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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

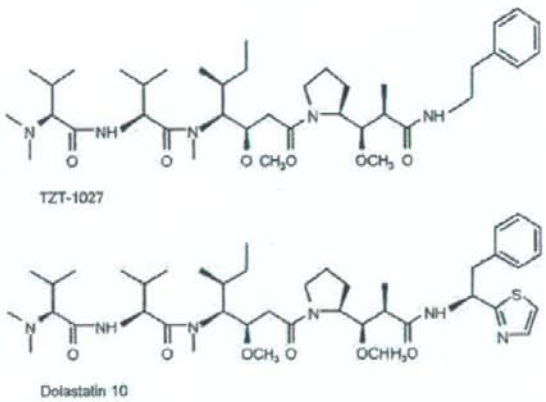


**Fig. 1.** Structural formulae of TZT-1027 and dolastatin 10.

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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.

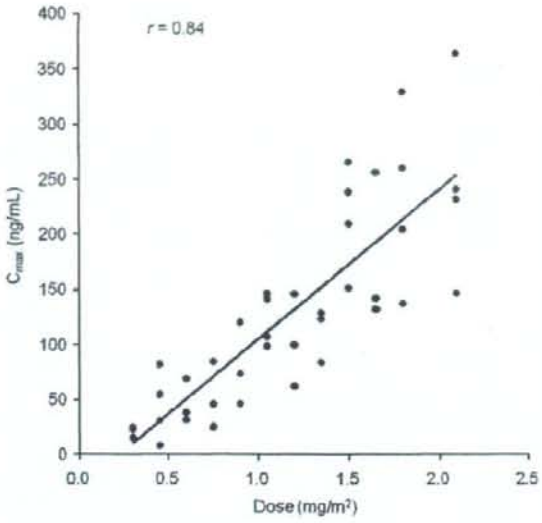


Fig. 2. Correlation between dose and maximum concentration ($C_{max}$) at the first administration.

## 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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# Vinorelbine plus gemcitabine followed by docetaxel versus carboplatin plus paclitaxel in patients with advanced non-small-cell lung cancer: a randomised, open-label, phase III study

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## Summary

**Background** Platinum-containing two-drug combinations improve survival and cancer-related symptoms in patients with advanced non-small-cell lung cancer (NSCLC). However, survival benefit is modest and platinum-containing regimens cause substantial toxic effects. We did a prospective randomised open-label phase III study to compare an experimental platinum-free, triplet, sequential regimen of vinorelbine plus gemcitabine followed by docetaxel with the standard platinum-containing, doublet regimen paclitaxel plus carboplatin in patients with advanced NSCLC.

**Methods** Between March, 2001, and April, 2005, patients with stage IIIB (positive pleural effusion) or IV NSCLC, performance status 0 to 1, and adequate organ function, were randomly assigned to experimental treatment or to standard treatment. Randomisation was done centrally by use of a dynamic balancing algorithm. Patients were stratified by weight loss, lactate dehydrogenase concentration, and disease stage. Patients in the experimental group were scheduled to receive intravenous vinorelbine (25 mg/m²) plus gemcitabine (1000 mg/m²) on days 1 and 8 every 21 days for three cycles, followed by intravenous docetaxel (60 mg/m²) on day 1 every 21 days for three cycles. Patients in the standard group were scheduled to receive intravenous paclitaxel (225 mg/m²) plus carboplatin (area under the curve=6) for 3 h on day 1, every 21 days for six cycles. The primary endpoint was overall survival, and secondary endpoints were progression-free survival, response, and toxic effects. Analyses were by intention to treat. This trial is registered with ClinicalTrials.gov, number NCT00079287.

**Findings** Of the 401 patients enrolled and randomised in the trial, five patients in the experimental group and three in the standard group were ineligible for analysis; thus 196 patients in the experimental group and 197 in the standard group were included in analyses. Patient characteristics were well-balanced between the two groups with regard to major prognostic factors. Median overall survival was 13·6 months (range 12·0–16·4) in the experimental group versus 14·1 months (11·9–17·5) in the standard group (p=0·97). 49 of 196 patients (25%) in the experimental group had a partial response compared with 73 of 197 patients (37%) in the standard group (p=0·012). There were no complete responses. Median progression-free survival was 5·5 months (95% CI 4·9–6·3) in the experimental group compared with 5·8 months (5·3–6·1) in the standard group (p=0·74). The incidence of grade 3 and 4 neutropenia, neuropathy, arthralgia, and myalgia was lower in the experimental group than in the standard group, although the incidence of pulmonary toxic effects was higher.

**Interpretation** Although platinum-containing regimens remain the standard treatment for advanced NSCLC, non-platinum regimens could provide equivalent efficacy with a different toxicity profile.

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See Reflection and Reaction page 1116

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## Introduction

Lung cancer is the leading cause of cancer death worldwide and a growing concern in an ageing society.[1] Non-small-cell lung cancer (NSCLC) accounts for 85% of lung cancer histology. Several third-generation agents are available for the treatment of NSCLC, including docetaxel, paclitaxel, gemcitabine, and vinorelbine, and the combination of one of these agents with a platinum compound (ie, cisplatin or carboplatin) has been considered the standard treatment option for advanced NSCLC on the basis of several randomised studies.[2–4]

Combination chemotherapy containing cisplatin has substantial toxic effects, including vomiting and renal impairment, making treatment of elderly patients or outpatients with this agent difficult. Carboplatin has fewer toxic effects than cisplatin, although it still causes vomiting and myelosuppression. Non-platinum, two-drug combinations using third-generation agents have shown an equivalent outcome compared with platinum-containing regimens in patients with NSCLC.[5,6] In the newer non-platinum combinations, vinorelbine plus gemcitabine has shown activity and a good toxicity profile.[7,8] Vinorelbine plus gemcitabine has also shown

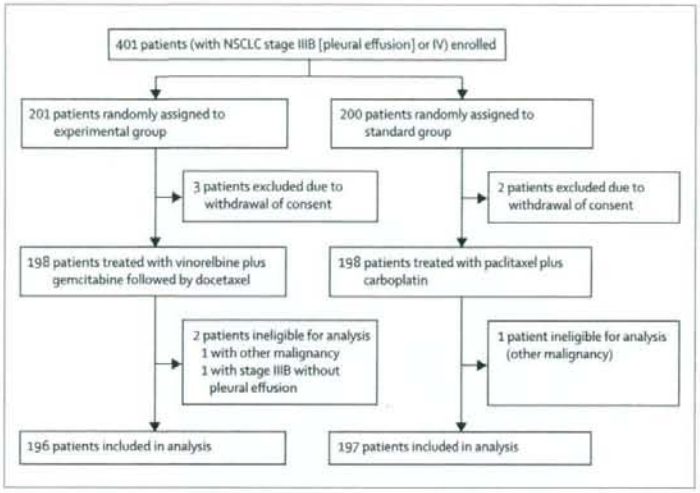


*Figure 1:* **Trial profile**

significantly better survival than vinorelbine plus carboplatin in a randomised trial.[9] Docetaxel is active against NSCLC and shows survival benefit in both chemotherapy naive patients, and patients previously treated with chemotherapy.[10,11] Docetaxel might be effective against subpopulations of lung-cancer cells (clones) resistant to first-line chemotherapy,[12] and some residual resistant clones might be eradicated by sequential administration of docetaxel before they grow and relapse.

