of 0–2. Additional eligibility criteria were as follows: they had not undergone surgery for LS-SCLC nor had been treated with a radiation field, not including elective nodal irradiation, because the significance of omitting elective nodal irradiation remains unclear (6). Written informed consent was obtained from all patients before treatment. Each patient underwent the following studies: chest radiography and fiberoptic bronchoscopy, complete blood count and biochemical tests, a computed tomography (CT) scan of the thorax and abdomen, a CT scan or magnetic resonance imaging of the brain, and a radionuclide bone scan, or positron emission tomography. Positron emission tomography was used for a few patients (7%) who were treated after 2001 according to the physician's preference. Bone marrow aspiration or biopsy was performed in cases of neutropenia and thrombocytopenia.

Radiation therapy technique

TRT was carried out with linear accelerators, and the energy of 6–10 MV photons was used. The TRT fields were changed from anteroposterior-posteroanterior fields to parallel opposed oblique fields after 30 Gy in the twice-daily regimen and 36–40 Gy in the once-daily regimen. Most patients (83%) that were eligible for this study were treated using conventional fluoroscopic simulation techniques at the start of the TRT, and CT simulation techniques were used only for the planning of the boost fields. The other 17% were treated using CT simulation techniques throughout the entire TRT. The other planning techniques were similar to those in our previous report on non–small-cell lung cancer (7). TRT was administered twice daily (1.5 Gy per fraction, with a 6 h or more interval between fractions) for a total dose of 45 Gy in 3 weeks or once-daily (1.8–2.0 Gy per fraction) for a total dose of 39.6–66 Gy in 4–7 weeks. After the TRT, prophylactic cranial irradiation (PCI) was administered to the patients who had a complete or near-complete response (10). The PCI consisted of 24 Gy in 2 Gy per fractions or 25 Gy in 2.5 Gy per fractions once daily, 5 days per week.

All patients who entered the clinical trial were treated with the AHF regimen. However, there were no adequate rationale for a decision about a patient's TRT dose and fractionation. The TRT dose and fractionation was decided according to the physician's preference.

Chemotherapy

In principle, the patients were treated with four cycles of chemotherapy and received at least one cycle of chemotherapy concurrent with TRT. The chemotherapy was given in a 28-day cycle in the concurrent phase and a 21-day cycle in the sequential phase. The most commonly used regimens were cisplatin/etoposide, carboplatinum/etoposide, and cisplatin/irinotecan. As a general rule, the cisplatin/etoposide regimen consisted of cisplatin (80 mg/m^2 intravenously) on day 1 and etoposide (100 mg/m^2 intravenously) on Days 1, 2, and 3. The carboplatinum/etoposide regimen consisted of carboplatinum (area under the blood concentration-time curve: 5 intravenously) on Day 1 and etoposide (100 mg/m^2 intravenously) on Days 1, 2, and 3. The cisplatin/irinotecan regimen was only performed sequentially with TRT and consisted of cisplatin (80 mg/m^2 intravenously) on Day 1 and irinotecan (60 mg/m^2 intravenously) on Days 1, 8, and 15.

Study design and statistical analysis

All available radiation records and charts were reviewed to assess patient and tumor characteristics and the details of treatment and outcome. Tumor response was classified in accordance with the Response Evaluation Criteria in Solid Tumors criteria (9). Complications were graded in accordance with the National Cancer Institute's Common Toxicity Criteria, version 3.0 (10). The date of the last follow-up was defined as the last recorded information available for the patient. Only 3 patients were lost to follow-up. Survival was measured from the start date of any treatment to the date of the last follow-up or death from any cause. Local failure, defined as locoregional progression on CT (including the primary tumor and the bilateral mediastinal and ipsilateral hilar lymph nodes), was measured from the start date of any treatment to the date of the first evidence of locoregional disease progression. Concurrent local and distant failures were scored as local failures for the first failure sites. Progression-free survival was measured from the start date of any treatment until the date of local or distant failure.

Overall survival (OS), overall local, and overall progression-free survival were calculated using Kaplan-Meier estimates. Subgroup analysis was used to compare the outcomes among the three groups, in which the total radiation doses were 45 Gy with AHF, <54 Gy with SF, and ≥54 Gy with SF, using the log–rank test. Moreover, sex, age at diagnosis, performance status, disease stage (I, II, vs. III), PCI (yes vs. no), total chemotherapy cycles (<3 vs. ≥3), concurrent chemotherapy (yes vs. no), and the duration of TRT (<40 days vs. ≥40 days) were also assessed for their impact on OS using the log–rank test. Fisher's exact test was used for comparisons of categorical data. Cox's proportional hazards model was used for multivariate analysis. $p < 0.05$ was considered significant.

RESULTS

Patient and treatment characteristics

A total of 127 patients were enrolled into the study. The median total dose of TRT with the once-daily regimen was 54 Gy; therefore, we divided the patients that had been treated with the once-daily regimen into two groups using the median total dose of 54 Gy for the subgroup analysis. The characteristics of the 127 eligible patients are shown in Table 1. Fifteen patients (40%) from the AHF group entered a clinical trial, but no patients from the other two groups did. The baseline characteristics were balanced in terms of sex, performance status, stage, and chemotherapy cycles. However, there was a slight imbalance in age; the patients in the AHF group tended to be younger than those in the other two groups, and the rate of patients older than age 75 years was lower than in the other two groups, but these differences were not significant ($p = 0.15$). There were significant differences in the rate of patients that received concurrent chemotherapy and PCI among the three groups ($p = 0.012$, $p < 0.001$, respectively). Fifty-five (43%) patients were alive at the time of this analysis, and the median follow-up time of the surviving patients was 33 months (range, 2–118 months). The median follow-up time of the surviving patients was 34 months (range, 16–96 months) for the AHF group, 67 months (range, 12–91 months) for the SF <54 Gy group, and 22 months (range, 2–118 months) for the SF ≥54 Gy group. There were no significant differences in the median follow-up time of the surviving patients among the three groups ($p = 0.32$).

As a result, 84% received four or more cycles of chemotherapy. Eight percent received three cycles, and 8% received less than two cycles either because the patient refused continuation

Table 1. Patient and tumor pretreatment characteristics

Characteristic	Prescription group: AHF group (n = 37)	SF <54 Gy group (n = 29)	SF ≥54 Gy group (n = 61)	p value*
Age (y)	58 (40–68)	70 (51–82)	66 (29–81)	
≥75 (%)	0 (0%)	3 (10%)	5 (8%)	0.15
Sex (%)				0.59
Male	30 (81%)	25 (86%)	54 (82%)	
Female	7 (19%)	4 (14%)	7 (18%)	
Performance status				0.29
0	13 (35%)	7 (25%)	20 (33%)	
1	24 (65%)	20 (68%)	36 (59%)	
2	0 (0%)	2 (7%)	5 (8%)	
Stage				0.20
I	0 (0%)	1 (3%)	6 (10%)	
II	4 (11%)	0 (0%)	4 (7%)	
IIIA	22 (59%)	11 (38%)	24 (39%)	
IIIB	11 (30%)	17 (59%)	27 (44%)	
CHT cycles	3.9 (2–5)	3.7 (1–6)	3.9 (1–6)	0.72
≥3 cycles	33 (89%)	27 (93%)	56 (92%)	
Concurrent CHT	37 (100%)	23 (79%)	56 (92%)	0.012*
Total dose (Gy)	45 (45)	50 (39.6–52.2)	56 (54–63)	<0.001*
Duration of TRT (days)	21 (19–27)	41 (30–56)	43 (36–59)	<0.001*
PCI	24 (65%)	6 (21%)	18 (30%)	< 0.001*

Abbreviations: AHF = accelerated hyperfractionation; SF = standard fractionation; CHT = chemotherapy; TRT = thoracic radiotherapy; PCI = prophylactic cranial irradiation.

Age and total dose data are presented as the median value. CHT cycles data are presented as the mean value. The numbers in square brackets indicate the range of age and CHT cycles.

* Fisher's exact test.

of the chemotherapy or their leukocyte or platelet counts or renal function did not return to levels at which chemotherapy could be performed. Most patients (91%) received at least one cycle of concurrent chemotherapy with TRT, whereas the remaining 9% only received sequential chemotherapy because the radiation field sizes of these patients were too large as the primary tumor was located in the inferior lobe or the primary tumor was so bulky that concurrent chemoradiotherapy was considered to carry a high risk of severe radiation pneumonitis (11). All patients received at least one cycle of platinum-based agents/etoposide regimen regardless of the TRT regimen. The cisplatin/irinotecan regimen was only performed sequentially with TRT for 24% of patients in the AHF group and 16% of the SF ≥54 Gy group.

Tumor response

Table 2 shows the tumor response in each group. The overall response rate was 94% (95% confidence interval [CI] 91–98%, 58% complete response rate [CI 50–67%], and 36% partial response rate [CI 28–45%]) for all eligible patients. There was a significantly lower rate of complete response in the SF <54 Gy group than in the AHF and SF ≥54 Gy groups (p = 0.018 and 0.0062, respectively). There was a significantly higher rate of complete response in the AHF group than in the SF ≥54 Gy group (p = 0.042).

Table 2. Tumor response in each group

Response	Prescription group: AHF (n = 37) No.	%	SF <54 Gy (n = 29) No.	%	SF ≥54 Gy (n = 61) No.	%	p value*
Overall response	34	92%	27	93%	59	97%	0.32
CR	27	73%	11	38%	36	59%	0.02*
PR	7	19%	16	55%	23	38%	
SD	0	0%	1	3%	2	3%	
PD	3	8%	1	3%	0	0%	

Abbreviations: AHF = accelerated hyperfractionation; SF = standard fractionation; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease.

