Ⅳ 基礎研究

副作用予測のためのバイオマーカー

Biomarkers for the prediction of adverse events

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Key words：6-MP, thiopurine methyltransferase (TPMT), irinotecan, carboxylesterase (CES), uridine diphosphate glycosyltransferase (UGT) 1A1, DPD, CYP3A4, CYP2D6

はじめに

抗がん剤の化学療法において，重篤な副作用が出現する投与量と効果が期待される投与量とが近接していることが多い，治療域の狭い薬剤であることから，効果と薬物有害反応は同様に重要である．副作用が予測されることは，患者および医療者にとって有益である．

近年，分子生物学の進歩により種々のバイオマーカーが明らかにされてきた．本稿では，これによる副作用予測について述べる．

1 6-Mercaptopurine (6-MP) と thiopurine methyltransferase (TPMT)

6-mercaptopurine (6-MP) は，急性白血病の治療に用いられる薬剤である．Hypoxanthine-guanine phosphoribosyltransferase により 6-thioguanine (6-TGN) へ変換され，TIMP は IMP dehydrogenase を阻害することにより，adenylic acid や guanine ribonucleotide の生合成を阻害することで細胞増殖を抑制し，抗がん作用を示す．

一方，thiopurine methyltransferase (TPMT) によりメチル化されることで，不活化される．この酵素は遺伝子型により酵素活性の低下亢進があることが知られている．TPMT 活性が低下することで，不活化される 6-MP が相対的に低下するため，活性型である 6-TGN 濃度が増加し，高度の骨髄抑制をもたらすことがわかっている．白人における TPMT 活性の分布が調べられ，集団の 11％が若干低活性を示し，300 人中 1 人程度の割合でほとんど TPMT 活性が検出されなかった．これには活性欠損型遺伝子 *2, *3A, *3C, 6 が関連している．また，日本人の TPMT 欠損者は約 4,000 人に 1 人で，欧米人での約 1,000 人に 1 人に比し低頻度である．赤血球の本酵素活性が測定され，赤血球 TPMT 活性に基づく個別化薬物療法が可能と考えられた．

2 Irinotecan と副作用予測

Irinotecan は，大腸がん，肺がん，卵巣がん，乳がん，悪性リンパ腫などに適応をもつ topoisomerase I 阻害薬である．特に結腸/直腸がんや肺小細胞がんでは標準レジメンを構成する薬剤である．

Irinotecan はプロドラッグであり，肝，血中，腫瘍内で carboxylesterase (CES) による代謝活性化を受け，抗がん活性の本体 7-ethyl-10-hydroxycamptotecin (SN-38) に変換される．がん細

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胞へ取り込まれたSN-38はtopoisomerase I (top1)を阻害する．top1阻害により，DNAの複製または転写を阻害しDNA合成阻害を起こすことで，細胞増殖抑制すなわち抗腫瘍効果を発揮する．SN-38は，肝細胞にてuridine diphosphate glycosyltransferase(UGT)[主にはfamily 1, polypeptide A1(UGT1A1)]にてグルクロン酸抱合されて不活化され，10-O-glucuronyl-SN-38(SN-38G)となる．SN-38Gは水様性の物質で，肝細胞より胆汁を介して小腸へと排泄され，糞便となり体外へ排出される．SN-38Gの肝細胞への取り込みおよび肝細胞からの排泄には，多数のトランスポーターが関与している．腸管中のSN-38Gは刷子縁に存在する腸内細菌のもつβ-glucuronidaseによって脱抱合され，再びSN-38となることがある．その一部は腸肝循環を介して体循環へと吸収される．また，一部はirinotecanからcytochrome P450 3A4 (CYP3A4)により，不活化代謝物7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin(APC)へ変換される経路もある(図1)．

SN-38は，同時にCPT-11の血液毒性や下痢などの副作用をもたらす．

好中球減少は，SN-38のarea under the concentration-time curve(AUC)が関与している．薬物代謝が遅延した場合にAUCが増加し，それにより好中球減少の重症化が起こる．

遅発性下痢は，SN-38による粘膜傷害が一因と考えられる．排泄の際にごく一部が不活化されずにSN-38の形のままで排泄される場合や，前述のように腸管内で腸内細菌の働きでSN-38Gから脱抱合されSN-38になる場合が考えられる．遅発性の軟便/水様便/排便回数の増加のような症状に対しては，ロペラミドの投与を行い，場合によっては大量投与も行われる．下痢が長期化，重症化した場合には，十分な補液管理を行うとともに，感染合併が考えられるときや好中球減少がある場合には，抗生剤の投与も行う必要がある．

1) Carboxylesterase(CES)

CES1およびCES2について遺伝子多型が報告されている．CES1については，irinotecanの薬物動態への影響はまだ研究の途上にある．CES2については，日本人でも遺伝子多型の報告があり，そのうち*CES*5*(1A>T, Met 1 Leu)で多型のない場合より12%の酵素の発現低下が報告されている．また，*CES*2*(100C>T, Arg 34 Trp)または*CES*5*をヘテロ接合体でもった場合，AUCにおいてSN-38とSN-38G対比が低下する(irinotecanからSN-38とSN-38Gへの代謝効率が低下している)という報告がある．他の多型についてはirinotecanの代謝や副作用との関連は認められなかった．

2) Uridine diphosphate glycosyl-transferase family 1, polypeptide A1 (UGT1A1)