We previously did a phase II trial of a sequential, non-platinum, triplet combination consisting of three cycles of vinorelbine (25 mg/m²) plus gemcitabine (1000 mg/m²) followed by three cycles of docetaxel (60 mg/m²). The resulting outcomes—21 of 44 patients [47·7%] had partial response, median overall survival was 15·7 months, and 1-year survival was 59%—were encouraging.[13] Therefore, we designed this phase III trial to identify whether vinorelbine plus gemcitabine followed by docetaxel offers better survival than the standard paclitaxel plus carboplatin regimen.

## Methods

### Patients

All patients enrolled in this study had histologically or cytologically confirmed NSCLC (categorised as squamous cell, large cell, adenocarcinoma, or NSCLC not otherwise specified), with stage IIIB (positive pleural effusion) or stage IV (no brain metastases) disease according to the International Staging System. Other eligibility criteria included: measurable or assessable disease; Eastern Cooperative Oncology Group performance status of 0 or 1; neutrophil count of at least $1·5\times10^9$ cells per L; platelet count above institutional lower limits of normal; haemoglobin concentration of a least 90 g/L; serum creatinine concentrations less than the institutional upper limit of normal (ULN) and a calculated or measured creatinine clearance of at least 50 mL/min; bilirubin, aspartate aminotransferase (AST) or alanine aminotransferase (ALT), and alkaline phosphatase concentrations of 2×ULN or less, or 4×ULN or less if the patient had liver metastases. Patients were excluded if they had grade 2 or higher peripheral neuropathy or previous chemotherapy or biological therapy. Stratification at the time of registration was by weight loss (<5% *vs* ≥5% from measurements taken 6 months before enrolment), disease stage (IIIB *vs* IV), and serum lactate dehydrogenase concentration (normal *vs* abnormal). All patients provided written informed consent. This protocol was approved by the institutional review boards of all participating institutions and of the data centre (Translational Research Informatics Centre, Kobe, Hyogo, Japan).

### Treatment

Patients were randomly assigned to either the experimental regimen or the standard regimen (figure 1). Central randomisation to each group was applied by use of a dynamic balancing algorithm to obtain a good balance between groups in terms of the stratified factors. Randomisation was done centrally by members of the Japan Multi-National Trial Organisation (JMTO) data centre at the Translational Research Informatics Centre, Kobe, Hyogo, Japan. After obtaining written informed consent, patients were registered via fax, and, if eligibility was confirmed, patients were allocated to one of the treatment groups by computer. Neither patients nor physicians were blinded to allocated treatment. In the experimental group, patients were assigned intravenous vinorelbine (25 mg/m²) and gemcitabine (1000 mg/m²) on days 1 and 8 every 21 days for three cycles. Single-agent docetaxel (60 mg/m²) was subsequently given intravenously on day 1 every 21 days for a further three cycles. Premedications, such as antiemetic agents or corticosteroids, were given as needed. All patients were assigned 8 mg of dexamethasone orally before docetaxel administration. The standard regimen consisted of intravenous paclitaxel (225 mg/m²) plus carboplatin (area under the curve [AUC]=6) for 3 h on day 1. Treatment was repeated every 3 weeks for six cycles. Patients in the standard group were assigned premedication with dexamethasone, diphenhydramine, and ranitidine or cimetidine. Use of additional antiemetics was left to the physician's judgment. Erythropoietin-stimulating agents were not approved in Japan for chemotherapy-related anaemia, and were thus not used. G-CSF was permitted at any time during the study except for prophylactic use in both groups. In the absence of progressive disease or intolerable toxic effects, patients in both groups were treated for six cycles.

Complete blood-cell count was checked either on the treatment day or the day before planned treatment during

| | Experimental group (N=196) | Standard group (N=197) |
|---|---|---|
| Median age (range), years | 64 (39-81) | 65 (33-81) |
| Sex, n (%) | | |
| Men | 143 (73) | 136 (69) |
| Women | 53 (27) | 61 (31) |
| Smoking, n (%) | | |
| Non-smokers | 47 (24) | 51 (26) |
| Former smokers | 52 (27) | 55 (28) |
| Smokers | 88 (45) | 82 (42) |
| Unknown | 9 (5) | 9 (5) |
| Histology, n (%) | | |
| Squamous cell | 46 (23) | 30 (15) |
| Adenocarcinoma | 130 (66) | 149 (76) |
| Other | 20 (10) | 18 (9) |
| Stage, n (%) | | |
| IIIB | 33 (17) | 35 (18) |
| IV | 163 (83) | 162 (83) |
| Performance status, n (%) | | |
| 0 | 79 (40) | 78 (40) |
| 1 | 117 (60) | 119 (60) |
| Weight loss, n (%) | | |
| <5% | 160 (82) | 161 (82) |
| ≥5% | 36 (18) | 36 (18) |
| LDH concentration, n (%) | | |
| Normal | 141 (72) | 142 (72) |
| Abnormal | 55 (28) | 55 (28) |

Experimental treatment=vinorelbine and gemcitabine followed by docetaxel. Standard treatment=paclitaxel and carboplatin. LDH=lactate dehydrogenase.

*Table 1:* **Characteristics of assessable patients**

| | Treatment | | p value |
|---|---|---|---|
| | Experimental group (N=196) | Standard group (N=197) | |
| Tumour response, n (%) | | | |
| Complete | 0 (0) | 0 (0) | – |
| Partial | 49 (25) | 73 (37) | – |
| No change | 90 (46) | 76 (39) | – |
| Progressive disease | 32 (16) | 20 (10) | – |
| Non assessable | 25 (13) | 28 (14) | – |
| Overall response (95% CI), % | 25 (19·1-31·7) | 37·1 (30·3-44·2) | 0·012 |
| Progression-free survival (PFS) | | | |
| Median (95% CI), months | 5·5 (4·9-6·3) | 5·8 (5·3-6·1) | – |
| 1-year PFS | 15·4% | 12·0% | – |
| 2-year PFS | 6·7% | 5·8% | – |
| HR* (95% CI) | 0·966 (0·79-1·19) | 1† | 0·742 |
| Overall survival (OS) | | | |
| Median (95% CI), months | 13·6 (12·0-16·4) | 14·1 (11·9-17·5) | |
| 1-year OS | 57·1% | 56·6% | |
| 2-year OS | 28·7% | 30·1% | |
| HR* (95% CI) | 0·966 (0·78-1·27) | 1† | 0·974 |

Experimental treatment=vinorelbine and gemcitabine followed by docetaxel. Standard treatment=paclitaxel and carboplatin. HR=hazard ratio. *Adjusted for disease stage, weight loss, and lactate dehydrogenase concentration. †Reference group.