* Fisher's exact test.

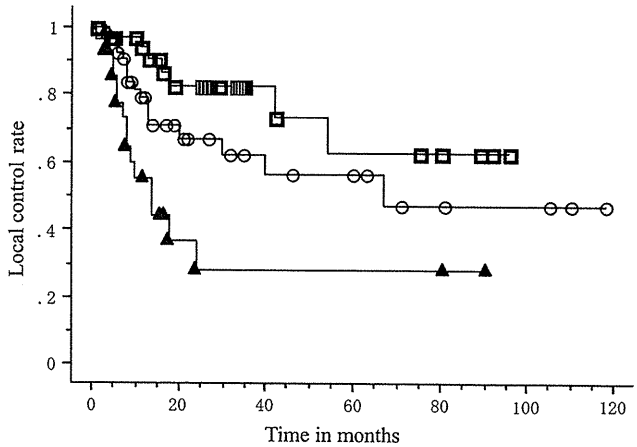

Fig. 1. The local control rates for patients with a total dose of 45 Gy with accelerated hyperfractionation (□), <54 Gy with standard fractionation (▲), and ≥54 Gy with standard fractionation (○).

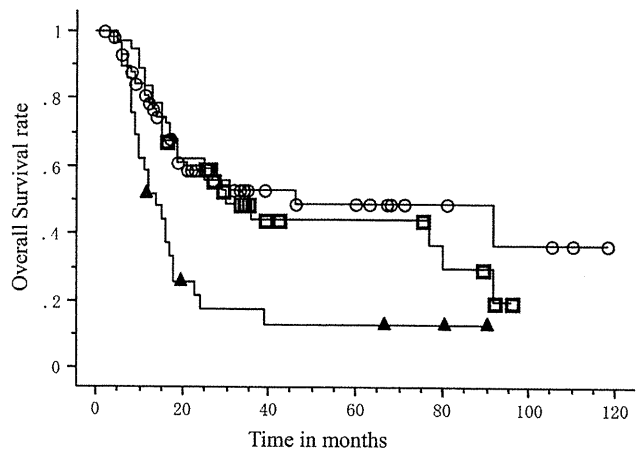

Fig. 2. Overall survival for patients with a total dose of 45 Gy with accelerated hyperfractionation (□), <54 Gy with standard fractionation (▲), and ≥54 Gy with standard fractionation (○).

Local control and progression-free survival

Figure 1 shows the local control rates for each group. The 3-year local control rates were 61.1% (CI 50.3–71.9%) for all eligible patients, 81.3% (CI 67.2–95.5%) for the AHF group, 27.7% (CI 5.0–50.4%) for the SF <54 Gy group, and 61.2% (CI 44.8–77.6%) for the SF ≥54 Gy group. The local control rate was also significantly lower for the SF <54 Gy group than the AHF and SF ≥54 Gy groups (p = 0.0016 and 0.011, respectively). Local control for the AHF group tended to be superior to that for the SF ≥54 Gy group, although no statistically significant difference was found (p = 0.096).

The 3-year progression-free survival rates were 28.1% (CI 19.5–36.7%) for all eligible patients, 37.5% (CI 21.5–53.5%) for the AHF group, 7.5% (CI 0–17.5%) for the SF <54 Gy group, and 33.2% (CI 19.7–46.7%) for the SF ≥54 Gy group. Progression-free survival was also significantly lower for the SF <54 Gy group than for the AHF and SF ≥54 Gy groups (p = 0.015 and 0.013, respectively). Progression-free survival was similar in the AHF group and the SF ≥54 Gy group (p = 0.80).

Overall survival

Figure 2 shows the survival curves for each group. The median survival time of all eligible patients was 24.0 months (CI 18.1–29.9 months). The median survival times were 30.0 months (CI 16.3–43.7 months) for the AHF group, 14.0 months (CI 6.6–21.4 months) for the SF <54 Gy group, and 41.0 months (CI 33.9–48.1 months) for the SF ≥54 Gy group. The 3-year survival rates were 41.2% (CI 31.6–50.8%) for all eligible patients, 44.1% (CI 26.5–61.7%) for the AHF group, 13.8% (CI 0–27.3%) for the SF <54 Gy group and 53.1% (CI 38.6–67.6%) for the SF ≥54 Gy group. There was a significantly lower rate of OS in the SF <54 Gy group than in the AHF and SF ≥54 Gy groups (p = 0.0018 and 0.00036, respectively). OS for the SF ≥54 Gy group seemed to be slightly superior to that for the AHF group, although no statistically significant difference was found (p = 0.64).

Factors associated with overall survival

Table 3 shows the effects of patient characteristics, disease factors, and treatment parameters on OS according to univariate analysis. To evaluate further the independent effects of disease stage, chemotherapy cycles, concurrent chemotherapy, PCI,

Table 3. Factors associated with overall survival according to univariate analysis

Factors	No. of patients	3-year OS (95% CI)	p value
Sex			0.367
Male	109	40.8% (33.1–48.5)	
Female	18	41.8% (36.1–47.5)	
Age (y)			0.652
<75	119	40.6% (33.2–48.0)	
≥75	8	48.6% (43.6–53.6)	
PS			0.546
0, 1	120	42.0% (34.6–49.4)	
2	7	28.6% (23.6–33.6)	
Stage			0.016*
I, II	15	80.0% (78.3–81.7)	
III	112	35.7% (28.3–43.1)	
CHT cycle			0.033*
<3	11	43.4% (35.8–51.0)	
≥3	116	20.0% (14.2–25.8)	
Concurrent CHT			0.026*
Yes	116	43.7% (36.1–51.3)	
No	11	20.0% (17.3–22.7)	
Treatment group			<0.001*
AHF	37	44.1% (26.5–61.7)	
SF ≥54 Gy	29	53.1% (38.6–67.6)	
SF <54 Gy	61	13.8% (0–27.3)	
Duration of RT (days)			0.821
<40	66	41.3% (31.0–51.6)	
≥40	61	40.0% (29.8–50.2)	
PCI			0.089
Yes	48	48.1% (36.6–59.6)	
No	79	35.6% (31.6–39.6)	

Abbreviations: CI = confidence interval; PS = performance status; CHT = chemotherapy; NA = not applicable; AHF = accelerated hyperfractionation; SF = standard fractionation; RT = radiation therapy; PCI = prophylactic cranial irradiation.

* Statistically significant.

Table 4. Factors associated with overall survival according to multivariate analysis

Factors	Hazard ratio of death (95% CI)	*p* value
Stage (I, II vs. III)	0.24 (0.074–0.78)	0.017*
CHT cycle (<3 vs. ≥3)	0.50 (0.24–1.07)	0.073
Concurrent CHT (yes vs. no)	0.61 (0.29–1.31)	0.20
Treatment group (AHF vs. SF ≥54 Gy SF 54 Gy)	NA	0.033*
PCI (yes vs. no)	0.75 (0.43–1.31)	0.31

Abbreviations: CHT = chemotherapy; NA = not applicable; AHF = accelerated hyperfractionation; SF = standard fractionation.
* Statistically significant.

and treatment group on OS, a multivariate Cox proportional hazards regression analysis was performed. This analysis included those factors that had displayed a p value <0.10 in the univariate analysis. As a consequence, disease stage and treatment group remained significant factors in the multivariate analysis (Table 4).

Toxicity

Documentation concerning toxicity data was not available for 6 patients (2 in each group), which left 121 patients assessable for toxicity. Only late toxicity ≥Grade 2 was assessed from the available information of each chart. There were only 2 treatment-related deaths (one in the SF <54 Gy group and the other in the SF ≥54 Gy group). Both patients died of radiation pneumonitis. Five patients developed Grade 2 radiation pneumonitis, 4 in the SF <54 Gy group and 1 in the SF ≥54 Gy group. Apart from the toxicities described, no other information about late toxicity was noted in the charts.

DISCUSSION

In our study, the comparison of overall, progression-free, and local control survival rates and the rate of complete response suggested that TRT administered with a total dose of <54 Gy by once-daily regimen was more disadvantageous than TRT treated with a total dose of ≥54 Gy in a once-daily regimen or a total dose of 45 Gy administered using the AHF regimen, and the difference was statistically significant for all outcomes. These results clearly demonstrate that radiation intensification improves the complete response rate and local control and that improved local control translates into improved OS. Furthermore, these results also suggest the importance of a high dose of radiation when using a once-daily regimen.