UGT1A1の遺伝子には，多くの遺伝子多型が報告されている．日本人においては，*UGT1A1*28*と*UGT1A1*6*が最も重要な遺伝子多型である．*UGT1A1*28*は，プロモーター領域にある(TA)の繰り返し配列の変異(通常6回であるものが7回となる)でタンパク合成が低下し，酵素活性が減少する(ホモ接合体で70%)．この多型は，(非抱合型)高ビリルビン血症を示すGilbert症候群の原因遺伝子の一つとされている．

Irinotecanの代謝において，SN-38からSN-38Gへのグルクロン酸抱合が低下することにより，活性型であるSN-38の増加をもたらす[5]．Irinotecanと*UGT1A1*28*について，レトロスペクティブな研究で好中球減少と下痢を含む重篤な副作用との関連が指摘された．Irinotecanの最大耐用量を明らかにするためのプロスペクティブな研究が行われ，多型のない群の最大耐用量が850 mg/bodyであったのに対して，ヘテロに*UGT1A1*28*をもつ群では，700 mg/body (34%)，ホモに*UGT1A1*28*をもつ群では，400 mg/body(40%)と少ないことが報告された．また，*UGT1A1*28*多型のない群の最大耐用量は，これまでに示されていた耐用量の350 mg/m²よりはるかに多く，また，*UGT1A1*6*はCrigler-Najjar syndrome type IIで発見された遺伝子多型で，同様にグルクロン酸抱合能の低下が認められる．

日本人においては，頻度が少ないため副作用予測としての有効性は高いとはいえないと考えられる．

5-FU系薬剤としてcapecitabine，tegafur配合剤(UFT，TS-1)がある．これらは，プロドラッグで，体内で5-FUへ変換されて効果を示す薬剤である．これらにおいても，DPD活性低下がある場合副作用リスクは高い可能性がある．

4 Tamoxifenと副作用予測

Tamoxifenは，エストロゲンレセプターにエストロゲンと競合的に結合することで抗エストロゲン作用を示し，エストロゲンレセプター陽性乳がんの治療において重要な薬剤である．Tamoxifenの代謝は，CYP3A4によりN-desmethyl-tamoxifenとなりCYP2D6によりendoxifenとなる経路と，CYP2D6により4-OH-tamoxifenとなりCYP3A4によりendoxifen.となる経路が主なものである．4-OH-tamoxifenとendoxifenは，エストロゲン依存性の細胞増殖抑制能がtamoxifenの30-100倍とされている．

CYP2D6には60以上のalleleがあり，活性の低下や増強が起こることが報告されている．活性が低下する*CTP2D6*3, 4, 5*の遺伝子多型をもつ場合，tamoxifenを内服して定常状態となったときのendoxifenの定常状態での血中濃度が，ホモの多型の場合，野生型の約25％へ低下していたとする報告があるが，日本人での頻度は7％程度で白人の20数％より少ない．アジア人に比較的多い(40％程度)*CTP2D6*9, 10*の場合においては，不安定型酵素による活性低下が報告されている．Endoxifenの定常状態での血中濃度は低下型ホモの場合では，30-40％へ低下していた．一方，*CTP2D6*9*においては活性が高くなる．酵素活性が高くなると，ホットフラッシュなどの有害事象により治療コンプライアンスが悪化するという報告もある．しかしながら，代謝にはCYPsの他の酵素(CYP2C9, CYP2C19やCYP2B6)も関連していることと，併用薬(CYP2D6阻害薬など)によっても強く影響を受けることも明らかとなっている．CYP2D6の酵素活性のみで，副作用や治療効果を予測することはできないと考えられる．

おわりに

薬剤，特に抗がん剤使用において副作用は，薬理作用や副反応として避けることのできない事象ともいえるものである．これを予測できれば，最小の副作用で最大の治療効果を得ることにもつながる．しかしながら，まだ発展途上といわざるをえない．代謝経路に関与する酵素やトランスポーターなどは，副作用予測を可能とする可能性の高い因子である．今回述べたもの以外でも，pazopanibによるCYP3A4やUGT1A1阻害，P-glycoprotein(Pgp)やbreast cancer resistance protein(BCRP)への影響，nilotinibのUGT1A1阻害などが，*in vitro*のデータとして示されているが，いずれも臨床に与える影響は明らかとなっていない．現在，米国FDAにおいて，添付文書において用量調節副作用予測のため検査することが推奨されているのは，6-MPとirinotecanのみである．今後のさらなる研究により，副作用予測可能となることを期待したい．

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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 non-malignant 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.

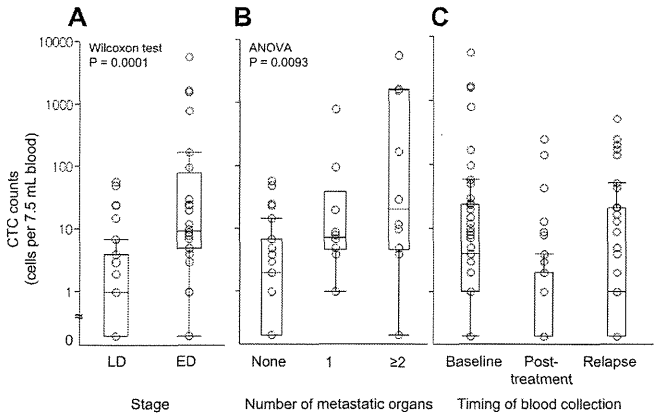

FIGURE 1. Box plots were drawn using the minimum and maximum values and the 25th, 50th, and 75th percentiles. *A*, Circulating tumor cell (CTC) levels at the baseline and the disease stage. ED, extensive disease; LD, limited disease. *B*, CTC levels at the baseline and number of metastatic organs. *C*, 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

