Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. CD133 and CD87 expression and sort position in SBC-7 cell line.

Fig. S2. The expression levels of aldehyde dehydrogenase 1A1 (ALDH1A1) in each subpopulation by western blotting.

Fig. S3. Growth curves of each subpopulation.

Fig. S4. The cell surface expression levels of MDR1 on each subpopulation by flow cytometry.

Fig. S5. Hematoxylin-eosin staining of xenograft tumors.

Fig. S6. The cell viability of CD133+/CD87− cells and CD133−/CD87− cells in the SBC-9 after treatment with cisplatin, etoposide or paclitaxel.

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Carboplatin- or Cisplatin-Based Chemotherapy in First-Line Treatment of Small-Cell Lung Cancer: The COCIS Meta-Analysis of Individual Patient Data

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ABSTRACT

Purpose
Since treatment efficacy of cisplatin- or carboplatin-based chemotherapy in the first-line treatment of small-cell lung cancer (SCLC) remains contentious, a meta-analysis of individual patient data was performed to compare the two treatments.

Patients and Methods
A systematic review identified randomized trials comparing cisplatin with carboplatin in the first-line treatment of SCLC. Individual patient data were obtained from coordinating centers of all eligible trials. The primary end point was overall survival (OS). All statistical analyses were stratified by trial. Secondary end points were progression-free survival (PFS), objective response rate (ORR), and treatment toxicity. OS and PFS curves were compared by using the log-rank test. ORR was compared by using the Mantel-Haenszel test.

Results
Four eligible trials with 663 patients (328 assigned to cisplatin and 335 to carboplatin) were included in the analysis. Median OS was 9.6 months for cisplatin and 9.4 months for carboplatin (hazard ratio [HR], 1.08; 95% CI, 0.92 to 1.27; $P = .37$). There was no evidence of treatment difference between the cisplatin and carboplatin arms according to sex, stage, performance status, or age. Median PFS was 5.5 and 5.3 months for cisplatin and carboplatin, respectively (HR, 1.10; 95% CI, 0.94 to 1.29; $P = .25$). ORR was 67.1% and 66.0%, respectively (relative risk, 0.98; 95% CI, 0.84 to 1.16; $P = .83$). Toxicity profile was significantly different for each of the arms: hematologic toxicity was higher with carboplatin, and nonhematologic toxicity was higher with cisplatin.

Conclusion
Our meta-analysis of individual patient data suggests no differences in efficacy between cisplatin and carboplatin in the first-line treatment of SCLC, but there are differences in the toxicity profile.

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INTRODUCTION

Small-cell lung cancer (SCLC) accounts for approximately 15% of all lung cancers. At presentation, approximately 70% of patients are diagnosed as having extensive disease and the remaining patients are diagnosed as having limited disease.[1]

The main international guidelines recommend platinum-based chemotherapy as the standard of care for first-line therapy of SCLC.[2-4] However, whether cisplatin or carboplatin are equally effective in the treatment of SCLC is still contentious. These two platinum compounds have different toxicity profiles. Cisplatin is associated with more GI adverse effects, neurotoxicity, and renal function impairment, and its administration requires a prolonged hydration,[5,6] but carboplatin is associated with more myelosuppression.

Although the mechanisms of action are similar, it is unclear whether carboplatin and cisplatin have the same clinical efficacy. For some tumors such as ovarian cancer, randomized studies[7,8] supported the use of carboplatin instead of cisplatin; for other tumors, such as germ cell and head and neck tumors, cisplatin is superior to carboplatin.[9] Several meta-analyses have addressed the issue of cisplatin-based versus carboplatin-based chemotherapy in the first-line treatment of advanced non–small-cell lung cancer. Cisplatin-based regimens resulted in slightly superior outcomes compared

with carboplatin-based chemotherapy in terms of objective response rate (ORR) and, in certain subgroups, prolonged overall survival (OS), without being associated with a significant increase in toxic effects.[10,11]

With the aim of comparing the efficacy of cisplatin versus carboplatin in the first-line treatment of SCLC, we conducted a meta-analysis of individual patient data (COCIS; Carboplatin- or Cisplatin-Based Treatment for SCLC) on patients enrolled onto randomized trials comparing the effectiveness of these two compounds.

PATIENTS AND METHODS

Identification of Eligible Trials

A literature search was performed in December 2008 and was updated in June 2009 to identify all published and unpublished randomized trials comparing cisplatin- and carboplatin-containing chemotherapy as first-line treatment of patients with SCLC.[12] The search was performed by using PubMed, EMBASE, MEDLINE, and the Cochrane Database. Proceedings of the main international meetings (American Society of Clinical Oncology, European Society for Medical Oncology, European Cancer Conference, and World Conference on Lung Cancer) were searched from 2005 onward. The following key words were used: "small cell lung carcinoma," "carboplatin," "cisplatin," and "randomized trial."

Data Collection and Study Quality

Individual patient data were requested for all patients within each of the four identified trials. A list of the types of data collected is available in Appendix Table A1 (online only). Before performing the analyses, data from each study were carefully checked and verified for coherence with the original publications: database quality was good for all of the eligible studies.

Statistical Methods

All of the analyses planned and prespecified in the meta-analysis protocol were performed according to the intention-to-treat principle. All the analyses were stratified by trial, and all tests were two-sided.

The primary end point was OS, defined as the time between date of random assignment and date of death or last date of follow-up for censored patients. OS curves were estimated by using the Kaplan-Meier technique and were compared by using the stratified log-rank test. Median follow-up was calculated according to the inverted Kaplan-Meier technique.[13]

Because the meta-analysis was based on individual patient data, heterogeneity of treatment effect on OS among trials was assessed by the likelihood ratio of two trial-stratified models, one with trial-specific treatment estimates and one with overall treatment estimates.[14] Under the null hypothesis of no heterogeneity, this statistic follows approximately a χ^2 distribution on J − 1 degrees of freedom (where J is the total number of

Table 1. Characteristics of the Four Randomized Trials Included in the Meta-Analysis

Variable	Joss et al[21]	Skarlos et al[22]	Okamoto et al[23]	Lee et al[24]
Treatment schedule				
Cisplatin arm	Cisplatin 30 mg/m^2 days 1–3 + doxorubicin 40 mg/m^2 day 1 + etoposide 100 mg/m^2 days 1-3 Followed (usually after 17-21 days) by cyclophosphamide 1,000 mg/m^2 day 1 + methotrexate 20 mg/m^2 days 14, 17 + vincristine 1.4 mg/m^2 day 1 + lomustine 40 mg/m^2 day 1	Cisplatin 50 mg/m^2 days 1-2 + etoposide 100 mg/m^2 days 1-3 every 3 weeks up to six cycles	Cisplatin 25 mg/m^2 days 1-3 + etoposide 80 mg/m^2 days 1-3, every 3-4 weeks up to four cycles	Cisplatin 60 mg/m^2 day 1 + etoposide 120 mg/m^2 day 1; 100 mg/m^2 twice a day orally days 2-3 every 3 weeks up to six cycles
Carboplatin arm	Carboplatin 80 mg/m^2 day 1 + teniposide 80 mg/m^2 day 1 once per week	Carboplatin 300 mg/m^2 day 1 + etoposide 100 mg/m^2 days 1-3 every 3 weeks up to six cycles	Carboplatin AUC 5 day 1 + etoposide 80 mg/m^2 days 1-3 every 3-4 weeks up to four cycles	Carboplatin AUC 5 day 1 + gemcitabine 1,200 mg/m^2 days 1 and 8 every 3 weeks up to six cycles
Radiotherapy	N/A	LD: OR → Chest RT (concurrent with third cycle) and PCI ED: CR → Chest RT (concurrent with third cycle) and PCI	N/A	LD: OR → Chest RT CR also PCI
Primary end point	N/S	N/S	Overall survival	Overall survival
Planned sample size	N/S	N/S	220	241
Actual sample size	59	143	220	241
Start of accrual	September 1989	September 1987	September 1998	January 1999
End of accrual	September 1991	November 1991	January 2004	October 2001
Median follow-up, months[13]	N/A (all patients dead)	26.3	58.9	24.0
No. of deaths recorded	59 (100%)	111 (78%)	203 (92%)	216 (90%)
Eligibility criteria				
Age limitations, years	N/S	< 75	≥ 70 (PS 0-2) < 70 (PS 3)	Both < 70 and ≥ 70
PS	0-3	0-2	0-2 (≥ 70 years) 3 (< 70 years)	0-2 (ED) ≥ 2 (LD)
Stage	ED	ED LD	ED	ED (PS 0-2) Poor prognosis LD (PS ≥ 2 and/or increased ALP)

Abbreviations: ALP, alkaline phosphatase; AUC, area under curve; CR, complete response; ED, extensive disease; LD, limited disease; N/A, not applicable; N/S, not specified; OR, objective response; PCI, prophylactic cranial irradiation; PS, performance status; RT, radiotherapy.

trials). Findings of the meta-analysis are depicted in classic Forest plots, with point estimates and 95% CIs for each trial and for the studies overall; diamond size is proportional to study size.

Further exploratory analyses were performed in the subgroups and were based on the main baseline patients' characteristics of sex, age (younger than 70 years *v* 70 or older), stage (limited *v* extensive), and Eastern Cooperative Oncology Group (ECOG) performance status (0 to 1 *v* 2 to 3) to describe possible heterogeneity of treatment effect. An interaction test was also performed.

Secondary end points were progression-free survival (PFS), ORR, and treatment toxicity. PFS was defined as the time between date of random assignment and date of progression, or date of death for patients without progression, or last date of follow-up for censored patients. PFS analyses were similar to those for OS. ORRs were compared by using the stratified Mantel-Haenszel χ^2 test for combining two-by-two tables, and the Breslow-Day test was used to detect differences in treatment effect among the trials.[14] For ORR, patients achieving a complete response or partial response were considered as responders, and all others were considered as nonresponders.

Toxicity variables were dichotomized as (1) any grade (grade 1 to 5) versus no toxicity and (2) severe (grade 3 to 5) versus no/mild toxicity (grade 0 to 2). Toxicity rates were compared by using the stratified exact tests; Zelen's exact test was used to detect differences in toxicity effects among the trials,[15] and the pooled odds ratio with 95% CI was estimated by means of the exact method.

Statistical analyses were performed by using S-PLUS (S-PLUS 6.0 Professional, release 1; Insightful Corporation, Seattle, WA) and SAS 9.2 (SAS Institute, Cary, NC); the graphs were generated by using SigmaPlot 8.0 for Windows (SPSS, Chicago, IL) and R 2.13 (R Foundation for Statistical Computing, Vienna, Austria) software packages. Exact tests were performed by using StatXact 7 (Cytel Software, Cambridge, MA).

