**Table 3.** Derived pharmacokinetic parameters of lapatinib (including 95% confidence intervals)

| Dose (mg/day)[a] | Geometric mean $C_{max}$ (ng/ml) | | Mean $CSS_{max}$ (ng/ml) | Median $t_{max}$ (h) | | Geometric mean AUC (h ng/ml)[b] | | Median $t_{1/2}$ (h) | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 21 | Day 21 | Day 1 | Day 21 | Day 1 | Day 21 | Day 1 | Day 21 |
| 900 | 1011 (694–1472) | 1895 (1319–2721) | 857 (386–1234) | 4.0 (2.0–6.0) | 4.0 (3.0–6.0) | 17 577 (11 812–26 154) | 29 272 (21 618–39 638) | 12.9 (10.1–18.3) | 23.1 (9.8–38.2) |
| 1200 | 1027 (474–2227) | 1715 (965–3048) | 820 (226–1308) | 3.5 (2.1–6.0) | 3.6 (3.0–7.9) | 15 441 (7410–32 176) | 25 680 (13 728–48 038) | 11.5 (10.1–19.5) | 16.9 (15.1–34.3) |
| 1600 | 1538 (1042–2268) | 3111 (1937–4996) | 1899 (818–4357) | 4.0 (2.0–8.0) | 5.1 (0.9–8.0) | 26 361 (17 519–39 665) | 51 099 (28 674–91 062) | 13.9 (9.6–18.0) | 26.2 (12.9–48.3) |
| 1800 | 1227 (465–3242) | 2333 (927–5870) | 1528 (586–3393) | 3.9 (3.0–8.0) | 3.9 (3.0–7.3) | 32 841 (18 884–57 114) | 39 451 (14 909–104 391) | 15.7 (11.0–133.1) | 21.8 (18.5–104.5) |

AUC, area under the plasma drug concentration–time curve; $C_{max}$, maximum serum concentration; $CSS_{max}$, mean steady state maximum serum concentration; $t_{max}$, time to reach $C_{max}$; $t_{1/2}$, terminal half-life.
[a]Six patients at 900, 1200 and 1600 mg/day and five at 1800 mg/day.
[b]Day 1, AUC from 0 to infinity; Day 21, AUC from 0 to 24 h.

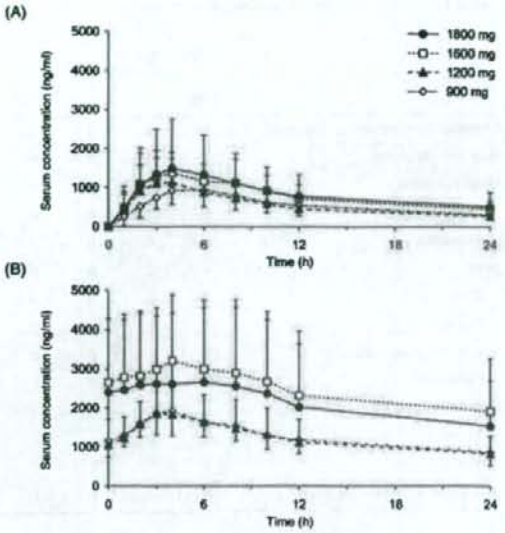


**Figure 1.** Serum concentrations of lapatinib at each dose level as detected on (A) Day 1 and (B) Day 21.

Steady-state serum concentrations of lapatinib generally increased with dose, 820 ± 448 ng/ml at 1200 mg dose level and 1899 ± 1356 ng/ml at 1600 mg dose level (Table 3). Both concentrations exceeded the half maximal inhibitory concentration values for *in vitro* tumor growth (14). The median $t_{1/2}$ after repeat dose was 16.9 h (range, 15.1–34.3) at 1200 mg dose level and 26.2 h (range, 12.9–48.3) at 1600 mg dose level.

The fraction of urinary excretion of lapatinib was <0.1% of the dose, suggesting that none or negligible amount of drug is excreted in urine.

Comparison of on-treatment $C_{max}$ and $AUC_{0-24}$ values obtained in Japanese and western patients are shown in Fig. 2 (43,44).

## Efficacy

Among 24 patients, the best overall response was assessed as partial response (PR) in two patients (8.3%), stable disease (SD) in 12 patients (50.0%), progressive disease in eight patients (33.3%) and indeterminate in two patients (8.3%).

Of the two patients with PR, the first was a 73-year-old man with NSCLC (squamous cell carcinoma) with prior docetaxel and gemcitabine treatment, who received lapatinib 900 mg/day. PR was assessed by CT scan with 41% shrinkage on Day 49. Time to progression was 191 days. The second patient was a 55-year-old woman with trastuzumab-resistant breast cancer (invasive ductal carcinoma; hormone receptor-negative, ErbB-2 3+). Disease progressed after doxorubicin and cyclophosphamide/docetaxel therapy, was

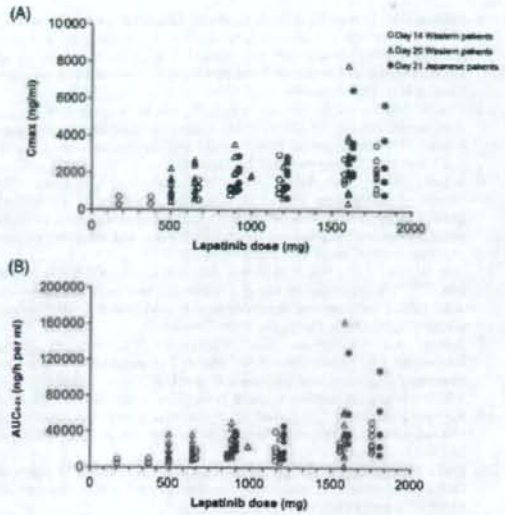


**Figure 2.** Relation between dose of lapatinib and exposure: comparison of (A) maximum serum concentration ($C_{max}$) and (B) area under the plasma drug concentration–time curve from 0 to 24 h ($AUC_{0-24}$) after dosing on Day 21 (our study, Japanese patients) and Days 14 and 20 (US studies, western patients).

stable with doxifluridine, and progressed with trastuzumab. Following treatment with lapatinib 1600 mg/day, the tumor shrank by 41% on Day 21. Time to progression was 133 days.

Among the patients with SD, three (two with NSCLC and one with colorectal cancer) were stabilized for >6 months and three (two with NSCLC and one with cervical cancer) were stabilized for 3–6 months and therefore were considered as having a durable response.

## DISCUSSION

The dual ErbB-1/-2 inhibitor lapatinib taken orally once daily for ≥21 days was well tolerated at doses of 900–1600 mg in Japanese solid tumor patients. Adverse events were mostly mild in nature, and only four grade ≥3 drug-related adverse events were noted, in three patients (three events of Grade 3 diarrhea and one Grade 3 γ-GTP increase). No NCI-CTC Grade 4 adverse events were observed. Grade 1–2 diarrhea occurred in some patients other than those who experienced Grade 3 diarrhea; for these, supportive therapy was given and fully recovered in all cases. Grade 1/2 drug-related nausea and vomiting were experienced only by patients at higher dose levels of lapatinib, with Grade 2 symptoms only seen at 1800 mg dose level.

The types and incidences of drug-related adverse events in Japanese patients were similar to those reported from studies conducted in healthy volunteers (18) and two overseas Phase I studies, the latter including a parallel study in western patients that used similar dose administration and dose-escalation schedules (43,44). In that study as well as in ours, diarrhea and rash were the most frequently noted drug-related adverse events. Adverse events were generally mild (Grade 1–2), transient and reversible on dose delay or interruption. Headache, which was common in western patients (18), was reported only by one patient at 1600 mg dose level. 1800 mg/day was considered as MTD, at which Grade 3 diarrhea and γ-GTP increase were observed.

Skin-related adverse events of lapatinib were similar to those reported for other agents that target ErbB-1; rash is also a common adverse event associated with the ErbB-1 tyrosine kinase inhibitors gefitinib (46–49) and erlotinib (7,50), as well as the anti-ErbB-1 antibody cetuximab (51). Patients who received these medications also experienced diarrhea (7,46–50). These adverse events occurred at a similar frequency in our study as in two overseas Phase I studies (43,44).

Apart from one event of γ-GTP increase, no Grade ≥3 abnormal laboratory test suggestive of liver dysfunction was noted. Therefore, drug-related liver abnormality was generally less frequently seen with lapatinib compared with gefitinib (48,49).

Hematologic toxicity was uncommon and limited to cases of anemia. This finding is similar to those of the Phase I biomarker study (44) and studies of gefitinib (48,49,52).

None of the patients developed interstitial lung disease, which is an adverse event reportedly associated with gefitinib (53,54) and occurs in 5.8% of Japanese patients (55). However, because of the limited number of patients in our study, further studies are required to assess safety of lapatinib in this regard.

Cardiotoxicity is a known adverse event associated with trastuzumab therapy and might be related to ErbB-2 inhibition (2,56); however, we found no evidence of drug-related cardiac dysfunction in our study.

PK parameters such as $C_{max}$ and $AUC_{0-24}$ in this study were analyzed and their means and 95% confidence intervals compared with those obtained at similar doses (900–1800 mg) in two overseas Phase I studies (43,44). As can be seen in Fig. 2, the values were comparable among the three studies. However, large inter-patient variations were noted, especially in Japanese patients, and these might have contributed to higher mean values. On the other hand, no clear pharmacokinetic differences were apparent between Japanese and non-Japanese subjects, suggesting that values obtained overseas can be extrapolated to the Japanese population.

The dose recommended for further clinical studies outside Japan, 1500 mg/day, can be used for Phase II studies in Japan. We base this recommendation on the similar PK profiles of lapatinib in Japanese and western patients, evidence of antitumor activity at doses of ≥900 mg/day, and an MTD of 1800 mg/day.

To conclude, lapatinib, taken continuously as once-daily oral therapy at 900–1600 mg, was well tolerated in Japanese

patients with solid tumors. The safety and PK profiles shown in this study are similar to those in Phase I studies conducted in western patients. Phase II studies to determine the efficacy of lapatinib against a range of tumors are now in progress.

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## Conflict of interest statement

The author, Hironobu Minami, receives honoraria from GlaxoSmithKline. The authors, Masayuki Kanezaki, Akihira Mukaiyama, and Yoshiyuki Minamide are employed by GlaxoSmithKline.