Because SCLC has high radiation sensitivity (12), recent pattern of care studies have shown that the traditional modest doses of TRT that are used in once-daily 1.8- to 2-Gy fractions are also widely used for LS-SCLC in Japan and Turkey (4, 5). However, although response rates are high, local control rates have been poor in this TRT setting. Intensifying the radiotherapy effect by accelerating its delivery was one of the initial strategies explored in prospective trials. Turrisi *et al.* (2) randomly assigned 471 LS-SCLC patients to either 45 Gy in 5 weeks (1.8 Gy once-daily for 25 fractions) or 45 Gy in 3 weeks (1.5 Gy twice-daily for 30 fractions) beginning with the first of four cycles of PE. The 5-year survival rate was 26% with accelerated TRT compared with 16% for the conventional TRT ($p = 0.04$), and the accelerated TRT arm was also superior to conventional TRT in local tumor control ($p = 0.06$). These data strongly suggest that attempts designed at improving local tumor control can favorably impact on the long-term outcome of patients with LS-SCLC. However, despite the significant improvement in long-term survival, only 10% of patients with LS-SCLC received a twice-daily regimen (3). Moreover, a second trial performed by the North Central Cancer Treatment Group reported negative results with a twice-daily regimen, although overall treatment times and total radiation doses were identical in each arm of the North Central Cancer Treatment Group trial (13). Therefore, different strategies were considered that might increase the local control rate with chemoradiotherapy. Accelerated fractionation via the concomitant boost technique uses once-daily irradiation early in the course of treatment and then twice-daily irradiation toward the end. Komaki *et al.* (14) reported a Phase 1 study (Radiation Therapy Oncology Group 97-12) using this regimen to improve local control by increasing the dose of TRT given with concurrent cisplatin/etoposide without causing acute severe esophagitis. They found that 61.2 Gy was the maximum tolerated dose, and there was a suggestion of improvement in the estimated short-term survival rate (18 months) by dose escalation from 50.4 Gy to 61.2 Gy (25% and 82%, respectively). Roof *et al.* (15) also showed a clear dose–response curve between 54 and 63 Gy with a once-daily regimen, although they did not find a significant difference in outcome because of their small sample size of 54 patients.

Obviously, there are problems that limit the interpretation of a single institutional retrospective review. We recognize the imbalance among our three groups. The rates of patients receiving PCI and concurrent chemotherapy were significantly different among the three groups (Table 1), although the multivariate analysis proved that these factors were not associated with OS. Another difficulty associated with retrospective reviews is the accurate assessment of toxicity. The rate of Grade 2-4 toxicities in our study was lower than those reported elsewhere. Although the patient charts were thoroughly scrutinized, the documentation concerning complications may not have been as thorough as it would have been in a prospective trial.

Whether twice-daily TRT to 45 Gy in 3 weeks is superior to a higher total dose than traditional modest doses delivered with a once-daily regimen is still unclear. Our results did not find a significant difference in outcome between the AHF group with a total dose of 45 Gy and the SF group with a total dose of ≥54 Gy. However, there was a significantly higher

rate of complete response in the AHF group compared with the SF ≥54 Gy group ($p = 0.042$), and the local control for the AHF group tended to be superior to that for the SF ≥54 Gy group. These results indicate that in the once-daily regimen much more than 54 Gy is necessary to achieve local control at the same level as 45 Gy with the AHF regimen. Despite the significantly higher rate of complete response and local control in the AHF group compared with the SF ≥ 54 Gy group, progression-free survival and OS were similar between the AHF group and the SF ≥54 Gy group ($p = 0.80$ and 0.64, respectively). We think that the reason was our small sample size. On the other hand, these data indicated that patients in the AHF group died from systemic disease as did those in the SF ≥54 Gy group, despite the better local control in the AHF group compared with the SF ≥54 Gy group. Therefore, another chemotherapy strategy such as integrating newer chemotherapy agents (16) or dose-intense regimens using either growth factors or stem-cell support (17) may be necessary for the platform of the curative approach to LS-SCLC.

Further intensification of TRT regimens such as a Phase III trial is under development by the Cancer and Leukemia Group B and the Radiation Therapy Oncology Group (18). This randomized trial is designed to compare three TRT approaches with four cycles of PE, with the three regimens being 45 Gy in 3 weeks (1.5 Gy twice-daily for 30 fractions), 70 Gy in 7 weeks (2.0 Gy once-daily for 35 fractions), and 61.2 Gy in 5 weeks (1.8 Gy accelerated fractionation via concomitant boost). We think that this trial will demonstrate the optimal method of radiation dose intensification. However, at this time, 45 Gy twice-daily TRT should be considered as the standard treatment for LS-SCLC because there are no Phase III once-daily trials that have shown better outcomes than the twice-daily regimen.

In conclusion, this analysis suggests that disease stage and treatment groups that are stratified according to 45 Gy with AHF, <54 Gy with SF, and ≥54 Gy with SF are independent factors associated with improved OS in patients with LS-SCLC and the potential importance of a high dose of radiation when using a once-daily regimen. However, there are problems that limit the interpretation of our single institutional retrospective review. There were some prognostic differences in the three groups compared, especially in the rates of patients receiving PCI and concurrent chemotherapy. A future prospective study of TRT regimens in the setting of chemoradiotherapy for LS-SCLC is needed to establish optimal radiation doses and fractionation, and such a study is under development by the Cancer and Leukemia Group B and Radiation Therapy Oncology Group.

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Original Article

Common Arm Comparative Outcomes Analysis of Phase 3 Trials of Cisplatin + Irinotecan Versus Cisplatin + Etoposide in Extensive Stage Small Cell Lung Cancer

Final Patient-Level Results From Japan Clinical Oncology Group 9511 and Southwest Oncology Group 0124

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BACKGROUND: Southwest Oncology Group 0124 was a large North American phase 3 trial that failed to confirm a survival benefit for cisplatin/irinotecan over cisplatin/etoposide in patients with extensive stage small cell lung cancer (SCLC). These results were contrary to Japan Clinical Oncology Group 9511, a phase 3 trial exclusively in Japanese patients. Because 0124 and 9511 used identical treatment regimens and similar eligibility criteria, patient-level data were pooled from both trials, and a common arm analysis was performed to explore potential reasons for the divergent results. **METHODS:** Patients with documented extensive stage SCLC and adequate end-organ function were randomized to intravenously receive either cisplatin 60 mg/m^2 Day 1 + irinotecan 60 mg/m^2 Days 1, 8, and 15 every 4 weeks or cisplatin 80 mg/m^2 Day 1 + etoposide 100 mg/m^2 Days 1-3 every 3 weeks. Demographic and outcome data were compared among 805 patients enrolled in 9511 and 0124 receiving identical treatment using a logistic model adjusted for age, sex, and performance status (PS). **RESULTS:** Of 671 patients in 0124, 651 eligible patients were included, as were all 154 patients from 9511. Significant differences in sex and PS distribution as well as toxicity were seen between trials. There were also significant differences in response rates (87% vs 60%, $P<.001$) and median overall survival (12.8 vs 9.8 months, $P<.001$) when the cisplatin/irinotecan arms from both trials were compared. **CONCLUSIONS:** Significant differences in patient demographics, toxicity, and efficacy were identified in the 9511 and 0124 populations. These results, relevant in the current era of clinical trials globalization, warrant: 1) consideration of differential patient characteristics and outcomes among populations receiving identical therapy; 2) utilization of the common arm model in prospective trials; and 3) inclusion of pharmacogenomic correlates in cancer trials where ethnic/racial differences in drug disposition are expected. ***Cancer* 2010;116:5710–5.** *© 2010 American Cancer Society.*

KEYWORDS: small cell lung cancer, chemotherapy, extensive stage, cisplatin, irinotecan.

Lung cancer represents the most common cause of malignant disease globally. Almost 1.4 million new cases of lung cancer are diagnosed annually worldwide, with nearly 1.2 million deaths.[1] Small cell lung cancer (SCLC) is a unique subtype of lung cancer that accounts for approximately 15% of all new cases.[2] Unfortunately, most SCLC patients die from the disease, due commonly to systemic metastasis (defined as "extensive stage").[3,4] Over the past 20 years, standard therapy for most patients with extensive stage SCLC has been either carboplatin or cisplatin in combination with etoposide.[5]

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This paradigm was challenged in 2002, when the results of the Japanese phase 3 study Japan Clinical Oncology Group 9511, comparing etoposide/cisplatin with cisplatin/irinotecan in 174 patients, demonstrated that tumor response, progression-free survival (PFS), and overall survival (OS) rates were significantly higher in the cisplatin/irinotecan group.[6] It must be noted that 9511 was stopped early at interim analysis by its data safety monitoring board when prospectively prespecified efficacy parameters were met.

Subsequently, the Southwest Oncology Group conducted a large phase 3 trial (0124) involving 671 patients that used virtually the same eligibility criteria and treatment regimens as the Japanese trial to confirm the results of 9511 in North American patients.[7] As reported previously, 0124 found no statistical differences in tumor response, PFS, and OS between the 2 arms, contrary to the results of 9511.

To explore potential reasons for the divergent results of these identically designed phase 3 trials, a pooled comparative outcomes analysis inclusive of patient-level data from both trials was conducted.

MATERIALS AND METHODS

Patients in both trials had cytologically or histologically confirmed SCLC and clinical evidence of extensive stage disease (defined by distant metastasis, contralateral hilar-node metastasis, or malignant pleural effusion). Eligibility criteria for both trials were similar and have been previously reported. Patients were randomly assigned to receive either etoposide/cisplatin or cisplatin/irinotecan. The cisplatin/irinotecan regimen consisted of 4 cycles of 60 mg of irinotecan per square meter of body surface area on Days 1, 8, and 15 and 60 mg of cisplatin per square meter on Day 1. Cycle length for this arm was 4 weeks. The etoposide/cisplatin regimen consisted of 4 cycles of 100 mg of etoposide per square meter on Days 1, 2, and 3 and 80 mg of cisplatin per square meter on Day 1. Cycle length for this arm was 3 weeks.

All patients underwent evaluations every cycle that included an assessment of symptoms, a physical examination, a complete blood count, and blood chemistry studies. Tumor response was assessed after every 2 cycles. In the 0124 trial, tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors, whereas in the 9511 trial, the World Health Organization criteria were used.[8] OS was calculated as the time between trial registration and death or date of last contact. PFS was calculated as the time between trial registration and death or progression, with censoring if alive without progression at last contact.