Table 2. Baseline Characteristics of the Patients by Treatment Group

Characteristic	Cisplatin-Based (n = 328) No.	%	Carboplatin-Based (n = 335) No.	%	All Patients (N = 663) No.	%
Clinical trial						
Okamoto et al[23]	110	33.5	110	32.8	220	33.2
Lee et al[24]	120	36.6	121	36.1	241	36.3
Skarlos et al[22]	71	21.6	72	21.5	143	21.6
Joss et al[21]	27	8.2	32	9.6	59	8.9
Age, years						
Median	67		66		67	
Range	27-85		36-86		27-86	
< 70	192	58.5	194	57.9	386	58.2
> 70	136	41.5	141	42.1	277	41.8
Sex						
Male	255	77.7	261	77.9	516	77.8
Female	73	22.3	74	22.1	147	22.2
Stage						
Limited disease	107	32.6	103	30.7	210	31.7
Extended disease	221	67.4	232	69.3	453	68.3
ECOG performance status						
0	37	11.3	42	12.5	79	11.9
1	204	62.2	193	57.6	397	59.9
2	66	20.1	77	23.0	143	21.6
3	21	6.4	23	6.9	44	6.6

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

RESULTS

Characteristics of the Trials

Of the nine publications evaluated at the initial stage, five were excluded for the following reasons: two because of data included in another article[16,17]; one because it was not a randomized trial[18]; one because it was a randomized phase II noncomparative trial[19]; and one because of a preplanned, systematic cross-over[20] (Appendix Fig A1, online only). The remaining four trials were eligible with a total of 663 patients: one trial was conducted in Switzerland,[21] one in Greece,[22] one in Japan,[23] and one in the United Kingdom.[24] The results of all four trials have already been published in full-length articles.

Thanks to the efforts of the principal investigators and data centers, individual patient data were available for all four eligible trials. Main characteristics of the four trials are described in Table 1. All four trials compared carboplatin- versus cisplatin-based doublets with the exception of the Joss et al trial,[21] in which a carboplatin doublet (considered the experimental arm) was compared with an alternating cisplatin-based schedule that included seven different drugs (considered the standard arm).

Patient Characteristics and Treatment Outcomes

Of the 663 eligible patients, 328 patients (49.5%) were assigned to cisplatin and 335 (50.5%) to carboplatin. Baseline characteristics of

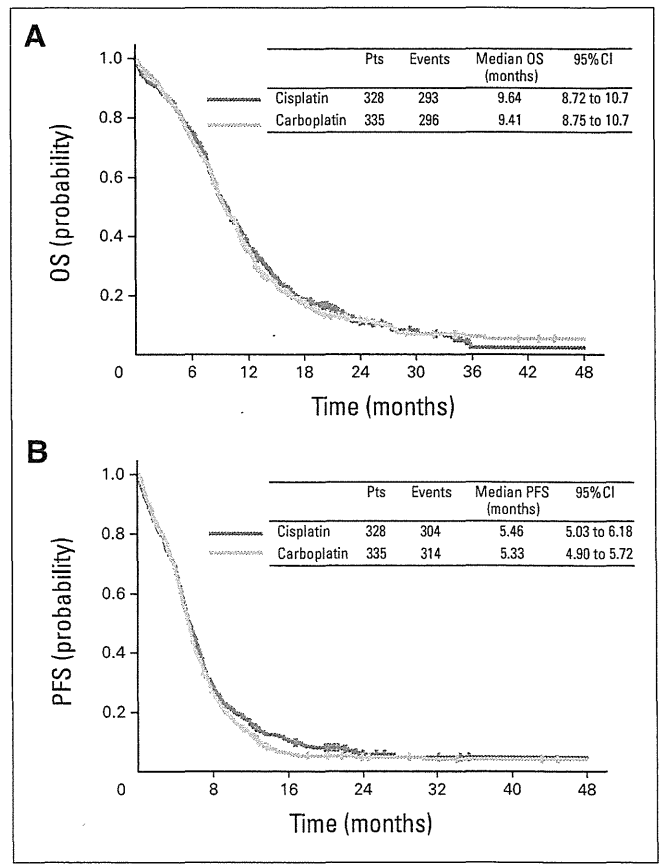

Fig 1. (A) Overall survival (OS) and (B) progression-free survival (PFS) curves by treatment arm. Pts, patients.

the 663 patients are described in Table 2. Median age was 67 years (range, 27 to 86 years). Most of the patients were males (78%) and had a good performance status (0 or 1 in 72%). Two trials[21,23] were limited to extensive disease, and the UK trial[24] allowed the inclusion of patients with limited disease who had a poor prognosis defined by poor performance status and/or high levels of alkaline phosphatase. The Greek trial[22] was the only trial that allowed the inclusion of patients with limited disease independent of their prognosis.

In the two trials[22,24] that enrolled patients with limited disease, thoracic radiotherapy was administered to 123 patients (32.1%), with similar proportions in the two treatment arms (34.2% in the cisplatin arm and 30.1% in the carboplatin arm). Information about prophylactic cranial irradiation was available in the same two trials[22,24]: prophylactic cranial irradiation was administered to 23.0% of patients, again with similar proportions in the two treatment groups (23.3% in the cisplatin arm and 22.8% in the carboplatin arm).

Median follow-up according to the Schemper and Smith method[13] was 31.9 months (29.4 months in the cisplatin arm and 31.9 months in the carboplatin arm). OS curves for patients according to treatment arms are shown in Figure 1A. Overall, 589 deaths were recorded (89%), with median OS of 9.6 months in the cisplatin arm and 9.4 months in the carboplatin arm. The corresponding hazard ratio (HR) was 1.08 (95% CI, 0.92 to 1.27; $P = .37$ with the log-rank test stratified by trial). The 6-month survival rate was 75.3% and 72.7% and the 1-year survival rate was 36.2% and 35.0% for cisplatin and carboplatin, respectively. As shown in Figure 2A, there was evidence of heterogeneity among the four trials ($P = .062$; $I^2 = 59\%$) with the Swiss trial reporting high HR values. A sensitivity analysis was performed excluding the Swiss trial,[21] and the heterogeneity disappeared ($P = .801$; $I^2 = 0\%$). With the exclusion of that trial, the HR was 1.01 (95% CI, 0.85 to 1.19; $P = .94$). Survival analysis by subgroups is shown in Figure 3A; there was no evidence of significant heterogeneity among subgroups of treatment effect around the overall effect.

PFS curves for patients according to assigned treatment are shown in Figure 1B. Overall, 618 progressions were recorded (93%), with median PFS equal to 5.5 and 5.3 months for cisplatin and carboplatin, respectively. The corresponding HR was 1.10 (95% CI, 0.94 to 1.29; $P = .25$ with a log-rank test stratified by trial). The 6-month PFS was 45.4% and 40.8% and the 1-year PFS was 16% and 12.2% for patients assigned to cisplatin and carboplatin, respectively. A Forest plot of treatment effect on PFS is shown in Figure 2B; there was statistically significant heterogeneity ($P < .001$; $I^2 = 81\%$) with the Swiss trial reporting high HR values. A sensitivity analysis was performed excluding the Swiss trial,[21] and heterogeneity disappeared ($P = .477$; $I^2 = 0\%$). With the exclusion of that trial, the HR was 1 (95% CI, 0.85 to 1.19; $P = .95$). PFS analysis by subgroups is shown in Figure 3B; there was no evidence of heterogeneity among subgroups of treatment effect

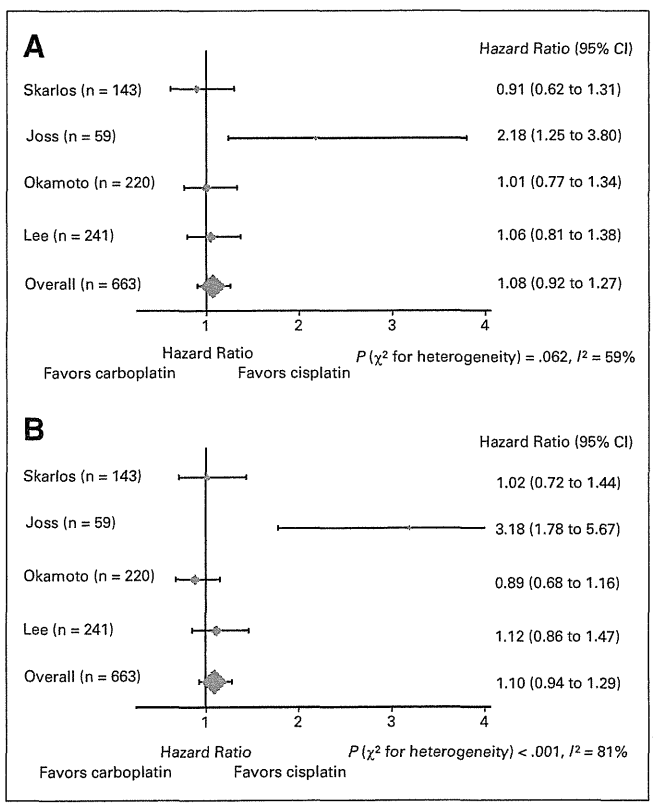

Fig 2. Forest plot of (A) overall survival and (B) progression-free survival by trial.

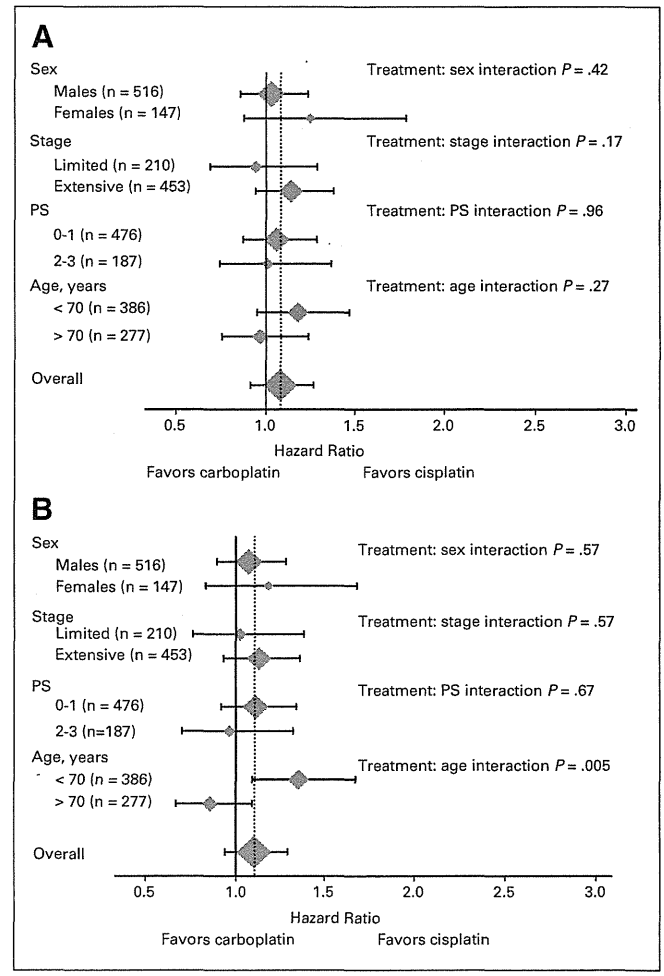

Fig 3. Forest plot of (A) overall survival and (B) progression-free survival by patients' subgroups. PS, performance status.