*Table 2:* **Treatment outcomes**

each of the cycles. During the vinorelbine plus gemcitabine cycles, serum AST and ALT were assessed. If neutrophil count was less than $1·5\times10^9$ cells per L, platelet count less than $100\times10^9$/L, or AST or ALT more than 100 IU/L on day 1 of each cycle, vinorelbine plus gemcitabine administration was delayed by a week. If neutrophil count was less than $1·0\times10^9$ cells per L, platelet count less than $70\times10^9$/L, or AST or ALT more than 100 IU/L, vinorelbine plus gemcitabine was not given on day 8. Docetaxel administration was delayed by a week when the neutrophil count was less than $1·5\times10^9$ cells per L or platelet count was less than $75\times10^9$/L on day 1 of each cycle. Treatment dose was decreased to 80% if grade 4 leucocytopenia or neutropenia were present, if platelet count was less than $20\times10^9$/L, or if other unacceptable toxic effects, including grade 3 neutropenic fever or grade 3 or higher non-haematological toxic effects, were present during the preceding treatment cycle. The first cycle of docetaxel was given at full dose even if toxic effects were noted in the previous vinorelbine plus gemcitabine cycles. The dose of docetaxel was decreased to 80% only when toxic effects were noted subsequent to docetaxel administration.

Dose modifications for paclitaxel and carboplatin were consistent with the Southwest Oncology Group Trial (SWOG) S0003.[14] In brief, if the neutrophil nadir was less than $0·5\times10^9$ cells per L, the platelet nadir less than $50\times10^9$/L, or the patient had febrile neutropenia, the dose of carboplatin was decreased to an AUC of 5. If a patient developed grade 2 neurotoxicity at any time during a cycle, the dose of paclitaxel was decreased to 200 mg/m². Chest pain or arrhythmia during infusion resulted in immediate discontinuation and patient assessment. Patients with symptomatic arrhythmias, atrioventricular block (except first degree), or a documented ischaemic event discontinued the study.

### Pretreatment and follow-up assessments

Baseline assessment and staging consisted of a physical examination; chest radiography; brain, chest, and abdominal CT or MRI; complete blood-cell count and serum chemistry; a bone scan if clinically indicated; and an electrocardiogram. A physical examination and complete blood work-up were done before each cycle. Scans or radiographs used to assess response were obtained every two cycles. Once treatment was finished, a follow-up assessment was done every 3 months.

### Response and toxicity criteria

Patients were assessed every two cycles for an objective response, according to the Response Evaluation Criteria in Solid Tumors.[15] Confirmed responses required repeat measurements at a minimum of 4 weeks. Responses

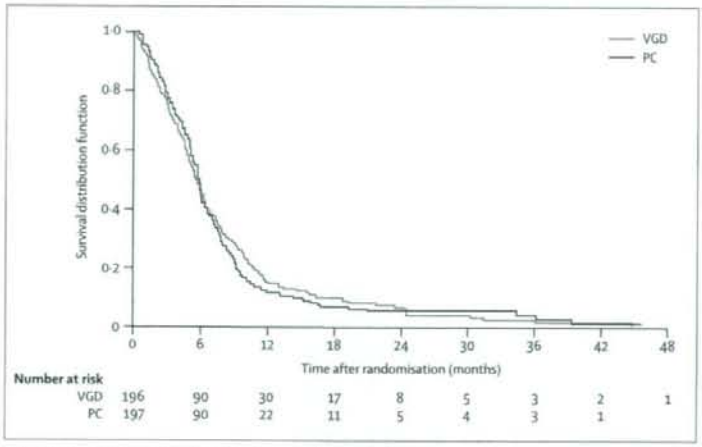


*Figure 2:* **Kaplan-Meier estimates of progression-free survival**
VGD=vinorelbine and gemcitabine followed by docetaxel. PC=paclitaxel and carboplatin.

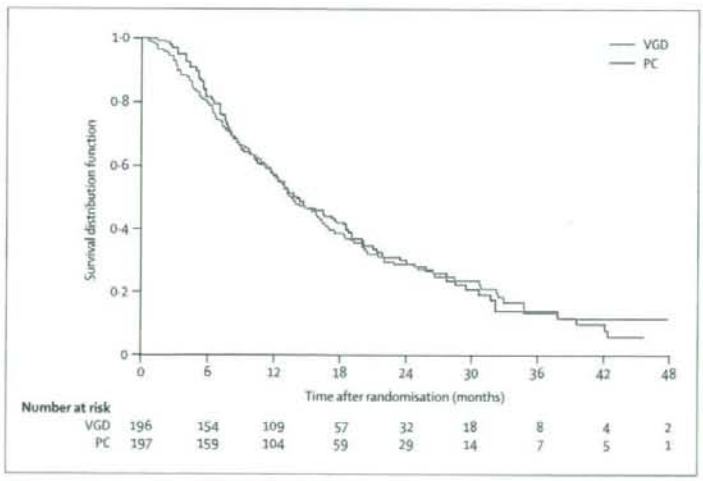


*Figure 3:* **Kaplan-Meier estimates of overall survival**
VGD=vinorelbine and gemcitabine followed by docetaxel. PC=paclitaxel and carboplatin.

were assessed by attending physicians, and a central review was not done for response evaluation. Grading of toxic effects was done in accordance with the US National Cancer Institute Common Toxicity Criteria, version 2.0.[16] Patients were removed from the study as a result of progression of disease, toxic effects, or at the patient's request. Appropriate procedures were undertaken for all unexpected or fatal toxic effects.

### Statistical analyses

The primary objective of this study was to determine whether the experimental regimen (vinorelbine and gemcitabine followed by docetaxel) produced a survival advantage compared with the standard regimen (paclitaxel plus carboplatin) in patients with advanced NSCLC. The primary endpoint was overall survival. Secondary endpoints were progression-free survival, response, and toxic effects. Analyses were done by intention to treat.