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# Phase I study of TZT-1027, a novel synthetic dolastatin 10 derivative and inhibitor of tubulin polymerization, given weekly to advanced solid tumor patients for 3 weeks

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**TZT-1027 is a novel synthetic dolastatin 10 derivative that inhibits tubulin polymerization. A phase I study was conducted to determine the maximum tolerated dose (MTD) of TZT-1027, and to assess its pharmacokinetic profile in Japanese patients with advanced solid tumors following administration of the drug weekly for 3 weeks. Eligible patients had advanced solid tumors that failed to respond to standard therapy or for which no standard therapy was available, and met the following criteria: performance status ≤2 and acceptable organ function. The MTD was defined as the highest dose at which more than two-thirds of the patients experienced grade 4 hematological toxicity or grade 3/4 non-hematological toxicity during weekly TZT-1027 administration for 3 weeks. Forty patients were enrolled in the present study. Twelve doses between 0.3 and 2.1 mg/m² were evaluated. Grade 4 neutropenia was the principal dose-limiting toxicity (DLT). At a dose of 2.1 mg/m², two patients developed DLT: one patient developed grade 4 neutropenia, grade 3 myalgia, and grade 4 constipation, and the other one developed grade 4 neutropenia and grade 3 constipation. At a dose level of 1.8 mg/m², toxicity was acceptable and no DLT was observed. The area under the curve and maximum concentration of TZT-1027 tended to increase linearly with the dose. The DLT observed were neutropenia, myalgia, and constipation, and the MTD was 2.1 mg/m². The recommended dose for a phase II study was determined to be 1.8 mg/m² for the drug administered weekly for 3 weeks. (*Cancer Sci* 2009; 100: 316–321)**

TZT-1027 (*N*²-[*N*,*N*-dimethyl-L-valyl]-*N*-([1S,2R]-2-methoxy-4-([2S]-2-([1R, 2R]-1-methoxy-2-methyl-3-oxo-3-([2-phenylethyl]-amino)propyl)-1-pyrrolidinyl)-1-([1S]-1-methylpropyl)-4-oxobutyl)-*N*-methyl-L-valinamide) is a synthetic analog of dolastatin 10, a compound isolated from the marine mollusk *Dolabela auricularia*.[1,2] The chemical structures of TZT-1027 and dolastatin 10 are shown in Figure 1.

In *in vitro* studies, TZT-1027 was found to exhibit time-dependent cytotoxicity superior to that of many other antitumor agents against a variety of murine and human tumor cell lines.[3] TZT-1027 exhibited antitumor activity against p-glycoprotein-overexpressing cell lines established from colon cancer H116 and breast cancer-resistant protein-positive cell lines established from lung cancer PC-6, and was more potent than vincristine, paclitaxel, and docetaxel against these cell lines. The efficacy of TZT-1027 has been attributed to its inhibition of tubulin polymerization. TZT-1027, which is believed to interact with the same domain on tubulin as the vinca alkaloid-binding region, inhibits the polymerization of microtubule proteins and the binding of GTP to tubulin.[4] In *in vivo* studies, intravenous injection of TZT-1027 has been shown to potently inhibit the growth of P388 leukemic cells and several solid tumors in mice, and to prolong the survival of the animals, and its antitumor efficacy has been shown to be superior or comparable to that of the reference agents dolastatin 10, cisplatin, vincristine, and 5-fluorouracil.[5] Furthermore, in xenograft models, TZT-1027 reduced intratumoral blood perfusion 1 to >24 h after its administration, thereby producing hemorrhagic necrosis of the tumors.[6–8] Thus, TZT-1027 exerts its antitumor activity both through direct cytotoxicity and by selective blockade of tumor blood flow, resulting in marked antitumor activity. In animal toxicology studies, TZT-1027 exhibited little or no neurotoxic potential, in marked contrast to vincristine and paclitaxel, which are antimicrotubule agents that have been shown in controlled animal studies to exert peripheral neurotoxicity.[9] However, at high doses of TZT-1027, myocardial toxicity was observed in rats and monkeys. It was estimated that the drug exerts its effects in a time-dependent manner because of the pattern of its cytocidal effects. The results of assessment in murine models of P388 leukemia and B16 melanoma indicate that simple dosing at short intervals would be the most suitable dosing schedule.

On the basis of this consideration, single dosing (a session of 1-h intravenous drip infusion followed by a 4-week period of observation) was conducted first in humans as a phase I study, and the present study was planned on the basis of the data from the single-dosing study. The previous single-dose phase I study

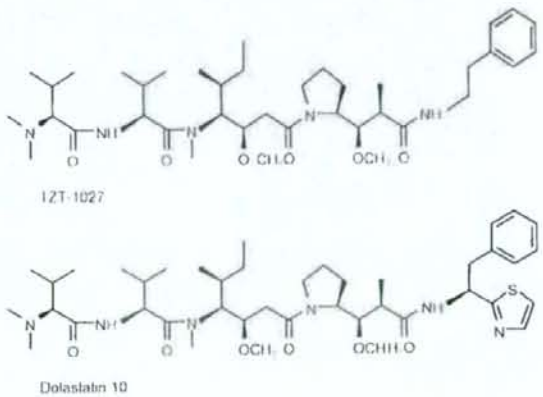


Fig. 1. Structural formulae of TZT-1027 and dolastatin 10.

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involved 23 patients and was conducted using doses in the range of 0.15–1.35 mg/m$^2$. The major hematological toxicity was neutropenia (all patients = grade 3). Nonhematological toxicities included anorexia, malaise, nausea, and alopecia. The maximum tolerated dose (MTD) was not determined. One patient with sarcoma showed partial response (PR). Three patients with non-small-cell lung cancer (NSCLC) showed a >50% tumor reduction; however, this did not satisfy the criteria for PR, as the duration of the response was short.[10]

The present study, a phase I repeated-dose administration study of TZT-1027, was conducted according to a schedule consisting of weekly administration of the drug for 3 weeks followed by a 4-week observation period.

## Patients and Methods

**Study design.** The present study, an open-label, dose-escalating phase I study, was conducted in Japanese patients with solid tumors to determine the MTD, identify the recommended dose for phase II studies, and assess the pharmacokinetic profile of TZT-1027. The study and the written consent form were approved by the Institutional Review Board. All patients provided informed consent before study entry. The study was conducted in accordance with the Good Clinical Practice Guidelines and the Declaration of Helsinki.

**Patient eligibility.** Patients with histologically or cytologically confirmed solid tumors that were refractory to standard therapy or for which no effective therapy was available were eligible to participate in the present study. Other inclusion criteria included: no prior chemotherapy or radiotherapy within 4 weeks of study entry (within 2 weeks of study entry in the case of hormone drugs and antimetabolites); age ≥15 years and ≤75 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤2; life expectancy at least 3 months; adequate bone marrow function with hemoglobin ≥9.5 g/dL, white blood cell (WBC) count 4000–12 000/mm$^3$, and platelet count ≥100 000/mm$^3$; normal hepatic function with serum bilirubin ≤1.5 mg/dL and serum aspartate aminotransferase and alanine aminotransferase ≤2.0 times the upper limit of the respective normal ranges; and adequate renal function with serum creatinine ≤ the upper limit of the respective normal range. All patients signed a written informed-consent form. Exclusion criteria included the presence of symptomatic brain metastases or pulmonary fibrosis, history of severe cardiac disorder (including severe atrial or ventricular arrhythmia or heart block), and pregnancy.

**Treatment and dose escalation.** TZT-1027 was given intravenously over 60 min in 250 mL saline. TZT-1027 was administered three times at weekly intervals (days 1, 8, and 15). The 4-week period after the third administration was designated as the observation period. The second and third administrations were conducted after confirmation of a WBC of 3000/mm$^3$ or more and neutrophil count of 1500/mm$^3$ or more. When these parameters did not meet the above-described criteria, the administration was delayed until they met the criteria; if, however, the criteria were not met even after 2 weeks of the final administration, the drug administration was discontinued altogether. If tumor regression was recognized and the patients recovered from adverse events by 4 weeks after the third administration (on day 15), re-administration at the same dose was allowed. Patients in whom the three weekly administrations of TZT-1027 failed for reasons other than dose-limiting toxicity (DLT) were replaced.

The starting dose was 0.3 mg/m$^2$, and the dose was increased up to 2.1 mg/m$^2$ (Table 1). The total dose of the three sessions (0.3 mg/m$^2$ × 3) was lower than 1.05 mg/m$^2$, which was lower than the 1.35 mg/m$^2$ used in the single-dose study. The safety of the maximum dose used (i.e. 1.35 mg/m$^2$) was confirmed in the single-dose phase I study carried out prior to the present study in Japan. According to the dose-escalation plan shown in Table 1, the dose was increased gradually to the maximum allowable dose (MAD). MAD was defined as the dose at which grade 3 or more severe hematotoxicity or grade 2 or more severe cardiac, hepatic, renal, or pulmonary toxicity appeared in two-thirds of patients. The MAD was reached at a dose of 1.5 mg/m$^2$; however, it was judged that estimation of the MTD is required for estimation of the recommended dose for phase II studies. Under approval by the Efficacy Safety Assessment Committee, the dose could be increased according to the protocol.

**Table 1. Number of TZT-1027 administrations**

| Dose of TZT-1027 (mg/m$^2$) | Number of patients | Number of administrations | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 0.30 | 3 | 0 | 0 | 3 |
| 0.45 | 4 | 0 | 0 | 4 |
| 0.60 | 3 | 0 | 0 | 3 |
| 0.75 | 3 | 0 | 0 | 3 |
| 0.90 | 3 | 0 | 0 | 3 |
| 1.05 | 4 | 1 | 0 | 3 |
| 1.20 | 3 | 0 | 0 | 3 |
| 1.35 | 3 | 0 | 0 | 3 |
| 1.50 | 3 | 0 | 0 | 3[†] |
| 1.65 | 3 | 0 | 1 | 2 |
| 1.80 | 4 | 1 | 0 | 3 |
| 2.10 | 4 | 2 | 1 | 1 |
| Total | 40 | 4 | 2 | 34 |

[†]One patient had five administrations.

Maximum tolerated dose was defined as the minimum dose at which DLT appeared in at least two-thirds of the patients, and the recommended dose was defined as one dose level lower than the MTD. DLT was defined as follows: (i) grade 4 neutropenia; (ii) grade 4 leukopenia; (iii) grade 4 thrombocytopenia; and (iv) grade 3/4 non-hematological toxicity, excluding nausea and vomiting. When grade 4 leukopenia was confirmed, administration of granulocyte colony stimulating factor (G-CSF) was allowed. When grade 4 thrombocytopenia appeared, platelet transfusion was allowed.

Toxicity was assessed using the Adverse Drug Reaction Criteria of the Japan Society for Cancer Therapy.[11] The criteria are approximately similar to the Common Toxicity Criteria adopted by the National Cancer Institution in the USA.

**Treatment assessment.** Baseline assessment, including a complete medical history, physical examination, vital signs, ECOG performance status, blood counts, serum biochemistry, and urinalysis, was conducted to assess patient eligibility and had to be completed 5 days before the start of treatment.

During the TZT-1027 administration period and the subsequent 4-week observation period, routine biochemistry, hematology, and urinalysis were carried out weekly. Electrocardiograms were recorded before the first administration and after the third administration of TZT-1027, and at the end of the observation period. The left ventricular ejection fraction was assessed before TZT-1027 administration, after the third administration of the drug, and 2 weeks into the observation period. Chest X-rays were obtained at least twice: before the start of treatment and at the end of the observation period. Imaging examinations, including computed tomography, were obtained as necessary for evaluating the antitumor effects of the drug. Tumor response was evaluated according to Criteria for the Evaluation of Direct Effects of Solid Cancer Chemotherapy of the Japan Society for Cancer Therapy.[12]

**Pharmacokinetic sampling, assay, and analysis.** The pharmacokinetic profile of TZT-1027 was evaluated after the first and third administration. Blood samples were collected immediately

before the drip infusion, at the end of the drip infusion, and 3, 6, and 24 h after the drip infusion. All blood samples were centrifuged immediately after sampling at 2 000 g for 10 min at 4°C, and the plasma samples were stored at −20°C until analysis. Plasma concentrations were determined using the liquid chromatography–mass spectrometry method.