Table 3. Toxicity

Toxicity	Patients With Toxicity Information	Any Grade						Severe Toxicity (grade ≥ 3)					
		Cisplatin (%)	Carboplatin (%)	Exact OR	95% CI	P*	P† for Homogeneity	Cisplatin (%)	Carboplatin (%)	Exact OR	95% CI	P*	P† for Homogeneity
Leucopenia	655	74	77	1.22	0.81 to 1.88	.357	< .001	34	34	0.96	0.67 to 1.37	.863	< .001
Neutropenia	458	86	90	1.53	0.81 to 2.92	.177	.397	64	73	1.74	1.07 to 2.83	.021	.999
Anemia	512	84	89	1.72	0.99 to 3.03	.049	.046	16	25	1.73	1.12 to 2.89	.011	< .001
Platelets	512	39	71	3.36	2.83 to 6.34	< .001	< .001	14	42	3.78	2.86 to 7.19	< .001	< .001
Nausea/vomiting	655	72	63	0.66	0.47 to 0.93	.013	.012	6	3	0.49	0.21 to 1.11	.066	.999
Stomatitis	655	25	21	0.78	0.52 to 1.17	.239	.065	1	< 1	0.24	0.01 to 3.32	.320	.999
Diarrhea	458	19	22	1.23	0.76 to 2.00	.415	.999	2	2	0.99	0.18 to 5.40	.999	.999
Constipation	239	39	51	1.58	0.92 to 2.73	.091	.999	3	5	1.51	0.35 to 7.48	.749	.999
Neurotoxicity	416	19	7	0.29	0.14 to 0.58	< .001	.243	1	< 1	0.35	0.01 to 7.27	.569	.999
Renal toxicity	415	25	10	0.34	0.19 to 0.61	< .001	.787	1.5	5	0.28	0.01 to 3.78	.351	.540
Toxic deaths	655	—	—	—		—	—	1.9	1.5	0.80	0.19 to 3.18	.769	.101

Abbreviation: OR, odds ratio.
*Exact text stratified by trial.
†Exact test for homogeneity of odds ratios.

around the overall effect, with the exception of a significant interaction with age, favoring cisplatin-based treatment in younger patients and carboplatin-based treatment in older patients.

ORR was 67.1% (220 of 328; exact 95% CI, 61.8% to 71.9%) with cisplatin and 66.0% (221 of 335; exact 95% CI, 60.7% to 70.8%) with carboplatin ($P = .83$ stratified by clinical trial). Relative risk of ORR was 0.98 (95% CI, 0.84 to 1.16). The test for heterogeneity was significant ($P = .035$; $I^2 = 65\%$). In this case, heterogeneity also disappeared after excluding the Swiss trial[21] ($P = .611$, $I^2 = 0\%$).

In the Japanese trial,[23] one patient assigned to the cisplatin arm was not eligible for toxicity analysis: no chemotherapy was administered because delirium occurred after registration. In the UK trial,[24] one patient in each arm did not start treatment, and neither patient was eligible for toxicity analysis. Finally, the data center of the Swiss trial[21] was not able to retrieve the toxicity information used for original publication. However, all the information that was available was used for this analysis. Overall, 655 of the 663 patients were included in the toxicity analysis, although information was not available for all adverse effects (Table 3). Carboplatin-containing chemotherapy is associated with more myelosuppression, with a significantly higher incidence of severe neutropenia, anemia, and thrombocytopenia. Patients treated with cisplatin had significantly more nausea/vomiting, neurotoxicity, and renal toxicity. Heterogeneity among studies was found for some adverse effects, probably due to the different drugs and doses used.

DISCUSSION

The COCIS meta-analysis of individual patient data shows that carboplatin-based regimens appear to be equally effective in terms of OS, PFS, and ORR compared with cisplatin-based combinations for the first-line therapy of SCLC, differing only in their toxicity profiles. Because of the small sample sizes of SCLC trials comparing carboplatin- with cisplatin-based chemotherapy, the COCIS meta-analysis allowed us to overcome the problem of reduced statistical power. The upper CI of the HR for OS (1.27) is higher than the margin usually considered acceptable for defining noninferiority. However, after excluding the Swiss trial,[21] the upper CI becomes 1.19, so we can rule out that risk of death with carboplatin is more than 20% worse than with cisplatin. These data support the increased use of carboplatin instead of cisplatin as part of standard treatment for SCLC.

A potential limitation of the COCIS meta-analysis is the difference in treatment schedules among the trials, especially considering that our results for all outcomes considered are burdened by a statistically significant heterogeneity. Sensitivity analysis suggested that the primary source of this heterogeneity was the Swiss study,[21] the only one that showed statistically significant superiority of cisplatin, which is different from the results of all the other trials. When this study was excluded from the analysis, the test for heterogeneity did not reach statistical significance. In the Swiss study, however, a great disparity is apparent between the treatment arms. Patients randomly assigned to the cisplatin arm received an alternating schedule of seven different drugs versus patients randomly assigned to carboplatin plus teniposide, which appeared substantially weaker. However, the overall results of the COCIS meta-analysis were not substantially affected by this trial, because it randomly assigned 59 patients, representing only 8.9% of all patients included in our meta-analysis. Of the remaining trials, two[22,23] compared platinum-based doublets that differed only for the platinum compound (carboplatin plus etoposide *v* cisplatin plus etoposide), although in one study,[24] the treatment arms also differed in the platinum companion (gemcitabine *v* etoposide). We recognize that these differences may contribute to the clinical heterogeneity of the meta-analysis. However, clinical heterogeneity may improve the generalizability of the observed results. In other words, the consistently similar efficacy between treatments in the three trials comparing cisplatin- and carboplatin-based doublets and the absence of statistical heterogeneity in the analysis excluding the Swiss trial represent relevant evidence for the choice of a platinum compound in clinical practice.

Another bias could be the role of thoracic radiotherapy in the group of patients with limited disease. However, the accrual of these patients was well balanced in both treatment groups. In the two trials[22,24] enrolling patients with limited disease, thoracic radiotherapy

was administered to a similar proportion of patients in the two treatment groups.

Another possible bias of this meta-analysis is related to the different doses of cisplatin and carboplatin used in the eligible trials. Cisplatin dose ranged from 60 to 100 mg/m^2 given in one dose or fractionated in 2 to 3 days, and carboplatin dose was based on either body surface area (at 80 or 300 mg/m^2) or on area under the curve 5. The carboplatin dose (80 mg/m^2) used in the Swiss trial was low and may explain the inferior outcome of the carboplatin arm. The cisplatin dose investigated in the UK trial (60 mg/m^2) was at the inferior limit of the activity dose to allow the enrollment of patients with poor prognosis. However, to date, no evidence exists of a dose-response effect associated with platinum agents within the range of the doses used in these studies, except for the low carboplatin dose used in the Swiss trial. Therefore, it is unlikely that these minor differences in platinum doses affected our findings.

As expected from literature and from clinical experience with the two drugs, the range of toxicity of the two platinum agents was different. Carboplatin-based regimens were associated with more cases of grade 3 to 4 hematologic toxicities. To date, the availability of granulocyte colony-stimulating factors and erythropoietins could also improve the control of corresponding hematologic toxicities.[25,26] Cisplatin-based therapies were associated with more nonhematologic toxicities of any grade. Considering that all eligible trials started accrual during the 1980s and 1990s, it is likely that with the introduction of newer and more effective antiemetic agents,[27] the incidence of nausea and vomiting associated with intermediate- to high-dose cisplatin can be further ameliorated. Grade less than 3 neurotoxicity and renal toxicity were statistically worse in cisplatin-based chemotherapy. Despite low or moderate intensity in the majority of patients, this toxicity could affect the quality of life of many patients.

We did not address the end point of health-related quality of life because only two trials[21,24] included this evaluation. Moreover, the tools used were different. Overall, in those two trials, there was no significant difference in quality of life at the different assessment points that could be attributed to treatment.

Before collecting data from individual studies for the COCIS meta-analysis, we performed a meta-analysis based on literature data.[28] Individual patient data permit us to draw more definite conclusions than in the previous analysis for the reasons given by Piedbois and Buyse.[29] In fact, the general results are substantially similar but, in contrast to meta-analysis based on abstracted data, the individual patient data approach of the COCIS meta-analysis allows the investigator to evaluate the reliability of the randomization methods, check the trial data, repeat the original or perform other analyses, and update the patients' outcomes. Furthermore, availability of individual data allowed subgroup analysis with exploratory intent. No evidence of significant differences in OS between cisplatin and carboplatin according to sex, stage, performance status, or age were apparent. Unfortunately, caution is needed to use this information for managing patients with limited disease because the majority of patients with limited disease included in this meta-analysis had bulky disease or poor prognosis. In other words, only a small group of patients had limited disease, and we think that no definite conclusions should be drawn in this subgroup of patients.

In our opinion, the question of which platinum compound to use is a relevant clinical issue, particularly in patients with SCLC who have a poor prognosis. This is the first and only individual patient data meta-analysis in which we collected all the available trials that addressed this issue and all of them have been published as full-length articles. On the basis of our results, the choice of the platinum compound for first-line treatment of patients with SCLC in clinical practice should take into account the expected toxicity profile, age, the patient's organ function, and the patient's comorbidities.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Correlations between serial pro-gastrin-releasing peptide and neuron-specific enolase levels, and the radiological response to treatment and survival of patients with small-cell lung cancer

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ABSTRACT

Introduction: To investigate whether decrease in the serum levels of pro-gastrin releasing peptide (ProGRP) and neuron-specific enolase (NSE) were correlated with the radiological response in patients with small-cell lung cancer (SCLC).
Methods: Of the 196 patients, we retrospectively reviewed 118 patients elevated baseline levels of ProGRP and NSE prior to the initial therapy (IT) who survived for more than 1 month. The radiological response was assessed by Response Evaluation Criteria in Solid Tumors (RECIST 1.1).
Results: Decrease in the serum ProGRP was strongly correlated with the decrease of the sum of the tumor diameters (SOD) before the third course ($\rho = 0.50$) and after the fourth course ($\rho = 0.42$) of IT. Decrease in the serum NSE was weakly correlated with the decrease of the SOD after the fourth course ($\rho = 0.27$), but not before the third courses ($\rho = 0.22$). In the receiver operating characteristic (ROC) curves predicting 1-year survivors, the area under the curve (AUC) for percent changes in serum ProGRP before the third course were significantly larger than those for NSE (0.714 vs. 0.527, $p = 0.004$).
Conclusions: Percent changes in serum ProGRP showed better correlation to SOD and prognostic impact than that of NSE.