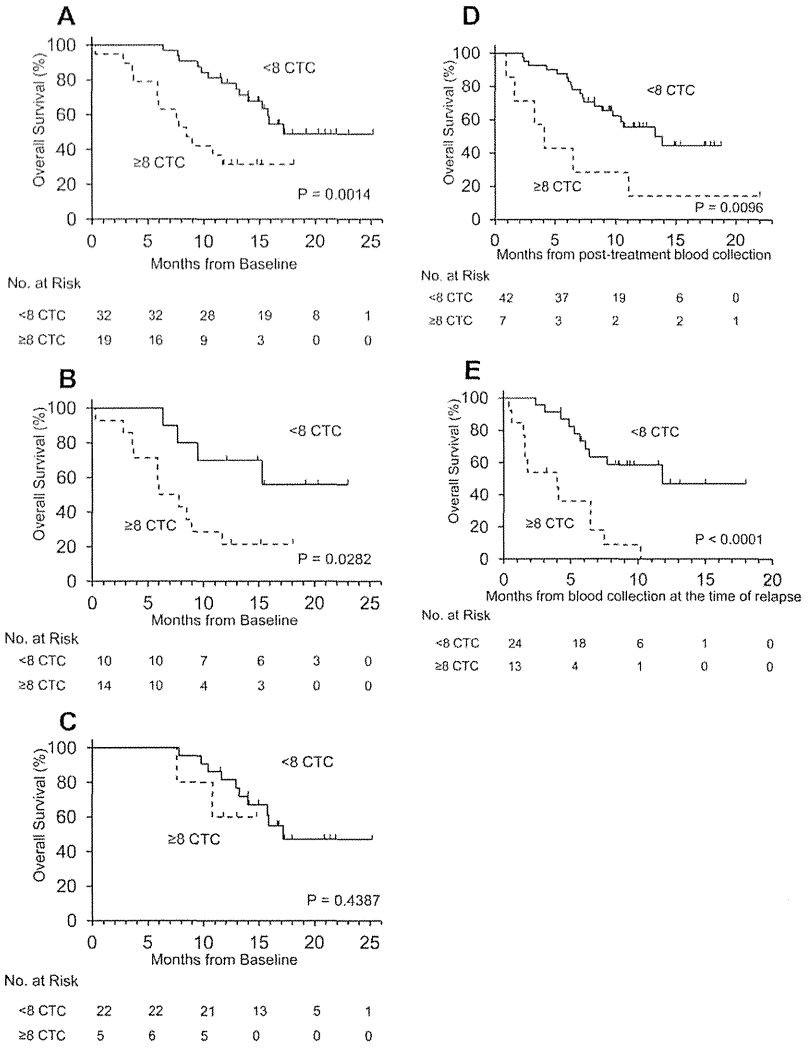

FIGURE 2. Kaplan-Meier curves for overall survival in patients with <8 and ≥8 circulating tumor cells (CTCs) at the baseline in the full set of data (*A*), extensive disease subset (*B*), and limited disease subset (*C*). Kaplan-Meier curves for posttreatment survival and postrelapse survival in patients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

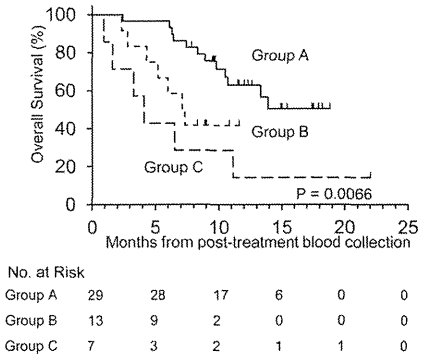

FIGURE 3. Kaplan-Meier curves for posttreatment survival in three groups, including patients in whom the baseline and posttreatment circulating tumor cell (CTC) levels remained at <8 (group A), patients in whom the CTC level converted from ≥8 to <8 cells (group B), and patients in whom the posttreatment 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).

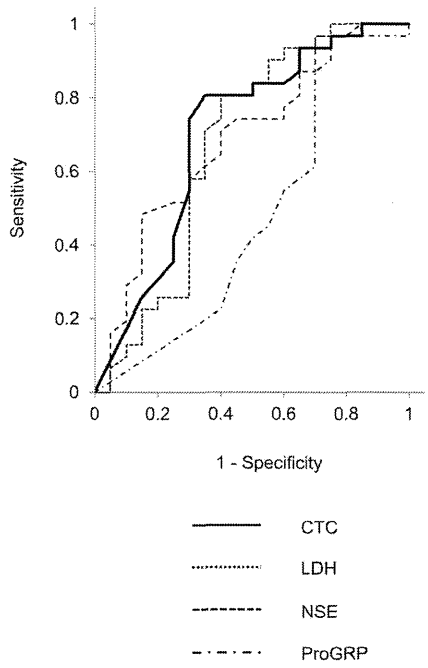

FIGURE 4. Receiver operator characteristics curve analysis for predicting 1-year survivors. The area under the curve is 0.70 (95% confidence interval [CI], 0.52–0.83) for the circulating tumor cell (CTC) level at baseline, 0.67 (95% CI 0.49–0.82) for serum lactate dehydrogenese (LDH) at baseline, 0.68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