Pharmacokinetic analysis of data from individual plasma samples was made using standard model-independent (non-compartmental) methods. The following pharmacokinetic parameters were calculated: area under the curve (AUC), maximum concentration ($C_{max}$), half-life ($T_{1/2}$), mean residence time, and total clearance.

## Results

**Patient characteristics.** The demographic characteristics of the patients are shown in Table 2. Forty patients (28 men and 12 women) with a median age of 60 years were enrolled in the present study. The most frequently encountered tumor type was NSCLC.

All patients were included in the assessment of safety. The patients in whom TZT-1027 could be administered only once or twice for reasons other than DLT were considered to be unevaluable for DLT and replacement patients were added for administration of the same dose. TZT-1027 could be administered three times in 34 patients.

The drug was administered only twice in two patients; administration was discontinued because of DLT in one of these patients (1.65 mg/m$^2$), and because of increased tumor size in the other patient (2.1 mg/m$^2$). Drug administration was discontinued after the first administration in four patients because of DLT in two of these patients (2.1 mg/m$^2$) and lack of fulfillment of the hematological criteria for further drug administration (neutrophil count <1500/mm$^3$ or WBC count <3000/mm$^3$) in the remaining two patients at 1.05 and 1.8 mg/m$^2$, respectively.

**Table 2. Patient characteristics**

| Characteristic | n |
|---|---|
| Patients | 40 |
| Sex | |
| Male | 28 |
| Female | 12 |
| Median age (years) | 60 (range 25–74) |
| Performance status | |
| 0 | 16 |
| 1 | 18 |
| 2 | 6 |
| Tumor type | |
| Lung | 17 |
| Soft tissue | 4 |
| Esophagus | 3 |
| Pancreas | 2 |
| Colorectum | 2 |
| Thymoma | 2 |
| Mesothelioma | 2 |
| Stomach | 1 |
| Breast | 1 |
| Carcinoid | 1 |
| Bile duct | 1 |
| Rectum | 1 |
| Duodenum | 1 |
| Pharynx | 1 |
| Mediastinum | 1 |
| Previous treatment | |
| Chemotherapy | 30 |
| Radiotherapy | 3 |
| Surgery | 2 |
| Combination | 5 |

**Dose-limiting toxicity.** As shown in Table 1, 12 different doses of TZT-1027, ranging from 0.3 to 2.1 mg/m$^2$, were administered. Three to four patients were treated at each dose.

Dose-limiting toxicity appeared in two patients at 2.1 mg/m$^2$. One was a 59-year-old man with malignant mediastinal tumor who developed grade 4 neutropenia/leukopenia, grade 3 myalgia, and grade 4 constipation. He had received chest radiotherapy as pretreatment. On day 4 after drug administration, he developed grade 3 myalgia. On day 5 after drug administration, ileus appeared. On day 8 he developed grade 4 leukopenia (700/mm$^3$) and grade 4 neutropenia (272/mm$^3$). On days 9–12, G-CSF was administered, with improvement of the leukopenia and neutropenia. The myalgia and ileus subsided on days 11 and 20, respectively. The other patient was a 73-year-old male patient with NSCLC who developed grade 3 constipation and grade 4 neutropenia. He had received chest radiotherapy and docetaxel administration as pretreatment. On day 8 after the drug administration, grade 4 neutropenia was detected. On day 9, grade 3 constipation occurred. On days 8–12, G-CSF was administered, with improvement of the neutropenia. The constipation also subsided on day 16.

As DLT appeared in two-thirds of the patients at 2.1 mg/m$^2$, the dose was determined to be the MTD. At 1.8 mg/m$^2$, which was one dose level lower than 2.1 mg/m$^2$, no patients were noted with DLT, and the toxicity was also within the tolerated range. Based on these observations, this dose was judged as the recommended dose for phase II studies. DLT in other patients included grade 4 neutropenia, which occurred in one patient after three administrations of TZT-1027 at 1.5 mg/m$^2$, and in one patient after two administrations of TZT-1027 at 1.65 mg/m$^2$. None of the patients developed febrile neutropenia. There were no treatment-related deaths.

**Hematological toxicities.** Neutropenia was the major DLT of TZT-1027. Hematological toxicities as a function of the total numbers of patients and courses of TZT-1027 are shown in Table 3. Grade 4 neutropenia was observed at doses of 1.5 mg/m$^2$. The severity grade of neutropenia tended to increase with increased dose. G-CSF was used in only two patients who developed DLT at 2.1 mg/m$^2$, whereas the neutrophil count improved spontaneously in the other patients. Both anemia and thrombocytopenia were relatively mild. There was only one event of grade 3 thrombocytopenia at a dose of 2.1 mg/m$^2$.

**Nonhematological toxicities.** Table 4 shows the overall drug-related non-hematological toxicities observed. The common non-hematological toxicities were malaise, nausea, vomiting, and constipation. The most frequently observed toxicity was malaise, and phlebitis was rare in the present study. The DLT were grade 3/4 constipation and grade 3 myalgia at a dose of 2.1 mg/m$^2$. Concerning the myalgia, grade 2 myalgia was recorded in another patient at 2.1 mg/m$^2$. Three patients developed peripheral neuropathy, grade 1 at 1.35 and 1.65 mg/m$^2$, and grade 2 at 2.1 mg/m$^2$. There were no cases of cardiovascular toxicity.

**Pharmacokinetic studies.** The pharmacokinetics of TZT-1027 were assessed in all patients at the first administration and in 34 patients at the third administration. The pharmacokinetic parameters determined during the first and third administrations of TZT-1027 are shown in Table 5. The maximum plasma TZT-1027 concentration occurred at the end of the 1-h infusion. The plasma concentrations during the third administration were almost the same as those during the first administration. No evidence of accumulation was observed during the third administration.

The $C_{max}$ and AUC tended to increase with the dose, whereas the changes in $T_{1/2}$ did not show any dose-dependent tendency (Table 5; Fig. 2). The correlation between pharmacokinetics (AUC and $C_{max}$) and hematological toxicity (decrease in the percentage neutrophil count from baseline) showed that the



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**Table 3. Hematological toxicities**

| Dose (mg/m²) | No.patients | Leucopenia | | | | Neutropenia | | | | Hemoglobin decreased | | | Thrombocytopenia | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grade | | | | Grade | | | | Grade | | | Grade | | | |
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| 0.30 | 3 | 1 | | | | | 1 | | | | | | | | | |
| 0.45 | 4 | 1 | | | | 1 | | | | 1 | | 1 | | | | |
| 0.60 | 3 | 1 | 1 | | | | 2 | | | 1 | 1 | | | | | |
| 0.75 | 3 | 1 | 1 | | | | | 1 | | | 1 | | | | | |
| 0.90 | 3 | 3 | | | | 1 | 1 | | | | 1 | | | | | |
| 1.05 | 4 | 2 | 1 | | | | 1 | 1 | | 1 | 1 | | | | | |
| 1.20 | 3 | | 2 | 1 | | | 2 | 1 | | | 3 | | 1 | | | |
| 1.35 | 3 | | 2 | 1 | | | 2 | 1 | | | 2 | 1 | | | | |
| 1.50 | 3 | 1 | 1 | 1 | | | 1 | 1 | 1 | 1 | | | | | | |
| 1.65 | 3 | 1 | 1 | 1 | | | 1 | | 1 | | 1 | | | | | |
| 1.80 | 4 | | 3 | 1 | | 1 | 1 | 2 | | | 1 | 1 | 1 | | | |
| 2.10 | 4 | | | 2 | 1 | | | 1 | 2 | | 1 | | | | 1 | |
| Total | 40 | 11 | 12 | 7 | 1 | 3 | 12 | 8 | 4 | 4 | 12 | 3 | 2 | 0 | 1 | 0 |

**Table 4. Nonhematological toxicities reported most frequently (>5%)**

| Dose (mg/m²) | No. patients | Malaise | | | | Nausea/vomiting | | | Alopecia | | | Constipation | | | | Phlebitis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grade | | | | Grade | | | Grade | | | Grade | | | | Grade | | |
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 2 | 3 | 4 |
| 0.30 | 3 | | | | | | | | 1 | | | | | | | | | |
| 0.45 | 4 | 1 | | | | | | | 1 | | | | | | | | | |
| 0.60 | 3 | | | | | | | | 1 | | | | | | | | | |
| 0.75 | 3 | 1 | | | | 1 | | | | | | 1 | | | | | | |
| 0.90 | 3 | | | | | 2 | | | | | | | | | | | | |
| 1.05 | 4 | 2 | | | | 2 | | | 1 | | | | | | | | | |
| 1.20 | 3 | 1 | | | | | | | 1 | | | | | | | | | |
| 1.35 | 3 | 1 | 1 | | | | 1 | | | | | | | | | | | |
| 1.50 | 3 | 1 | | | | 1 | | | | | | | | | | 2 | | |
| 1.65 | 3 | 2 | | | | 1 | | | 1 | | | | | | | | | |
| 1.80 | 4 | | | | | | 1 | | 1 | | | | | | | 1 | | |
| 2.10 | 4 | 3 | | | | | | | 1 | | | | | 1 | 1 | | | |
| Total | 40 | 12 | 1 | 0 | 0 | 7 | 2 | 0 | 8 | 0 | 0 | 1 | 0 | 1 | 1 | 3 | 0 | 0 |

**Table 5. Pharmacokinetic parameters of TZT-1027 at the first administration**

| Dose (mg/m²) | No. patients | $C_{max}$ (ng/mL) Mean (CV%) | AUC (ng h/mL) Mean (CV%) | $Cl_{tot}$ (l/h/m²) Mean (CV%) | $T_{1/2}$ (h) Mean (CV%) | MRT (h) Mean (CV%) |
|---|---|---|---|---|---|---|
| 0.30 | 3 | 21.3 (24.4) | 49.1 (24.3) | 6.4 (27.0) | 3.4 (7.6) | 2.4 (16.0) |
| 0.45 | 4 | 44.3 (71.7) | 125.4 (86.0) | 6.9 (93.8) | 3.7 (21.8) | 3.2 (35.5) |
| 0.60 | 3 | 46.6 (43.0) | 132.1 (65.5) | 5.8 (50.3) | 4.1 (20.4) | 3.1 (26.2) |
| 0.75 | 3 | 52.2 (57.7) | 153.0 (77.6) | 7.2 (66.0) | 3.9 (31.2) | 3.1 (26.1) |
| 0.90 | 3 | 80.5 (46.5) | 209.6 (60.0) | 5.4 (52.3) | 3.3 (32.5) | 2.4 (24.6) |
| 1.05 | 4 | 123.9 (19.3) | 401.1 (37.5) | 2.9 (30.1) | 5.8 (44.8) | 4.6 (59.3) |
| 1.20 | 3 | 103.2 (40.8) | 276.7 (57.4) | 5.4 (54.3) | 3.9 (47.7) | 2.8 (40.9) |
| 1.35 | 3 | 112.4 (22.0) | 325.2 (17.7) | 4.3 (19.1) | 4.8 (15.4) | 3.1 (4.8) |
| 1.50 | 3 | 219.1 (27.2) | 652.9 (28.3) | 2.5 (33.9) | 5.6 (25.2) | 3.6 (16.6) |
| 1.65 | 3 | 177.3 (38.9) | 527.7 (30.2) | 3.3 (27.5) | 5.1 (22.1) | 3.5 (27.8) |
| 1.80 | 4 | 233.6 (34.9) | 731.2 (45.8) | 2.8 (40.1) | 5.4 (16.0) | 3.7 (28.7) |
| 2.10 | 4 | 246.5 (36.3) | 991.8 (50.8) | 2.5 (37.8) | 7.8 (28.2) | 6.9 (41.5) |

AUC, area under the curve; $C_{max}$, maximum concentration; $Cl_{tot}$, total clearance; MRT, mean residence time; $T_{1/2}$, half-life.

neutrophil count tended to decrease as AUC and $C_{max}$ increased ($r = 0.58$ and 0.58, respectively).