We calculated our sample size based on an anticipated median overall survival of 8·0 months in the standard group[17] and an expected 40% increase in median overall survival (to 11·2 months) in the experimental group (chosen on the basis of the findings of our previous phase II study of the experimental regimen, which showed a median survival of 15·7 months[13]). This difference in median overall survival is equivalent to a 2-year survival of 12·5% in the standard group compared with 22·6% in the experimental group (HR 0·714). On the basis of these assumptions, we calculated that we would need 200 patients per group to detect such a difference, with a power of 0·85 using a two-sided Log-rank test at a significance level of 0·05. Survival curves were estimated by the product-limit method and compared by use of the Log-rank test, stratified by predetermined prognostic factors.[18,19] Cox regression analysis was used to estimate hazard ratios (HRs) for overall survival and progression-free survival.[20] Fisher's exact test was used to test the difference between treatment groups for response and toxic effects. Unless otherwise indicated, all reported p values are two sided. A planned interim analysis was done by the data monitoring committee when 300 patients had been enrolled, with early study termination to occur if the null hypothesis of no difference for the experimental group was rejected at the one-sided 0·0025 level.

### Role of the funding source

This trial was sponsored by the JMTO, and members of JMTO were responsible for the design, set-up, and data collection of the trial. All authors had full access to the raw data in the study and the corresponding author had final responsibility for the decision to submit for publication.

## Results

401 patients were enrolled in the trial between March 29, 2001, and April 13, 2005, from 45 institutions in Japan. Eight patients (2·0%) were ineligible for analysis: five withdrew informed consent, two had other malignancies, and one had stage IIIB disease without pleural effusion. Thus, 393 patients were eligible for analysis, 196 in the experimental group and 197 in the standard group. Patient characteristics are listed in table 1. Although the proportion of patients with adenocarcinoma histology was higher in the standard group than in the experimental group, there was no significant difference between the two groups.

Overall response (ie, best confirmed response during study treatment) was 25% (49 of 196 patients with PR) in the experimental group and 37% (73 of 197 patients with PR) in the standard group (p=0·012; table 2). No patients

had a complete response. 17 of 196 patients (8·7%) had a partial response after three cycles of treatment with vinorelbine plus gemcitabine, as reported by the attending physicians. The difference in response between the two groups was larger in patients with squamous-cell histology (15% [seven of 46 patients] in the experimental group *vs* 63% [19 of 30 patients] in the standard group; p<0·0001) than in patients with adenocarcinoma histology (26% [34 of 130 patients] in the experimental group *vs* 32% [47 of 149 patients] in the standard group; p=0·356), and the proportion of patients with squamous-cell histology who had progressive disease was higher in the experimental group (33% [15 of 46]) than in the standard group (13% [four of 30]). The comparison of response by the Mantel-Haenszel test (adjusted for the distribution imbalance of histology) was also significant (p=0·007). Median progression-free survival was 5·5 months (95% CI 4·9–6·3) in the experimental group and 5·8 months (5·3–6·1) in the standard group (p=0·742; figure 2 and table 2), and median overall survival was similar between groups, at 13·6 months (12·0–16·4) in the experimental group and 14·1 months (11·9–17·5) in the standard group (p=0·97; figure 3 and table 2). For overall survival, the HR was 1·06 (95% CI 0·80–1·41; p=0·688) in patients with adenocarcinoma histology and 0·94 (0·56–1·57; p=0·802) in patients with squamous-cell histology. For progression-free survival, the corresponding values were 0·98 (0·77–1·25; p=0·848) and 1·04 (0·65–1·68; p=0·861), respectively. Thus, there was no interaction between treatment and histology ($p_{interaction}$=0·794 and 0·773, respectively). In terms of other factors (ie, age, sex, smoking history, Eastern Cooperative Onology Group performance status, weight loss, disease stage, and lactate dehydrogenase concentration), there were no significant interactions between treatment and factor (data not shown).

196 patients in the experimental group and 197 patients in the standard group were assessable for toxic effects (table 3). The standard regimen resulted in a significantly increased incidence of grade 3 or 4 neutropenia, neuropathy, arthralgia, and myalgia compared with the experimental regimen. However, the incidence of pulmonary toxic effects was significantly higher in the experimental group than in the standard group. Only one patient assigned the standard regimen developed grade 1 to 4 drug-related pneumonitis compared with 17 patients assigned the experimental regimen (p<0·0001). Of these 17 patients, 14 developed pneumonitis during vinorelbine plus gemcitabine treatment, whereas the remaining three patients had pneumonitis during docetaxel treatment. Almost all patients improved with corticosteroids. There was no significant difference in neutropenic fever, anaemia, and thrombocytopenia between the two groups. Treatment-related death occurred in two patients. One patient had pneumonitis after the fourth cycle of the experimental regimen. Despite improvement of pneumonitis with corticosteroids, steroid-induced exacerbation of hepatitis C, followed by deterioration of pneumonitis, resulted in death due to respiratory failure. Another patient died of pneumonia after the fourth cycle of the experimental regimen.

| | Treatment | | p value |
|---|---|---|---|
| | Experimental group (N=196), n (%) | Standard group (N=197), n (%) | |
| **Haematological toxic effects** | | | |
| Leucopenia | 79 (40·3) | 89 (45·2) | 0·359 |
| Neutropenia | 116 (59·2) | 137 (69·5) | 0·035 |
| Neutropenic fever | 23 (11·7) | 24 (12·2) | 1·000 |
| Thrombocytopenia | 6 (3·1) | 14 (7·1) | 0·106 |
| Anaemia | 9 (4·6) | 16 (8·1) | 0·214 |
| **Non-haematological toxic effects** | | | |
| Allergic reaction | 0 (0) | 4 (2·0) | 0·123 |
| Fatigue | 10 (5·1) | 14 (7·1) | 0·528 |
| Constipation | 3 (1·5) | 7 (3·6) | 0·337 |
| Nausea | 8 (4·1) | 17 (8·6) | 0·097 |
| Vomiting | 2 (1·0) | 6 (3·0) | 0·284 |
| Anorexia | 16 (8·2) | 22 (11·2) | 0·394 |
| Neuropathy (motor) | 1 (0·5) | 8 (4·1) | 0·037 |
| Neuropathy (sensory) | 1 (0·5) | 19 (9·6) | <0·0001 |
| Arthralgia | 0 (0) | 17 (8·6) | <0·0001 |
| Myalgia | 0 (0) | 14 (7·1) | <0·0001 |
| Dyspnoea | 11 (5·6) | 3 (1·5) | 0·032 |
| Drug-related pneumonitis | 9 (4·6) | 1 (0·5) | 0·011 |
| Pneumonia | 14 (7·1) | 1 (0·5) | 0·0004 |
| Liver dysfunction | 6 (3·1) | 5 (2·5) | 0·771 |

Experimental treatment=vinorelbine and gemcitabine followed by docetaxel. Standard treatment=paclitaxel and carboplatin.