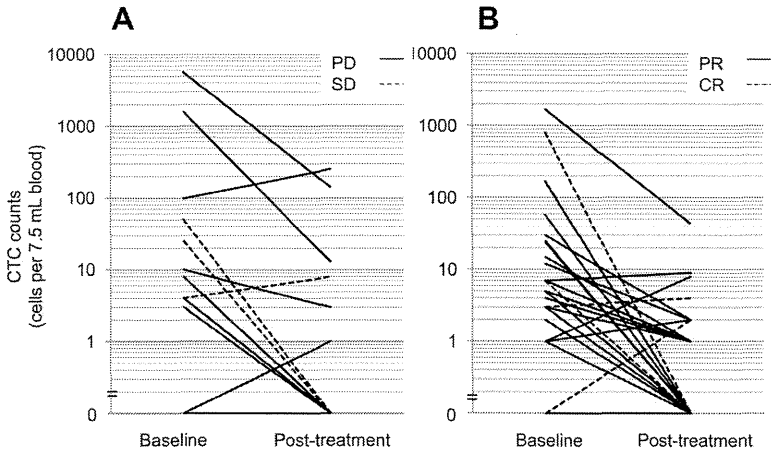

FIGURE 5. Relationship between radiologic response and the changes in the circulating tumor cell (CTC) level. *A*, Baseline and posttreatment CTC levels in patients showing PD (solid line) and SD (dotted line). *B*, Baseline and posttreatment CTC levels in patients showing PR (solid line) and CR (dotted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

ses.[3,7,11,15] Consistent with these reports, the detection rate in the SCLC patients in our study was 68.6%. Given that approximately half of our patients had nonmetastatic disease, we consider that CTCs are detected in a high percentage of cases of SCLC. Higher CTC counts have been reported as an indicator of the presence of distant metastases, such as bone metastasis in prostate cancer[20] and liver metastasis in colorectal cancer.[11] In patients with NSCLC, the CTC levels reportedly correlated with the number of organs showing metastatic involvement, and higher CTC levels are predictive of liver and bone metastasis.[15] Our results also showed an association between the CTC levels and the presence of metastasis, especially to the liver.

The CTC cutoff level (8 CTCs per 7.5 ml of blood) in our study to discriminate between groups with a favorable and unfavorable prognosis was higher than that reported for other tumors. In metastatic breast cancer, the cutoff level of 5 was chosen by comparing the median PFS and the Cox proportional HR for each threshold from 1 to 10,000 CTCs. The same cutoff was also shown to be correlated with the OS.[7] The cutoff of five cells was then applied to metastatic castration-resistant prostate cancer and was well validated to be predictive of the OS.[14] In metastatic colorectal cancer, the cutoff level of three cells was chosen by correlating the baseline CTC level with the response at the first follow-up imaging study. The cutoff level was well validated to be predictive of both the OS and PFS in a subsequent validation cohort.[11] Our cutoff level was based on a comparison of the Cox proportional HR for OS. The differences in the cutoff levels may be attributable to the statistical method used for choosing the optimal cutoff level or might reflect the highly metastatic potential of SCLC itself. In addition, we observed the prognostic significance of the baseline CTC only in the ED subset or patients treated by only chemotherapy in the subset analyses. As the previous studies in other malignancies have been conducted only in patients with metastatic disease, another study for ED-SCLC will be required to validate our results.

Conversion from an unfavorable baseline CTC level to a favorable follow-up CTC level reportedly has a strong impact on the survival. Patients with such conversion showed a favorable OS, statistically similar to that in patients with a persistent favorable CTC level in breast, prostate, and colorectal cancers.[7,11,14] In contrast, our study showed a relatively small impact of such conversion on the survival in SCLC patients. This difference might reflect the nature of SCLC itself, known to be aggressive and to rarely be in a dormant state.[2,3] A lower CTC level might be an appropriate treatment goal if minimal residual cancer cells after treatment had a larger impact on the survival in SCLC patients. Chemotherapeutic agents active against SCLC are as yet limited, and the classic platinum doublet with etoposide or irinotecan remains the standard first-line treatment regimen. Treatment options for relapsed SCLC are further limited to several cytotoxic agents,[21,22] and no molecular-targeted agents have yet been approved.[23] These limitations in treatment modalities might be related to the small impact of conversion after first-line treatment. NSE and ProGRP are commercially available serum biomarkers and are used as markers for monitoring of SCLC patients. They have been reported to be highly sensitive and specific for the diagnosis of SCLC, and elevated levels of these markers at baseline have been shown to be associated with poor prognosis.[24–26] LDH has also been reported to have prognostic significance in patients of SCLC.[27] We showed that the baseline CTC level showed a good discriminatory power for predicting the prognosis in SCLC patients, similar to serum NSE and LDH, and furthermore, that the baseline CTC level was probably a better predictor of survival than the serum ProGRP, by receiver operator characteristics curve analysis.

The treatment response was reported to be associated with the CTC level at the time of imaging in breast cancer.[28] In colorectal cancer, the CTC level measured 3 to 5 weeks after the initiation of therapy had a relatively low sensitivity (27%) for predicting PD.[11] In our study, we found no correlation between the results of the response assessment using the RECIST criteria and the baseline CTC level, posttreatment CTC level, or change in the CTC level associated with treatment. The changes in the tumor size might not always be related to the changes in the outflow of tumor cells from the tumors.

The major limitation of this study was that the study population was small. The threshold value was derived from a cohort at a single institution and not validated in an independent validation cohort. In addition, our study included not only patients receiving chemotherapy alone but also patients treated by chemoradiotherapy. Because the treatment goals are different for chemotherapy and chemoradiotherapy, that is, palliation versus cure, separate derivation studies will be required to choose the optimal CTC cutoff level.