**Response evaluation.** The antitumor activity was assessed in all patients, with 16 patients showing no change. One patient with invasive thymoma who had previously received the cisplatin, vincristine, doxorubicin plus etoposide regimen, gemcitabine plus vinorelbine, and thoracic radiation at 40 Gy showed PR at 1.5 mg/m². Although administration of TZT-1027 was discontinued after the fifth administration (see Discussion) in this patient due to DLT (grade 4 neutropenia), the duration of PR was 183 days.

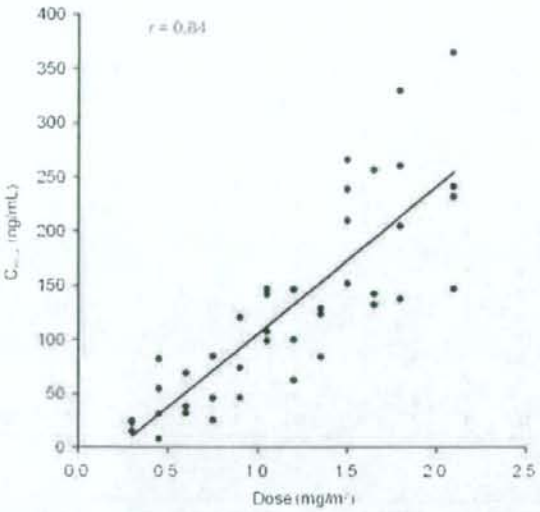


**Fig. 2.** Correlation between dose and maximum concentration ($C_{max}$) at the first administration.

## Discussion

Cellular tubulin is a well-established target for anticancer agents. Although currently available antitubulin agents, including the taxanes and vinca alkaloids, are highly effective anticancer agents, their clinical usefulness is limited due to their high rates of intrinsic or acquired resistance and systemic toxicities. Thus, it is important to develop newer agents targeting the tubulin and microtubule system that may be effective against tumors resistant to the existing anticancer agents and having an improved toxicity profile. A number of potent cytotoxic compounds have been discovered over the past decade, and candidate anticancer agents originating from marine life have been examined in human clinical trials. Of these compounds, dolastatin 10 and dolastatin 15 have been evaluated extensively in clinical studies. Cemadotin, an analog of dolastatin 15, was evaluated in phase I studies by several administration schedules and was found to cause neutropenia as a major DLT, apart from cardiac toxicity and hypertension.[13] Tasidotin, another dolastatin 15 analog, was also found to be associated with the DLT of neutropenia, ileus, and elevated transaminase levels.[14,15] Phase I studies of dolastatin 10 revealed that the drug caused neutropenia as a DLT.[16,17]

TZT-1027 was developed with the goal of obtaining the potent antitumor activity of the parent compound, but with reduced toxicity. In mice, intravenous injection of TZT-1027 showed efficacy equivalent to or greater than that of dolastatin 10. At the beginning of the present study, there were only data from a single-dose study in humans. Thus, the present study was the first repeated-dose phase I study conducted in humans. For this reason, TZT-1027 was, as a rule, administered three times at weekly intervals. The administration period was followed by a 4-week period of observation with the aim of confirming recovery of the patients from any toxicity. The administration was judged to be beneficial in the patients in whom no serious toxicity was noted and tumor regression was recognized after the three administrations. The drug was allowed to be continued even after the 4-week observation period only in the above patients. Because one patient with invasive thymoma experienced 70% tumor regression during the 4-week observation period, it was administered twice more until the patient developed the DLT of grade 4 neutropenia. This patient showed tumor regression by approximately 80% at the maximum, which confirmed PR.

The most frequently encountered DLT was grade 4 neutropenia, which either resolved spontaneously without treatment or could be reversed by administration of G-CSF. Other DLT included grade 4 leukopenia, grade 3 myalgia, and grade 3 and 4 constipation. However, peripheral neurological disturbance was mild, and it was considered that the toxicity of this antitubular agent resembled that of the vinca alkaloids rather than that of the taxanes. With regard to the pharmacokinetics, the AUC and $C_{max}$ increased with the dose. It was considered from the blood concentrations of the drug after the first and third administrations that the drug did not show accumulation.

On the basis of the results of the present study, some repeated-dose phase I studies were conducted after the present study. In the Netherlands, a repeated-dose study on days 1 and 8 of a 3-week course was conducted in patients with solid tumors. The dose of TZT-1027 was escalated to 2.7 mg/m$^2$, which produced neutropenia and infusion arm pain as DLT. The recommended dose of TZT-1027 for phase II studies was determined to be 2.4 mg/m$^2$.[18] In Japan also, a phase I study was conducted with the drug administered on days 1 and 8 of a 3-week course. Eighteen patients were enrolled in the study. Neutropenia was the principal DLT. Phlebitis was the most frequently encountered non-hematological toxicity. The recommended dose was determined to be 1.5 mg/m$^2$. This recommended dose was lower than that determined in the phase I study in the Netherlands.[19]

The recommended dose determined in the present study was 1.8 mg/m$^2$, which is also lower than that determined in the Netherlands study. The results of the pharmacokinetic parameters of TZT-1027 were similar between the present study and the Netherlands study. Therefore, it might be difficult to explain the difference in the recommended dose from the point of view of only pharmacokinetics. The possible difference might be the severity of bone marrow toxicity. In the present study, three of four patients at 2.1 mg/m$^2$ and one of four patients at 1.8 mg/m$^2$ could not receive TZT-1027 administration on day 8 on schedule. In a phase II study of TZT-1027 carried out in patients with treated soft-tissue sarcoma in the USA,[19] the dose used was 2.4 mg/m$^2$. Dose reduction to 1.8 mg/m$^2$ was required in approximately 40% of the patients, suggesting that 2.4 mg/m$^2$ may be a slightly high dose for patients who have received chemotherapy.

Some reports have shown that TZT-1027 exerts both considerable vascular effects and a direct cytotoxic effect, resulting in its strong antitumor activity,[20,21] and that TZT-1027 enhances the antitumor effect of ionizing radiation.[22] Clinical development of TZT-1027 in the future may include systemic treatment as a new anticancer drug with antiangiogenesis effects, and simultaneous combined use of the drug with radiation as a radiation sensitizer.

In conclusion, in the present study the MTD and recommended dose of TZT-1027, a synthetic analog of the natural marine product dolastatin 10, were determined to be 2.1 and 1.8 mg/m$^2$, respectively, for Japanese patients with advanced solid tumors, with the drug administered on days 1, 8, and 15. TZT-1027 showed less neurotoxicity than other tubulin inhibitors. These results suggest that TZT-1027 might be a promising new tubulin polymerization inhibitor that is generally well tolerated when administered on a weekly dosing schedule.

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# Phase I/II Pharmacokinetic and Pharmacogenomic Study of *UGT1A1* Polymorphism in Elderly Patients With Advanced Non–Small Cell Lung Cancer Treated With Irinotecan

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**This phase II study investigated the recommended dose (RD) of irinotecan (CPT-11) by dose escalation in elderly (≥70 years) chemotherapy-naive Japanese patients with advanced non–small cell lung cancer. *UGT1A1*28* and **6* polymorphisms and pharmacokinetics were also investigated. Thirty-seven patients received the RD, 100 mg/m$^2$ of intravenous CPT-11, on days 1 and 8 of each 3-week cycle in phase II. The overall response rate was 8.1%. The median survival time was 441 days, and time to progression was 132 days. A significant correlation was observed between the incidence of grade 3/4 neutropenia and area under the time-concentration curve (AUC) values of SN-38. A reduction in AUC ratios ($AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$) and a rise in incidence of grade 3/4 neutropenia were observed with increase in polymorphism. The regimen was well tolerated and provided good disease control and promising survival effects. An analysis of the influence of *UGT1A1*28* and **6* polymorphisms provides useful information for the prediction of CPT-11-related hematological toxicity.**

Lung cancer is the most common fatal cancer in Japan and in Western countries.[1] The majority of cases of advanced non-small cell lung cancer (NSCLC) are found among patients aged >65 years, and the number of such cases is predicted to rise with increases in the numbers of the elderly.[2,3]

Chemotherapy has been shown to yield better results than best supportive care in NSCLC patients in terms of survival and quality of life.[4] Platinum-based regimens containing a third-generation agent, including irinotecan (CPT-11), taxanes, gemcitabine (GEM), and vinorelbine (VNR), have been the mainstream treatment for patients with NSCLC.[5] However, these regimens have been associated with high toxicity while providing no survival benefit in elderly patients. Several prospective randomized trials have investigated optimal chemotherapy in patients aged ≥70 years with advanced NSCLC.[6–9] The regimens investigated have included VNR monotherapy,[6] GEM plus VNR vs. VNR alone,[7] VNR vs. GEM vs. VNR plus GEM,[8] and docetaxel (DOC) vs. VNR.[9] The results of the Elderly Lung Cancer Vinorelbine Italian Study (ELVIS) led to the recommendation that VNR monotherapy be used as first-line therapy in elderly patients with advanced NSCLC.[6] On the basis of these studies, and given that GEM is less active than VNR, many researchers now recommend VNR monotherapy.

CPT-11 is a semi-synthetic camptothecin derivative with topoisomerase I–inhibiting activity.[10–12] CPT-11, a prodrug, is converted to its active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin), by carboxylesterase, which is 100- to 1,000-fold more cytotoxic than CPT-11. Further hepatic metabolism by uridine diphospho-glucuronosyl-transferases (UGTs) converts SN-38 to its inactive metabolite, SN-38 glucuronide (SN-38G).[10–12]

Phase III clinical studies on CPT-11 conducted in NSCLC patients have included a comparison we made of CPT-11

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monotherapy, a cisplatin-plus-vindesine group (VDS-P), and a cisplatin-plus-CPT-11 (IP) group.[13] The response rate in the CPT-11 monotherapy group in a subset of elderly patients (aged 70–75 years) in that study was 40.0%, similar to that in the VDS-P group (43.5%). Moreover, the response rate was higher in the IP group (60.9%) than in those undergoing either of the other two regimens. Interestingly, survival time was better in the CPT-11 monotherapy group (44.3 weeks) than in the VDS-P group (35.7 weeks). As for adverse events in this subset of elderly patients, although the incidence of diarrhea tended to be higher in the CPT-11 monotherapy group, leukopenia, neutropenia, nausea/vomiting, and anorexia were all mild. Because these findings suggested that CPT-11 monotherapy might be a useful regimen in elderly patients with NSCLC, the regimen was investigated in this prospective study.

