with response to gefitinib treatment (Paez et al. 2004; Lynch et al. 2004). These *EGFR* mutations were predominantly found in Japanese lung cancer patients (about 25–40%) (Paez et al. 2004; Kosaka et al. 2004; Shigematsu et al. 2005; Tokumo et al. 2005; Endo et al. 2005) when compared to USA patients (about 8–10%) (Paez et al. 2004; Lynch et al. 2004; Pao et al. 2004; Shigematsu et al. 2005) or European patients (Shigematsu et al. 2005; Marchetti et al. 2005). Although original two groups have sequenced whole *EGFR* gene, they found no mutation within C-terminal of *EGFR* (Paez et al. 2004; Lynch et al. 2004).

C-terminal domain of the EGFR plays an integral role in regulation of the kinase. In particular, kinetic analyses of the EGFR indicated that the C-terminal domain modulated receptor function by virtue of repressing kinase activity in the absence of autophosphorylation (Bertics and Gill 1985; Bertics et al. 1988). To determine *EGFR* mutation status at C-terminal domain in Japanese lung carcinoma, we investigated *EGFR* gene status by direct sequences. The findings were compared to the clinico-pathologic features of lung cancer.

Materials and methods

Patients and samples

The study group included 374 lung cancer patients who had undergone surgery at the Department of Surgery II, Nagoya City University Medical School between 1997 and 2006. Mean age was 65.1 years old and median age was 67 years old. The lung tumors were classified according to the general rule for clinical and pathological record of lung cancer in Japan. All tumor samples were immediately frozen and stored at −80°C until assayed. We have also investigated *EGFR* SNP status for 24 NSCLC patients who were treated with gefitinib for their recurrent diseases after they had undergone surgery at the National Hospital Organization, Kinki-chuo Chest Medical Center. The clinical and pathological characteristics of the 398 lung cancer patients were as follows; 270 (67.8%) were male 128 were female. Two hundred and sixty-eight (67.3%) were diagnosed as adenocarcinoma, and 130 were diagnosed as other types of carcinoma. Two hundred and sixty (65.3%) were smoker and 138 were non-smoker. Of 374 patients from Nagoya City University, 218 (57.8%) were stage I.

PCR assays for *EGFR* mutations

Total RNA was extracted from lung cancer tissues and adjacent non-malignant lung tissues using Isogen kit (Nippon gene, Tokyo, Japan) according to the manufacturers' instructions. RNA concentration was determined by spectrophotometer and adjusted to a concentration of 200 ng/ml. About ten cases were excluded because tumor cells were too few to sufficiently extract tumor RNA. RNA (1 μg) was reverse transcribed by Superscript II enzyme (Gibco BRL, Gaithersburg, MD, USA) with 0.5 μg oligo $(dT)_{12-16}$ (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). The reaction mixture was incubated at 42°C for 50 min and then at 72°C for 15 min. We then used 1 μl of each DNA for PCR analyses. The PCR reactions were performed using LA-Taq kit (Takara Bio Inc., Shiga, Japan) in à 25-μl reaction volume. The primer sequences for *EGFR* gene for C-terminal domain (exon 23–28) were as follows: the forward primer, 5-GGGAGTTGATGACCTTTGGA-3 and the reverse primer, 5-TTCTGCATTTTCAGCTGTGG-3 (875 bp). The cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 45 s, 58°C for 45 s, 72°C for 60 s. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA, USA). Genomic DNA was extracted from lung cancer tissues ($n = 91$) and adjacent peripheral leukocyte ($n = 20$) using Wizard SV Genomic DNA purification Systems (Promega) according to the manufacturers' instructions. The primer sequences for *EGFR* gene at exon 25 were as follows: the forward primer, 5-TAAGGC ACCCACATCATGTCA-3 and the reverse primer, 5-TGG ACCTAAAAGGCTTACATCAA-3 (Paez et al. 2004). The cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 45 s, 64°C for 45 s, 72°C for 45 s. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA, USA). These samples were sequenced by ABI prism 3100 analyzer (Applied Biosystems Japan Ltd., Tokyo, Japan) and analyzed by BLAST and chromatograms by manual review. The results of *EGFR* mutation statuses at kinase domain were already reported (Endo et al. 2005; Sasaki et al. 2005, 2006).

Statistical analysis

Statistical analyses were done using the Mann–Whitney *U* 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 χ^2 test. The overall survival of lung cancer patients was examined by the Kaplan–Meier methods, and differences were examined by the Log-rank test. All analysis was done using the Stat-View software package (Abacus Concepts Inc., Berkeley, CA, USA), and was considered significant when the *P* value was less than 0.05.

Results

EGFR gene mutation status in Japanese lung cancer patients

We have sequenced for C-terminal of *EGFR* gene from 286 NSCLC samples. Of 286 patients, from direct sequencing using cDNA samples, we found only one mutation at exon 25 (G3034C, D1012H). Matched normal lung tissues showed wild type sequence suggested this mutation was somatic (Fig. 1). This patient was male, non-smoker with well differentiated adenocarcinoma. Pathological stage was T2N0 (stage Ib). The patient also had the deletion type mutation in exon 19. We have additionally sequenced at exon 25 of *EGFR* gene from 88 NSCLC samples. However, from direct sequencing using genomic DNA samples, no mutation was found. Comparison of protein sequences indicated that D1012 was highly conserved with other erbB family protein, such as Her2 and erbB4 (Fig. 2).