1. Introduction

Small cell lung cancer (SCLC) is characterized by rapid tumor growth and early metastatic spread. SCLC is one of the most chemosensitive and radiosensitive solid tumors, and neuroendocrine differentiation is considered to be an important feature of this disease [1]. However, most patients with these tumors are found to show tumor recurrence during follow-up examinations [2].

Neuron-specific enolase (NSE) has been used as a marker for the diagnosis and therapeutic monitoring of SCLC [3,4]. Recently, pro-gastrin-releasing peptide (ProGRP) has also been reported as a promising marker for SCLC. Gastrin-releasing peptide (GRP), the mammalian counterpart of amphibian bombesin, is a gut hormone that was originally isolated from the porcine stomach, and is widely distributed throughout the mammalian nervous system [5]. ProGRP is a precursor of GRP that reportedly functions as an autocrine growth factor for SCLC cells; ProGRP shows remarkable stability as compared to GRP [6,7].

Measurement of tumor marker concentrations is more convenient, cheaper and easier than measurement of the target lesions by the standard imaging modalities specified in the RECIST. With the emergence of reliable markers, serologic tumor marker responses may be a useful endpoint in the clinical setting for SCLC patients undergoing treatment.

It has been suggested that the percent changes in tumor marker levels during initial therapy for SCLC might not only be useful for assessment of the prognosis [8,9], but may also be correlated with the percent decrease in the sum of the tumor diameters calculated according to the RECIST guidelines. There have been no reports of investigation of the correlation between the serial changes in tumor marker levels and the radiological responses/survival in patients with SCLC.

The aim of the present study was to analyze the correlation between the kinetics of the serum ProGRP and NSE concentrations,

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and the percent decrease in the sum of the tumor diameters in patients with SCLC.

2. Materials and methods

2.1. Patients

Between September 2002 and April 2008, 196 patients were diagnosed as having SCLC at the Shizuoka Cancer Center. Of these, 166 patients received initial therapy, and we retrospectively reviewed the data of 118 of these patients with baseline ProGRP and NSE values of more than the upper limits of the normal (ULN) were included in the present study (Fig. 1). The following inclusion criteria were set for this study; patients with pathologically proven SCLC who had received initial therapy (including chemotherapy or chemoradiotherapy), had measurable lesions and elevated baseline levels of ProGRP and NSE before the start of the initial therapy, and had survived for more than 1 month; Eastern Cooperative Oncology Group performance status (ECOG PS) of 3 or less; adequate bone marrow, hepatic and renal functions; and no other serious underlying diseases. Histological and cytological diagnoses were performed according to the criteria in the WHO classification [10]. The study was conducted with the approval of the local ethics committee of our institution. We obtained consent for participation in the study from each of the patients.

Serum ProGRP and NSE concentrations were measured at the baseline, at the start of every treatment course and after completion of the final course of the initial therapy. The ProGRP concentration was measured using an ELISA kit (FUJIREBIO Inc., Tokyo, Japan), while the NSE concentration was measured using a solid-phase RIA method (SRL Inc., Tokyo, Japan).

The upper limits of the percentiles of healthy individuals for ProGRP and NSE were 46 pg/mL and 10 ng/mL respectively. Tumor marker response was evaluated as the percent change in the serum ProGRP and NSE concentrations. A standard evaluation, including the patient's medical history, physical examination and routine laboratory testing, was performed before each treatment.

The radiological response was assessed by RECIST 1.1 [11]. An a CT (computed tomography) scan with 5-mm slice thickness and contrast enhancement was performed at the baseline and after every two cycles of treatment during the initial therapy. Response was defined as a partial response (PR). Non-response was defined as stable disease (SD) or progressive disease (PD). The SOD was regarded as indicating the overall tumor burden.

2.2. Statistical methods

In our study, subjects with missing data were excluded from the statistical analysis.

To reduce the potential bias arising from the fact that some patients died too early to receive IT, the six patients who died prior to 1 month (30 days) after the start of IT were excluded from the analysis. To identify the best prognostic tumor marker for the 1-year survivors, ROC curve and the corresponding AUC were calculated for each of the percent change in tumor markers and SOD. The Spearman ρ correlation coefficient was also calculated for the correlation analysis. All statistical analyses were performed using the SPSS statistical software (SPSS Inc., Chicago, IL, USA).

3. Results

The characteristics of the patients ($n = 118$) are listed in Table 1. The median patient age at the start of the initial therapy was 66 years (range, 43–84 years). Most patients received chemotherapy alone ($n = 82$), and 103 patients had a good PS (0/1). At the staging assessment performed before the start of the third cycle of the initial therapy, 89 (75.4%) of the 118 patients showed partial response, 2 (1.7%) showed progressive disease, and 21 (17.8%) showed stable disease.

Table 1
Patients characteristics.

Number of patients	118
Sex: female/male	22/96
Age: median (range)	66 (43–84)
ECOG PS: 0/1/2/3	33/70/13/2
Treatment, CTx/CRT	82/36
Disease extend LD/ED	45/73
Response: CR/PR/SD/PD/NE	0/89/21/2/6
Median overall survival time (month [95%CI])	18.6 [13.1–21.0]

CTx, chemotherapy; CRT, chemoradiotherapy; ECOG, Eastern Cooperative Oncology Group; PS, performance status; LD, limited stage; ED, extensive stage; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

The kinetics of actual values and percent change in the serum ProGRP and NSE levels during the initial therapy are shown in Table 2. We included 118 patients who had elevated baseline levels of both ProGRP and NSE in our study. Twenty-seven patients had missing data in both tumor markers before the second course and the total number of patients analyzed were 91 patients. Nineteen and twenty patients had missing data in ProGRP and NSE before the third course, respectively ($n = 99$ and 98). Twenty-nine patients had missing data in both tumor markers before the fourth course ($n = 89$). Twelve and 12 patients had missing data in ProGRP and NSE after completion of the fourth course, respectively ($n = 107$ and 106). The ratios of the median baseline levels/cutoff value for ProGRP (13.1) were markedly higher than those for NSE (4.2) ($p = 0.003$). While the median ProGRP level decreased to less than the ULN after the completion of fourth course of treatment, the median NSE level decreased to less than the ULN before the second course of treatment. The median percent changes in serum ProGRP and NSE were similar, with the levels of both decreasing by about 80% before the second and third course of therapy.

The percent change in the serum ProGRP level and the percent decrease in the SOD were significantly correlated at before the third course, with a Spearman correlation coefficient of 0.504 ($p < 0.0001$). Meanwhile, the percent change in the serum NSE level demonstrated a weak, although significant, correlation with the percent decrease in the SOD at before the third course, with a Spearman correlation coefficient of 0.229 ($p < 0.027$). At baseline, both serum ProGRP and NSE values were correlated with the SOD to assess tumor burden for response determination, with Spearman ρ values of 0.42 ($p < 0.001$) and 0.48 ($p < 0.0001$), respectively (Table 3).

The median percent decrease in the SOD at second course was 51.3% (range, −66 to 98%). The ratio of patients with decrease of ProGRP ($n = 90$), NSE ($n = 95$), and SOD ($n = 112$) values from baseline were 90%, 96%, and 94% respectively.

A waterfall plot demonstrating the percent changes in the serum ProGRP and NSE before the third course of treatment are shown in Fig. 2A and B, respectively. A median decrease (range −267.7 to 99.3%) of the serum ProGRP of 84.1% was observed before the third course of the initial therapy.

The percent changes in serum ProGRP before the third course of the initial therapy was better discriminating power in identified those patients with 1-year survivors reaching AUCs in the ROC curve. The AUCs for percent changes in serum ProGRP before the third course [0.740 (95%CI: 0.639–0.842)] was significantly larger than those before the third course NSE [0.538 (0.419–0.658)] ($p = 0.0044$) [12]. The AUCs for SOD before the third course was 0.681(0.571–0.792). Furthermore, the AUCs for the percent changes of the combination of serum ProGRP and NSE was 0.69 (95%CI: 0.57–0.79).

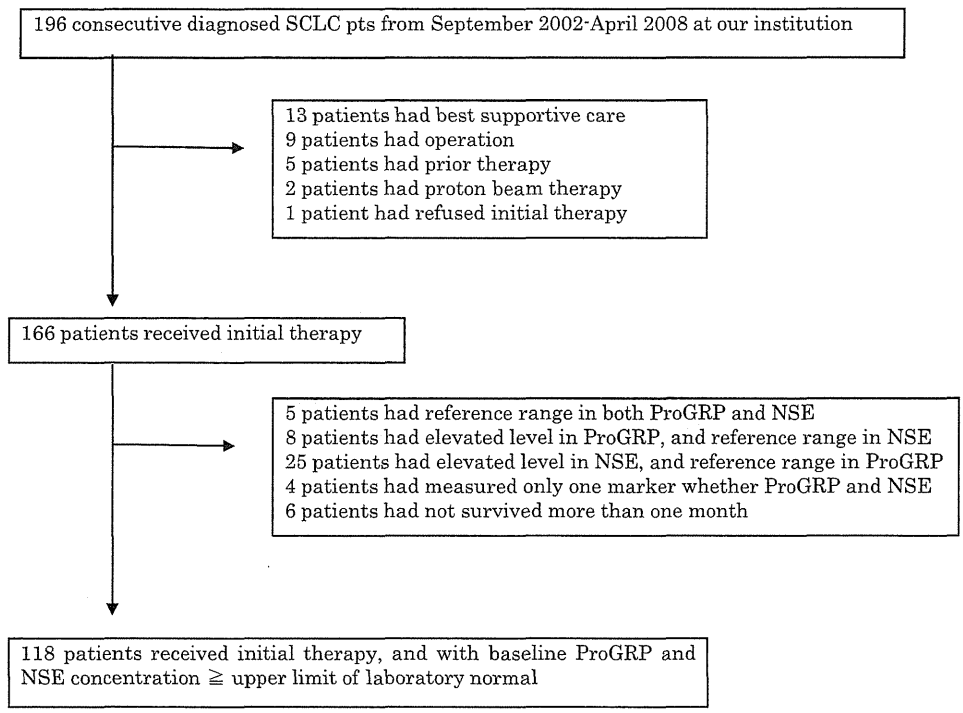

Fig. 1. A flow-diagram of the patients included in the analysis.