Severe CPT-11-associated diarrhea and myelosuppression have been reported as dose-limiting toxicities (DLTs).[14,15] These effects correlate significantly with the area under the time-concentration curve (AUC) values of CPT-11 and its active metabolite SN-38 and glucuronized SN-38.[14,15] Among UGT isoforms, *UGT1A1* is believed to be responsible for SN-38 glucuronidation and is also thought to be involved in the large inter-individual variations seen in SN-38 pharmacokinetics.[16] Several studies have reported a correlation between the adverse effects of CPT-11 and the presence of *UGT1A1* polymorphisms including *UGT1A1*28* and *UGT1A1*6*.[17–19] Ethnic differences have also been reported in the distribution of these polymorphisms, with higher incidences of *UGT1A1*6* occurring in Asians (including Japanese) than in Caucasians.[20–22] This suggests that *UGT1A1* polymorphism is an important determining factor in the efficacy and toxicity of CPT-11 and that pharmacogenetics-guided dosing of CPT-11 may help to individualize the dose of CPT-11 and moderate its toxicity in cancer patients.

We performed phase I and II studies involving CPT-11 monotherapy on days 1 and 8 of a 3-week cycle in elderly patients with NSCLC to determine the DLT, maximum-tolerated dose (MTD), and recommended dose (RD) and to investigate the antitumor effect and safety of the RD. Further, a prospective analysis of *UGT1A1* mutations was performed, and we investigated the relationship between the presence of these polymorphisms and the occurrence of adverse events. We also analyzed the variation in the pharmacokinetics of CPT-11 and its metabolites in elderly patients.

## RESULTS

### Patient characteristics

Between April 2003 and March 2006, 46 patients with stage IIIB/IV NSCLC were enrolled. In the overall study population, 76% of the patients (35 of 46) had stage IV disease, and 69.5% (32 of 46) had adenocarcinoma. Twelve patients were enrolled and treated in phase I. Six patients were treated at dose level 1 (60 mg/m$^2$), three patients at dose level 2 (80 mg/m$^2$), and three patients at dose level 3 (100 mg/m$^2$). DLT of persistent grade 2 leukopenia was observed in one patient at dose level 1, and an additional three patients were enrolled at this dose level. No further DLTs were observed in these patients or in patients receiving 80 or 100 mg/m$^2$. Therefore the MTD was not reached in this study, and the RD was set at 100 mg/m$^2$, in accordance with the study protocol described in "Methods."

In phase II, 34 additional patients were treated at 100 mg/m$^2$, making a total of 37 patients treated with the RD. Table 1 shows the selected baseline demographics and disease characteristics of the patients treated with the RD. There were 25 men and 12 women, with a median age of 76 years (range: 71–88).

The median number of treatment cycles in phase II was 4.0 (range: 1–18); 37.8% of patients (14 of 37) received five or more cycles, and the percentage of patients with 6-month or longer treatment was ~22%. The relative dose intensity was 90.0%. Twenty-five of the 37 patients went on to second-line therapy comprising gefitinib (in 7 patients, 28%), different regimens of CPT-11 (7 patients, 28%), carboplatin/paclitaxel (4 patients, 16%), DOC (3 patients, 12%), GEM (3 patients, 12%), and S-1/cisplatin (1 patient, 4%).

### Response and survival

All 37 patients (including 3 patients in phase I) who received the RD were evaluated to determine the overall response rate. The overall response rate was 8.1% (complete response (CR): 0, partial response (PR): 3; 3/37, 95% confidence interval: 1.7–21.9), and the disease control rate was 21.6% (8/37, 95% confidence interval: 9.8–38.2). The median survival time (MST) was 441 days after a median follow-up of 440 days, and the 1-year survival rate was 56.8% (Figure 1). The median time to progression (TTP) was 132 days.

### Toxicity

In phase I, persistent grade 2 leukopenia was observed in one patient who received treatment at level 1, and the second cycle could not be started until day 30. This adverse event was therefore regarded as a DLT. Adverse events that occurred in phase II are summarized in Table 2. The most frequently observed hematological toxicity (grade 3/4) was neutropenia (27.0%).

**Table 1 Demographics of patients treated with irinotecan 100 mg/m$^2$**

| Characteristic | No. of patients ($N$ = 37) | % |
|---|---|---|
| Sex | | |
| Male | 25 | 68 |
| Female | 12 | 32 |
| Age (years) | | |
| Median | 76.0 | |
| Range | 71–88 | |
| Performance status | | |
| 0 | 11 | 30 |
| 1 | 26 | 70 |
| Histology | | |
| Adenocarcinoma | 25 | 68 |
| Other | 12 | 32 |
| Stage | | |
| IIIB | 10 | 27 |
| IV | 27 | 73 |

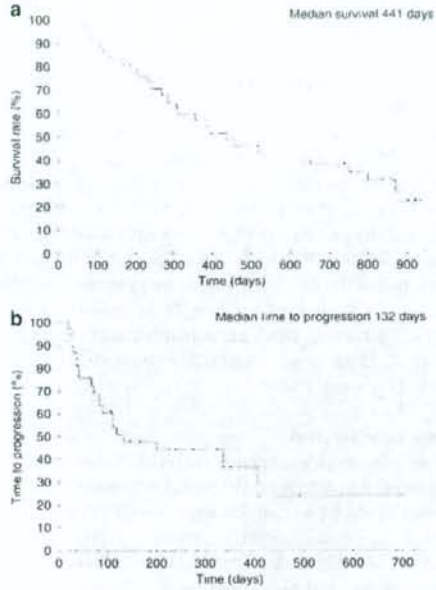


**Figure 1** Elderly patients with advanced NSCLC treated with irinotecan. (**a**) Kaplan–Meier overall survival curve and (**b**) time-to-progression curve.

**Table 2 Summary of adverse events in phase II (all courses)**

| | CPT-11 dose: 100 mg/m² (*N* = 37) | |
|---|---|---|
| Adverse event, patients | Any event | Grade 3/4 (%) |
| Leukopenia | 26 | 9 (24.3) |
| Neutropenia | 28 | 10 (27) |
| Anemia | 27 | 4 (10.8) |
| Thrombocytopenia | 1 | 1 (2.7) |
| Febrile neutropenia | 0 | 0 (0) |
| Diarrhea | 28 | 3 (8.1) |
| Nausea | 23 | 4 (10.8) |
| Vomiting | 13 | 0 (0) |
| Anorexia | 31 | 9 (24.3) |
| Fatigue | 14 | 1 (2.7) |

Adverse events were assessed using National Cancer Institute Common Toxicity Criteria.

Frequently observed nonhematological toxicities (grade 3/4) included nausea (10.8%), anorexia (24.3%), and diarrhea (8.1%). Grade 4 toxicity (neutropenia) occurred in one patient who received treatment at level 3. Treatment-related death occurred in one patient, due to interstitial pneumonia.

### Relationship of *UGT1A1**6 and *28 polymorphisms to pharmacokinetics and toxicity of CPT-11

The analysis of *UGT1A1* genotypes was performed in the 36 patients who had provided informed consent, and their

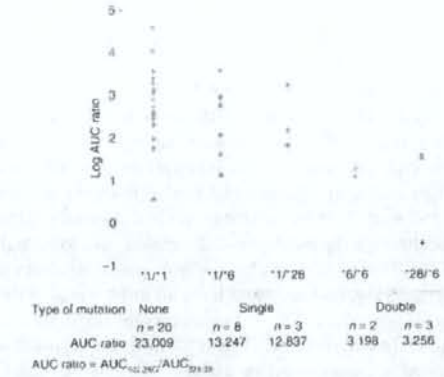


**Figure 2** Comparison of area under the time-concentration curve (AUC) ratios by type of polymorphism in 36 patients treated with 100 mg/m² of irinotecan. The pharmacokinetic profile of irinotecan was affected to similar extents by **28* heterozygous and **6* heterozygous mutations, and by **6* homozygous and **6/*28* heterozygous mutations. The lines indicate geometric mean and the *y*-axis represents the log scale.

**Table 3 Relationships between polymorphisms and adverse events and pharmacokinetic profile by type of *UGT1A1* polymorphism**

| | *UGT1A1*28* or *UGT1A1*6* mutation | | | |
|---|---|---|---|---|
| | No mutation (*n* = 20) | Single (*n* = 11) | Double (*n* = 5) | *P* |
| Adverse events (no. of patient (%)) | | | | |
| Leukopenia grade 3 or 4 | | | | |
| First cycle | 0 (0%) | 3 (27%) | 2 (40%) | 0.006[a] |
| All cycles | 3 (15%) | 3 (27%) | 3 (60%) | 0.046[a] |
| Neutropenia grade 3 or 4 | | | | |
| First cycle | 1 (5%) | 2 (18%) | 2 (40%) | 0.039[a] |
| All cycles | 3 (15%) | 3 (27%) | 4 (80%) | 0.008[a] |
| AUC ratio[b] | 23.009 | 12.949 | 3.233 | 0.001[c] |

Adverse events were assessed using National Cancer Institute Common Toxicity Criteria.

[a]Jonckheere–Terpstra test; [b]AUC ratio = $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$; [c]Cochran–Armitage test.

polymorphisms are categorized and listed in Figure 2. Double mutations of *UGT1A1*28* and **6* (**6/*6* and **28/*6*) were detected in 5 of 36 patients (14%), and single mutations of *UGT1A1*28* or **6* were found in 11 of 36 patients (31%). No mutation was detected in 20 of 36 patients (55.6%). No *UGT1A1*28/*28* was found in homozygous patients.

Pharmacokinetic analyses were performed in the first cycle of treatment at a dosage of 100 mg/m², and the $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ ratios of the *UGT1A1*28* and **6* polymorphisms were compared (Figure 2). The $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ was 23.009 in the wild-type group. In the single-mutation group, the AUC ratios were 12.837 and 13.247 in **28* heterozygous and **6* heterozygous patients, respectively. In the double-mutation group, the ratios were 3.198 and 3.256 in **6* homozygous and **6/*28* heterozygous patients, respectively.

**Table 4 Relationship between adverse events and pharmacokinetic profile during the first cycle of irinotecan treatment**

| Adverse event | Pharmacokinetic parameter | Spearman's rank correlation ρ (*P* value) |
|---|---|---|
| Leukopenia | CPT-11 $AUC_{0-inf}$ | 0.463 (<0.001) |
| | CPT-11 $C_{max}$ | 0.384 (0.001) |
| | SN-38 $AUC_{0-inf}$ | 0.542 (<0.001) |
| | SN-38 $C_{max}$ | 0.513 (<0.001) |
| Neutropenia | CPT-11 $AUC_{0-inf}$ | 0.449 (<0.001) |
| | CPT-11 $C_{max}$ | 0.314 (0.017) |
| | SN-38 $AUC_{0-inf}$ | 0.587 (<0.001) |
| | SN-38 $C_{max}$ | 0.59 (<0.001) |

AUC, area under the time-concentration curve; $C_{max}$, peak plasma concentration; CPT-11, irinotecan.