In exon 18 or exon 21, 52 patients had the missense point mutations (1 G719S, 3 G719C, 48 L858R and 2 L861Q). Four patients had exon 20 insertion mutations, and 52 patients had exon 19 deletion mutations. Of these 111 patients, 40 were male and 71 were female. Seventy-nine were non-smokers and 32 were smokers. One hundred and four patients had adenocarcinoma, four had squamous cell carcinoma and three had adenosquamous cell carcinoma. Thus *EGFR* mutation statuses at exon 18–21 were significantly correlated with gender ($P < 0.0001$), tobacco-smoking ($P < 0.0001$) and pathological subtypes (adenocarcinoma vs. non-adenocarcinoma, $P < 0.0001$).

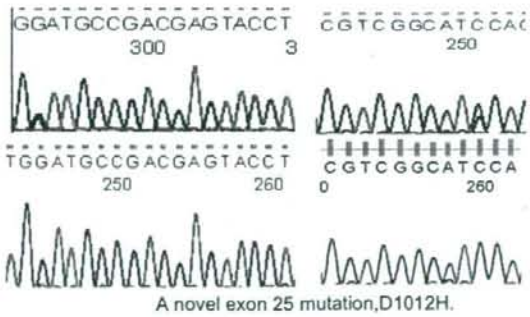

Fig. 1 A novel mutation, D1012H, at exon 25. *Left upper*; forward sequence from lung cancer samples. *Left lower*; forward sequence from adjacent normal lung tissue. *Right upper*; reverse sequence from lung cancer samples. *Right lower*; reverse sequence from adjacent normal lung tissue

HumanEGFR	1007	MDDVVDADEYL	1017
MouseEGFR	1007	MEDVVDADEYL	1017
Her2	1015	MGDLVDAEEYL	1025
ErbB4	1013	LEDMMDAEEYL	1023

Fig. 2 Comparison of protein sequences indicated that D1012 was highly conserved with other erbB family protein, such as Her2 and erbB4

EGFR polymorphism at exon 25

During sequencing of the *EGFR* C-terminal domain in lung cancer samples, a sequence difference in exon 25 (C2982T; D994D) was found (Fig. 3). Of 398 patients, 194 patients had the *EGFR* polymorphism. The sequencing results from adjacent peripheral leukocyte showed the same results. One hundred and thirty-six were male and 58 were female. Sixty-seven were non-smokers and 127 were smoker. One hundred and twenty-eight patients had adenocarcinoma and 66 had other types of lung cancers. Of 374 patients from Nagoya City University, 180 (48.1%) had the polymorphism. The polymorphism did not correlate with pathological stages ($P = 0.5400$). The *EGFR* polymorphism ratio was significantly higher in lymph node positive NSCLC (66/115, 57.4%) than in lymph node negative NSCLC (114/259, 44%, $P = 0.0168$).

The polymorphism did not correlate with gender ($P = 0.3457$), smoking status ($P = 0.9552$), pathological subtypes ($P = 0.5734$) and *EGFR*-TK mutation status of lung cancer ($P = 0.7447$) (Table 1). The *EGFR* polymorphism ratio was significantly higher in younger NSCLC ($\leq$60, 56.8%) than in older NSCLC (>60, 45.6%, $P = 0.0467$), although the P value was marginal.

Relationship between clinical courses of lung cancer patients treated with gefitinib and *EGFR* polymorphism

The overall survival of gefitinib treated lung cancer patients with follow-up through December 30, 2006, was studied in reference to the *EGFR* polymorphism status. Of 377

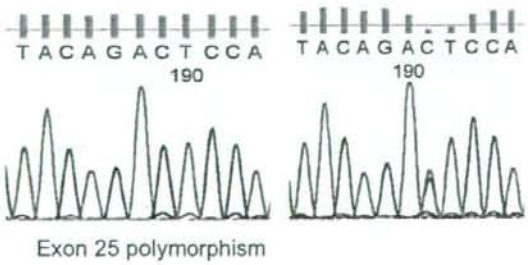

Fig. 3 The sequence results of *EGFR* exon 25. *Left*; wild type (CC). *Right upper*; heterozygous SNP (CT)

Table 1 Clinico-pathological data of 398 lung cancer patients

Factors	EGFR		*P* value
	CC patients (%)	CT + TT patients (%)	
pStage			
I	116 (59.8)	102 (56.7)	0.5400
II–IV	78 (40.2)	78 (43.3)	
Lymph node (meta)			
Negative	145 (74.7)	114 (63.3)	0.0168
Positive	49 (25.3)	66 (36.7)	
Smoking			
Non-smoker	71 (34.8)	67 (34.5)	0.9552
Smoker	133 (65.2)	127 (65.5)	
Pathological subtype			
Adenocarcinoma	140 (68.6)	128 (66.0)	0.5734
Others	64 (31.4)	66 (34.0)	
EGFR mutation			
Positive	63 (30.8)	57 (29.4)	0.7447
Negative	141 (69.2)	137 (70.6)	
Age			
≤60	48 (23.5)	63 (29.9)	0.0467
>60	156 (76.5)	131 (70.1)	
Gender			
Male	134 (65.7)	136 (70.1)	0.3457
Female	70 (34.3)	58 (29.9)	