*Table 3:* **Grade 3 and 4 toxic effects occurring in ≥3% of patients in at least one group**

The median number of cycles delivered was six (range one to six) for the experimental regimen and four (one to six) for the standard regimen. There was no difference in the number of patients receiving four or more cycles between the groups. The proportion of patients receiving six cycles was significantly higher in the experimental group (97 of 196 [49%]), than in the standard group (57 of 197 [29%]; p<0·0001). The proportion of patients who needed a dose reduction was 29% (57 or 196) in the experimental group and 51% (100 of 197) in the standard group (p<0·0001).

128 of 196 patients (65%) in the experimental group and 133 of 197 patients (68%) in the standard group received post-protocol chemotherapy of any type. In the experimental group, 44 of 196 patients (22%) received paclitaxel plus carboplatin, 35 (18%) received gefitinib, 17 (9%) received additional docetaxel, nine (5%) received vinorelbine plus gemcitabine, and 23 (12%) received other regimens. In the standard group, 40 of 197 patients (20%) received gefitinib, 38 (19%) received docetaxel, 23 (12%) received vinorelbine plus gemcitabine, six (3%) received additional paclitaxel plus carboplatin, and 26 (13%)

received other regimens as second-line chemotherapy. In both groups, around 35% of patients received more than one additional line of chemotherapy. Gefitinib was used as any line treatment after the study protocol in 75 of 196 (38%) patients in the experimental group and in 78 of 197 (40%) patients in the standard group, respectively.

## Discussion

This study assessed whether a non-platinum, sequential, triplet (vinorelbine and gemcitabine followed by docetaxel) regimen[13,21] produced a survival advantage compared with a standard platinum-containing regimen in patients with advanced NSCLC. The experimental regimen did not result in better overall survival than the standard regimen of paclitaxel plus carboplatin.

Although some baseline imbalances existed in terms of histology between the two groups, histology (adenocarcinoma *vs* others) was not an independent prognostic factor for overall survival (adjusted HR 0·96 [95% CI 0·73–1·27]; p=0·80) and the effect of imbalance on the endpoints was small. The proportion of patients receiving six cycles of treatment was higher in the experimental group than in the standard group; however, a median number of four cycles (range one to six) with standard treatment is the usual standard of care, and therefore, it is unlikely that the difference in number of cycles affected the outcomes of the study.

Although there was no difference between the regimens in terms of efficacy, the experimental, non-platinum regimen did show some benefits compared with the platinum-containing regimen. The regimen was well tolerated and 97 of 196 (49%) patients completed the planned six cycles. Furthermore, the incidence of grade 4 neutropenia, grade 3 or 4 neuropathy, arthralgia, and myalgia was significantly higher in the standard group; however, the incidence of pulmonary toxicity was higher in the experimental group. Of the 17 (8·7%) cases of grade 1 to 4 drug-related pneumonitis in the expermental group, 14 (82%) occurred during treatment with vinorelbine plus gemcitabine. In another Japanese study using vinorelbine plus gemcitabine, grade 3 or higher drug-related pneumonitis occurred in two of 62 patients (3%), resulting in one death.[21] A few cases of drug-related pneumonitis have also been reported when vinorelbine or gemcitabine is combined with cisplatin.[5] Interstitial lung disease due to inhibitors of epidermal growth factor receptors (EGFR) is also problematic in Japan,[22,24] either because of ethnic differences in drug-related pneumonitis or greater vigilance in diagnosing pneumonitis in Japan. Further studies are thus crucial.

Because docetaxel is active as second-line chemotherapy, sequential administration of docetaxel after other chemotherapy regimens might be effective for clones resistant to previous chemotherapy.[12] Edelman and colleagues[25] did a randomised phase II trial of carboplatin plus gemcitabine followed by paclitaxel, or cisplatin plus vinorelbine followed by docetaxel. Both regimens resulted in survival data comparable to platinum-based two-drug combinations and few patients showed an improvement in response with sequential taxane therapy.[25] In the present study, of the patients who achieved partial response in the experimental group, about a third (17 of 49) had their best response during treatment with vinorelbine plus gemcitabine, whereas nearly two-thirds (32 of 49) achieved partial response with docetaxel monotherapy. Although we should be careful when interpreting these data, because central review for response assessment was not done and the protocol specified response assessment every two cycles, which might have been too early to detect the real response to treatment with vinorelbine plus gemcitabine, data from this study suggest that alternative sequential therapy could be effective for NSCLC if highly active regimens are selected and administered in the optimum sequence. Preliminary findings from a randomised phase III study comparing immediate with delayed second-line chemotherapy in patients with stage IIIB or IV NSCLC suggest that median overall survival might be improved by giving docetaxel immediately after completion of a full course of first-line treatment (median overall survival 11·9 months for immediate docetaxel and 9·1 months for delayed docetaxel; p=0·071).[26] Pemetrexed has comparable efficacy to docetaxel as second-line chemotherapy.[27] Pemetrexed plus cisplatin showed similar overall survival to gemcitabine plus cisplatin in chemotherapy-naive patients with advanced NSCLC, and overall survival was better with pemetrexed plus cisplatin than with gemcitabine plus cisplatin in adenocarcinoma and large-cell carcinoma histology.[28] By contrast, a significant improvement in overall survival was shown with gemcitabine plus cisplatin in patients with squamous-cell histology.[28] Maintenance chemotherapy with pemetrexed after four cycles of platinum-based chemotherapy improved overall survival compared with supportive care in patients with non-squamous NSCLC, with a median survival of 14·4 months for pemetrexed and 9·4 months for the placebo group.[29] Furthermore, a subgroup analysis of a randomised trial of maintenance gefitinib after chemotherapy versus chemotherapy alone showed a significantly better overall survival favouring gefitinib in patients with adenocarcinoma.[30] Although the present study did not show better survival with the experimental regimen than with the standard regimen of carboplatin plus paclitaxel, further study of sequential chemotherapy in selected patients with stage IIIB or IV NSCLC is warranted.

The present study was done as a JMTO-SWOG common-arm trial with identical eligibility, staging, response, and toxicity criteria to SWOG S0003.[14] Dose, schedule, and dose modifications for paclitaxel and carboplatin were consistent with SWOG S0003. Patient baseline characteristics were similar in the two studies. Overall survival in patients

treated with paclitaxel plus carboplatin was better in the current study than in the SWOG S0003 trial (median overall survival 14·1 months [95% CI 11·9–17·5] *vs* 9 months, respectively).[14] The prolonged overall survival in the current study compared with the SWOG trial might have been due to post-study treatment.[31] About two-thirds of patients in each group received poststudy chemotherapy, with most receiving gefitinib. Docetaxel is the only cytotoxic chemotherapy that has been shown to prolong overall survival compared with supportive care alone in patients with NSCLC who have received previous chemotherapy, although some other agents have shown activity in this population.[32] The EGFR inhibitor erlotinib also prolongs survival in previously treated patients with NSCLC.[33] Furthermore, placebo-controlled trials have shown that EGFR gene mutations are also prognostic factors, irrespective of the EGFR inhibitor used as treatment.[33] More EGFR gene mutations have been reported in Japanese patients with NSCLC than in US patients.[34] Thus, biological differences in lung cancer might exist between Japanese and US patients.