There has been an increasing interest in several aspects of CTCs. First, measurement of the CTC levels has been expected to guide decision making, such as determining the timing of changing, continuing, or discontinuing the current treatment, or identifying appropriate candidates for adjuvant chemotherapy.[29–31] Second, CTC analysis is anticipated to provide samples for biomarker analysis. Monitoring of human EGFR-related 2-positive CTCs in breast cancer patients during human EGFR-related 2-targeted therapy[32–34] and analysis of androgen receptor gene alterations in the CTCs of prostate cancer patients[35,36] have been reported. In addition, the newly developed CTC analyzer shows a high detection power for CTCs and was used for the analysis of *EGFR*-gene alterations in the CTCs from patients with NSCLC.[37,38] These studies have established a new role for CTC analysis as a noninvasive method of tumor profiling or target monitoring during treatment with molecular-targeted agents. Although few molecular-targeted agents currently available are active against SCLC, the high detection rate of CTCs in cases of SCLC might provide an opportunity for the screening of active drugs and accelerate the development of new therapeutic strategies.

In conclusion, this study showed that CTCs are readily detectable by the CellSearch system in patients with SCLC and that the CTC levels before and after treatment had strong

prognostic significance. A large prospective multiinstitutional validation study is required to confirm our results.

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First Case of Combined Small-Cell Lung Cancer with Adenocarcinoma Harboring *EML4-ALK* Fusion and an Exon 19 *EGFR* Mutation in Each Histological Component

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A 72-year-old male exsmoker of 60-pack-years had undergone a high anterior resection, followed by chemotherapy (Leucovorin+5-Fluorouracil, S-1, FOLFOX-4+Bevacizumab, FOLFIRI+Bevacizumab) for rectal cancer with liver and sacral bone metastases 6 years ago. Because a nodal shadow had appeared in the right lower lobe of the lung, despite the disappearance of the liver and sacral metastases, he was referred to our department for a treatment of the pulmonary nodule.

Computed tomography showed an irregular nodule in the right lower lobe, which was confirmed as active by positron emission tomography, although there were no active lesions on the liver or sacral bone (Fig. 1*A*). The pulmonary lesion was assumed to be primary lung cancer, and right lower lobectomy with lymphadenectomy was performed. The cut sections revealed a whitish solid nodule encircled by a gray-whitish component with a maximum diameter of 4.5 cm (Fig. 1*B*). The central component was pathologically diagnosed as small-cell lung cancer (SCLC), which was 30% of the entire tumor, and the surrounding area was adenocarcinoma (70%) with papillary, acinar and lepidic components (formerly nonmucinous bronchioloalveolar carcinoma, 10%; Fig. 2 *A– C*). Both the components showed immunoreactivity to thyroid transcriptional factor 1, whereas synaptophysin and CD56 were detected only in the SCLC component. The pathological stage was finally determined to be IB. Each of the components was separately examined for mutations of epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) by the direct sequencing method. A deletion in exon 19 of *EGFR* was detected only in the lepidic component, whereas only the SCLC component harbored variant 1 of echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* fusion (Fig, 3*A*, *B*) and those were confirmed by immunohistochemistry (Fig. 3*C*, *D*). Figure. 3*E* shows gene mapping of the mutations in each component.

DISCUSSION

Gene mutations in tyrosine kinases play crucial roles in the pathogenesis of adenocarcinoma. Tumors with the *EGFR* gene, the most well-known tyrosine kinase which

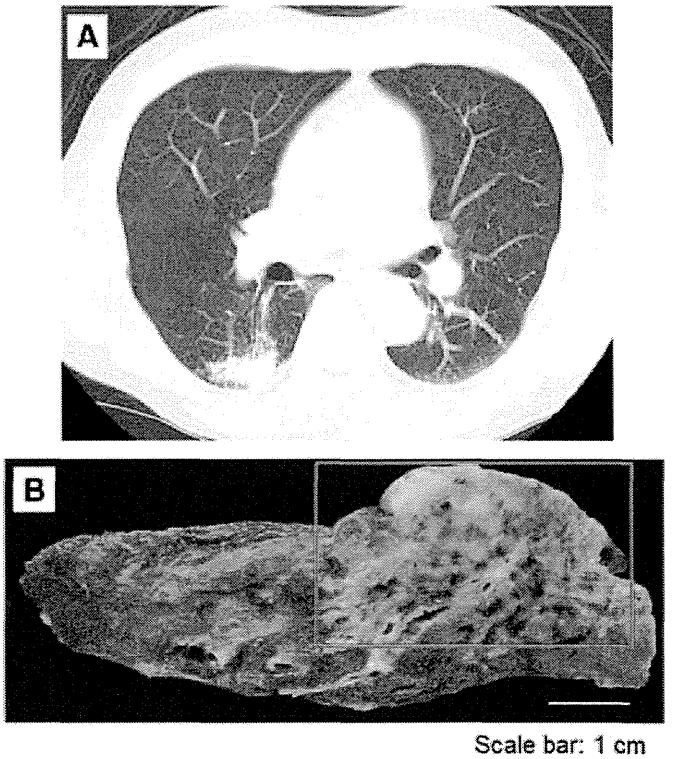

FIGURE 1. *A,* Computed tomography showing an irregular nodule in the right lower lobe of lung. *B,* Cut sections of the tumor are seen by the encircled part.