NS not significant, *Adeno* adenocarcinoma

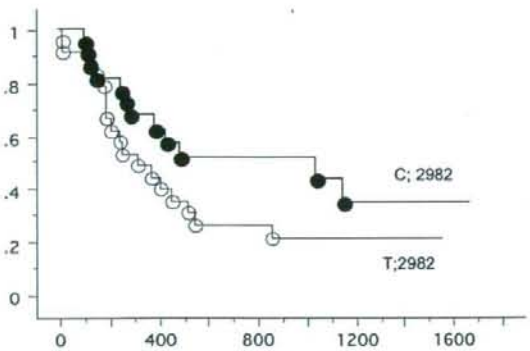

Fig. 4 The overall survival of 46 gefitinib untreated lung cancer patients was studied in reference to the *EGFR* polymorphism (C2982T) status. The prognosis was not significantly different between the patient with EGFR wild type (CC) ($n = 22$, 12 were dead) than the patient with *EGFR* polymorphism (CT or TT) ($n = 24$, 18 were dead) (Log-rank test, $P = 0.1471$)

patients from Nagoya City University, 22 were treated with gefitinib therapy. Total 46 gefitinib treated patients were investigated with the C2982T polymorphism statuses. In this analysis, 24 patients had *EGFR* polymorphism (CT or TT). The prognosis was not different between in *EGFR* wild type patients (CC; 12/22 were dead) and in *EGFR* polymorphism patients (CT + TT; 18/24 were dead) ($P = 0.1471$) (Fig. 4).

Discussion

We have found a novel D1012H (G3034C) mutation at C-terminal domain of *EGFR* gene. This mutation was very rare (0.2%) somatic mutation. We also obtained findings that C2982T *EGFR* polymorphism was existed in 49% of Japanese lung cancer, and the *EGFR* polymorphism ratio was significantly higher in lymph node positive NSCLC (57.4%) than in lymph node negative NSCLC (44%). However, none of other clinico-pathological factors were correlated with the polymorphism. The *EGFR* polymorphism ratio was significantly higher in younger NSCLC, although the *P* value was marginal.

In this report, we found a novel somatic *EGFR* mutation (D1012H) within *EGFR*-C-terminal domain. The-C-terminal phosphorylation domain of the EGFR is believed to regulate protein kinase activity as well as mediate the assembly of signal transduction complexes (Lee et al. 2006). It was shown that truncation of the C-terminal domain enhanced the affinity of the nucleotide binding site for TNP-ATP, suggesting that the C-terminal autophosphorylation domain of the EGFR modulates the nucleotide-binding properties of the protein TK domain (Cheng and Koland 1996). In addition, the computational analyses, based on the three-dimensional structure of EGFR's kinase domain suggested that direct contact between the kinase and a segment from the C-terminal regulatory domains inhibits enzymatic activity (Landau et al. 2004). More recently, it has been reported that EGFR C-terminal sequences 1005–1017 and di-leucine motif (1,010) LL (1,011) are essential in EGFR endocytosis (Wang et al. 2007). Graduate truncation within 991–1044 of EGFR showed progressively lower EGF-induced EGFR endocytosis with most significant effects observed for residues 1005–1017 (Wang et al. 2007). The residues 1005–1017 were also required for EGFR internalization triggered by non-ligand-induced receptor internalization. Comparison of protein sequences indicated that D1012 was highly conserved with other erbB family protein, such as Her2 and erbB4, suggested that the sequence was important. However, D1012H mutation was found in only one patient from our cohort and this patient also had deletion mutation in exon 19. This finding would indicate that D1012H mutation was lacking of strong impact in EGFR function in Japanese lung cancers.

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 (Zhang et al. 2006; Fukushima et al. 2006; Kang et al. 2005; Shintani et al. 1999; Liu et al. 2008). In this study, we detected a polymorphism in exon 25 of the *EGFR* C-terminal domain at nucleotide 2982, codon 994 (Asp), which changed nucleotide 2982 from C to T, without amino acid substitution. The *EGFR* polymorphism ratio was significantly higher in lymph node positive NSCLC than in lymph node negative NSCLC. Thus C2982T polymorphism might be associated with the aggressive behavior of lung cancers. It remains verified whether the *EGFR* C2982T changes *EGFR* expression or function (Zhang et al. 2006; Fukushima et al. 2006). Even if there is no amino acid change, the *EGFR* polymorphism identified here might led to difference in *EGFR* gene transcription, mRNA stability, or translation, or could be a genetic marker of another risk-associated genotype. Shintani et al. (1999) 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. These might be explanation for higher *EGFR* polymorphism ratio in younger NSCLC, probably correlated with early onset of lung cancers. However, if we used cut-off value of 65 or 66 years old, the *EGFR* polymorphism ratio was not different between old and young patients.

In summary, *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.

Acknowledgments The authors would like to thank Mrs. Emi Sugiyama for his excellent technical assistances. This work was supported by AstraZeneca Research Grant 2004, Grand-in-Aid for Research in Nagoya City University (2006), and Grants-in-Aid for Scientific Research, Japan Society for the Promotion of Science (JSPS) (Nos. 19390367, 18390381, 18659407).