Neutropenic fever has also been shown to be more common in Japanese patients than in US patients (12% *vs* 3%).[14] Similar findings were obtained when European and US data were compared with a Japanese phase III study that used 200 mg/m² of paclitaxel plus carboplatin AUC of 6.[35] The difference in these toxicities might be due to pharmacogenomics. Another possibility is the difference in the method of measuring serum creatinine concentrations. In Japan, most institutions use an enzymatic method,[36] whereas the colorimetric Jaffe method is more frequently used in the USA.[37] The enzyme method tends to give lower serum creatinine concentrations resulting in a higher carboplatin dose when using Calvert formula.[38] Because clinical trials of cancer chemotherapy are being done internationally, caution should be paid to these medical differences.

Platinum-containing regimens remain the standard treatment for advanced NSCLC. However, the non-platinum regimen used in this study could still provide equivalent efficacy with a different toxicity profile, increasing the options available to patients.

#### Contributors

MK was the chief investigator of the trial. KKu, MK, MO, MF, and KF designed the trial and wrote the protocol. KKu, MK, MO, YN, KKo, KM, and YF enrolled patients. ST was responsible for data management and statistical analysis. KKu, KM, and ST took part in writing the report. All authors reviewed and approved the report.

#### Trial co-investigators

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#### Conflicts of interest

KKu has received honoraria from Eli Lilly, Sanofi-Aventis, and Chugai. All other authors declared no conflicts of interest.

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# EGFR Polymorphism of the Kinase Domain in Japanese Lung Cancer

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***Background.*** **Mutations of the epidermal growth factor receptor (EGFR) gene at kinase domain have been reported in non-small-cell lung cancer (NSCLC), and some common somatic mutations in EGFR have been examined for their ability to predict sensitivity to gefitinib or erlotinib. However, EGFR mutations at exon 20 have been reported to predict resistance to gefitinib therapy.**

***Materials and methods.*** **We investigated the EGFR mutations and/or polymorphism statuses at kinase domain in 303 surgically treated non-small cell lung cancer (NSCLC) cases. One hundred ninety-four adenocarcinoma cases were included. The presence or absence of EGFR polymorphism of kinase domains was analyzed by direct sequences. We have also investigated EGFR polymorphism status at exon 20 for 23 NSCLC patients who had undergone surgery followed by treatment with gefitinib at the National Hospital Organization, Kinki-chuo Chest Medical Center.**

***Results.*** **EGFR mutations at kinase domain were found in 75 of 303 lung cancer patients. During sequencing of EGFR tyrosine kinase domain in tumors, 86 EGFR polymorphism (G2607A) cases were identified at exon 20. G2067A polymorphism was significantly higher in nonadenocarcinomas (37.4%) than in adenocarcinoma (25.3%, $P = 0.0415$). The polymorphism status did not correlate with gender, smoking (never smoker *versus* smoker), and EGFR mutations. In 46 total gefitinib treated NSCLC patients, there was a tendency toward better prognosis in EGFR wild type (GG) patients than AG + AA patients. EGFR polymorphism in Japanese lung cancers seemed to be less frequent than Caucasian lung cancers.**

***Conclusions.*** **EGFR-tyrosine kinase polymorphism might be associated with clinicopathological background of lung cancers.** 

***Key Words:*** **EGFR; lung cancer; polymorphism; exon 20.**

## INTRODUCTION

Lung cancer is a major cause of death from malignant diseases, due to its high incidence, malignant behavior, and lack of major advancements in treatment strategy [1]. There is much accumulated evidence that epidermal growth factor receptor (EGFR) and its family members are strongly implicated in the development and progression of numerous human tumors, including lung cancer [2, 3]. The EGFR tyrosine kinase inhibitor, gefitinib, was approved in Japan for the treatment of non-small cell lung cancer (NSCLC) in 2002. Phase II and III trials have shown partial responses in 8% to 12% of unselected patient with progressive NSCLC after chemotherapy [4, 5], especially a higher response in the never-smoker, female, and of Asian ethnicity (more than 20%) [4, 6, 7]. Original two reports showed that EGFR mutation status at tyrosine kinase (TK) domain in NSCLC patients was correlated with the clinicopathological features related to good response to gefitinib [8, 9]. These EGFR mutations were predominantly found in Japanese lung cancer patients (about 25–40%) [8, 10–13] compared with U.S.A. patients (about 8% to 10%) [8, 9, 11, 14] or European patients [11, 15]. Actually, EGFR mutations in lung cancer have been correlated with clinical response

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to gefitinib therapy in vivo and in vitro [8, 9, 14]. However, EGFR mutations at exon 20 have been reported to predict resistance to gefitinib therapy [16, 17]. During sequencing of the EGFR tyrosine kinase domain in lung cancers, an EGFR polymorphism (G2607 A) was identified at exon 20 [17]. This EGFR single nucleotide polymorphism (SNP) was significantly higher in lung cancer (83.6%) than control (73.5%) in the Caucasian population [18]. To determine this EGFR polymorphism status and correlation with clinicopathological features in Japanese lung carcinoma, we investigated EGFR gene status by direct sequences. The findings were compared with the clinicopathological features of lung cancer.

## MATERIALS AND METHODS

### Patients

The study group included 303 lung cancer patients who had undergone surgery at the Department of Surgery II, Nagoya City University Medical School between 1997 and 2005. Mean age was 65.2 y and median age was 66 y. We have also investigated EGFR SNP status for 23 NSCLC patients who had undergone surgery followed by treated with gefitinib at the National Hospital Organization, Kinki-chuo Chest Medical Center. The lung tumors were classified according to the general rule for clinical and pathological record of lung cancer in Japan [19]. All tumor samples were immediately frozen and stored at −80°C until assayed.

The clinical and pathological characteristics of the 303 lung cancer patients were as follows: 209 (69.0%) were male and 94 were female; 194 (64.0%) were diagnosed with adenocarcinoma, and 109 were diagnosed with other types of carcinoma; 205 (67.7%) were smokers and 98 were nonsmokers.