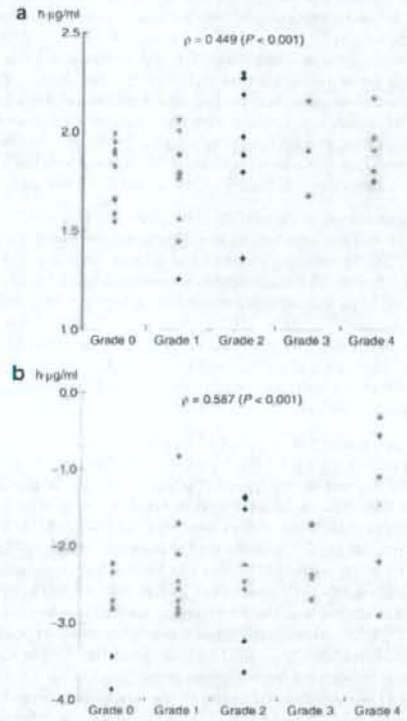


**Figure 3** Correlation between neutropenia and pharmacokinetic profile: (**a**) CPT-11 $AUC_{0-inf}$ and (**b**) SN-38 $AUC_{0-inf}$. The lines indicate geometric mean and the *y*-axis represents the log scale. AUC, area under the time-concentration curve.

The $AUC_{SN-38G}/AUC_{SN-38}$ ratio was highest in the wild-type group, lower in the single-mutation group, and least in the double-mutation group. Although the number of patients was insufficient to establish statistical significance, the $AUC_{SN-38G}/AUC_{SN-38}$ ratios of *6 heterozygous patients were nearly equivalent to those of *28 heterozygous patients, and those of *6 homozygous patients were nearly equivalent to those of *6/*28 heterozygous patients.

The association of *UGT1A1*28* and *6 polymorphisms with grade 3/4 hematological toxicity or AUC ratio was investigated during the first cycle of therapy. Significant correlations were observed between *UGT1A1*28* and *6 polymorphisms and AUC ratio ($P = 0.001$) and between *UGT1A1*28* and *6 polymorphisms and grade 3/4 hematological toxicity (Table 3). When the same association was examined through all cycles, a similar correlation between the incidence of grade 3/4 hematotoxicity and polymorphisms was observed (Table 3).

The relationship between adverse events and pharmacokinetic profile was further analyzed (Table 4). All five parameters correlated well with the frequency of grade 3/4 leukopenia and neutropenia ($P < 0.001$). The correlation between neutropenia and pharmacokinetic profile (CPT-11 $AUC_{0-inf}$ and SN-38 $AUC_{0-inf}$) is shown in Figure 3. Both of these parameters correlated with neutropenia (CPT-11 $AUC_{0-inf}$: $\rho = 0.449$ ($P < 0.001$), SN-38 $AUC_{0-inf}$: $\rho = 0.587$ ($P < 0.001$)). The pharmacokinetic parameters of SN-38 appeared to correlate more significantly than those of CPT-11.

## DISCUSSION

In this study, CPT-11 was administered on days 1 and 8 every 3 weeks in elderly patients (aged ≥70 years) with NSCLC, and the DLT, MTD, and RD were determined. The efficacy and safety of this regimen were investigated at the RD. In addition, the results were compared prospectively with the results of pharmacokinetic analysis and exploratory analysis of *UGT1A1* gene polymorphisms.

The results showed low antitumor effect for CPT-11 (response rate, 8.1%). The disease control rate was 21.6%. However, the TTP in this study was 132 days. This was longer than that observed in the phase III study we conducted.[13] Although the incidences of grade 3 or higher leukopenia, neutropenia, and anorexia were >20%, other adverse events occurred less frequently, and tolerability was acceptable. Also, the median number of treatment courses was four, and 22% of the patients were able to undergo prolonged treatment (more than eight courses). Almost all the doses of CPT-11 were administered as planned (dose intensity, 90%), and 25 patients were able to proceed to second-line therapy. As a result, an MST of 441 days was achieved. Because the MST was longer than predicted at the start of this study, the median follow-up time was also longer (440 days). These findings suggest that the regimen tested in this study is feasible and appropriate in elderly patients.

The high tolerability of this regimen contrasts with the results of a phase III comparative study of DOC monotherapy vs. VNR monotherapy in elderly patients (West Japan Thoracic Oncology Group Trial 9904)[9] conducted in Japan at around the same time. The response rate of 8.1% in our study was lower than that achieved with DOC monotherapy (22.7% in the West Japan Thoracic Oncology Group study). However, the survival time (14.3 months) was better in our study than that reported

in the West Japan Thoracic Oncology Group study. Moreover, the incidences of grade 3/4 neutropenia and leukopenia were 83 and 58%, respectively, with DOC,[9] which were higher than those in this study. These results indicate that this CPT-11 regimen should be considered as an option for first-line therapy in elderly patients with NSCLC.

To the best of our knowledge, this is the first prospective study with NSCLC patients that has explored the association between *UGT1A1* polymorphisms and the clinical effects of CPT-11 treatment. The $AUC_{SN-38G}/AUC_{SN-38}$ ratios were 23.009 in the wild-type group, 12.837 and 13.247 in the single-mutation group, and 3.198 and 3.256 in the double-mutation group, with the AUC ratio decreasing from wild-type to single-mutation to double-mutation groups. Furthermore, the individual AUC ratios in **6* heterozygous patients were similar to those in **28* heterozygous patients, and those in **6* homozygous patients were similar to those in **6/*28* heterozygous patients, although the number of patients in this study was too small to establish statistical significance.

Among the adverse events occurring during the first course of treatment, a correlation was observed between the incidence of grade 3/4 leukopenia or neutropenia and the AUC and peak plasma concentration of SN-38, as has been reported previously in relation to serious adverse reactions.[17-19] The results also showed that the incidence of grade 3/4 leukopenia and neutropenia was lowest in the wild-type group, higher in the single-mutation group, and highest in the double-mutation group of *UGT1A1*. We consider our classification of polymorphisms of *UGT1A1* as single-mutation and double-mutation appropriate.

The 100 mg/m$^2$ dose of intravenous CPT-11 on days 1 and 8 every 3 weeks was well tolerated in this prospective phase II study. These results suggest that this CPT-11 regimen should be considered as one of the options for first-line therapy in elderly patients with NSCLC. A phase III study has been scheduled to clarify the effect of *UGT1A1* mutations on response to CPT-11 therapy.

## METHODS

**Eligibility criteria.** Chemotherapy- and radiotherapy-naive patients with histologically or cytologically proven stage IIIB/IV NSCLC were enrolled. Other eligibility criteria included age ≥70 years; measurable and assessable disease; Eastern Cooperative Oncology Group performance status of 0–1; an expected survival duration of ≥12 weeks; adequate bone marrow function (leukocyte count 4,000–12,000/mm$^3$; hemoglobin concentration ≥9.5 g/dl; platelet count ≥100,000/mm$^3$); serum creatinine at or below the institutional upper limits of normal level; total bilirubin level ≤1.5 mg/dl; and aspartate aminotransferase and alanine aminotransferase levels ≤100 IU. Laboratory tests were performed within 7 days of enrollment in the study. Exclusion criteria included the presence of symptomatic brain metastasis or apparent dementia; active concomitant malignancy; massive pleural effusion or ascites; active infection; severe heart disease or elevated electrocardiogram abnormality; uncontrolled diabetes mellitus; ileus; pulmonary fibrosis; diarrhea; or bleeding tendency. Written informed consent was obtained from all the participants. Institutional Review Board approval was obtained for the study protocol at each institution.

**Treatment schedule.** CPT-11 was administered intravenously over 1.5 h on days 1 and 8 of each 3-week cycle. In the phase I study, the starting dose, 60 mg/m$^2$ (level 1), was increased in 20-mg/m$^2$ increments to 100 mg/m$^2$ (level 3). The dosage of 100 mg/m$^2$ was used as the upper limit because this is the approved dosage for NSCLC in Japan. Dose escalation was carried out on the basis of toxicities encountered during cycle 1 of therapy. A cohort of at least three patients was treated at each dose level. If none of the first three patients experienced DLTs, the dose was escalated to the next level. If one of the three patients experienced DLTs, additional patients were enrolled at the same dose level to a total of at least six patients. The MTD was defined as the dose level below the one at which at least 33% of the patients experienced DLTs, defined as febrile neutropenia (neutrophil count <1,000/mm$^3$ and fever ≥38.5°C), grade 4 neutropenia lasting >4 days, grade 3 or 4 leukopenia or anemia, grade 3 or 4 thrombocytopenia, or nonhematological toxicity (except electrolyte abnormality, nausea, anorexia, fatigue, or alopecia). A delay in the second CPT-11 administration of >7 days during the first cycle or >4 weeks between cycles was also categorized as a DLT. The RD was defined as the dose level below the MTD. If the MTD was not achieved at 100 mg/m$^2$, then 100 mg/m$^2$ was considered to be the RD because this is the dose that is used in clinical practice for nonelderly NSCLC patients.

**Evaluation.** In the phase II study, the efficacy and toxicity of CPT-11 monotherapy were evaluated at the RD. Tumor size was assessed by computed tomography at intervals of ≥6 weeks. Tumor response was categorized as CR, PR, stable disease, or progressive disease according to Response Evaluation Criteria in Solid Tumors.[23] Response rate was defined as CR plus PR. Disease control rate was defined as CR plus PR plus stable disease, including "shown no progression for 6 months." In order to be assigned a status of PR, the change in tumor size had to be confirmed by repeat assessments performed no less than 4 weeks after the criteria for response are first met. As for stable disease, it had to be confirmed by an assessment performed at least once after study enrollment but not earlier than 6 weeks. All tumor assessments were carried out by an investigator, and subsequently reviewed by the external response review committee. Toxicity was graded in accordance with the National Cancer Institute Common Toxicity Criteria, version 2 (ref. 24).

**Pharmacokinetic assay.** Venous blood for pharmacokinetic analysis was collected in sodium-heparinized and -evacuated tubes on day 1 of cycle 1, before CPT-11 infusion, at the end of infusion, and at 1, 2, 4, 7, and 24 h after infusion. The concentrations of unchanged CPT-11, SN-38, and SN-38G in plasma were determined using high-performance liquid chromatography,[25] and the $AUC_{0-inf}$ and peak plasma concentration were calculated using WinNonlin Version 4.1 (Pharsight, Mountain View, CA). The AUC ratio of SN-38G to SN-38 ($AUC_{SN-38G}/AUC_{SN-38}$) was calculated as a surrogate marker for *UGT1A1* activity involved in SN-38 glucuronidation.

**UGT1A1 genotyping assay.** *UGT1A1* polymorphisms were categorized into three groups: wild-type (**1/*1*), homozygous (**28/*28*, **6/*6*, **28/*6*), and heterozygous (**1/*28*, **1/*6*). Ando *et al.*[26] have reported that serious adverse events are associated with double-heterozygous (**28/*6*) as well as homozygous (**28/*28*, **6/*6*) polymorphisms. Sai *et al.*[27] also showed that the $AUC_{SN-38G}/AUC_{SN-38}$ ratio in patients with **28/*6* was similar to that in patients with **28/*28* and significantly lower than that in patients in the wild-type group.[22] On the basis of these two reports, we defined patients with *UGT1A1*28/*6*—along with those having the homozygous genotype of *UGT1A1*28/*28* or *UGT1A1*6/*6*—as the double-mutation group. Patients with the heterozygous genotype of either *UGT1A1*28* or *UGT1A1*6* were defined as the single-mutation group. Patients with no *UGT1A1*28* or *UGT1A1*6* mutations were defined as the no-mutation group.