Conflict of interest None declared.

References

Bertics PJ, Gill GN (1985) Self-phospholylation enhances the protein-tyrosine kinase activity of the epidermal growth factor receptor. J Biol Chem 260:14642–14647

Bertics PJ, Chen WS, Hubler L, Lazar CS, Rosenfeld MG, Gill GN (1988) Alteration of epidermal growth factor receptor activity by mutation of its primary carboxyl-terminal site of tyrosine self-phosphorylation. J Biol Chem 263:3610–3617

Cheng K, Koland JG (1996) Nucleotide binding by the epidermal growth factor receptor protein-tyrosine kinase. Trinitrophenyl-ATP as a spectroscopic probe. J Biol Chem 271:311–318

Endo K, Konishi A, Sasaki H, Takada M, Tanaka H, Okumura M, Kawahara M, Sugiura H, Kuwabara Y, Fukai I, Matsumura A, Yano M, Kobayashi Y, Mizuno K, Haneda H, Suzuki E, Iuchi K, Fujii Y (2005) Epidermal growth factor receptor gene mutation in non-small cell lung cancer using highly sensitive and fast TaqMan PCR assay. Lung Cancer 50:375–384

Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereuslova A, Dong RP, Baselga J (2003) Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol 21:2237–2246

Fukushima T, Favereaux F, Huang H, Shimizu T, Yonekawa Y, Nakazato Y, Ohagki H (2006) Genetic alterations in primary glioblastomas in Japan. J Neuropathol Exp Neurol 65:12–18

Ginsberg RJ, Kris K, Armstrong G (1993) Cancer of the lung. In: Principles and practice of oncology, 4th edn. Lippincott, Philadelphia, pp 673–682

Kang D, Gridley G, Huang WY, Engel LS, Winn DM, Brown LM, Bravo-Otero E, Wu T, Diehl SR, Hayes RB (2005) Microsatellite polymorphisms in the epidermal growth factor receptor (EGFR) gene and the transforming growth factor-alpha (TGFA) gene and risk of oral cancer in Puerto Rico. Pharmacogenet Genomics 15:343–347

Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T (2004) Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 64:8919–8923

Kris MG, Natale RB, Herbst RS, Lynch TJ, Prager D, Belani CP, Schiller JH, Kelly K, Spiridonidis H, Sandler A, Albain KS, Cella D, Wolf MK, Averbuch SD, Ochs JJ, Kay AC (2003) Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non small cell lung cancer: randomized trial. JAMA 290:2149–2158

Landau M, Fleishman SJ, Ben-Tal N (2004) A putative mechanism for downregulation of the catalytic activity of the EGF receptor via direct contact between its kinase and C-terminal domains. Structure 12:2265–2275

Lee NY, Hazlett TL, Koland JG (2006) Structure and dynamics of the epidermal growth factor receptor C-terminal phopshorylation domain. Protein Sci 15:1142–1152

Liu G, Gurubhagavatula S, Zhou W, Wang Z, Yeap BY, Asomaning K, Su L, Heist R, Lynch TJ, Christiani DC (2008) Epidermal growth factor receptor polymorphisms and clinical outcomes in non-small-cell lung cancer patients treated with gefitinib. Pharmacogenomics J 8:129–138

Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Branniga BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. New Engl J Med 350:2129–2139

Marchetti A, Martella C, Felicioni L, Barassi F, Salvatore S, Chella A, Camplese PP, Iarussi T, Muchilli F, Mezzetti A, Cuccurullo F, Sacco R, Buttitta F (2005) EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Onlol 23:857–865

Miller VA, Kris MG, Shah N, Patel J, Azzoli C, Gomez J, Krug LM, Pao W, Rizvi N, Pizzo B, Tyson L, Venkatraman E, Ben-Porat L, Memoli N, Zakowski M, Rusch V, Heelan RT (2004) Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. J Clin Oncol 22:1103–1109

Nicolson RI, Gee JM, Harper ME (2001) EGFR and cancer prognosis. Eur J Cancer 37:S9–S15

Onn A, Correa AM, Gilcrease M, Isobe T, Massarelli E, Bucane CD, O'Reilly MS, Hong WK, Fidler IJ, Putnum JB, Herbst RS (2004) Synchronous overexpression of epidermal growth factor receptor and HER2-neu protein is a predictor of poor outcome in patients with stage I non-small cell lung cancer. Clin Cancer Res 10:136–143

Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Seller WR, Johnson BE, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304:1497–1500

Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkari I, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kri M, Varmus H (2004) EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and elrotinib. Proc Natl Acad Sci USA 101:13306–13311

Sasaki H, Endo K, Konishi A, Takada M, Kawahara M, Iuchi K, Matsumura A, Okumura M, Tanaka H, Kawaguchi T, Shimizu T, Takeuchi H, Yano M, Fukai I, Fujii Y (2005) EGFR mutation status in Japanese lung cancer patients: genotyping analysis using LighyCycler. Clin Cancer Res 11:2924–2929

Sasaki H, Shimizu S, Endo K, Takada M, Kawahara M, Tanaka H, Matsumura A, Iuchi K, Haneda H, Suzuki E, Kobayashi Y, Yano M, Fujii Y (2006) EGFR and erbB2 mutation status in Japanese lung cancer patients. Int J Cancer 118:180–184