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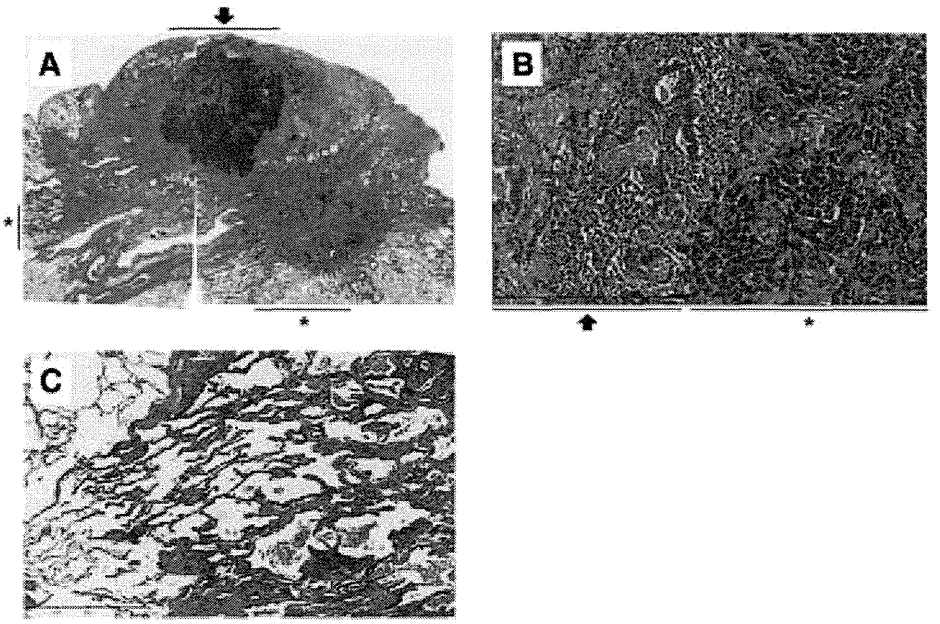

FIGURE 2. Microscopic findings. *A*, Microscopic findings of the tumor consisting of SCLC (arrow) surrounded by adenocarcinoma with papillary, acinar and lepidic components (asterisk). Highly magnified images of (*B*) the SCLC (asterisk), the adenocarcinoma (arrow), and (*C*) the lepidic components. SCLC, small-cell lung cancer.