Study Design and Statistical Analysis

The primary objective of both studies was to compare the survival in patients with extensive SCLC treated with etoposide/cisplatin (standard arm) with that in comparable patients treated with the cisplatin/irinotecan (experimental) on an intent-to-treat basis. As 0124 and 9511 protocols used identical treatment regimens and similar eligibility criteria, patient-level data from both trials were pooled to explore potential reasons for the divergent results. Final results of both trials have previously been reported. Of 671 patients in 0124, 651 were eligible and included in this analysis, as were all 154 patients from 9511. Patient demographics, toxicity, and outcomes were compared among 805 patients receiving identical treatment using a common arm analysis. OS and PFS as compared between the Japan and US trials for both treatment arms in the combined sample were analyzed using Cox proportional hazards regression, adjusted for age, sex, and performance status. A logistic model adjusted for age, sex, and performance status was used to compare response to treatment between the 2 trials for the 2 treatment arms. The existence of possible interactions between trial (Japan Clinical Oncology Group vs Southwest Oncology Group) and treatment arm was evaluated for all endpoints, using data pooled over both arms. Significance was set at $P<.05$.

RESULTS

Patient Demographics

Median age in 9511 and 0124 was 61 and 62 years, respectively. There were proportionally more men in 9511 (86%, n = 132) compared with 0124 (57%, n = 370). There were more patients with Zubrod performance status 0 in 0124 (211, 32%) compared with 9511 (19, 12%). Demographics are summarized in Table 1.

Toxicity

Common arm comparisons of select attributable hematologic toxicities are summarized in Table 2. Regardless of treatment arm, patients in 9511 experienced significantly more hematologic toxicity consisting of neutropenia, leucopenia, and anemia than 0124. Other than a difference in infection rates in the cisplatin/etoposide arm, no

Table 1. Patient Demographics

Characteristic	JCOG-9511			SWOG-0124		
	Cisplatin + Etoposide	Cisplatin + Irinotecan	Total	Cisplatin + Etoposide	Cisplatin + Irinotecan	Total
Age, y						
Median	63	63	63	63	62	63
Minimum	41	30	30	35	22	22
Maximum	70	70	70	86	86	86
Male sex	90%	82%	86%	56%	58%	57%
PS						
0	12%	13%	12%	33%	32%	32%
1	75%	79%	77%	66%	67%	66%
2	13%	8%	10%	0%	0%	0%

JCOG-9511 indicates Japan Clinical Oncology Group 9511 trial; SWOG-0124, Southwest Oncology Group 0124 trial; PS, performance status.
Comparisons of the JCOG and SWOG populations with respect to differences in sex and PS were significant by chi-square test ($P < .0001$ for both comparisons).

Table 2. Common Arm Comparative Toxicity Analysis

≥Grade 3 Toxicity	Cisplatin + Etoposide			Cisplatin + Irinotecan		
	JCOG-9511	SWOG-0124	*P*	JCOG-9511	SWOG-0124	*P*
Infection	3 (4%)	52 (16%)	.01	4 (5%)	36 (11%)	.23
Neutropenia	71 (92%)	220 (68%)	<.001	49 (65%)	107 (34%)	<.001
Leukopenia	41 (53%)	109 (34%)	.006	20 (27%)	57 (18%)	.04
Anemia	25 (32%)	39 (12%)	<.001	21 (28%)	18 (6%)	<.001

JCOG-9511 indicates Japan Clinical Oncology Group 9511 trial; SWOG-0124, Southwest Oncology Group 0124 trial.

differences in nonhematologic toxicities between the 2 trials were seen.

Treatment Delivery and Dose Intensity

In the original 9511 and 0124 papers, there were no significant differences reported between the 2 arms in terms of treatment delivery. A preliminary common arm comparison of treatment delivery and dose intensity (DI) was performed in the current analysis. These results are summarized in Table 3. There were no clear differences in the proportion of patients completing all 4 cycles of therapy. However, a higher proportion of patients completed all 4 cycles of etoposide/cisplatin in 9511 versus 0124 (38% vs 29%). A more modest difference was seen in the cisplatin/irinotecan arm (29% vs 23%). When comparing the published DI data (9511 vs 0124), there was a numerical difference in the proportion of irinotecan (80.4% vs 66%) and cisplatin (95.3% vs 78%) DI.

Efficacy

Common arm comparisons of efficacy endpoints including response rate, PFS, OS are summarized in Table 4 and Figure 1. Ten patients (2 from Japan Clinical Oncology Group and 8 from Southwest Oncology Group) were excluded from the analysis of treatment response because they did not receive treatment. Significant differences in response rates were seen in the common arm comparisons when evaluated in multivariate logistic regression models, which enabled adjustment for age, sex, and performance status. Specifically, for the etoposide/cisplatin arm, response rates were 68% in 9511 and 57% in 0124 ($P = .02$). For the cisplatin/irinotecan arm, response rates were 87% for the 9511 and 60% in 0124 ($P<.001$). In an expanded logistic regression model that pooled the data for both treatment arms, there was a significant arm by trial interaction, indicating that the difference in response between the Japanese and US patients is significantly greater in the cisplatin/irinotecan arm patients. (P value for interaction = .03)

There were no differences in PFS and OS for the etoposide/cisplatin arm across trials. However, significant differences were seen for OS for the cisplatin/irinotecan arm. Specifically, median OS was 12.8 months for 9511 and 9.9 months for 0124 ($P<.001$, adjusted for age, sex, and performance status via Cox proportional hazards regression). Similarly, 1-year survival rates were 58% and 41%, respectively. The 1-month numerical difference in PFS in the cisplatin/irinotecan arm was not statistically significant. Kaplan-Meier survival curves of OS common arm comparisons in the cisplatin/irinotecan arm are shown in Figure 1. In a multivariate proportional hazards regression model including trial (Japan vs United States) treatment arm, age, sex, and performance status, the interaction between trial and treatment arm is significant, confirming that the survival difference by site (Japan vs United States) depends on treatment arm (P value for interaction term = .01). A performance status of 0 (vs 1 or 2) was also independently prognostic for increased survival in multivariate modeling ($P<.001$). Age and sex were not.

Table 3. Common Arm Analysis of Treatment Delivery and Dose Intensity

Treatment Arm	P + E	P + I
Completed all 4 cycles		
JCOG-9511	55/77 (71.4%)	53/77 (68.8%)
SWOG-0124	218/327 (66.6%)	213/324 (65.8%)
Completed 4 cycles without dose modification		
JCOG-9511	29/77 (38%)	22/77 (29%)
SWOG-0124	94/327 (29%)	76/324 (23%)
Reported average dose intensity[a]		
JCOG-9511	E: 83.9%; P: 84.6%	I: 80.4%; P: 95.3%
SWOG-0124	E: 78%; P: 81%	I: 66%; P: 78%

P indicates cisplatin; E, etoposide; I, irinotecan; JCOG-9511, Japan Clinical Oncology Group 9511 trial; SWOG-0124, Southwest Oncology Group 0124 trial.

[a] Percentage of total planned dose.

DISCUSSION

This common arm comparison of 9511 and 0124 using pooled patient-level data provides unique insights into potential reasons for the divergent results of these trials. In addition, this analysis highlights the issue of whether in the current era of clinical trials globalization, the results of randomized oncology studies conducted outside the Unites States are directly translatable to North American populations.[9] These issues obviously have regulatory implications.

This analysis is unparalleled because 0124 was designed a priori as a confirmatory trial for 9511, albeit accruing from a different ethnic patient pool. The design of the 0124 protocol was modeled directly on 9511, including similar eligibility criteria and identical treatment dose schedules. The observed differences in demographics, toxicity, and efficacy outcomes between these trials can be attributed to many factors, some of which were previously discussed in the 0124 paper. With the pooled multivariate analysis, we were able to investigate (and rule out) the possibility that the different outcomes between trials in the cisplatin/irinotecan arms were attributable to clear differences in patient populations with respect to sex and performance status. Our analysis of both survival and response showed that although performance status was prognostic for survival, the differences between trials in the cisplatin/irinotecan arm persisted even after adjusting for this imbalance.

Other potential factors included the smaller sample size and/or the early stopping of 9511, which may have overestimated the treatment effect.[10]

This common arm analysis demonstrates that the principal difference in OS was seen only in the cisplatin/irinotecan arms. The control etoposide/cisplatin arms in both 0124 and 9511 had identical OS results. In the context of irinotecan-based therapy, 1 hypothesis that has been posited is that there are inherent genetic differences related to genes involved in irinotecan drug disposition

Table 4. Common Arm Analysis of Efficacy

Efficacy Measure	Cisplatin + Etoposide			Cisplatin + Irinotecan		
	JCOG-9511	SWOG-0124	*P*	JCOG-9511	SWOG-0124	*P*
Response rate	68%	57%	.01	87%	60%	<.001
Median PFS, mo	4.7	5.2	.18	6.8	5.8	.6
Median OS, mo	9.4	9.1	.5	12.8	9.9	<.001
One-year survival rate	38%	34%		58%	41%	

JCOG-9511 indicates Japan Clinical Oncology Group 9511 trial; SWOG-0124, Southwest Oncology Group 0124 trial; PFS, progression-free survival; OS, overall survival.