Table 2
The kinetics and actual measurement values of the serum ProGRP and NSE.

	ProGRP		NSE	
	AC (pg/ml)	PC (%)	AC (ng/ml)	PC (%)
Baseline Median [range]	600.5 [45–45,200]		41.5 [12.0–850.0]	
Before 2nd course Median [range]	97.6 [9.1–12,900]	−74.1 [−99.0 to 1405]	8.0 [2.4–64.0]	−75.2 [−96.3 to 60.3]
Before 3rd course Median [range]	68.2 [9.2–13,200]	−84.0 [−99.3 to 267.7]	8.1 [0.9–84.0]	−76.2 [−98.8 to 107.9]
Before 4th course Median [range]	64.0 [1.9–14,500]	−88.5 [−99.7 to 804.8]	7.8 [3.7–120.0]	−75.5 [−98.0 to 144.9]
After completion of 4th course Median [range]	42.8 [7.6–11,600]	−88.8 [−99.0 to 3642]	7.9 [4.2–480.0]	−70.9 [−97.5 to 638.5]

PC: percent change, AC: actual measurement value. Cut off value of ProGRP: 46 pg/ml, cut off value of NSE: 10 ng/ml.

The sensitivity and '1 minus specificity' were 0.714 and 0.238, respectively, for an 80% decrease of the serum ProGRP before the third course of the initial therapy (black arrow). Since, sensitivity plus '1 minus specificity' maximized at level of 80%, this is the optimal cutoff value for the detection of 1-year survival (Fig. 3).

4. Discussion

In the present study, we showed a stronger correlation between the ProGRP response and RECIST response than that between the NSE response and RECIST response to the initial therapy.

Table 3
Correlation with percent change in the serum ProGRP level and the percent decrease in the SOD.

Response		Percent change of tumor marker			
		ProGRP (%)		NSE (%)	
		Before the 3rd course	After the 4th course	Before the 3rd course	After the 4th course
Percent change of SOD[b]	Before the 3rd course	0.50 (<0.0001)[a]		0.22 (0.27)[a]	
	After the 4th course		0.42 (<0.0001)[a]		0.27 (0.005)[a]

[a] Spearman's rho (ρ) coefficient (p-value).
[b] Sum of the tumor diameters assessed by RECIST 1.1.

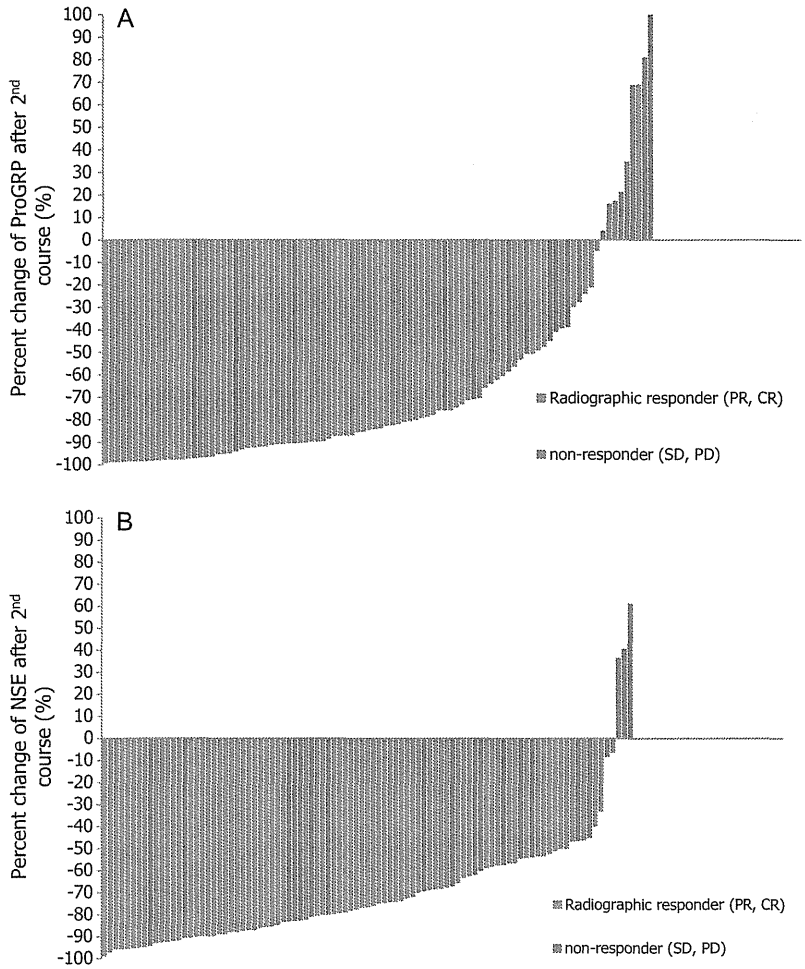

Fig. 2. A Waterfall plot demonstrating the percent changes of the serum ProGRP (A) and NSE (B) before the 3rd course of IT stratified by the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) category (patients with complete or partial response vs. patients with stable or progressive disease).

The percent change in serum ProGRP before the third course of the initial therapy was a significantly better predictor of 1-year survival period than that in serum NSE. Furthermore, a significant correlation between the percent changes in serum ProGRP and the SOD was observed.

Several reports have been published on the usefulness of measuring tumor makers at the baseline. Shibayama et al. [13] reported that while measurement of ProGRP is more sensitive than that of NSE for the diagnosis of SCLC, NSE is superior to ProGRP as a prognostic factor. In another study, EWA Wójcik et al. [8] reported that elevated NSE at the baseline is an unfavorable prognostic factor in limited disease (LD)-SCLC patients.

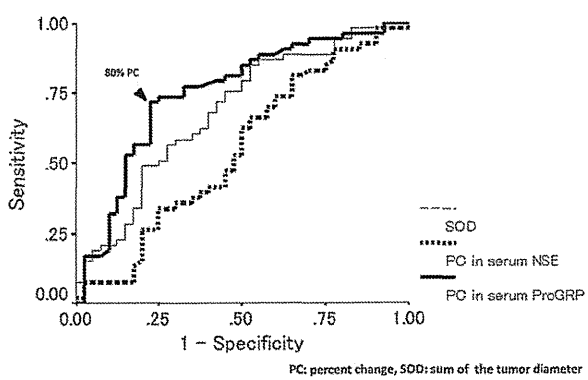

Fig. 3. Receiver-operating-characteristic curves for determination of the 1-year survival rates in relation to the percent changes of the serum ProGRP, NSE and SOD before the 3rd course of IT. PC, percent change; SOD, sum of the tumor diameter.

Some studies have reported that the relationship of serum levels of ProGRP or NSE at relapse with response to salvage therapy [14,15]. Recently, Hirose et al. [16] have reported the serum level of NSE, not ProGRP, at relapse is a useful predictive marker for complete response to salvage chemotherapy and a useful prognostic factor after relapse in patients with SCLC.

As for serial monitoring of the serum concentrations of these tumor markers, Niho et al. [14] reported that the serial measurements of the serum levels of ProGRP reflect the disease course of patients with SCLC most accurately. Yamaguchi et al. have suggested the existence of an excellent correlation between the changes in serum ProGRP levels and the therapeutic response in SCLC patients. It has also been reported that the ratios of the mean levels/cutoff value for ProGRP are markedly higher than those for NSE, indicating that ProGRP is a more reliable tumor marker than NSE [17]. However, it still remains to be clarified whether the percent changes in serum tumor marker levels accurately reflect radiographic changes as assessed by RECIST.

We tested the hypothesis that the percent changes in serum ProGRP and NSE concentrations relative to the baseline values might be useful predictors of survival, and that these changes might be

correlated with the percent decrease in SOD of the target lesions as calculated by RECIST, version 1.1.

A study by Holdenrieder et al. suggested that ProGRP-kinetics, the percent changes from the start of the first course to the start of the second course, and from the start of the first course to the start of the third course of treatment clearly discriminated among the response groups, whereas no such correlations were observed for serum NSE [9]. Their suggestion was in agreement with our results. However, the following must be considered. First, it is not clear whether the percent changes in serum tumor marker (ProGRP, NSE) levels were accurately correlated with the sum of the tumor diameters, especially in the case of ProGRP, in their study. Second, the response to therapy was classified according to the WHO classification. Meanwhile, we assessed the tumor responses using the RECIST 1.1. The RECIST guideline has been widely adopted by academic institutions, cooperative groups and the industry for trials in which the primary endpoints are set as objective response or progression. In addition, regulatory authorities accept RECIST as the gold standard for such assessments [11]. Some studies have reported that the relationship of serial tumor marker monitoring with tumor response and survival in germ cell tumors [18–20], pancreatic carcinoma [21], ovarian tumor [22], prostate cancer [23].

Radiographically measurable lesions occur frequently in hepatocellular carcinoma and non-small cell lung cancer, and some recent reports have indicated a good correlation between the percent changes in tumor marker levels and radiographic responses [24–26]. ProGRP was identified as a surrogate marker of Bcl-2 amplification and changes correlated with changes in tumor volume [27]. In our study, the gradual decrease in actual measurement value of the serum ProGRP but rapid decrease of serum NSE might be explained by the differences in the mechanism of release of these two markers from the tumor into the serum. ProGRP is an autocrine marker [28], whereas release of NSE into the blood is caused by the destruction of tumor cells containing NSE [29].

The present study has several limitations. Retrospective selection of patients, exclusion of those patients with tumor marker levels within normal limits, and exclusion of missing data results in an inherent selection bias in our analysis. The clinical stage distribution and seroprevalence of ProGRP in our study were similar to those described in previous reports [7,30]. In patients with tumor marker levels within normal limits, their responses cannot be assessed by monitoring of serum ProGRP, and routine imaging methods need to be employed.

In our study, we showed that the tumor marker response was well correlated with the radiological response in SCLC patients. Tumor marker assessment, as compared to CT evaluation, represents a more objective, reproducible and quantitative method. In addition, the costs and exposure to radiation are reduced, and exposure to contrast medium is avoided.

The potential applications of tumor marker assessment might include identification of candidates for maintenance therapy and early detection of recurrence. Furthermore, tumor marker responses might be the ideal endpoint and surrogate marker for assessment of the treatment efficacy in clinical trials. These issues should be evaluated and validated in a prospective study of serial serum ProGRP monitoring.

5. Conclusion

In conclusion, the correlation between the percent changes in serum ProGRP and SOD was stronger than those between NSE and SOD. The percent changes in serum ProGRP before the third course of the initial therapy had a better discriminatory power to identify 1-year survivors.

Conflict of interest statement

None declared.

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Prognostic Impact of Circulating Tumor Cells in Patients with Small Cell Lung Cancer

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Background: Enumeration of circulating tumor cells (CTCs) may be valuable for prognostic assessment in lung cancer patients. In this study, we report the clinical significance of CTCs in small cell lung cancer (SCLC).