Shigematsu H, Lin L, Takahashi T, Numura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD, Gazdar AF (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutation in lung cancers. J Natl Cancer Inst 97:339–346

Shintani S, Matsuo K, Crohin CC, McBride J, Tsuji T, Donoff RB, Posner M, Todd R, Wong DT (1999) Intragenic mutation analysis of the human epidermal growth factor receptor (EGFR) gene in malignant human oral keratinocytes. Cancer Res 59:4142–4147

Thatcher N, Chang A, Purvish P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Tan EH, Pemberton K, Archer V, Carroll K (2005) Gefitinib plus best supportive care in previously treated patients with refractory advance non-small-cell lung cancer: result from a randomized, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 366:1527–1537

Tokumo M, Toyooka S, Kiura K, Shigematsu H, Tomii K, Aoe M, Ichimura K, Tsuda T, Yano M, Tsukuda K, Tabata M, Ueoka H, Tanimoto M, Date H, Gazder AF, Shimizu N (2005) The relationship between epidermal growth factor receptor mutation and clinicopathological feature in non-small cell lung cancers. Clin Cancer Res 11:1167–1173

Zhang W, Stabile LP, Keohavong P, Romkes M, Grandis JR, Traynor A, Siegfried JM (2006) Mutation and polymorphism in the EGFR-TK domain associated with lung cancer. J Thorac Oncol 1:635–646

Wang Q, Zhu F, Wang Z (2007) Identification of EGF receptor C-terminal sequence 1005–1017 and di-leucine motif (1010) LL(1011) as essential in EGF receptor endocytosis. Exp Cell Res 313:3349–3363

Efficacy, toxicity and cost analysis for non-platinum triplet (gemcitabine and vinorelbine, followed by docetaxel) vs. platinum-based chemotherapy in IIIB/IV non-small-cell lung cancer: single-institution experience

H. TADA, MS, RN, *Department of Clinical Trial Design and Management, Translational Research Center, Kyoto University Hospital, Shogoin Kawahara-cho, Sakyo-Ku, Kyoto,* S. MATSUI, PHD, *Department of Pharmacoepidemiology, Graduate School of Medicine and Public Health, Kyoto University, Yoshida Konoe-cho, Sakyo-ku, Kyoto,* M. KAWAHARA, MD, PHD, *Department of Internal Medicine, National Hospital Organization Kinki-chuo Chest Medical Center, Nagasone-cho, Sakai, Osaka,* S. HOSOE, MD, PHD, *Hosoe Clinic, Jusohigashi, Yodogawa-Ku, Osaka,* C. HAMADA, PHD, *Department of Management Science, Faculty of Engineering Tokyo University of Science, Kagurazaka, Shinjuku-Ku, Tokyo,* & M. FUKUSHIMA, MD, PHD, *Department of Clinical Trial Design and Management, Translational Research Center, Kyoto University Hospital, Shogoin Kawahara-cho, Sakyo-Ku, Kyoto, Japan*

TADA H., MATSUI S., KAWAHARA M., HOSOE S., HAMADA C. & FUKUSHIMA M. (2008) *European Journal of Cancer Care* **17**, 120–126

Efficacy, toxicity and cost analysis for non-platinum triplet (gemcitabine and vinorelbine, followed by docetaxel) vs. platinum-based chemotherapy in IIIB/IV non-small-cell lung cancer: single-institution experience

A new non-platinum sequential triplet combination chemotherapy regimen, comprising gemcitabine (1000 mg/m^2) and vinorelbine (25 mg/m^2), followed by docetaxel (60 mg/m^2), was compared in terms of efficacy, toxicity and cost with platinum-based chemotherapy regimens (comprising cisplatin plus one or more other anti-tumour drugs) for the treatment of advanced non-small-cell lung cancer in a matched, small-sample size, case–control study. Patients were selected from a single institution. Patients in the platinum and non-platinum groups were matched for clinical stage (IIIB/IV), performance status (0/1), age and sex. For the non-platinum and platinum groups, the overall response rates were 40% and 47%, and the median survival times were 14 and 14.5 months respectively. The most common grade 3–4 toxicity was neutropenia (27%) in the non-platinum group and nausea/vomiting (67%) in the platinum group. The total treatment cost did not differ significantly between the two groups. The non-platinum sequential triplet combination chemotherapy regimen studied was shown to be as effective as the traditional cisplatin-based combination chemotherapy regimen, and was associated with less toxicity.

Keywords: non-small-cell lung cancer, vinorelbine, gemcitabine, docetaxel, cisplatin.

Correspondence address: Harue Tada, Department of Clinical Trial Design and Management, Translational Research Center, Kyoto University Hospital, 54 Shogoin Kawahara-cho, Sakyo-Ku, Kyoto, 606-8507, Japan (e-mail: haru.ta@kuhp.kyoto-u.ac.jp).