### Polymerase Chain Reaction (PCR) Assays for EGFR Mutations

Genomic DNA was extracted using Wizard SV Genomic DNA purification Systems (Promega, Madison, WI) according to the manufacturer's instructions. The primers and TaqMan MGB probe were designed with Primer Express 2.0 software (Applied Biosystems, Foster City, CA). The sequences of 13 allele-specific probes and primer sets used in the TaqMan PCR assay were already shown and the results were already reported [13]. The direct sequencing for EGFR genes was performed for 91 cases at Dana-Farber Cancer Institute. Most of the results from sequencing were already reported by Paez *et al.* [8]. Other cases were genotyped using LightCycler and also sequenced [20, 21]. The PCR reactions were performed using LA-Taq kit (Takara Bio Inc., Shiga, Japan) in a 50 $\mu$L reaction volume. The primer sequences for EGFR gene at exon 20 were as follows: forward primer, 5-ATCGCATTCATGCGTCTTCA-3 and reverse primer, 5-ATCCCCATGGCAAACTCTTG-3 (378 bp). The cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 64°C for 30 s, and 72°C for 45 s. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA). These samples were sequenced by ABI prism 3100 analyzer (Applied Biosystems Japan Ltd., Tokyo, Japan) and analyzed by BLAST and chromatograms by manual review.

### Statistical Analysis

Statistical analyses were done using the Mann-Whitney *t*-test for unpaired samples and Wilcoxon's singed rank test for paired samples. Linear relationships between variables were determined by means of simple linear regression. Correlation coefficients were determined by rank correlation using Spearman's test and $\chi 2$ test. The overall survival of lung cancer patients was examined by the Kaplan-Meier method, and differences were examined by the Log-rank test. All analyses were done using the Stat-View software package (Abacus Concepts Inc. Berkeley, CA), and a $P$ value $< 0.05$ was considered significant.

## RESULTS

### GFR Gene Mutation Status in Japanese Lung Cancer Patients

Of 303 patients from Nagoya City University, in exon 19, 33 patients had the deletion type mutation. In exon 18 or exon 21, 41 patients had the missense point mutations (1 G719S, 2 G719C, 36 L858R, and 2 L861Q). One patient had exon 20 insertion mutation. Of these 75 patients, 26 were male and 49 were female; 55 were nonsmokers and 20 were smokers; 72 patients had adenocarcinoma, 1 had squamous cell carcinoma, and 2 had adenosquamous cell carcinoma. Thus EGFR mutation status at exon 18 to 21 was significantly correlated with gender ($P < 0.0001$), tobacco-smoking ($P < 0.0001$) and pathological subtypes (adenocarcinoma *versus* nonadenocarcinoma, $P < 0.0001$). Of 303 patients from Nagoya City University, 176 (58.1%) were Stage I. There was a tendency toward higher EGFR mutation in Stage I (50/176, 28.4%) than in Stage II to IV (25/127, 19.7%, $P = 0.1052$).

### EGFR Polymorphism at Exon 20

During sequencing of the EGFR-TK domain in lung cancer samples, a sequence difference in exon 20 (G2607A; Q787Q) was found in tumors that defined a previously identified SNP (refSNP ID: rs 10251977) in the EGFR-TK gene (Fig. 1). Of 303 patients, 86 patients had the EGFR polymorphism; 57 were male and 29 were female; 60 were nonsmokers and 26 were smokers; 49 patients had adenocarcinoma and 37 had

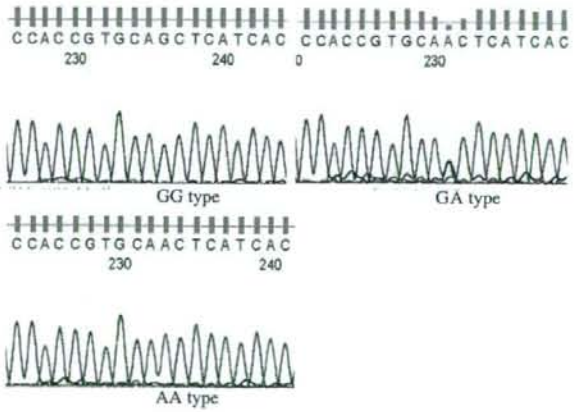


**FIG. 1.** The sequence results of *EGFR* exon 20. Left upper; wild type (GG). Right upper; heterozygous SNP (GA). Left lower; homozygous SNP (AA). (Color version of figure is available online).

**TABLE 1**

**Clinicopathological Data of 303 Lung Cancer Patients**

| Factors | EGFR | | |
|---|---|---|---|
| | GG patients | GA + AA patients | *P* value |
| Mean age (years) 65.2 ± 9.6 | 86 | 217 | |
| pStage | | | |
| I | 47 (26.7%) | 129 (73.3%) | 0.5187 |
| II–IV | 39 (30.7%) | 88 (69.3%) | |
| Smoking | | | |
| Nonsmoker | 26 (26.5%) | 72 (73.5%) | 0.6837 |
| Smoker | 60 (29.3%) | 145 (70.7%) | |
| Pathological subtype | | | |
| Adenocarcinoma | 49 (25.3%) | 145 (74.7%) | 0.0415 |
| Others | 37 (37.4%) | 72 (62.6%) | |
| EGFR mutation | | | |
| Positive | 19 (25.3%) | 56 (73.7%) | 0.5566 |
| Negative | 67 (29.4%) | 161 (70.6%) | |
| Age | | | |
| =<60 | 17 (20.7%) | 65 (79.3%) | 0.0853 |
| >60 | 69 (31.2%) | 152 (68.8%) | |
| Gender | | | |
| Male | 57 (27.3%) | 152 (82.7%) | 0.3141 |
| Female | 29 (20.9%) | 65 (79.1%) | |

other types of lung cancers. G2607A polymorphism was significantly higher in nonadenocarcinomas (37/109; 37.4%) than in adenocarcinoma (49/194; 25.3%, $P = 0.0415$). However, the polymorphism did not correlate with gender ($P = 0.5820$), smoking status ($P = 0.7789$), pathological stages ($P = 0.5077$), and EGFR-TK mutation status of lung cancer ($P = 0.5566$) (Table 1). Previous report from the United States demonstrated that the G2607A polymorphism was found from 102/122 (83.6%) patients. EGFR polymorphism (G2607A) in our Japanese lung cancers was less frequent than Caucasian lung cancers ($P < 0.0001$).

### Relationship Between Clinical Courses of Lung Cancer Patients Treated with Gefitinib and EGFR Polymorphism

The overall survival of gefitinib untreated lung cancer patients from Nagoya City University, with follow-up through December 30, 2006, was studied in reference to the EGFR polymorphism status. Of 303 patients from Nagoya City University, 23 were treated with gefitinib therapy. A total of 46 gefitinib treated patients were investigated for G2607 polymorphism status. In this analysis, 11 patients had EGFR polymorphism (AG or AA). There was a tendency toward better prognosis in EGFR wild type patients (GG; 21/35 were deceased) than in EGFR polymorphism patients (AG + AA; 9/11 were deceased) ($P = 0.0653$) (Fig. 2).