Methods: In total, 51 consecutive patients newly diagnosed as having SCLC and starting chemotherapy or chemoradiotherapy were prospectively enrolled. Blood samples were drawn at the baseline, after chemotherapy, and at relapse. CTCs were isolated using the CellSearch System (Veridex LLC). Thresholds of 1 to 100 cells at the baseline were systematically correlated with the overall survival. The optimal cutoff was determined by comparing the Cox proportional hazard ratios (HRs).

Results: Two or more CTCs were detected at baseline in 35 patients (68.6%; 95% confidence interval, 55.0–79.7). The HR signifying the difference between the unfavorable (more than or equal to threshold) and favorable (less than threshold) groups was maximal at the threshold of 8 CTCs (HR, 3.50; 95% confidence interval, 1.45–8.60). Patients with ≥8 CTCs had worse survival than those with <8 CTCs at baseline ($p = 0.0014$). Patients with ≥8 CTCs posttreatment or at relapse also showed worse survival than those with <8 CTCs ($p = 0.0096$ and <0.0001). Patients whose baseline and posttreatment CTC levels remained <8 tended to show better survival than those whose CTC level converted from ≥8 to <8 cells ($p = 0.0288$) or whose posttreatment CTC level was ≥8 cells ($p = 0.0047$).

Conclusions: CTCs were highly detectable in SCLC, and higher CTC levels were strongly associated with worse survival. Consistently favorable CTC levels were associated with favorable outcomes.

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Small cell lung cancer (SCLC) accounts for 15% of all lung cancer diagnoses and is characterized by aggressive tumor growth, often presenting with metastases in the regional lymph nodes and distant organs. Because SCLC is highly sensitive to chemotherapy and radiotherapy, early diagnosis followed by appropriate treatment can be expected to yield favorable outcomes.[1,2] Circulating tumor cells (CTCs) are known to circulate in the peripheral blood in patients with several types of malignancies,[3–6] while rarely being detected (0.3–1.0%) in healthy control subjects or patients with nonmalignant diseases.[3,7,8] The CellSearch system (Veridex LLC, Raritan, NJ) is a well-validated system for quantitative evaluation of CTCs, in which CTCs are immunomagnetically captured using an antibody against epithelial cell adhesion molecules (EpCAMs).[9,10] A growing body of evidence suggests the existence of a correlation between CTC level as measured by the CellSearch system and the progression-free survival (PFS) and overall survival (OS) in patients with metastatic breast, colorectal, castration-resistant prostate, and non-small cell lung cancers (NSCLC).[7,11–15] In SCLC, the detection rate of CTCs by the Cell Search system has been reported to be relatively high, with 67 to 86% of the patients being reported to have ≥2 CTCs per 7.5 ml of blood.[8,16,17] However, the prognostic impact of CTCs and their relationship to the presence of metastases in patients with SCLC remain unknown. We conducted this study to evaluate the relationship of CTC levels to the disease extent and prognosis and to determine the optimal CTC level cutoff for predicting the outcomes in SCLC patients.

METHODS

Study Design

This prospective study was conducted at two institutions (Shizuoka Cancer Center and Hyogo College of Medicine) to evaluate the usefulness of measurement of the CTC

levels for predicting the OS. Patients with chemotherapy-naive, pathologically confirmed SCLC scheduled to commence first-line standard chemotherapy with or without thoracic radiotherapy were eligible. All patients were enrolled at the Shizuoka Cancer Center and had an Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0 to 2. The institutional review boards at each center approved the study protocol, and all patients provided written informed consent. Before the start of the new treatment, the patients underwent an evaluation of metastatic sites by means of standard imaging studies, including contrast-enhanced computed tomography of the chest to lower abdomen, contrast-enhanced magnetic resonance imaging of the brain, and bone scan or positron emission tomography, along with the collection of blood sampled for counting of the baseline CTCs. The post-treatment blood samples were collected 3 weeks after completion of the last chemotherapy cycle or completion of sequential thoracic radiotherapy. The samples were collected 2 weeks after relapse had been diagnosed by imaging and before administration of the second-line chemotherapy. The sampling date could be adjusted depending on the type of treatment and the visit schedule, with allowance for ±2 weeks. Reevaluations of the disease status were conducted using the same techniques as those applied at the baseline, every 8 to 12 weeks, depending on the type of treatment the patient had received and the treatment schedule. Disease status was assessed according to the RECIST[18] by examiners with no knowledge of the CTC levels. Serum lactate dehydrogenese (LDH) levels and the levels of other biomarkers, including neuron-specific enolase (NSE) and progastrin-releasing peptide (ProGRP), were measured at the same time point as the baseline CTC measurement. The blood samples for the serum biomarker measurements were obtained by venous puncture, and the sera were stored at −40°C until use. The ProGRP concentration was measured using an ELISA kit (FUJIREBIO Inc., Tokyo, Japan), and the NSE concentration was measured using the radioimmunoassay solid-phase method (SRL Inc., Tokyo, Japan).

Counting of CTCs

Blood samples were drawn into 10-ml vacuum tubes (CellSave, Immunicon, Huntingdon Valley, PA). Samples were maintained at room temperature, mailed overnight, and processed within 96 hours of collection. The results were reported quantitatively as the number of CTCs per 7.5 ml of blood. All CTC evaluations were performed without knowledge of the patient clinical status in one of two laboratories (Hyogo College of Medicine, Japan, or the laboratory of SRL Inc.). The CellSearch system was used for the CTC counting, the technical details of which, including accuracy, precision, linearity, and reproducibility, have been previously described.[3] CTCs were defined as EpCAM-isolated intact cells showing positive staining for cytokeratin and negative staining for CD45. At each time point, the favorable and unfavorable groups were defined as those with CTC levels less than or more than or equal to the selected threshold, respectively.

Statistical Analysis

The primary analysis was a comparison of the OS between the unfavorable and favorable groups stratified according to the selected threshold of CTC level. The study was designed to enroll 50 patients for a statistical power of 80% with a two-sided log-rank test at a level of 0.05 to detect an absolute difference of 40% points between the two groups in the 1-year estimates of OS (20% in the unfavorable group versus 60% in the favorable group). To select the threshold CTC level that most clearly distinguished patients with an unfavorable prognosis from those with a favorable prognosis, thresholds of 1 to 100 cells at baseline were systematically correlated with the OS. The Cox proportional hazard ratio (HR), goodness-of-fit, and discriminatory power of each threshold were compared. The Bonferroni correction was applied for multiple testing for 14 thresholds, and a p value of <0.0036 was set to obtain a statistical significance of $p < 0.05$. The goodness-of-fit of the model was assessed by the coefficient of determination (R^2) defined as 1 − {(log likelihood of the estimated model)/(log likelihood of the model with only the intercept)}. The discriminatory power was assessed by the accuracy rate ([AR] defined as the rate of correct diagnosis among all predictions of 1-year survivors) and the area under the receiver operator characteristics curve (AUROC). The treatment-free interval (TFI) was defined as the time between the completion of first-line chemotherapy and the diagnosis of relapse. Patients with a TFI of 90 days or more were considered to have treatment-sensitive disease, and those with a TFI of less than 90 days were considered to have treatment-refractory disease. For all survival analyses, the time to death was defined as the time between the date when the blood sample was obtained and the date of death or date of the last follow-up visit. Separate Kaplan-Meier survival plots were generated based on the CTC levels at baseline and the results in the follow-up blood collections. Survival curves were compared using the log-rank test. Cox proportional hazards regression was used to determine the HRs for the OS adjusted for age, gender, pretreatment stage (extensive disease [ED] versus limited disease [LD]), and ECOG-PS at the time of blood collection. The discriminatory power of the baseline CTC, LDH, NSE, and ProGRP for predicting 1-year survivors was compared by AUROC. The χ^2 test or Fisher exact test was used to compare categorical variables. For comparison of the means, the nonparametric Wilcoxon's test or analysis of variance was used. We tested the correlations between variables by calculating the Spearman's rank correlation coefficients. Calculations were carried out using the statistical program, JMP version 9.0 for Windows (SAS Institute Inc., Cary, NC).

RESULTS

Patient Characteristics

In total, 51 consecutive patients met the inclusion criteria and were prospectively enrolled between July 2009 and September 2010. The cutoff date for analysis was August 31, 2011. The median age of the patients was 67 years, and 44 of the patients (86.3%) were men (Table 1). Nineteen of the

TABLE 1. Baseline Characteristics

Characteristics	All (n = 51)	Extensive Disease (n = 24)	Limited Disease (n = 27)
Age, median (range)	67 (34–92)	66.5 (57–80)	68 (34–92)
Gender (female:male)	7:44	3:21	4:23
ECOG-PS, n (%)			
0	21 (41.2)	6 (25.0)	15 (55.6)
1	21 (41.2)	10 (41.7)	11 (40.7)
2	9 (17.6)	8 (33.3)	1 (3.7)
No. of organs with metastasis, median (range)	0.5 (0–3)	1 (0–2)	None
Brain metastasis, n (%)	7 (13.7)	7 (29.2)	None
Liver metastasis	8 (15.7)	8 (33.3)	None
Bone metastasis	3 (5.9)	3 (12.5)	None
Malignant effusion	12 (23.5)	11 (45.8)	1 (3.7)
Serum biomarkers (mean ± SE)			
NSE (ng/ml)	75.7 ± 24.3	131.2 ± 49.5	26.4 ± 5.5
ProGRP (pg/ml)	657.2 ± 205.7	1071.3 ± 419.1	289.0 ± 66.7
LDH (IU/L)	360.5 ± 79.9	529.7 ± 164.4	210.1 ± 8.8
Treatments, n (%)			
Chemotherapy alone	32 (62.7)	24 (100.0)	8 (29.6)
Chemoradiotherapy	19 (37.3)	None	19 (70.4)
Regimens, median cycle (range)	4 (1–6)	4 (1–6)	4 (1–5)
Cisplatin + etoposide, n (%)	23 (45.1)	16 (66.7)	7 (25.9)
Carboplatin + etoposide	21 (41.2)	2 (8.3)	19 (76.4)
Cisplatin + irinotecan	7 (13.7)	6 (25.0)	1 (3.7)

ECOG-PS, Eastern Cooperative Oncology Group performance status; SE, standard error; NSE, neuron-specific enolase; ProGRP, progastrin-releasing peptide; LDH, lactate dehydrogenese.