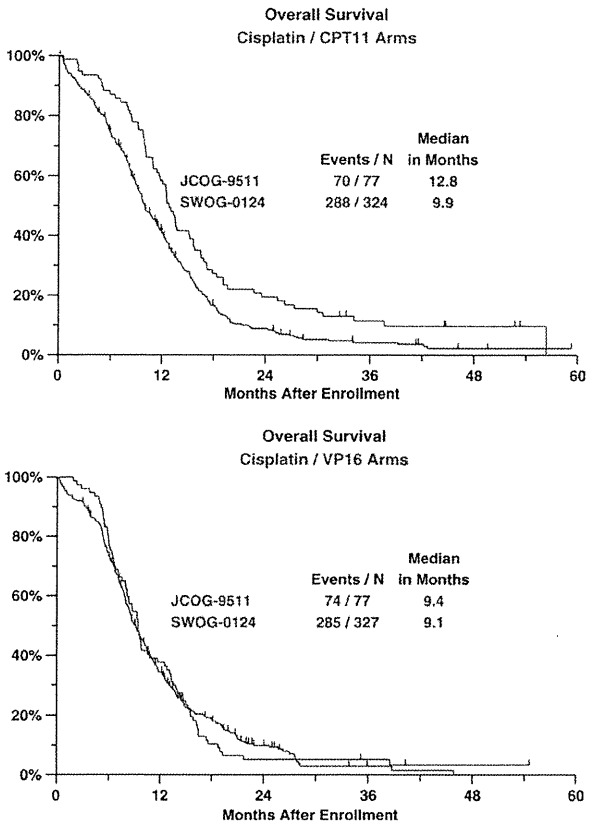

Figure 1. (*Top*) Overall survival analysis by trial is shown for the cisplatin and irinotecan (CPT-11) treatment arm. (*Bottom*) Overall survival analysis is shown for the cisplatin and etoposide (VP16) treatment arm. JCOG-9511 indicates Japan Clinical Oncology Group 9511 trial; SWOG-0124, Southwest Oncology Group 0124 trial.

between patient populations. Although a preliminary pharmacogenomic analysis of specimens from 0124 patients was performed to investigate some of these irinotecan-related genes, no specimens were available from the older 9511 trial for similar pharmacogenomic investigations. Hence, no direct comparison of relevant genotypes between trials is possible. However, insights on this issue can be derived from prior common arm joint collaborations between Southwest Oncology Group and Japanese investigators wherein patients with advanced nonsmall cell lung cancer were enrolled in Southwest Oncology Group and Japanese trials onto a common arm of paclitaxel and carboplatin.[11] In that experience, genes relevant to chemotherapy metabolism and transport were analyzed in both American and Japanese populations. Significant differences in toxicity, efficacy, and allelic distribution for genes involved in paclitaxel disposition or DNA repair were observed between Japanese and US patients, supporting the hypothesis that pharmacogenomics may in part be responsible for outcome divergence among patient populations. This may also partly explain the toxicity differences seen between the Japanese and North American populations, wherein Japanese patients apparently had increased hematologic toxicity (neutropenia, leucopenia, and anemia) in both treatment arms when compared with North Americans.

In addition, there appears to be some differences in the delivered DI in the cisplatin/irinotecan arms of both trials (as reported in the published papers). Specifically, more 9511 patients achieved a higher DI for both irinotecan and cisplatin as compared with 0124 patients. Enhanced DI for 9511 patients may potentially explain the differences in toxicity and efficacy between the trials. A more detailed and expansive analysis of dose delivery using individual patient data is required, but is beyond the scope of this article. Finally, it must be noted that other trials comparing similar chemotherapy regimens in SCLC have previously been published.[12,13] Some of us (P.N.L., R.N., and D.R.G.) have previously discussed these trials in the context of 0124 and 9511 in a recent editorial.[14] We refer readers to that editorial for additional details.

In conclusion, etoposide/cisplatin remains the reference treatment standard in North America. In Japan, cisplatin/irinotecan remains a standard treatment option. Significant differences in patient demographics, toxicity, and efficacy exist between Japanese and North American SCLC patients receiving identical treatment. These results, relevant in the current era of clinical trials globalization, warrant 1) consideration of differential patient characteristics and outcomes among patients receiving identical therapy, 2) utilization of the common arm model in prospective trials, and 3) inclusion of pharmacogenomic correlates in cancer trials where ethnic/racial differences in drug disposition are expected.

CONFLICT OF INTEREST DISCLOSURES

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Original Article

The Antitumor Activity of NK012, an SN-38–Incorporating Micelle, in Combination With Bevacizumab Against Lung Cancer Xenografts

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BACKGROUND: It has been demonstrated that NK012, a novel 7-ethyl-10-hydroxycamptothecin (SN-38)-incorporating polymeric micelle, exerts significantly more potent antitumor activity against various human tumor xenografts than irinotecan (CPT-11) (a water-soluble prodrug of SN-38). Combination therapy of anticancer agents with bevacizumab (Bv), an anti-vascualr endothelial growth factor humanized monoclonal antibody, has more potently inhibited tumor growth than either agent alone. In the current study, the authors examined the antitumor effect of NK012 in combination with Bv against human lung cancer. **METHODS:** Nude mice bearing lung adenocarcinoma (PC-14 or A549 xenografts) were administered NK012 at SN-38-equivalent doses of 5 mg/kg or 30 mg/kg in combination with or without Bv at 5 mg/kg. CPT-11 at a dose of 66.7 mg/kg was administered with or without Bv at a dose of 5 mg/kg in the same experimental model. To evaluate interaction with Bv, the pharmacokinetics and microvessel density in tumors that were treated on each regimen were analyzed. **RESULT:** In vitro, the growth-inhibitory effect of NK012 was 50-fold more potent than that of CPT-11 and was almost equivalent to that of SN-38. In vivo studies revealed that the combination of NK012 plus Bv had significantly greater antitumor activity against human lung cancer xenografts compared with NK012 alone (PC-14, $P = .0261$; A549, $P < .001$). The pharmacokinetic profile of NK012 revealed that coadministration of Bv did not interfere with the accumulation of NK012. **CONCLUSIONS:** In this study, significant antitumor activity was noted with NK012 in combination with Bv against lung cancer cells. The current results warrant the clinical evaluation of NK012 in lung cancer. *Cancer* 2010;116:4597–604. © *2010 American Cancer Society.*

KEYWORDS: NK012, drug-delivery system, lung cancer, lung adenocarcinoma, 7-ethyl-10-hydroxycamptothecin, SN-38, micelles, bevacizumab.

Lung cancer is the leading cause of cancer-related deaths worldwide, and nonsmall cell lung cancer (NSCLC), including adenocarcinoma, accounts for 75% to 80% of lung cancer cases.[1] Currently, cisplatin (CDDP)-based chemotherapy is the recommended first-line treatment for patients with advanced NSCLC.[2,3] Despite recent advances in the treatment of lung cancer, the prognosis for patients with NSCLC remains relatively poor, so attention currently is focused on finding novel agents, including new cytotoxic agents.

Irinotecan (CPT-11), a prodrug of 7-ethyl-10-hydroxycamptothecin (SN-38) (the active metabolite of irinotecan), which is a topoisomerase-I inhibitor, appears to be an effective agent against NSCLC when used as monotherapy or in combination with cisplatin.[4,5] Bevacizumab (Bv) is an antivascular endothelial growth factor (anti-VEGF) humanized monoclonal antibody. Bv reportedly is effective in various cancers, including colorectal cancer,[6] renal cell cancer,[7] and breast cancer.[8] Sandler et al reported that the addition of Bv to paclitaxel plus carboplatin in the treatment of NSCLC had a significant survival benefit.[9] In addition, Reck et al reported that the addition of Bv to gemcitabine plus cisplatin also had a significant clinical benefit in NSCLC.[10]

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NK012 is an SN-38-incorporating polymeric micelle and is categorized as a drug-delivery system. We previously demonstrated that NK012 accumulates more efficiently in various human tumor xenografts by using leaky tumor vessels and exerts significantly more potent antitumor activity against various human tumor xenografts compared with CPT-11.[11-17] Since the greater antitumor effect of NK012 may be attributed to its greater accumulation in the tumor using the leaky tumor vasculature, the addition of Bv to NK012 may hinder the efficient accumulation of NK012 in tumors because the permeability of tumor vasculature caused by VEGF is inhibited by Bv. In the current study, we evaluated the antitumor activity of NK012 administered in combination with Bv in experimental models.

MATERIALS AND METHODS

Drugs and Cells

NK012, an SN-38-incorporating polymeric micelle, and SN-38 were obtained from Nippon Kayaku Company, Ltd. (Tokyo, Japan), CPT-11 was purchased from Yakult Honsha Company, Ltd. (Tokyo, Japan), and Bv was purchased from Chugai Seiyaku Company, Ltd. (Tokyo, Japan). The human lung adenocarcinoma cell lines PC-14 and PC-9 kindly were provided by Dr. Y. Hayata (Tokyo Medical University, Tokyo, Japan). Human lung adenocarcinoma cell lines A549, NCI-H23, and NCI-H1975 were purchased from the American Type Culture Collection (Manassas, Va). These cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (Cell Culture Technologies, Gaggenau-Hoerden, Germany), penicillin (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (25 μg/mL; all from Sigma, St. Louis, Mo) in a humidified, 5% CO_2 atmosphere at 37°C.