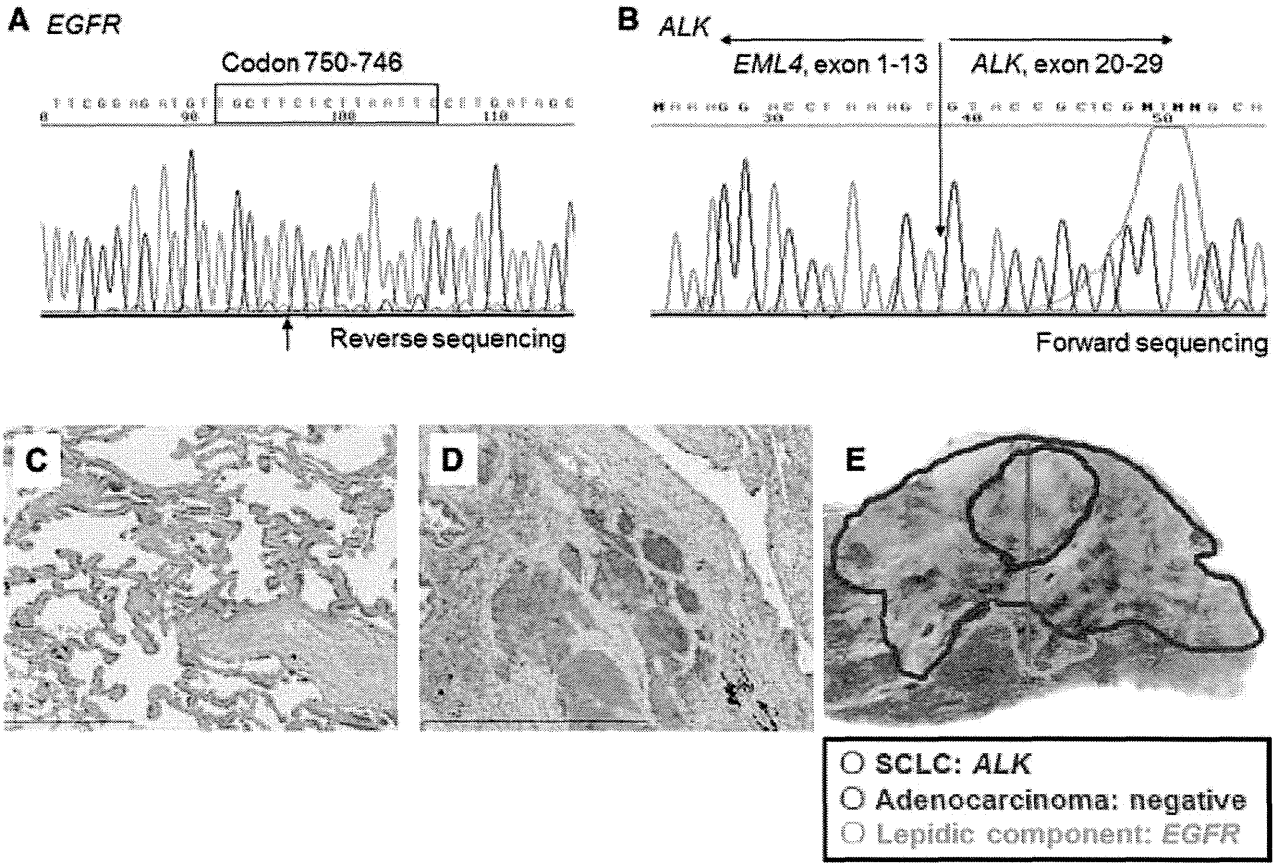

FIGURE 3. A direct sequence analysis revealing (*A*) a deletion in exon 19 of *EGFR* in the adenocarcinoma and (*B*) a variant 1 mutation of *EML4-ALK* in the SCLC. Immunoreactivity of the lepidic component to the deletion in exon 19 of EGFR using an antibody that specifically detects deleted EGFR (E746-A750del) (6B6, Cell Signaling, Danvers, MA) (*C*) and of the SCLC to ALK using primary antibody against ALK (5A4, Nichirei, Tokyo, Japan) (*D*). A polymer method was used for the immunohistochemical analysis, specifically, an intercalating antibody-enhanced polymer method was used for the detection of ALK. *E*, Gene mapping of the driver mutations in each component. EGFR, epidermal growth factor receptor; EML4-ALK, echinoderm microtubule-associated protein-like 4; SCLC, small-cell lung cancer; ALK, anaplastic lymphoma kinase.

harbors activating mutations in exon 19 and 21, can be successfully treated by EGFR-tyrosine kinase inhibitors (TKIs) in comparison to cytotoxic reagents.[1] The *EML4-ALK* fusion gene also possesses a transforming activity[2] and has attracted much attention because it might be a potential therapeutic target of ALK inhibitors in the treatment of adenocarcinoma.[3] Although *EGFR* mutations have already been identified in SCLCs (4%),[4] there are no reports on the *EML4-ALK* translocation in SCLCs. Intriguingly, *ALK* translocation was detected in the SCLC component in the present case, whereas the exon 19 *EGFR* mutation was shown only in the lepidic component.

Adenocarcinoma with sensitive *EGFR* mutations can transform into SCLC in the process of acquiring resistance to EGFR-TKIs.[5] This mechanism does not apply to the current case, because the patient had not received EGFR-TKIs. Although the complexity of the combined histology and driver mutations in the present case has not been elucidated, this phenomenon suggests that *ALK* rearrangements could be involved in the pathogenesis of SCLC, which could be successfully treated with ALK inhibitors.

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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 ≤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 $P = 0.041$).

Table 1. Individual patients' characteristics, treatment methods and outcome of the patients treated with concurrent chemoradiotherapy (CRT)

No.	Age (years)	Gender	PS	Stage	CTx	Response	RTx (timing)	RTx (Dose/Fr)	CTx compliance	RTx compliance	Failure site	PFS	OS
C-1	75	F	1	IIIA	CB(5)+ETP(80)2c	PR	From c2	39.6/22	Discontinuation +	Discontinuation +	WT	165	971
C-2	75	M	0	IIIA	CB(5)+ETP(80)3c	PR	From c2	44/22	Discontinuation +	4 days omission	Brain	547	1114
C-3	75	M	1	IIB	CD(80)+ETP(100)4c	PR	From c1	45/30	Dose reduction +	7 days omission	Brain	1790	2393+
C-4	76	M	1	IIB	CD(80)+ETP(100)4c	PR	From c1	45/30	Completed	2 days omission	Brain	214	2485
C-5	77	F	1	IIIB	CD(25)x3+ETP(80)1c→ CB(5)+ETP(80)3c	Near CR	From c2	45/30	Changed CTx regimen and dose reduction	Completed	Liver	201	359

No., number; PS, performance status; CTx, chemotherapy; RTx, radiotherapy; Fr, fraction; PFS, progression-free survival; OS, overall survival; F, female; M, male; CB, carboplatin; ETP, etoposide; c, cycle; CD, cisplatin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; NA, not available; WT, within the thorax; PF, progression-free.
The dose of carboplatin was indicated by area under the curve in parentheses.
The doses of etoposide and cisplatin were indicated by per body surface area in parentheses.

Patients tended to be female, have lower stage and have a poorer PS in concurrent CRT, although there is no significant difference.

All five patients treated with concurrent CRT exhibited a partial response (PR) and the response rate was 100%. Of the 15 patients treated with sequential CRT, 3 had a complete response (CR), 9 exhibited PR, 1 showed stable disease (SD), 1 developed progressive disease (PD) and 1 was not evaluable (NE). The response rate was 80%. The median PFS of concurrent and sequential CRT were 208 and 216 days, respectively (Fig. 1). There was no statistically significant difference between the PFS of the two treatment methods (log-rank $P = 0.9715$) and the two PFS curves almost overlapped each other.

Of the five patients treated with concurrent CRT, discontinuation of chemotherapy occurred in two (40%) and dose reductions were needed in two due to adverse events (40%). Moreover, discontinuation of radiotherapy occurred in one patient (20%) and omissions were needed in three (60%). Among the 15 patients treated with sequential CRT, 11 completed the whole treatment method without discontinuation, dose reduction and omission of chemotherapy/TRT. Dose reductions of chemotherapy were needed in two patients (13%), and one of the two patients was treated with etoposide (100 mg/m^2, days 1–3) plus cisplatin (25 mg/m^2, days 1–3). Discontinuation of chemotherapy occurred in two patients (13%) due to toxicities. Radiotherapy was completed without omission in all 11 patients who received sequential radiotherapy.

Table 4 shows the adverse events in patients treated with concurrent CRT and sequential CRT. Hematological toxicities, febrile neutropenia, fatigue and anorexia tended to be more frequent and severe in concurrent CRT than in sequential CRT. However, Grade 3 or more severe pneumonitis tended to be frequent in sequential CRT (four patients, 27%).