27 patients with LD had received chemoradiotherapy, while the remaining 8 patients could not receive radiotherapy for the following reasons and were treated by chemotherapy alone. The first patient was a 73-year-old man with a treatment history of thoracic chemoradiotherapy for esophageal cancer 6 years before the current treatment. Reirradiation was avoided because of the potential late adverse effects of radiotherapy. The second patient was a 79-year-old man with poor pulmonary functions who was scheduled for sequential radiotherapy after chemotherapy. However, his tumor progressed, with the development of contralateral pulmonary metastases after the first course of chemotherapy, and radiotherapy could not be administered. The remaining six patients had interstitial lung disease before the start of the treatment. Thoracic radiotherapy was withheld because of the potential risk of severe radiation pneumonitis. Twenty-four patients (47.1%) were still alive at the time of analysis. The median follow-up period for determining the survival was 13.0 months after the baseline blood sample collection. All 51 patients were evaluable for the baseline CTC level. Blood samples were not obtained during follow-up from two patients who died of interstitial lung disease and cancer progression. The remaining 49 patients were evaluable for the posttreatment CTC levels. The median time between the baseline and posttreatment blood collections was 3.4 months. Thirty-eight patients (74.5%) exhibited tumor progression; 37 were evaluable for the CTC level at the time of relapse, and 1 woman refused to provide blood samples.

Circulating Tumor Cells

Two or more CTCs were detected in 68.6% of the patients (95% confidence interval [CI], 55.0–79.7) at baseline, in 26.5% of the patients (95% CI, 16.2–40.3) posttreatment, and in 67.6% of the patients (95% CI, 51.5–80.4) at the time of relapse (Table 2). The CTC counts at baseline were higher in patients with ED, who showed a median of 9.5 cells (range, 0–5648), than in those with LD, who showed a median of 1 cell (range, 0–58; $p = 0.0001$, Figure 1*A*). Fourteen of the 16 patients (87.5%) who had a baseline CTC level of ≤1 had LD. The median CTC levels at baseline in patients with 0, 1, and ≥2 organs showing metastases were 2.0 (range, 0–58), 7.5 (1–799), and 21.0 (0–5648), respectively, showing a statistically significant correlation of the CTC count with the number of organs showing metastases (Spearman's rho, 0.72, $p < 0.0001$, Figure 1*B*). Patients with liver metastasis had higher CTC levels than those without liver metastasis (64 [range, 5–5648] versus 3 [range, 0–799]; $p = 0.0007$). There was no association between brain or bone metastasis and the CTC levels (data not shown).

Stratification According to Levels of Circulating Tumor Cells

The baseline CTC level was predictive of the OS when it was stratified by the threshold of 8 cells ($p = 0.0029$; Table 3). The Cox proportional HR signifying the difference between the unfavorable (more than or equal to threshold) and favorable (less than threshold) groups showed a waxing and waning pattern with the peak at the level of 8 CTCs. The HR associated with a CTC level of 8 cells was 3.50 (95% CI, 1.45–8.60) after adjustment for stage (ED or LD), age, gender, and ECOG-PS at the baseline. The Cox proportional hazard model at this level also showed a favorable goodness-of-fit and discriminatory power with the highest R^2, AR, and AUROC among all the thresholds examined. Thus, a cutoff level of 8 CTCs was chosen for the subsequent analyses. Analyses based on the stage (ED or LD) and therapy type

TABLE 2. CTC Levels at the Baseline, Posttreatment, and at the Time of Relapse

	Baseline	Posttreatment	At Relapse
Total[a]	51	49	38
Evaluable[b]	51	49	37
CTC, median (range)	4 (0–5648)	0 (0–253)	1 (0–510)
CTC, mean ± SE	203.2 ± 118.5	10.2 ± 5.9	44.6 ± 16.8
CTC ≥ 2, % (95% CI)	68.6 (55.0–79.7)	26.5 (16.2–40.3)	67.6 (51.5–80.4)

[a] Number of patients alive and evaluable.
[b] Number of patients with nonmissing data for CTCs at the time-point indicated.
CTC, circulating tumor cell; SE, standard error; CI, confidence interval.

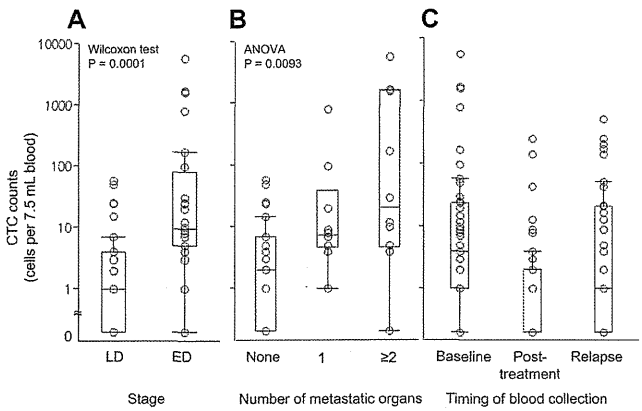

FIGURE 1. Box plots were drawn using the minimum and maximum values and the 25th, 50th, and 75th percentiles. *A*, Circulating tumor cell (CTC) levels at the baseline and the disease stage. ED, extensive disease; LD, limited disease. *B*, CTC levels at the baseline and number of metastatic organs. *C*, CTC levels at the baseline and the timing of blood sampling. *p* values calculated by Wilcoxon's test and analysis of variance (ANOVA) are presented.

TABLE 3. Baseline CTC and Prognosis

CTC Level[a]	Adjusted HR (95% CI)[b]	p^c	R^2	AR	AUROC (95% CI)
1	0.74 (0.26–2.40)	0.0604	0.06	0.49	0.55 (0.43–0.65)
2	0.67 (0.25–1.87)	0.0532	0.06	0.51	0.55 (0.42–0.67)
3	0.76 (0.27–2.11)	0.0606	0.06	0.55	0.58 (0.45–0.71)
4	0.85 (0.25–2.79)	0.0656	0.05	0.61	0.63 (0.48–0.75)
5	1.59 (0.61–4.29)	0.0481	0.06	0.67	0.68 (0.53–0.80)
6	2.97 (1.24–7.31)	0.0063	0.08	0.73	0.73 (0.58–0.84)
7	2.97 (1.24–7.31)	0.0063	0.08	0.73	0.73 (0.58–0.84)
8	3.50 (1.45–8.60)	0.0029	0.09	0.76	0.74 (0.59–0.85)
9	2.90 (1.20–7.04)	0.0072	0.08	0.73	0.71 (0.57–0.83)
10	2.41 (0.99–5.81)	0.0151	0.07	0.71	0.69 (0.54–0.80)
15	3.00 (1.19–7.40)	0.0079	0.08	0.71	0.68 (0.54–0.79)
25	2.02 (0.74–5.04)	0.0318	0.06	0.67	0.62 (0.50–0.73)
50	3.49 (1.23–9.79)	0.0107	0.08	0.67	0.62 (0.50–0.72)
100	3.97 (0.90–15.59)	0.0181	0.07	0.65	0.58 (0.48–0.67)

[a] CTC levels are expressed as the number of cells per 7.5 ml of blood.
[b] The Cox proportional hazard ratios were adjusted for stage, age, gender, and ECOG-PS at the baseline.
[c] The level of significance calculated by the Bonferroni method was $p < 0.0036$.
HR, hazard ratio; CTC, circulating tumor cell; CI, confidence interval; AR, accuracy rate for predicting 1-year survivors; AUROC, area under the receiver operator characteristics curve for predicting 1-year survivors.

(chemotherapy alone or chemoradiotherapy) showed that the prognostic significance of the CTC level was significant only in the ED subset and in the patients treated by chemotherapy alone (Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/JTO/A204).

Baseline CTC and Prognosis

Figure 2*A* shows the Kaplan-Meier curves for the OS according to the baseline CTC levels. Patients in the unfavorable group had significantly shorter survival than those in the favorable group ($p = 0.0014$). The 1-year survival rates and the median OS in the unfavorable and favorable groups were 31.6% versus 78.0% and 8.5 versus 17.2 months, respectively. The sensitivity, specificity, AR, and AUROC for predicting 1-year survivors using the cutoff level of 8 CTCs were 0.81, 0.65, 0.75, and 0.73 (95% CI, 0.58–0.84), respectively. The 1-year survival rates in the unfavorable and favorable groups were 21.4 and 70.0% ($p = 0.0282$), respectively, in the ED subset, and 60.0 and 81.6% ($p = 0.4387$), respectively, in the LD subset (Figures 2*B*, *C*).

Posttreatment CTC and Prognosis

During the posttreatment period, the CTC levels were measured in the 49 patients who were available for the evaluation. Of these 49 patients, 7 (14.3%) with ≥8 CTCs had a significantly shorter posttreatment survival than the remaining 42 (85.7%) with <8 CTCs ($p = 0.0096$, Figure 2*D*). The HR of the threshold CTC count adjusted for stage, age, and posttreatment PS was 2.76 (95% CI, 0.97–6.92, $p = 0.0562$). The median posttreatment survivals in the unfavorable and favorable groups were 4.1 and 13.9 months, respectively. At the time of relapse, CTC levels were measured in 37 patients. Of these 37 patients, the 13 (35.1%) with ≥8 CTCs had a significantly shorter postrelapse survival than the remaining 24 (64.9%) with <8 CTCs ($p < 0.0001$, Figure 2*E*). The HR of the threshold CTC adjusted for stage, age, TFI (<90 versus ≥90 days), and PS at the time of relapse was 6.20 (95% CI, 2.39–17.52, $p = 0.0002$). The median postrelapse survivals in the unfavorable and favorable groups were 4.0 and 11.8 months, respectively.

Posttreatment CTC Status and Prognosis

Among the 42 patients with posttreatment CTC levels of <8, 29 had a baseline CTC level also of <8 (group A), and in the remaining 13, the baseline CTC level was ≥8 (group B). Among the seven patients with posttreatment CTC levels of ≥8 (group C), four had a baseline CTC level also of ≥8, and the remaining three had a baseline CTC level of <8. As shown in Figure 3, the survival impact of conversion from an unfavorable to favorable CTC level was assessed by using the Kaplan-Meier curve for posttreatment survival according to the posttreatment CTC status. The median posttreatment survival was >18.8 months in group A, 7.2 months in group B, and 4.1 months in group C ($p = 0.0066$). The difference in the survival between group A and group C was significant ($p = 0.0047$ by log-rank test; level of significance calculated by the Bonferroni method, $p = 0.0166$). Conversely, there was no significant difference between group A and group B ($p = 0.0288$), or group B and group C ($p = 0.2489$). The HR adjusted for the pretreatment stage, posttreatment ECOG-PS, and TFI was 3.08 (95% CI, 1.03–8.90; $p = 0.0450$) in group B and 3.29 (95% CI, 1.01–10.07; $p = 0.0479$) in group C, both calculated using group A as the reference (Table 4).