Accepted 14 April 2007
DOI: 10.1111/j.1365-2354.2007.00816.x

European Journal of Cancer Care, 2008, **17**, 120–126

INTRODUCTION

Lung cancer is a major cause of deaths from cancer in Japan, the USA and the European Union. Non-small-cell lung cancer (NSCLC) accounts for about 80% of patients with lung cancer in Japan (Ministry of Health and Welfare 2000). Cisplatin (CDDP)-based chemotherapy has been shown to confer a certain survival benefit for patients with advanced NSCLC (Non-small Cell Lung Cancer Collaborative Group 1995), and use of CDDP has been found to be an independent predictor of survival (Grilli *et al.* 1993). A drawback of CDDP-based chemotherapy, however, is its serious toxicity; its side effects include severe nausea, vomiting, renal toxicity requiring adequate hydration, and neuropathy, which increases the difficulty associated with treating elderly patients and outpatients.

Recently, new chemotherapeutic agents, such as the taxanes, vinorelbine (VNR), gemcitabine (GEM), and several non-platinum combinations have been developed for the treatment of NSCLC. The new non-platinum combination of GEM plus VNR has been shown to be active for the treatment of NSCLC, and seems to be less toxic than platinum-based combinations, including those involving CDDP (Non-small Cell Lung Cancer Collaborative Group 1995; Lorusso *et al.* 1998; Feliu *et al.* 1999; Isokangas *et al.* 1999; Beretta *et al.* 2000; Chen *et al.* 2000; Frasci *et al.* 2000; Lorusso *et al.* 2000; Krajnik *et al.* 2000; Herbst *et al.* 2002; Gridelli *et al.* 2003). Treatment with docetaxel (DOC) alone has also been shown to confer a survival benefit, especially as a second-line treatment (Roszkowski *et al.* 2000). Recently, a new non-platinum sequential triplet combination, GEM plus VNR, followed by DOC, was evaluated for 44 chemotherapy-naïve patients with advanced NSCLC in a phase II study conducted by the Japan Multinational Trial Organization (JMTO). The response rate in that study was 47.7%, and the median survival time (MST) was 15.7 months, with a 1-year survival rate of 59%. Grade 3–4 neutropenia was seen in approximately 36% of patients during the GEM/VNR cycles, and in 39% of patients during the DOC cycles. Overall, only 2.3% of patients experienced grade 3–4 thrombocytopenia, and 4.5% experienced grade 3–4 anaemia (Hosoe *et al.* 2003). Given that this non-platinum combination has been found to be very active and well tolerated by patients, it is likely that the regimen will also be suitable for elderly patients and outpatients.

On the basis of these observations, we conducted a case-matched retrospective study as a part of the aforementioned phase II study to assess the non-platinum sequential triplet combination in terms of efficacy, safety and cost relative to platinum-based combinations.

PATIENTS AND METHODS

Patient selection

This study was performed as a part of the phase II trial (JMTO LC00-02) conducted by JMTO (Hosoe *et al.* 2003). The criteria for patient selection (eligibility and exclusion criteria) are summarized in Table 1. Fifteen patients who were enrolled in the phase II trial at the National Hospital Organization Kinki-chuo Chest Medical Center, who received non-platinum triplet chemotherapy (GEM and VNR, followed by DOC) during the period between May 2000 and February 2001, comprised the non-platinum group. For the platinum group, in order to ensure that the two groups were comparable, we selected 15 eligible patients from the pool of all NSCLC patients ($n = 124$) who received CDDP-based chemotherapy between April 1998 and February 2001 at the same institution, by matching each patient in the non-platinum group for stage (IIIB/IV), performance status (0/1), age and sex. The protocol of this study was approved by ethical committees at Kyoto University and the National Hospital Organization Kinki-chuo Chest Medical Center.

Chemotherapy regimens

Patients in the non-platinum group were first treated with both GEM (1000 mg/m^2) and VNR (25 mg/m^2) on days 1 and 8 of three cycles of 21 days each, followed by a further three cycles of 21 days each, during which DOC (60 mg/m^2) was administered on day 1 of each cycle (Hosoe *et al.* 2003). Patients in the platinum group received CDDP-

Table 1. Criteria for patient selection

Eligibility criteria
• Eastern Cooperative Oncology Group performance status 0–1
• Over 18 years old (no upper age limit)
• Stage IV or IIIB non-small-cell lung cancer [with malignant pleural effusion and/or pulmonary nodule(s) in the same lobe as the primary lesion]
• Unidimensionally measurable disease
Exclusion criteria
• Presence of apparent interstitial pneumonitis, massive pleural effusion requiring thoracentesis, uncontrollable diabetes mellitus, heart diseases, history of another cancer (excluding non-melanomatous skin cancer and *in situ* cervical cancer)
• Reduced bone marrow, pulmonary, renal or hepatic function
• Stage IIIB disease with pulmonary nodule(s) at the same lobe of the primary lesion (if they could be considered to indicate that the patient had undergone radiation therapy or operation)
• Presence of asymptomatic central nervous system metastases was not considered an exclusion criterion

based chemotherapy without any restrictions on other concomitant drugs, amount of medication, or number of treatment cycles.