## DISCUSSION

We obtained findings that G2607A EGFR polymorphism was significantly higher in nonadenocarcinomas than in adenocarcinomas. In addition, our analysis also suggested that there was a tendency toward better prognosis in EGFR wild type patients (GG) than in EGFR polymorphism patients (GA + AA) who were treated with gefitinib.

In this report, the EGFR SNP(G2607A) is not associated with somatic EGFR-TK mutation. Approximately 563 EGFR-SNPs have been identified in human genome according to the National Cancer for Biotechnology information database. However, there are few studies examining associations between EGFR SNPs and human disease [18, 22–25]. In this study, we detected a polymorphism in exon 20 of the EGFR-TK domain at nucleotide 2607, codon 787 (Gln), which changed nucleotide 2607 from G to A, without amino acid substitution. Previous reports suggested that EGFR exon 20 mutations were critical roles for gefitinib resistance. EGFR containing the exon 20 point mutation T790M were associated with resistance to gefitinib and erlotinib [16]. Greulich *et al.* reported that transformation by the D770_N771insNPG (exon 20) EGFR insertion mutant was remarkably insensitive to gefitinib and erlotinib, as inhibition of colony growth in soft agar required exposure to 100-fold higher concentrations (>1 mM) of these agents than was required to inhibit colony formation by cells expressing the EGFR missense mutants or deletion mutant [17]. Greulich *et al.* also reported that all three lung adenocarcinoma patient with known exon 20 insertion mutants of EGFR have failed to show a clinical response to treatment and have instead achieved only stable disease with erlotinib [17]. Actually, in this report, a weak association between G2607A polymorphism and the prognosis of gefitinib therapy was also found. This

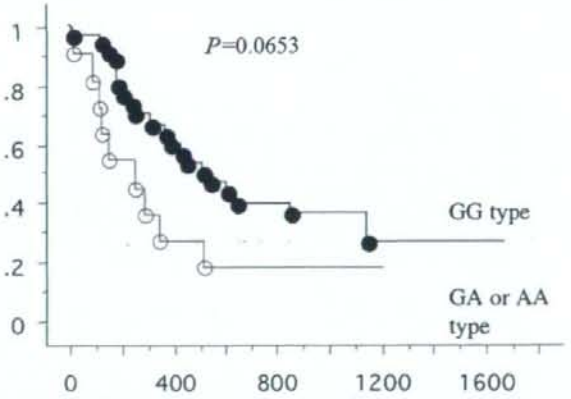


**FIG. 2.** The overall survival of 46gefitinib untreated lung cancer patients was studied in reference to the EGFR polymorphism (G2607A) status. There was a tendency toward better prognosis from patient with EGFR wild type (GG) ($n = 35$, 21 were deceased) than the patient with EGFR polymorphism (GA or AA) ($n = 11$, 9 were deceased) (log-rank test, $P = 0.0653$, Breslow-Gehan-Wilcoxon test; $P = 0.0174$).

might be explained because of the difference in gefitinib response between adenocarcinomas and other types of carcinomas. In our report, G2607 polymorphisms were lower in adenocarcinomas in the Japanese population. A larger number would help to determine the correlation between the G2607 polymorphism and gefitinib sensitivity.

A previous report showed a different G2607 frequency of distribution between Swiss and Japanese population with glioblastoma [22]. This polymorphism was found at a higher frequency in lung cancer patients than normal control [18]. Zhang *et al.* also suggested that no association was found between the EGFR-TK mutation and the G2607A SNP [18]. It remains to be verified whether the *EGFR* G2607A changes EGFR expression or function [18, 22]. Even if there is no amino acid change, the EGFR polymorphism identified here might lead to difference in EGFR gene transcription, mRNA stability or translation, or could be a genetic marker of another risk-associated genotype. Shintani *et al.* demonstrated that another EGFR-SNP at position 2073 was correlated with truncated EGFR transcription, which might interfere with EGFR three-dimensional structure and EGFR expression [24].

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ORIGINAL PAPER

# A novel *EGFR* mutation D1012H and polymorphism at exon 25 in Japanese lung cancer

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**Abstract**
*Introduction* Mutations of the epidermal growth factor receptor (*EGFR*) gene at kinase domain have been reported in non-small-cell lung cancer (NSCLC). However, *EGFR* mutations status at C-terminal domain has not been reported in detail.
*Materials and methods* We investigated the *EGFR* mutation and polymorphism statuses at C-terminal domain in 398 surgically treated NSCLC cases. Two hundred and sixty-eight adenocarcinoma cases were included. The presence or absence of *EGFR* mutation and polymorphism was analyzed by direct sequences.
*Results* A novel *EGFR* somatic mutation at exon 25 (G3034, D1012H) was found from 1 of 398 lung cancer patients. During sequencing of *EGFR* C-terminal domain in NSCLC, 194 *EGFR* polymorphism (C2982T) cases were identified at exon 25. The polymorphism statuses were not correlated with gender, smoking status (never smoker vs. smoker), pathological subtypes and *EGFR* mutations. The *EGFR* polymorphism ratio was significantly higher in younger NSCLC (≤60, 56.8%) than in older NSCLC (>60, 45.6%, $P = 0.0467$). The *EGFR* polymorphism ratio was significantly higher in lymph node positive NSCLC (57.4%) than in lymph node negative NSCLC (44%, $P = 0.0168$). In 46 total gefitinib treated NSCLC patients, exon 25 polymorphism was not correlated with prognosis.
*Conclusion* *EGFR* mutation at C-terminal in lung cancers seemed to be extremely rare, however, this D1012H mutation might be a role in EGFR function. *EGFR* polymorphism at exon 25 might be correlated with progression of NSCLC.

**Keywords** *EGFR* · D1012H · Lung cancer · Polymorphism · Exon 25

## Introduction

Lung cancer is a major cause of death from malignant diseases, due to its high incidence, malignant behavior and lack of major advancements in treatment strategy (Ginsberg et al. 1993). There are much accumulated evidences that epidermal growth factor receptor (*EGFR*) and its family member are strongly implicated in the development and progression of numerous human tumors, including lung cancer (Nicolson et al. 2001; Onn et al. 2004). The *EGFR* tyrosine kinase (TK) inhibitor, gefitinib, was approved in Japan for the treatment of non-small cell lung cancer (NSCLC) since 2002. Phase II and III trial have shown partial responses in 8–12% of unselected patient with progressive NSCLC after chemotherapy (Kris et al. 2003; Thatcher et al. 2005), especially higher response in never smoker, female and Asian ethnicity (more than 20%) (Kris et al. 2003; Fukuoka et al. 2003; Miller et al. 2004). Original two reports showed that *EGFR* mutation statuses at TK domain (exon 18–24) in NSCLC patients were correlated

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