In Vitro Growth-Inhibition Assay

PC-14, A549, NCI-H23, and NCI-H1975 cells were seeded in 96-well plates at a density of 10,000 cells per well in a final volume of 100 μL. Twenty-four hours after seeding, the medium was removed, and a graded concentration of SN38, NK012, and CPT-11 was added to the wells. Cultures were maintained in a CO_2 incubator for an additional 72 hours. Then, cell growth inhibition was measured by using a tetrazolium salt-based proliferation assay (WST assay; Wako Chemicals, Osaka, Japan). After removal of the medium, WST-8 solution (10 μL) and medium (90 μL) were added to the wells, and the plates were incubated at 37°C for 1 hour. The absorbance of the formazan product formed was detected at 450 nm in a 96-well spectrophotometric plate reader. Cell viability was measured and compared with that of the control cells. Each experiment was carried out in triplicate. Data were averaged and normalized against the nontreated controls to generate dose-response curves.

In Vivo Growth-Inhibition Assay

The animal experimental protocols were approved by the Committee for Ethics of Animal Experimentation, and the experiments were conducted in accordance with the Guidelines for Animal Experiments from the National Cancer Center.

Female BALB/c mice, 6 weeks old, were obtained from SLC Japan (Shizuoka, Japan). These mice were maintained in a laminar air-flow cabinet and were inoculated subcutaneously with 5×10^6 PC-14 cells or with 5×10^6 A549 cells in the flank region. When tumor volumes (TVs) reached approximately 100 mm^3, the mice were divided randomly into test groups of 5 mice per group (Day 0). The length (a) and width (b) of the tumor mass were measured twice weekly, and the TV was calculated as follows: $TV = (a \times b^2)/2$. The relative TV (RTV) at Day n was calculated as follows: $RTV = TV_n/TV_0$, where TV_n is the TV at Day n, and TV_0 is the TV at Day 0.

Experiment 1: Evaluation of the Antitumor Effect of NK012 and CPT-11

By comparing the data between NK012 and CPT-11, we evaluated their effects as single agents against PC-14 or A549 xenografts. The maximum tolerated dose (MTD) of NK012 (30 mg/kg)[11] or the MTD of CPT-11 (66.7 mg/kg)[18] was administered by intravenous injection into the tail vein on Days 0, 4, and 8.

Experiment 2: Evaluation of the Antitumor Effect of NK012 Alone and NK012 With Bv

By comparing the data between NK012 alone and NK012 plus Bv, we evaluated the combined effect of NK012 plus Bv against PC-14 xenografts. NK012 at a dose of 5 mg/kg was administered intravenously into the tail vein on Days 0, 4, and 8 with or without Bv. In addition, we evaluated the combined effects against A549 xenografts (NK012 [30 mg/kg intravenously] with Bv). When Bv was coadministered with each anticancer agent, Bv was administered intraperitoneally at a dose of 5 mg/kg on Days 0, 4, and 8.

Table 1. Fifty Percent Inhibitory Concentration Values of 7-Ethyl-10-Hydroxycamptothecin (SN-38), the SN-38–Incorporating Polymeric Micelle NK012, and Irinotecan in Various Human Lung Adenocarcinoma Cell Lines

	IC_{50} (μmol/L)[a]		
Cell Line	SN-38	NK012	CPT-11
PC-14	0.050±0.003	0.053±0.002	9.688±1.187
A549	0.506±0.029	0.883±0.840	48.153±4.641
PC-9	0.028±0.011	0.059±0.005	21.782±2.145
NCI-H23	0.025±0.005	0.060±0.002	5.223±1.586
NCI-H1975	0.047±0.084	0.082±0.002	6.330±0.432

IC_{50} indicates 50% inhibitory concentration; CPT-11, irinotecan.
[a] All values shown are the mean values±standard deviation.

Distribution Studies of Free SN-38, CPT-11, and NK012 in Tumors by High-Performance Liquid Chromatography

When the PC-14 TV reached approximately 100 mm^3, NK012 (30 mg/kg) or CPT-11 (66.7 mg/kg) was administered intravenously with or without Bv (5 mg/kg intraperitoneally). Twenty-four hours after the injection of NK012 or CPT-11, each tumor was excised under anesthesia. In other experiments, NK012 (5 mg/kg) was administered intravenously with or without Bv (5 mg/kg intraperitoneally), and each tumor was excised under anesthesia at 12 hours, 24 hours, 3 days, 7 days, 10 days, and 14 days after the injection of NK012. The tumor tissues were rinsed with physiologic saline; mixed with 0.1 M glycine-HCl buffer, pH 3.0, in methanol at 5% (weight/weight); and homogenized. To detect free SN-38 and CPT-11, the tumor samples (100 μL) were mixed with 20 μL 1 mM phosphoric acid in methanol (1:1) and 40 μL ultrapure water, and camptothecin was used as the internal standard (10 ng/mL for free SN-38, 12 ng/mL for CPT-11). The samples were vortexed vigorously for 10 seconds and filtered through an Ultrafree-MC centrifugal filter device (Millipore, Bedford, Mass). Reverse-phase high-performance liquid chromatography (HPLC) was conducted at 35°C on a Mightysil RP-18 GP column (150 × 4.6 mm; Kanto Chemical, Tokyo, Japan). Then, the samples were injected into an Alliance Water 2795 HPLC system (Waters, Milford, Mass) equipped with a Waters 2475 multi-λ fluorescence detector. Fluorescence originating from SN-38 was detected at 540 nm with an excitation wavelength of 365 nm.

For the detection of polymer-bound SN-38, SN-38 was released from the polymer as described previously.[11] In brief, 100-μL tissue samples were diluted with 20 μL methanol (50% [weight/weight]) and 20 μL NaOH (0.7 M). The samples were incubated for 15 minutes at room temperature. After incubation, 20 μL HCl (0.7 M) and 60 μL of internal standard solution were added to the samples and the hydrolysate was filtered. The filtrate was applied to the HPLC system. Polymer-bound SN-38 was determined by subtraction of nonpolymer-bound SN-38 from the total SN-38 in the hydrolysate.

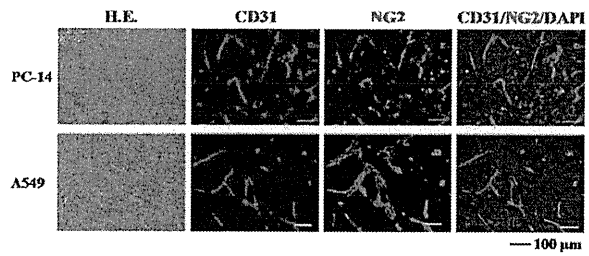

Figure 1. These photomicrographs are from the histologic examination of excised tumors from PC-14 and A549 xenografts that were stained with hematoxylin and eosin (H.E.) or analyzed by immunohistochemistry for cluster of differentiation molecule 31 (CD31) (also called platelet endothelial cell adhesion molecule 1) (red) for the chondroitin sulfate proteoglycan NG2 (green) and for 4′,6-diamidino-2-phenylindole (DAPI) (blue). Scale bars = 100 μm.

Immunofluorescence Study

At Day 14 after the injection of saline, Bv, NK012, or NK012 plus Bv, the PC-14 tumors were excised under anesthesia. Frozen sections of these tumors (10 μm) were fixed with 4% paraformaldehyde and washed with phosphate-buffered saline (PBS). After blocking with 5% skim milk (BD, Franklin Lakes, NJ) in PBS, the slides were incubated with anti-cluster of differentiation molecule 31 (anti-CD31) monoclonal antibody (1:100 dilution; PharMingen, San Diego, Calif) and anti-NG2 monoclonal antibody (1:1000 dilution; Chemicon, Temecula, Calif) for 1 hour. After washing with PBS, the slides were stained with Alexa 555-, Alexa 647-conjugated secondary antibodies, antirat (red) and antirabbit immunoglobulin G (green; 1:100 dilution; Invitrogen, Carlsbad, Calif), and 4′,6-diamidino-2-phenylindole (DAPI) for nuclear staining. Five areas were chosen randomly from each mouse (n = 2), and the fluorescence intensity was measured and analyzed with BZ-II ANALYZER software (Keyence, Osaka, Japan) for histologic quantification under fluorescence microscopy at 20-fold magnification.

Statistical Analysis

One-way fractional analyses of variance and multiple comparison tests (Scheffe and Bonferroni/Dunn)

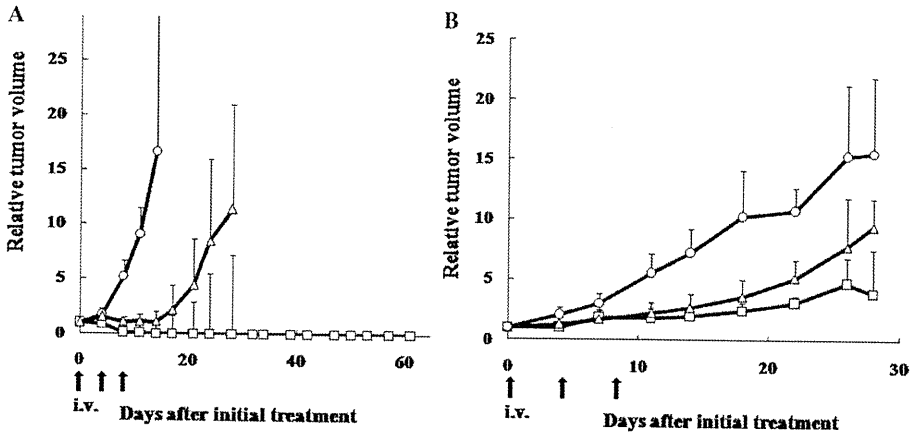

Figure 2. These graphs illustrate (A) the antitumor effects of the novel 7-ethyl-10-hydroxycamptothecin (SN-38)-incorporating polymeric micelle NK012 alone (5 mg/kg daily), bevacizumab (Bv) alone (5 mg/kg daily), and combined NK012 (5 mg/kg daily) plus Bv (5 mg/kg daily) against PC-14 tumor-bearing mice and (B) the effects of NK012 alone (30 mg/kg daily) and combined NK012 (30 mg/kg daily) plus Bv (5 mg/kg daily) against A549 tumor-bearing mice. Squares indicate NK012; open triangles, Bv; solid triangles, NK012 plus Bv; saline, circles. NK012 was administered intravenously (i.v.), and Bv was administered intraperitoneally (i.p.) on Days 0, 4, and 8. Each group included 5 mice. Points indicate mean values; bars, standard deviation.