Endpoints

The endpoints of this study were tumour response to chemotherapy, recurrence-free survival time, toxicity and cost of treatment. Haematological and non-haematological toxicity were evaluated using the National Cancer Institute's Common Toxicity Criteria, version 2.0. For patients who underwent two or more cycles of chemotherapy, the response was evaluated using the Response Evaluation Criteria in Solid Tumors (Therasse *et al.* 2000). The recurrence-free survival time refers to the period of time between the day treatments began and the day of recurrence or death. The recurrence-free survival time was censored at the end of follow-up. The total cost of treatment was evaluated using patients' receipts, and comprised the cost of chemotherapy (cost of drugs plus costs associated with drug administration) plus the cost of hospitalization, ambulatory care and supportive care for other adverse events or complications. The cost of granulocyte-colony stimulating factor (G-CSF) for each patient was also calculated. The average cost per month was calculated. The endpoints were evaluated during the period between the month in which the chemotherapy began and 1 month after the completion of chemotherapy.

Statistical methods

Differences between the characteristics of patients in the two groups were evaluated using the *t*-test for quantitative variables and Fisher's exact test for categorical variables. Comparisons of the response rate and the incidence of toxicity events between groups were carried out using Fisher's exact test. The survival rate was estimated for each group using the Kaplan-Meier method. Comparisons of survival between groups were performed using the log rank test. Monthly medical costs were compared using the Wilcoxon rank-sum test. Subgroup analyses were carried by dividing the patients into elderly (65 years or older) and non-elderly groups. Statistical analyses were performed using SAS version 8.0 (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics

The characteristics of patients participating in this study are summarized in Table 2. The distribution of these factors is almost identical between groups, because each patient in the platinum group was selected by matching each patient in the non-platinum group for stage (IIIB/IV), performance status (0/1), age and sex. With respect to histological diagnosis, the non-platinum group included a smaller number of patients with adenocarcinomas but a greater number of patients with large cell carcinomas than the platinum group. The dosage of CDDP in the platinum group was 70–80 mg/m^2 per day. The drugs combined with CDDP for treatment of patients in the platinum group are shown in Table 3. Eight patients received new anticancer agents (DOC: 6 patients; VNR: 2 patients) combined with CDDP, accounting for 53% of the platinum group as a whole. The mean number of chemotherapy cycles per patient was 3.9 for the non-platinum group and 3.1 for the platinum group.

The major reasons for discontinuing chemotherapy were patient refusal and the mental burden caused by adverse reactions in the platinum group, and disease progression in the non-platinum group. Adverse reactions were not a major factor for discontinuation of chemotherapy in the non-platinum group.

Table 2. Patient characteristics

Regimen characteristic	Non-platinum ($n = 15$) n (%)	Platinum ($n = 15$) n (%)
Sex*		
Male	11 (73)	11 (73)
Female	4 (27)	4 (27)
Median age in years (range)*	65 (42–74)	64 (48–76)
ECOG performance status*		
0	2 (13)	2 (13)
1	13 (87)	13 (87)
Stage*		
IIIB	3 (20)	3 (20)
IV	12 (80)	12 (80)
Histological diagnosis		
Adenocarcinoma	3 (20)	8 (53)
Squamous cell carcinoma	7 (47)	6 (40)
Large cell carcinoma	5 (33)	1 (7)

ECOG, Eastern Cooperative Oncology Group.
*Matching factor.

Table 3. Drugs apart from cisplatin used in CDDP-based chemotherapy regimens ($n = 15$)

Drug	n
Docetaxel	6
Vindesin	6
Vinorelbine	1
Mitomycin C	1
Mitomycin C + vinorelbine	1

Table 4. Response, toxicity and cost of platinum and non-platinum chemotherapy regimens

	Non-platinum (*n* = 15) *n* (%)	Platinum (*n* = 15) *n* (%)	*P*-value
Response			
Complete response	0	0	
Partial response	6	7	
Stable disease	5	6	
Progressive disease	4	2	
Response rate	6 (40)	7 (47)	1.000
MST (months)	14	14.5	0.264
(95% CI)	(8–14)	(11–31)	
Mean no. cycles administered	3.9	3.1	0.378
Grade 3–4 toxicity experienced	6 (40)	14 (93)	0.005
Haematological			
Neutropenia	4 (27)	2 (13)	0.651
Leukopenia	2 (13)	5 (33)	0.390
Non-haematological			
Nausea, vomiting	0 (0)	10 (67)	<0.001
Fatigue	0 (0)	3 (20)	0.224
Mean total treatment cost (yen/month)	475 372	443 979	0.147

MST, median survival time.

Response, toxicity and cost

All results regarding response, toxicity and cost are summarized in Table 4. The overall response rate was 40% for the non-platinum group and 47% for the platinum group ($P = 1.000$). The MST was 14 months for the non-platinum group and 14.5 months for the platinum group ($P = 0.264$). The frequency of grade 3–4 toxicity was 40% for the non-platinum group and 93% for the platinum group, which was statistically significant ($P = 0.005$). The most frequently observed grade 3–4 haematological toxicity was neutropenia (27%) in the non-platinum group and leucopenia (33%) in the platinum group. There were no grade 3–4 non-haematological toxicity events in the non-platinum group, but 67% of patients suffered from nausea/vomiting in the platinum group ($P < 0.001$). As an index of overall efficacy and toxicity, the number of responders who did not experience grade 3–4 toxicity was 3 (20%) in the non-platinum group and 0 (0%) in the platinum group ($P = 0.100$). The number of non-responders who experienced grade 3–4 toxicity was 3 (20%) in the non-platinum group and 7 (47%) in the platinum group ($P = 0.128$). The average total treatment costs per month were ¥475 372 (approximately US$4080) and ¥443 979 (approximately US$3810) for the non-platinum and platinum groups respectively ($P = 0.141$). The average hospitalization costs per month were ¥265 663 (approximately US$2290) and ¥266 415 (approximately US$2296) for the non-platinum and platinum groups respectively. There was no statistically significant difference between the two groups with respect to cost. Three patients in each group received G-CSF. The average costs for G-CSF per patient were ¥12 797 (approximately US$110) and ¥22 073 (approximately US$190) in the non-platinum and platinum groups respectively ($P = 0.366$).