Patients' Characteristics, Tumor Response, PFS, OS and Toxicity in Patients Treated with Etoposide Plus Carboplatin Followed by Sequential TRT

Twelve patients were treated with etoposide plus carboplatin followed by sequential TRT. The number of male patients, 10 (83%), was larger than that of the female patients, and the median age of the patients was 79 years. Eight patients (67%) had a PS of 0 and the remaining a PS of 1. All were smokers, and 10 patients (83%) were Stage IIIA or IIIB and the remaining Stage IIA or IIB.

With regard to the tumor response, CR was achieved by three patients, PR by eight and one patient was NE. The response rate was 91%.

The median PFS and OS were 244 and 601 days, respectively (Fig. 2). The median follow-up duration was 496 days. In terms of the first failure site during and after CRT, nine patients (75%) had experienced disease relapse at the time of data analyses. Five (42%) and two (17%) patients

Table 2. Individual patients' characteristics, treatment methods and outcome of the patients treated with sequential CRT

No.	Age (years)	Gender	PS	Stage	CTx	Response	RTx (dose/Fr)	CTx compliance	RTx compliance	Failure site	PFS	OS
S-1	75	M	0	IIIA	CB(5)+ETP(80)4c	PR	45/30	Completed	Completed	PF	2754+	2754+
S-2	75	M	0	IIIA	CD(25)x3+ETP(80)4c	SD	45/30	Completed	Completed	Brain	137	578
S-3	75	M	0	IIIA	CD(25)x3+ETP(100)4c	PD	50/25	Dose Reduction +	Completed	WT	143	769
S-4	76	M	1	IIIB	CB(5)+ETP(80)4c	PR	45/30	Dose Reduction +	Completed	WT and liver	414	652
S-5	76	M	1	IIIA	CB(5)+ETP(80)4c	CR	45/30	Completed	Completed	Brain	137	257
S-6	77	M	1	IIA	CB(5)+ETP(80)4c	PR	45/30	Completed	Completed	PF	442+	442+
S-7	77	M	0	IIIB	CD(25)x3+ETP(80)3c	PR	NA	Discontinuation +	NA	WT	243	454
S-8	78	M	1	IIIA	CB(5)+ETP(80)4c	PR	59/32	Completed	Completed	Brain	181+	181+
S-9	78	M	0	IIIA	CB(5)+ETP(80)4c	PR	45/30	Completed	Completed	Brain	181	550+
S-10	80	F	1	IIIA	CB(5)+ETP(80)1c	NE	NA	Discontinuation +	NA	WT	70	316+
S-11	80	M	0	IIIB	CB(5)+ETP(80)4c	CR	45/30	Completed	Completed	Brain	152	258
S-12	81	F	1	IIB	CB(5)+ETP(80)4c	PR	50/25	Completed	Completed	PF	1892+	1892+
S-13	83	M	1	IIIB	CB(5)+ETP(80)4c	CR	45/30	Completed	Completed	Brain	269	327
S-14	83	F	1	IIIA	CB(5)+ETP(80)4c	Near CR	50/25	Completed	Completed	Liver and lung	408	415+
S-15	92	M	0	IIIA	CB(5)+ETP(80)4c	PR	45/30	Completed	Completed	WT	218	383

The dose of carboplatin was indicated by area under the curve in parentheses.
The doses of etoposide and cisplatin were indicated by per body surface area in parentheses.

Table 3. Individual patients' characteristics, past history and complications of the patients treated with concurrent CRT and sequential CRT

No	Age (years)	Gender	PS	Stage	Past history	Complications
C-1	75	F	1	IIIA	–	Osteoarthritis
C-2	75	M	0	IIIA	–	Anal stenosis
C-3	75	M	1	IIB	Gastric ulcer	COPD, prostatic hypertrophy
C-4	76	M	1	IIB	Gastric ulcer	–
C-5	77	F	1	IIIB	–	Hypertension, hyperlipidemia, osteoporosis
S-1	75	M	0	IIIA	–	Arrhythmia, prostate cancer
S-2	75	M	0	IIIA	–	Gastric ulcer, hypertension
S-3	75	M	0	IIIA	–	Prostatic hypertrophy, abdominal aortic aneurism
S-4	76	M	1	IIIB	Abdominal aortic aneurism	IHD, DM, hypertension
S-5	76	M	1	IIIA	Abdominal aortic aneurism	Aortic dissection
S-6	77	M	1	IIA	Laryngeal cancer, brain hemorrhage	Hypertension
S-7	77	M	0	IIIB	Gout, gastritis	Hypertension, prostatic hypertrophy
S-8	78	M	1	IIIA	Bladder cancer, brain hemorrhage	Hypertension
S-9	78	M	0	IIIA	ASO, IHD, gastric ulcer	–
S-10	80	F	1	IIIA	IHD, pneumothorax, gout, renal failure	COPD
S-11	80	M	0	IIIB	Rectal cancer	–
S-12	81	F	1	IIB	–	IHD
S-13	83	M	1	IIIB	Asthma, gastric ulcer, colon cancer	Hypertension
S-14	83	F	1	IIIA	Uterine cancer	Hypertension
S-15	92	M	0	IIIA	–	Reflux esophagitis, hypertension

COPD, chronic obstructive pulmonary disease; IHD, ischemic heart disease; DM, diabetes mellitus; ASO, arteriosclerosis obliterans.