatment period and every 3–6 months after treatment. The tumor response was evaluated in accordance with the response evaluation criteria in solid tumors (RECIST; version 1.0) (12). Adverse events were evaluated in accordance with the common terminology criteria for adverse events (CTCAE; version 3.0) (13).

Statistical Analyses

To evaluate the difference between concurrent CRT and sequential CRT, in relation to the patients' characteristics, the χ^2 test, Fisher's exact test and the Mann–Whitney *U*-test were performed. To analyze the PFS and OS, survival curves were drawn using the Kaplan–Meier method. The PFS was calculated from the date of initiation of the treatment to the date of detection of disease progression or the date of death from any cause. The PFS was censored at the date of the last visit for those patients who were still alive without any documented disease progression. PFS were compared between concurrent CRT and sequential CRT using the log-rank test. The OS was calculated from the date of initiation of the treatment to the date of death. The OS was censored at the date of the last visit for those patients whose deaths could not be confirmed. *P* values of <0.05 were considered to be statistically significant. All statistical analyses were performed by the application of JMP version 8.0 for Windows (SAS Institute Inc., Cary, NC, USA).

RESULTS

Characteristics and Treatment Methods of the 20 Patients Treated with Chemoradiotherapy

Twenty patients 75 years of age or older and with Stage II–IIIB LD-SCLC were scheduled to be treated with concurrent or sequential CRT at the Shizuoka Cancer Center. During the same period, seven patients 75 years of age or older and with Stage II–IIIB LD-SCLC were excluded by the inclusion/exclusion criteria of CRT. The reasons for exclusion were interstitial lung disease in six patients and renal failure in one patient. Tables 1 and 2 show the individual patients' characteristics, treatment methods and outcome of the patients treated with concurrent and sequential CRT. Of these patients, 80% were men and their median age was 77 years. Forty percent of the patients had a PS of 0 and the remaining a PS of 1. The majority of the patients were smokers and 80% were Stage IIIA or IIIB.

Five patients were treated with concurrent CRT and 15 were scheduled to be treated with sequential CRT. Of the five treated with concurrent CRT, two received TRT from the first cycle of chemotherapy and three received TRT from the second cycle of chemotherapy. From the beginning, two were scheduled to receive TRT from the second cycle after the confirmation of toxicity in the first cycle. The other patient was also scheduled to receive TRT from the second cycle if the symptom due to tumor compression had not recovered by chemotherapy only. Two patients received etoposide (80 mg/m^2, days 1–3) plus carboplatin (AUC 5, day 1), two were administered etoposide (100 mg/m^2, days 1–3) plus cisplatin (80 mg/m^2, day 1) and one received etoposide (80 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3) as their chemotherapy regimen. Of these patients, one patient switched from etoposide (80 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3) to etoposide (80 mg/m^2, days 1–3) plus carboplatin (AUC 5, day 1) from cycle 2 due to Grade 4 hyponatremia and Grade 3 anorexia.

Of the 15 patients scheduled to be treated with sequential CRT, 12 received etoposide (80 mg/m^2, days 1–3) plus carboplatin (AUC 5, day 1), two received etoposide (80 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3) and one was administered etoposide (100 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3) as chemotherapy. Two patients could not receive TRT due to discontinuation of treatment during the chemotherapy period.

The planned TRT doses were 45 Gy in twice-daily fractions and 1.5 Gy per fraction in 12 patients, 50 Gy in a once-daily fraction and 2 Gy per fraction in three patients, and the other radiation doses in three patients. PCI was performed in Patient #C-5 and #S-13.

Table 3 shows the individual patients' characteristics, past history and complications of the patients treated with concurrent and sequential CRT. Generally, past history and complications were fewer and less severe in concurrent CRT, especially in terms of cardiopulmonary diseases.

Comparison of Patient Characteristics, Response, PFS, Compliance and Adverse Events Between Concurrent CRT and Sequential CRT

In terms of patient characteristics, (gender, age, PS, stage), the difference in age between concurrent CRT and sequential CRT is significant (Mann–Whitney *U*-test $P = 0.041$).