Genomic DNA was extracted from the peripheral blood mononuclear cells of the 3 patients who received the RD in phase I and from 33 patients in phase II. One patient did not consent to analysis of *UGT1A1* genotype. For genotyping of *UGT1A1*6* polymorphism, products were amplified by direct PCR sequencing using the primer 5'-AAGTAGGAGAGGGCGAACC-3' as described in ref. 26. Genotyping for the *UGT1A1*28* polymorphism was performed by subjecting amplified products to gel electrophoresis and determining the product size by migration rate, depending on the number of bases.

**Statistical analysis.** In the phase II study, the primary end point was the response rate. Secondary end points included survival time and 1-year survival rate. For achieving the ±15% confidence interval under an expected response rate of 25%, a total sample size of 33 patients was calculated as being required for the study.

The 95% confidence interval for treatment response was estimated according to *F*-distribution. Overall survival and cumulative TTP were determined using the Kaplan–Meier method. Overall survival time was calculated from the first day of therapy until the death of the patient or the last day that the patient was known to be alive. TTP was defined as the period from the first day of treatment to the date of (i) first evidence of any toxicity requiring discontinuation of protocol therapy, (ii) progressive disease, or (iii) death.

The Cochran–Armitage trend test was used for analyzing the trend of grade 3/4 adverse events across polymorphism types. Spearman's rank correlation test was used to assess the relationship between the grade of hematological toxicity and the pharmacokinetic profile in the first cycle. In this assessment, the grade according to the National Cancer Institute Common Toxicity Criteria was used as the continuous variable. The association between pharmacokinetic profiles and the type of polymorphism was assessed using the Jonckheere–Terpstra test. All analyses were performed using the SAS software, version 8.2 (SAS Institute, Cary, NC).

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**CONFLICT OF INTEREST**

The authors declared no conflict of interest.



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# Phase II Study of Combination Therapy with S-1 and Irinotecan for Advanced Non–Small Cell Lung Cancer: West Japan Thoracic Oncology Group 3505

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**Abstract** **Purpose:** To evaluate the efficacy and toxicity of combination therapy with the oral fluoropyrimidine formulation S-1 and irinotecan for patients with advanced NSCLC.

**Experimental Design:** Chemotherapy-naive patients with advanced NSCLC were treated with i.v. irinotecan (150 mg/m$^2$) on day 1 and with oral S-1 (80 mg/m$^2$) on days 1 to 14 every 3 weeks.

**Results:** Fifty-six patients (median age, 63 years; range, 40-74 years) received a total of 286 treatment cycles (median, 5; range, 1-15). No complete responses and 16 partial responses were observed, giving an overall response rate of 28.6% [95% confidence interval (95% CI), 17.3-42.2%]. Twenty-four patients (42.9%) had stable disease and 12 patients (21.4%) had progressive disease as the best response. The overall disease control rate (complete response + partial response + stable disease) was thus 71.4% (95% CI, 57.8-82.7%). Median progression-free survival was 4.9 months (95% CI, 4.0-6.4 months), whereas median overall survival was 15 months. Hematologic toxicities of grade 3 or 4 included neutropenia (25%), thrombocytopenia (3.6%), and anemia (3.6%), with febrile neutropenia being observed in four patients (7.1%). The most common nonhematologic toxicities of grade 3 or 4 included anorexia (14.3%), fatigue (8.9%), and diarrhea (8.9%). There were no deaths attributed to treatment.

**Conclusions:** The combination of S-1 and irinotecan is a potential alternative option with a favorable toxicity profile for the treatment of advanced NSCLC. This nonplatinum regimen warrants further evaluation in randomized trials.

Non–small cell lung cancer (NSCLC) is the leading cause of death related to cancer worldwide (1). Platinum-based chemotherapy is the standard first-line treatment for advanced NSCLC based on the moderate improvement in survival and quality of life it confers compared with best supportive care alone (2–4). The poor outlook even for patients with advanced NSCLC who receive such treatment has prompted a search for new chemotherapeutic agents and combination regimens.

S-1 is an oral fluorinated pyrimidine formulation that combines tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate in a molar ratio of 1:0.4:1 (5). Tegafur is a prodrug that generates 5-fluorouracil (5-FU) in blood largely as a result of its metabolism by cytochrome *P*450 in the liver. CDHP increases the plasma concentration of 5-FU through competitive inhibition of dihydropyrimidine dehydrogenase, which catalyzes 5-FU catabolism (6). CDHP also attenuates the cardiotoxic and neurotoxic effects of 5-FU by reducing the production of fluoro-β-alanine, the main catabolite of 5-FU (7, 8). Oxonate reduces the gastrointestinal toxicity of 5-FU. After its oral administration, oxonate becomes distributed selectively to the small and large intestine, where it inhibits the phosphorylation of 5-FU to fluoropyrimidine monophosphate catalyzed by orotate phosphoribosyltransferase within gastrointestinal mucosal cells, thereby reducing the incidence of diarrhea (9). In a phase II trial of S-1 as a single agent for treatment of advanced NSCLC, a response rate of 22% and a median survival time of 10.2 months were obtained in 59 patients without prior chemotherapy (10). Few severe gastrointestinal or hematologic adverse events were reported (10). Moreover, a phase II trial of S-1 plus cisplatin in advanced NSCLC patients revealed a response rate of 47% and a median survival time of 11 months (11).

Irinotecan is an inhibitor of DNA topoisomerase I. It has shown activity as a single agent in first-line chemotherapy for advanced NSCLC (12). Weekly administration of irinotecan

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**Translational Relevance**

Non–small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide. The dismal outlook for patients with advanced NSCLC treated with available therapies has prompted a search for new and more effective chemotherapeutic agents and combination regimens. S-1 is a new oral fluorinated pyrimidine formulation that combines tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate and has been found to exhibit marked antitumor activity in recent clinical trials with cancer patients, including those with NSCLC. We have now examined the therapeutic efficacy and toxicity of the combination of S-1 and irinotecan in chemotherapy-naive patients with advanced NSCLC. We found this drug combination to be active, with a response rate of 28.6%, median progression-free survival of 4.9 months, and median overall survival of 15 months, values that compare favorably with those reported for phase III studies of standard platinum-based doublet chemotherapy. Furthermore, toxicities were manageable, and in most instances, treatment could be continued in the outpatient setting. Our data indicate that the combination of S-1 and irinotecan is a promising alternative for treatment of advanced NSCLC. This nonplatinum regimen warrants further evaluation in randomized trials.

(100 mg/m$^2$) for 3 weeks followed by 1 week of rest yielded a response rate of 20.5% and a median survival time of 10.6 months in 132 patients with advanced NSCLC (13).

S-1 and irinotecan have both shown single-agent activity against a wide range of solid tumors, including NSCLC, and the combination of these two agents has manifested synergistic effects in tumor xenograft models *in vivo* (14). A phase I study examined administration of irinotecan at a dose of 150 mg/m$^2$ on day 1 and of S-1 at 80 mg/m$^2$ per day from days 1 to 14 of a 21-day cycle (15); it found no difference in pharmacokinetic variables for the two drugs relative to the expected values for S-1 or irinotecan administered as single agents. A subsequent phase II study in patients with advanced colorectal cancer showed that this combination was well tolerated and had marked antitumor activity (16). The safety or effectiveness of the combination of S-1 and irinotecan in patients with advanced NSCLC has not previously been reported.

We now present the results of a multicenter phase II trial of S-1 in combination with irinotecan for patients with previously untreated advanced NSCLC. The aims of this study were to determine the objective tumor response rate, overall and progression-free survival, and toxicity profile for such treatment.

## Materials and Methods

***Patient eligibility.*** The criteria for patient eligibility included a diagnosis of NSCLC confirmed either histologically or cytologically, clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy, such as those with malignant pleural effusion, pleural dissemination, malignant pericardial effusion, metastatic lesions in the same lobe of the primary lesion, or involvement of contralateral mediastinal or hilar lymph nodes), measurable disease, no prior chemotherapy, an age range of 20 to 74 y, an Eastern Cooperative Oncology Group performance status of 0 or 1, and a projected life expectancy of at least 3 mo. Other eligibility criteria for organ function included a leukocyte count of ≥3,000/mm$^3$, a neutrophil count of ≥1,500/mm$^3$, a platelet count of ≥100,000/μL, a serum bilirubin concentration of ≤1.5 mg/dL, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels of ≤2.5 times the upper normal limit, a normal serum creatinine level, and either a partial pressure of arterial oxygen of ≥65 torr or a peripheral oxygen saturation of ≥92%. Main exclusion criteria included active concomitant of any malignancy, symptomatic brain metastasis, interstitial pneumonia, watery diarrhea, obstructive bowel disease, heart failure, uncontrolled diabetes mellitus, active infection, and a past history of drug allergy. Written informed consent was obtained from all patients, and the study protocol was approved by the institutional ethics committee of each of the participating institutions.

***Study design and treatment.*** This was a multicenter, open-label, single-arm, phase II study. The primary end point of the study was the response rate, which determined the sample size. We chose a 35% response rate as a desirable target level and a 20% response rate as uninteresting with an $\alpha$ error of 0.05 and a power of 0.8, resulting in a requirement for 50 patients. Allowing for a patient ineligibility rate of 10%, we planned to enroll 55 patients.

Each treatment cycle consisted of the oral administration of S-1 (40 mg/m$^2$) twice daily for 2 wk, with a 90-min i.v. infusion of irinotecan (150 mg/m$^2$) on day 1 followed by a drug-free interval of 1 wk. S-1 was available as capsules containing 20 or 25 mg of tegafur. Patients were assigned based on body surface area to receive one of the following oral doses of S-1 twice daily: 40 mg (body surface area < 1.25 m$^2$), 50 mg (1.25 ≤ body surface area < 1.50 m$^2$), or 60 mg (body surface area ≥ 1.50 m$^2$). Courses of treatment were repeated every 21 d until the occurrence of tumor progression or unacceptable toxicity, refusal of the patient, or a decision by the physician to stop treatment.

If laboratory variables changed after the start of treatment so that they no longer met the eligibility criteria for the study, subsequent courses of treatment were withheld until the abnormality had resolved. If the abnormality had not resolved within 43 d, the patient was excluded from the study. The doses of both S-1 and irinotecan were reduced in the event of any of the following toxicities during the previous treatment cycle: neutropenia of grade 4 for >7 d, febrile neutropenia, thrombocytopenia of grade ≥4, and nonhematologic toxicity of grade ≥3. S-1 was reduced in subsequent courses from 60, 50, or 40 mg twice daily to 50, 40, and 25 mg twice daily, respectively. The dose of irinotecan was reduced by 25 mg/m$^2$ for subsequent courses. Once lowered, the doses of S-1 and irinotecan were not increased.

***Evaluation.*** Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (17). Tumors were measured by computed tomography within 2 wk before the first cycle of treatment and then every 4 wk. Patients were evaluable for response if they had a baseline exam and at least one follow-up exam and had received at least one cycle of treatment. A central radiological review was done to determine the eligibility of patients and the response to treatment. Response was confirmed at least 4 wk (for a complete or partial response) or 6 wk (for stable disease) after it was first documented. Progression-free survival was defined as the time from registration until objective tumor progression or death. Patients whose disease had not progressed at the time of discontinuation of the study treatment continued to be assessed until progression was documented. If a patient died without documentation of disease progression, the patients was considered to have had tumor progression at the time of death, unless there was sufficient documented evidence to conclude otherwise. Overall survival was defined as the time from registration until death from any cause. Progression-free and overall survival as well as the 1-y survival rate were estimated by the Kaplan-Meier method.