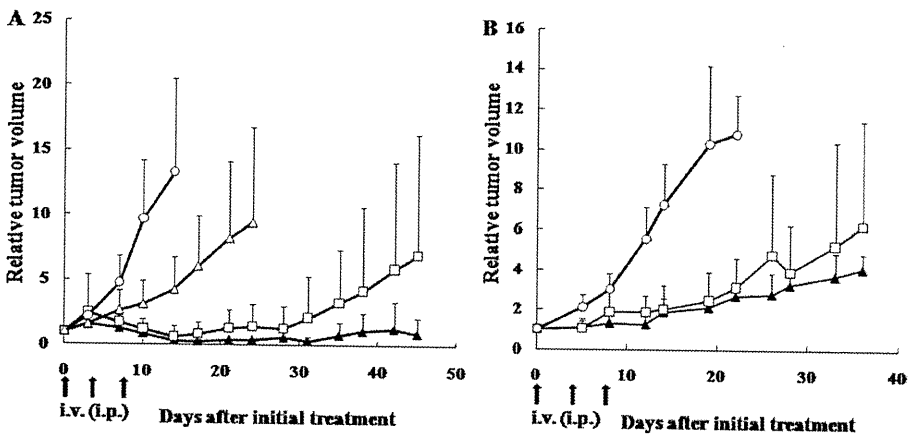

Figure 3. These graphs illustrate the antitumor effects of the novel 7-ethyl-10-hydroxycamptothecin (SN-38)-incorporating polymeric micelle NK012 alone or irinotecan (CPT-11) alone against (A) PC-14 (B) and A549 tumor-bearing mice. The treatment was initiated 11 days after PC-14 inoculation and 13 days after A549 inoculation. NK012 (30 mg/kg daily) (squares), CPT-11 (66.7 mg/kg daily) (triangles), or saline (circles) was administered intravenously (i.v.) on Days 0, 4, and 8. Each group included 5 mice. Points indicate mean values; bars, standard deviation.

conducted with StatView software (version 5.0; SAS Institute, Inc., Cary, NC) were used to compare the different treatment groups of xenografts. Data were expressed as the mean ± standard deviation. Data were analyzed with the Student t test when the groups had equal variance (F test) or with the Welch test when they had unequal variance (F test). P values $<.05$ were regarded as statistically significant. All statistical tests were 2-sided.

RESULTS

Sensitivity of Lung Cancer Cells to SN-38, NK012, and CPT-11

The 50% inhibitory concentration values of NK012 for the cell lines ranged from 0.059 μmol/L to 0.88 μmol/L. The growth-inhibitory effect of NK012 was 50-fold more potent than that of CPT-11 and was almost equivalent to that of SN-38 (Table 1).

Histologic Examination of PC-14 and A549 Xenografts

Hematoxylin and eosin staining of the tumors from PC-14 xenografts revealed that the tumors were poor in stroma, whereas the tumors from A549 xenografts appeared to be stroma-rich. Immunostaining of both tumor tissues with CD31 and NG2 indicated that vasculatures covered with pericytes were more abundant in the A549 xenografts than in the PC-14 xenografts (Fig. 1).

Antitumor Activity of NK012 and CPT-11 on Subcutaneous PC-14 and A549 Xenografts

Experiment 1: Comparison of the antitumor effect of NK012 and CPT-11

In PC-14 xenografts that were treated with NK012 at 30 mg/kg, the tumors started to shrink on Day 4, the tumors disappeared completely by Day 14, and there was no relapse during observation until 60 days after treatment (Fig. 2A). Comparison of the relative TV revealed that the antitumor activity of NK012 was significantly greater than that of CPT-11 ($P = .0267$). Conversely, the TV did not shrink in A549 tumor-bearing mice that were treated with NK012 (Fig. 2B). Although the antitumor activity of NK012 did not differ significantly from that of CPT-11 in A549 xenografts ($P = .0869$), a trend toward a superior antitumor effect against A549 tumors was observed in the NK012 treatment group.

Experiment 2: Comparison of the antitumor effect of NK012 alone and NK012 plus Bv

In PC-14 xenografts, the combination of 5 mg/kg NK012 with 5 mg/kg Bv resulted in a significantly greater inhibition of tumor growth compared with NK012 5 mg/kg alone ($P = .0261$) (Fig. 3A). Also in A549 xenografts, the combination of 30 mg/kg NK012 with 5 mg/kg Bv resulted in significant inhibition of tumor growth compared with NK012 30 mg/kg alone ($P < .0001$) (Fig. 3B).

Distribution Studies of Free SN-38, CPT-11, and NK012 in Tumors Using HPLC

In tumors that were obtained 24 hours after the injection of CPT-11 or NK012, the level of free SN-38 released from NK012 was significantly greater than the level of SN-38 converted from CPT-11 ($P = .003$) (Fig. 4A). Conversely, the level of free SN-38 released from treatment with NK012 plus Bv did not differ significantly from the level released from treatment with NK012 alone. The intratumor concentrations of polymer-bound SN-38 did not differ between NK012 plus Bv and NK012 alone

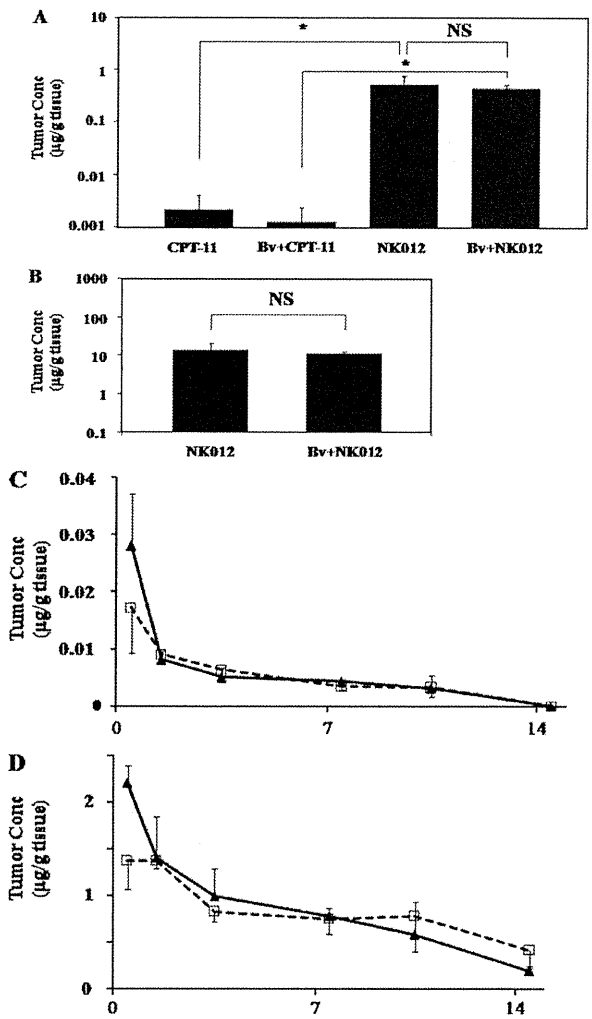

Figure 4. These charts illustrate pharmacokinetics in PC-14 tumor-bearing mice. (A) Polymer-unbound 7-ethyl-10-hydroxycamptothecin (free SN-38) in tumor was quantified by high-performance liquid chromatography (HPLC) 24 hours after the injection of irinotecan (CPT-11) (66.7 mg/kg), combined CPT-11 (66.7 mg/kg) plus bevacizumab (Bv) (5 mg/kg), the SN-38-incorporating micelle NK012 (30 mg/kg), or combined NK012 (30 mg/kg) plus Bv (5 mg/kg). (B) Polymer-bound SN-38 in tumor also was quantified by HPLC 24 hours after the injection of NK012 (30 mg/kg) or combined NK012 (30 mg/kg) plus Bv (5 mg/kg). Free SN-38 (C) and polymer-bound SN-38 (D) in tumor was quantified by HPLC at 12 hours, 24 hours, 3 days, 7 days, 10 days, and 14 days after the injection of NK012 (5 mg/kg daily) (squares) or combined NK012 (5 mg/kg daily) plus Bv (5 mg/kg daily) (triangles). Each group included 3 mice. Points indicate mean values; bars, standard deviation; asterisk, $P < .01$.