Discriminatory Power of CTCs and Serum Biomarkers for Predicting the Prognosis

Figure 4 shows the receiver operator characteristics curves for CTCs, and the serum levels of LDH, NSE, and ProGRP measured at the baseline. Data on survival at 1 year

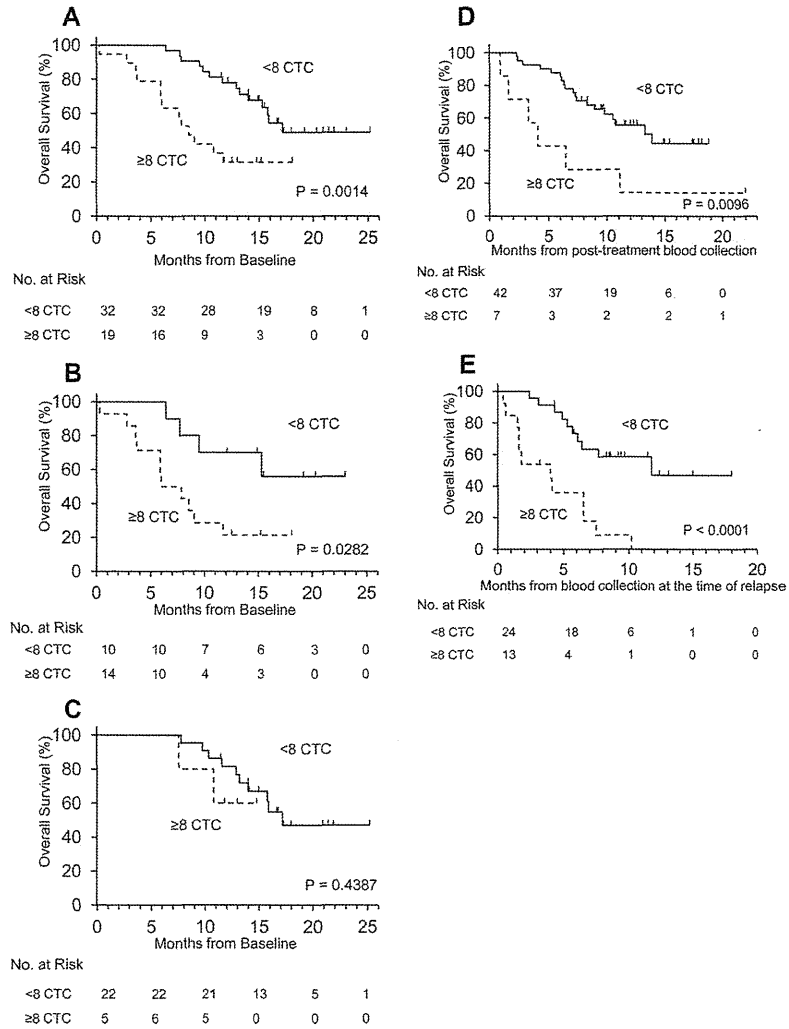

FIGURE 2. Kaplan-Meier curves for overall survival in patients with <8 and ≥8 circulating tumor cells (CTCs) at the baseline in the full set of data (*A*), extensive disease subset (*B*), and limited disease subset (*C*). Kaplan-Meier curves for posttreatment survival and postrelapse survival in patients with <8 and ≥8 CTCs posttreatment and at relapse (*D* and *E*). *p* values calculated by the log-rank test are presented.

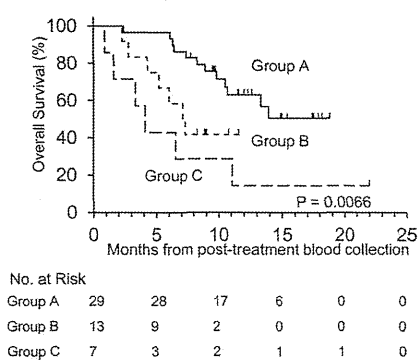

FIGURE 3. Kaplan-Meier curves for posttreatment survival in three groups, including patients in whom the baseline and posttreatment circulating tumor cell (CTC) levels remained at <8 (group A), patients in whom the CTC level converted from ≥8 to <8 cells (group B), and patients in whom the posttreatment CTC level was ≥8 cells (group C). *p* values calculated by the log-rank test are presented.

TABLE 4. Hazard Ratios of the Posttreatment Status of CTC Level

Posttreatment CTC Status	CTC Level[a]	*n*	MST (mo)	Adjusted HR (95% CI)[b]	*p*
Group A	<8–<8	29	NR	Reference	
Group B	≥8–<8	13	7.2	3.08 (1.03–8.90)	0.0450
Group C	≥8–≥8 or <8–≥8	7	4.1	3.29 (1.01–10.07)	0.0479

Group A: patients whose baseline and posttreatment CTC levels remained <8 cells; group B: patients whose CTC level converted from ≥8 to <8 cells; and group C: patients whose posttreatment CTC level was ≥8 cells.

[a] CTC levels are expressed as the number of cells per 7.5 ml of blood.

[b] The Cox proportional hazard ratios were adjusted for the pretreatment stage, posttreatment ECOG-PS, and treatment-free interval.

CTC, circulating tumor cell; HR, hazard ratio; MST, median survival time; CI, confidence interval; NR, not reached.

were available for all 51 patients. The baseline CTC level showed a favorable discriminatory profile, showing an AUROC of 0.70 (95% CI, 0.52–0.83), as compared with that of 0.67 (0.49–0.82) for LDH, 0.68 (0.52–0.82) for NSE, and 0.46

(0.29–0.64) for ProGRP. The differences in the AUROC among the parameters were not significant ($p = 0.1044$).

Radiologic Response and Changes in the CTC Levels

Assessment of the best radiologic response to the first-line treatment was performed using the RECIST criteria in 50 patients. One man died of interstitial lung disease before the follow-up imaging study. Figure 5 shows the baseline and posttreatment CTC levels in patients showing complete response (CR, $n = 6$), partial response (PR, $n = 27$), stable disease (SD, $n = 5$), and progressive disease (PD, $n = 12$). There was no significant difference between the CR/PR subsets and SD/PD subsets in the baseline CTC (median, 4 [range, 0–1683] versus 4 [range, 0–5648]; $p = 0.7337$ by the Wilcoxon's test) or posttreatment CTC (0 [0–44] versus 0.5 [0–253]; $p = 0.3370$) level. The numbers of patients with undetectable posttreatment CTCs or patients with lower posttreatment CTC levels than the baseline CTC levels were 4 (66.7%) in the CR group, 24 (88.9%) in the PR group, 4 (80.0%) in the SD group, and 7 (58.3%) in the PD group, with no significant differences among the groups showing the various treatment responses ($p = 0.2878$ by the χ^2 test).

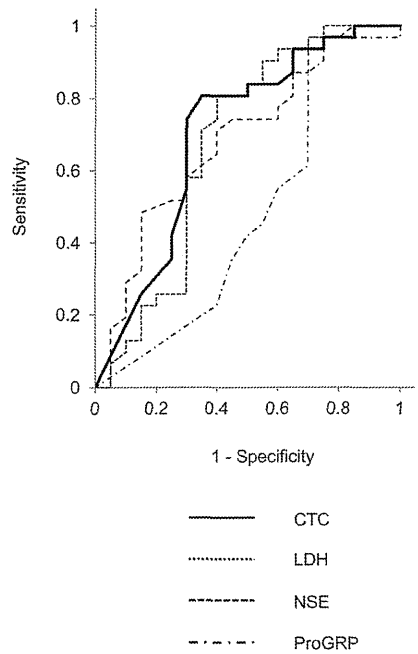

FIGURE 4. Receiver operator characteristics curve analysis for predicting 1-year survivors. The area under the curve is 0.70 (95% confidence interval [CI], 0.52–0.83) for the circulating tumor cell (CTC) level at baseline, 0.67 (95% CI 0.49–0.82) for serum lactate dehydrogenese (LDH) at baseline, 0.68 (95% CI 0.52–0.82) for serum neuron-specific enolase (NSE) at baseline, and 0.46 (95% CI 0.29–0.64) for serum progastrin-releasing peptide (ProGRP) at baseline.

DISCUSSION

This study is the first prospective evaluation of the optimal CTC cutoff to predict the OS in patients with chemotherapy-naive SCLC. First, we showed that the CTC level was strongly predictive of the OS, especially in the ED subset. Then, an optimal cutoff level, CTC count of ≥8 cells per 7.5 ml of blood was identified by comparing the Cox proportional HRs of various CTC levels for the OS. This cutoff level was also found to be valid for predicting the posttreatment survival and postrelapse survival in the same cohort. We also showed that the baseline CTC level had a high discriminatory power, similar to the serum NSE and LDH.

Circulating SCLC cells have been reported to show high expression levels of EpCAM,[19] which has been used as a key marker to isolate CTCs using the CellSearch system. The appropriateness of using the CellSearch system for detecting circulating SCLC cells was previously assessed by Hou et al.[16] They showed that 15 CTC samples obtained from patients with SCLC by the CellSearch system were neuroendocrine in nature (CD56 positive) and confirmed their neoplastic origin by immunohistochemical comparison of these cells with the cells obtained from matched tumor biopsy specimens. The detection rate (≥2 CTCs per 7.5 ml blood) of circulating SCLC cells by the CellSearch system in cases of SCLC is reportedly quite high, being 67 to 86%,[8,16] as compared with that in cases with other tumors with metasta-

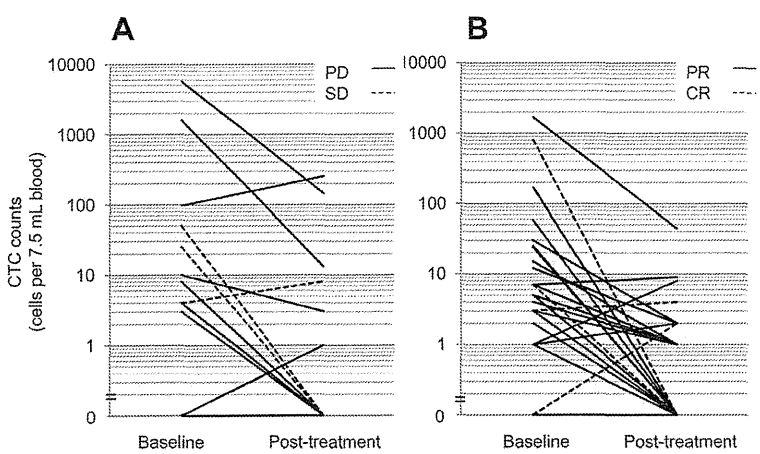

FIGURE 5. Relationship between radiologic response and the changes in the circulating tumor cell (CTC) level. *A*, Baseline and posttreatment CTC levels in patients showing PD (solid line) and SD (dotted line). *B*, Baseline and posttreatment CTC levels in patients showing PR (solid line) and CR (dotted line). PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.