**Table 1.** Characteristics of the 56 eligible patients

| Characteristic | No. patients |
|---|---|
| Median age, y (range) | 63 (40-74) |
| Sex | |
| Male | 46 (82%) |
| Female | 10 (18%) |
| Performance status (ECOG) | |
| 0 | 20 (36%) |
| 1 | 36 (64%) |
| Stage | |
| IIIB | 16 (29%) |
| IV | 40 (71%) |
| Histology | |
| Adenocarcinoma | 30 (54%) |
| Squamous cell carcinoma | 21 (38%) |
| Adenosquamous cell carcinoma | 1 (1.8%) |
| Large cell carcinoma | 1 (1.8%) |
| NSCLC, not specified | 3 (5.4%) |

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (version 3). All patients who received one dose of chemotherapy were assessable for toxicity. A clinical and laboratory assessment was done at least every 2 wk.

## Results

*Patient characteristics.* Between February and June 2006, a total of 59 patients were enrolled in the study at the 14 participating centers. Three patients did not receive treatment: one patient withdrew her consent, and two patients had a fall before treatment onset that resulted in a reduction in performance status. These three patients were thus not included in the analysis. The remaining 56 patients (46 men and 10 women) were eligible for the current analysis and their characteristics are summarized in Table 1. Their median age was 63 years, with a range of 40 to 74 years. Histologic analysis revealed that 30 patients (54%) had adenocarcinoma and 21 patients (38%) had squamous cell carcinoma. Forty patients (71%) had stage IV disease and the other 16 patients had stage IIIB disease (including 12 patients with malignant pleural effusion).

*Treatment administered.* Patients received a median of five cycles of treatment (range, 1-15), with 37 patients (66%) completing at least four cycles. Overall, 286 cycles of chemotherapy were delivered. The mean relative dose intensities of S-1 and irinotecan were 91% and 98%, respectively. Dose reductions were uncommon and were necessary according to the study protocol in only eight cycles (2.8% of total cycles) because of diarrhea in three patients, anorexia in two patients, vomiting in two patients, and an increase in serum ALT and AST levels in one patient. Treatment administration was delayed for at least 1 week because of toxicity in 12 cycles (4.2% of total cycles); the major causes of delayed administration were insufficient bone marrow function (six cycles with a leukocyte count of $<3{,}000/mm^3$ and one cycle with a platelet count of $<100{,}000/\mu L$) and nonhematologic toxicity (two cycles with fever in the absence of neutropenia, two cycles with an increase in serum ALT and AST levels, and one cycle with diarrhea).

**Table 2.** Overall response rate (Response Evaluation Criteria in Solid Tumors criteria) by independent radiologic assessment

| Response | No. patients (%) |
|---|---|
| Complete response | 0 (0) |
| Partial response | 16 (28.6) |
| Overall response | 16 (28.6; 95% CI, 17.3-42.2) |
| Stable disease | 24 (42.9) |
| Disease progression | 12 (21.4) |
| Not evaluable | 4 (7.1%) |

*Response and survival.* Four patients were not evaluable for response: three patients withdrew from the study after one treatment cycle and one patient did not have a measurable target lesion. There were 16 partial responses and no complete responses, yielding an overall response rate of 28.6% (Table 2). Twenty-four patients (42.9%) had stable disease, yielding an overall disease control rate (complete response + partial response + stable disease) of 71.4% [95% confidence interval (95% CI), 57.8-82.7%]. Twelve patients (21.4%) had progressive disease as the best response.

All 56 treated patients were assessable for progression-free survival and overall survival. With a median follow-up time of 14.9 months (range, 1.4-20.1 months), 25 patients were still alive. The progression-free survival curve is shown in Fig. 1; the median progression-free survival was 4.9 months (95% CI, 4.0-6.4 months). The curve for overall survival is shown in Fig. 2; the median overall survival time was 15 months (95% CI could not be estimated) and the 1-year survival rate was 63% (95% CI, 50-75%). No correlation was apparent between overall survival and sex, age, histology, disease stage, or smoking status.

*Toxicity.* The adverse events observed for all 56 treated patients are summarized in Table 3. The most frequently observed hematologic toxicity of grade 3 or 4 was neutropenia (14 cases, 25%). Four patients (7.1%) developed febrile neutropenia. Anemia or thrombocytopenia of grade 3 or 4 was less frequent, each occurring in 3.6% of patients. Nonhematologic toxicities were generally mild in intensity. The most common nonhematologic toxicities of grade 3 or 4 were anorexia (14.3%), fatigue (8.9%), diarrhea (8.9%), vomiting (3.6%), and an increase in serum ALT or AST levels (3.6%). Treatment was discontinued because of toxicity in only two of

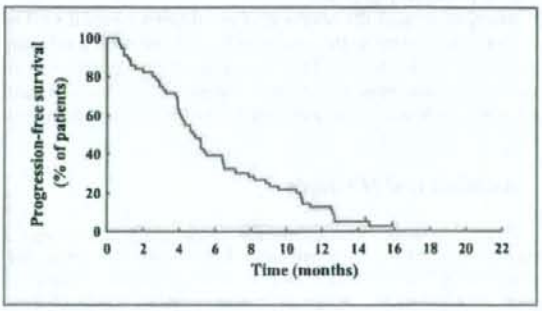


Fig. 1. Kaplan-Meier analysis of progression-free survival for all 56 treated patients.

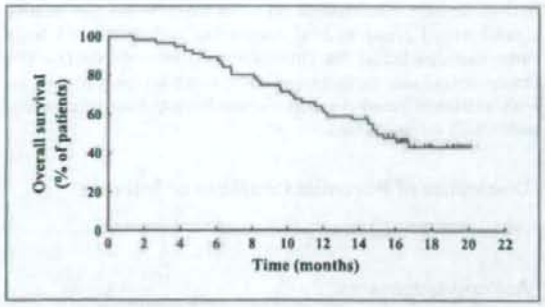


Fig. 2. Kaplan-Meier analysis of overall survival for all 56 treated patients.

the 56 patients (3.6%): in one patient because of pneumonitis (grade 3) and in the other because of prolonged anorexia (grade 3) and fatigue (grade 3). The patient with pneumonitis developed fever with hypoxemia after the fourth course of treatment. A computed tomographic scan of the chest revealed new ground-glass opacities distributed diffusely in both lungs. The patient responded well to steroid therapy and improved. No treatment-related deaths were observed.

## Discussion

Platinum-based doublet chemotherapy is the standard of care for most patients with advanced NSCLC (2–4). However, there continues to be reluctance on the part of both patients and treating physicians to accept the toxicity of platinum-based therapy given the associated small gain in survival. Active therapies with improved toxicity profiles are clearly needed in this setting. Since the introduction of active third-generation agents (docetaxel, paclitaxel, gemcitabine, vinorelbine, and irinotecan), many clinical trials have been undertaken to evaluate nonplatinum regimens based on these drugs in the hope that platinum analogues could be eliminated from the treatment of advanced NSCLC. A recent meta-analysis showed that these newer nonplatinum regimens are valid options for the treatment of advanced NSCLC because of their shown activity and good toxicity profiles (18). Currently, however, there is no single best treatment regimen for advanced NSCLC.

As first-line chemotherapy for advanced NSCLC, the oral fluoropyrimidine formulation S-1 administered as a single agent showed a response rate of 22% and a median survival time of 10.2 months with toxicities that were generally mild (10). Combinations of S-1 with other active agents with a different mechanism of action are being investigated with the aim of achieving a greater clinical benefit. Irinotecan and fluoropyrimidines were shown not to induce cross-resistance in both experimental and clinical settings (19). Preclinical studies have also found that the combination of irinotecan and 5-FU has antitumor activities that are additive to synergistic (20). Furthermore, a possible molecular mechanism for synergistic cytotoxicity of S-1 and irinotecan has been suggested by the observation that irinotecan reduces thymidylate synthetase activity in tumor xenografts and thereby facilitates the antitumor effect of S-1 (14). Recent phase II studies have shown that combination treatment with S-1 and irinotecan is highly active with acceptable toxicity in patients with advanced colorectal cancer or gastric cancer (16, 21). However, the activity of this combination in patients with NSCLC has not previously been documented.

We have now assessed the efficacy and safety of combined treatment with S-1 and irinotecan in patients with previously untreated advanced NSCLC. We found the combination to be active, with a response rate of 28.6%, median progression-free survival of 4.9 months, median overall survival of 15 months, and 1-year survival rate of 63%. Previous phase III studies of platinum-based doublets for the treatment of advanced NSCLC showed response rates of 17% to 33%, a median time to progression or progression-free survival of 3 to 5 months, and a median overall survival time of 7 to 14 months (22–25). Although there are limitations to comparisons of the results from different studies, the efficacy data in our study compare favorably with those reported in these previous phase III studies of platinum-based doublets.

The S-1-irinotecan regimen was well tolerated in the patients of the present study. With regard to hematologic toxicity, neutropenia of grade 3 or 4 occurred in only 25% of all treated patients without the prophylactic administration of granulocyte colony-stimulating factor. Anemia and thrombocytopenia of grade 3 or 4 were each observed in only two patients (3.6%). These results compare favorably with the toxicity profiles reported for platinum-based combinations in previous studies with NSCLC patients, in which higher frequencies of neutropenia (~80%), anemia (~20%), and thrombocytopenia (~23%) of grade 3 or 4 were observed (22–24). The only nonhematologic toxicity of grade 3 or 4 encountered in >10% of patients in the present study was anorexia (14.3%). Although irinotecan and S-1 have each been shown to increase the frequency of severe diarrhea, the incidence of diarrhea of grade 3 in the present study was only 8.9%, consistent with the findings of a recent phase II study of the combination of S-1 and irinotecan administered according to the same doses and schedule in patients with advanced colorectal cancer (16).

**Table 3.** Toxicity for all 56 treated patients according to the National Cancer Institute Common Toxicity Criteria (version 3)

| Toxicity | Grade | | | | Grade ≥3 (%) |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| Leukopenia | 9 | 10 | 5 | 0 | 8.9 |
| Neutropenia | 1 | 7 | 12 | 2 | 25.0 |
| Anemia | 31 | 19 | 1 | 1 | 3.6 |
| Thrombocytopenia | 23 | 2 | 2 | 0 | 3.6 |
| Febrile neutropenia | NA | NA | 4 | 0 | 7.1 |
| Anorexia | 25 | 10 | 8 | 0 | 14.3 |
| Fatigue | 18 | 12 | 4 | 1 | 8.9 |
| Diarrhea | 12 | 11 | 5 | 0 | 8.9 |
| Nausea | 27 | 11 | 1 | 0 | 1.8 |
| Vomiting | 12 | 4 | 2 | 0 | 3.6 |
| Stomatitis | 7 | 6 | 0 | 0 | 0 |
| Rash | 8 | 6 | 0 | 0 | 0 |
| Hyperbilirubinemia | 12 | 6 | 0 | 0 | 0 |
| Elevation of AST/ALT | 18 | 3 | 2 | 0 | 3.6 |
| Elevation of creatinine | 2 | 1 | 0 | 0 | 0 |
| Pneumonitis | 1 | 0 | 1 | 0 | 1.8 |

Abbreviation: NA, not applicable.