(Fig. 4B). At only 12 hours after injection, intratumor concentrations of polymer-bound SN-38 were significantly greater with NK012 alone than with NK012 plus Bv ($P = .015$). At this time point, however, there was no

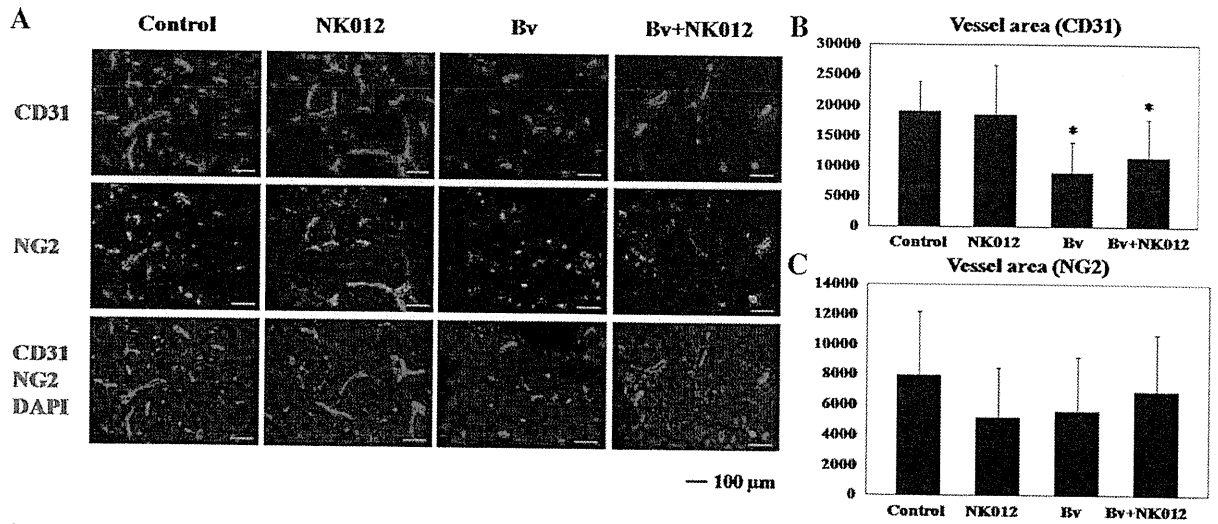

Figure 5. These photomicrographs reveal immunofluorescence staining of cluster of differentiation molecule 31 (CD31)-positive endothelial cells and the chondroitin sulfate proteoglycan NG2-positive pericytes. (A) Fourteen days after the injection of saline, bevacizumab (Bv), the novel 7-ethyl-10-hydroxycamptothecin (SN-38)-incorporating polymeric micelle NK012, or combined NK012 plus Bv, all tumors were excised from the mice. Frozen sections from these tumors (10 μm) were stained with anti-CD31 monoclonal antibody (red), anti-NG2 antibody (green), and 4′,6-diamidino-2-phenylindole (DAPI) (blue). Scale Bars = 100 μm. Histologic quantification under fluorescence microscopy at 20-fold magnification was performed (B) for CD31-positive areas and (C) for NG2-positive areas. Bars indicate standard deviation; asterisks, $P < .01$ compared with control.

difference in the intratumor concentration of free SN-38 between treatment with NK012 alone and treatment with NK012 plus Bv. Thereafter, the intratumor concentrations of both polymer-bound SN-38 and free SN-38 did not differ between treatment with NK012 alone and treatment with NK012 plus Bv (Fig. 4C,D).

Immunofluorescence Staining to Clarify the Antivascular Effect of Bv

Treatment with Bv in combination with or without NK012 significantly reduced the area of CD31-positive proliferating endothelial cells in the tumors compared with controls on Day 14 ($P < .01$) (Fig. 5A,B). Conversely, the area of NG2-positive pericytes was not significantly different between the groups (Fig. 5A,C).

DISCUSSION

The size of NK012 is approximately 20 nm in diameter, and NK012 is sufficiently large to avoid renal secretion. NK012 can evade nonspecific capture by the reticuloendothelial system in various organs, because the outer shell of NK012 is covered with polyethyleneglycol. Therefore, NK012 is expected to achieve a long plasma half-life, which permits large amounts of SN-38 to reach the tumor site through the enhanced permeability and retention effect.[19]

To date, we have reported that NK012 has significantly greater antitumor activity against various human tumor xenografts including, small cell lung cancer,[11,17] colorectal cancer,[14] renal cancer,[13] pancreatic cancer,[12] gastric cancer,[15] and malignant glioma,[16] compared with CPT-11. In the current study, NK012 also appeared to eradicate PC-14 xenografts completely, but not A549 xenografts. This difference may be because of differences in the sensitivity of each cell line to NK012 and in pericyte coverage on vasculatures. Less pericyte coverage reportedly to results in more leakiness of plasma substances; therefore, the degree of NK012 accumulation may be associated inversely with the degree of pericyte coverage.[20,21]

Angiogenesis, which permits tumors to grow and metastasize, plays a pivotal role in several pathologic disorders.[22] VEGF is 1 of the most potent positive regulators of angiogenesis[23] and is recognized as an attractive target in cancer therapy. Unlike normal vasculature, the microvessels of tumors are hyperpermeable to several substances, including macromolecules and nanoparticles. The permeability, interstitial fluid pressure, and numbers of microvessels are increased by VEGF-induced angiogenesis.[24,25] Anti-VEGF antibody administered in combination with chemotherapeutic agents, including doxorubicin,[26] topotecan,[27,28] paclitaxel,[29] and docetaxel,[30] resulted in more potent inhibition of tumor growth than either agent alone. However, it has not been clarified whether anti-VEGF antibody

administered in combination with drug-incorporating polymeric micelles has an additive effect. In the current study, we demonstrated that the combination of NK012 plus Bv had significantly greater antitumor activity against human lung adenocarcinoma cells (PC-14 and A549) compared with NK012 alone. The concentrations of either polymer-bound SN-38 or free SN-38 after the administration of NK012 plus Bv did not clearly differ from the concentrations after NK012 alone. In addition, after treatment with Bv, the area of vascular endothelial cells stained with CD31 was decreased significantly compared with controls. These results suggest that VEGF inhibition may not disturb NK012 accumulation in the tumors and that the direct effect of NK012 plus Bv produced an additional antitumor effect.

In the current study, we demonstrated that NK012 has significantly greater antitumor activity against human lung adenocarcinoma cells (PC-14 and A549) compared with CPT-11. Therefore, we believe that NK012 is a promising oncologic treatment for patients with NSCLC. In 2 individual phase 1 trials that were conducted in Japan and the United States, the toxic profile of NK012 was favorable, and the dose-limiting toxicity was neutropenia.[31,32] Diarrhea was mild; that is, even the worst diarrhea was grade 2 in the phase 1 setting.

In conclusion, the current study demonstrated the superior antitumor activity of NK012 against NSCLC cells compared with CPT-11. In patients with NSCLC, clinical trials of the combination of NK012 plus Bv may be warranted.

CONFLICT OF INTEREST DISCLOSURES

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Multinational Trials Reveal Striking Regional Differences

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Many multinational clinical trials have recently been conducted to enable the rapid accrual of patients and the use of registration data in multiple countries. Such trials often include multiple ethnicities, and regional differences sometimes affect the treatment results. Many factors can cause regional differences, including medical care, medical insurance, and clinicopathologic features, as well as pharmacogenomics. When discrepant data are observed between Asian and Caucasian populations, new clinical trials should be scheduled in specific populations. This commentary discusses three examples of such trials.

EGFR Tyrosine Kinase Inhibitors

During phase II trials of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib (Iressa), such as the Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL) 1 and 2 studies,[1,2] the response rate appeared to be higher among Japanese patients than among Caucasians.

> The frequency of *EGFR* mutation is 35% to 40% among East Asian populations vs less than 10% among Caucasian populations. Therefore, the strategy for treating NSCLC differs considerably between East Asians and Caucasians.

However, a subsequent placebo-controlled randomized phase III trial named the Iressa Survival Evaluation in Lung Cancer-or ISEL study-did not stratify patient populations according to Asian vs non-Asian. With an enrollment of 1,692 advanced or metastatic non-small cell lung cancer (NSCLC) patients who had undergone prior chemotherapy, the ISEL study was designed to investigate the effect on survival of gefitinib as a second- or third-line therapy. Overall survival was not statistically significantly different among the entire population (HR = 0.89, $P = .087$) or among patients with adenocarcinoma (HR = 0.84, $P = .089$).

Among the 342 patients of Asian origin, however, the median survival was significantly different from that of non-Asian patients (HR = 0.66, $P = .01$). On the other hand, the survival curves of EGFR tyrosine kinase inhibitor and placebo groups were completely superimposable in Caucasians.[3] Although the analysis was preplanned, these data represent a post-study stratification. Thus, the data were regarded as preliminary in many countries with regard to the approval of regulatory affairs.

The reason the response rate to gefitinib is higher among Asian populations has been explained pharmacogenomically as the presence of a higher EGFR mutation rate in this population.

The Iressa Pan-Asia Study (IPASS), comparing gefitinib vs standard chemotherapy, including carboplatin and paclitaxel, was conducted only in Asian patients with adenocarcinoma who had not received prior chemotherapy. These patients were either never-smokers or light smokers. The progression-free survival (PFS) period was significantly longer in the gefitinib arm, although the PFS crossed over at 6 months. (PFS before 6 months was better in the standard chemotherapy arm.) A clear advantage was observed in the gefitinib group among EGFR mutation-positive patients.[4] In two other Japanese randomized controlled trials comparing gefitinib vs standard chemotherapy in EGFR mutation-positive patients, gefitinib produced a significantly better PFS than chemotherapy.[5,6]

Based on these data, new algorithms for the treatment of NSCLC have been established. The frequency of EGFR mutation is 35% to 40% among East Asian populations vs less than 10% among Caucasian populations. Therefore, the strategy for treating NSCLC differs considerably between East Asians and