Table 5. Response and toxicity of platinum and non-platinum chemotherapy regimens for elderly patients

	Non-platinum (*n* = 8) *n* (%)	Platinum (*n* = 6) *n* (%)	*P*-value
Response			
Complete response	0	0	
Partial response	5	5	
Stable disease	2	1	
Progressive disease	1	0	
Response rate	5 (63)	5 (83)	0.580
Grade 3–4 toxicity experienced	5 (63)	5 (83)	0.580
Haematological			
Neutropenia	3 (38)	0 (0)	0.209
Leukopenia	2 (25)	2 (33)	1.000
Non-haematological			
Nausea, vomiting	0 (0)	4 (55)	0.015
Fatigue	0 (0)	1 (17)	0.429

We carried out further analysis on a subgroup of elderly patients (those aged 65 years or older; the non-platinum group: 8 patients, and the platinum group: 6 patients). The response and toxicity data for the elderly patient subgroups are summarized in Table 5. In this subgroup, the overall response rate was 63% for the non-platinum group and 83% for the platinum group ($P = 0.580$). The frequency of grade 3–4 toxicity was 63% for the non-platinum group and 83% for the platinum group ($P = 0.580$). The most frequently observed grade 3–4 haematological toxicity was neutropenia (38%) in the non-platinum group and leucopenia (33%) in the platinum group. There was no grade 3–4 non-haematological toxicity event in the non-platinum group, but 67% of patients in the platinum group suffered from nausea/vomiting; the difference was statistically significant ($P = 0.015$). The number of responders who did not experience grade 3–4 toxic events was 2 (25%) in the non-platinum group and 0 (0%) in the platinum group. The number of non-responders who experienced grade 3–4 toxic events was 2 (25%) in the non-platinum group and 0 (0%) in the platinum group.

DISCUSSION

Although CDDP-based chemotherapy has become established as a standard therapy for the treatment of patients

with advanced NSCLC with good performance status, it has the drawback of serious toxicity, causing such symptoms as severe nausea, vomiting and renal toxicity, and is thus not suitable for elderly patients and outpatients. Recent trials of new anticancer drugs have indicated that some non-platinum-based combinations are almost as active as CDDP-based chemotherapy regimens, but are less toxic. In particular, the GEM/VNR combination has been shown to be well tolerated by patients, and to be very active (Non-small Cell Lung Cancer Collaborative Group 1995; Lorusso *et al.* 1998; Feliu *et al.* 1999; Isokangas *et al.* 1999; Beretta *et al.* 2000; Chen *et al.* 2000; Frasci *et al.* 2000; Lorusso *et al.* 2000; Krajnik *et al.* 2000; Herbst *et al.* 2002; Gridelli *et al.* 2003), and thus might be a good alternative to CDDP-based chemotherapy regimens. A new non-platinum sequential triplet combination, GEM and VNR, followed by DOC, was recently evaluated in a JMTO phase II study, and was found to be well tolerated, with one of the highest response rates yet reported for treatment of advanced NSCLC (JMTO LC00-02; Hosoe *et al.* 2003). Given these findings, a phase III randomized trial (JMTO LC00-03) began in April 2001 to compare this non-platinum sequential triplet combination with a platinum combination (carboplatin/paclitaxel). This phase III trial is ongoing in collaboration with the Southwest Oncology Group's (SWOG) trial (S0003) (carboplatin/paclitaxel versus carboplatin/paclitaxel + tirapazamine), using the same protocol for the common control arm (carboplatin/paclitaxel) (Williamson *et al.* 2005). We thus conducted a case-matched retrospective study in a single institution as a part of the multi-institutional phase II trial (JMTO LC00-02). The purpose of the present study was, in the context of JMTO LC00-02, to assess this non-platinum sequential triplet combination in terms of efficacy, toxicity and treatment cost relative to platinum-based combinations comprising CDDP plus one or more other anticancer drugs for the treatment of advanced NSCLC. Consequently, the present study provides some of the first results concerning a comparison of the new non-platinum sequential triplet combination with platinum-based combinations.

The non-platinum group in the present study was a subgroup of patients involved in the JMTO LC00-02 phase II trial, which included 44 patients from 17 institutions (response rate of 47.7%, median survival time of 15.7 months) (Hosoe *et al.* 2003). We believe that the selected patients were representative of the phase II study population as a whole, because there was no significant difference in the distribution of outcomes and patient characteristics between the group as a whole and the selected patients.

In order to ensure comparability between the non-platinum and platinum groups, we sourced patients from a single institution, and selected each patient in the platinum group from the pool of all patients who received CDDP-based chemotherapy during the study period by matching for stage (IIIB/IV), performance status (0/1), age and sex, all of which are considered to be important prognostic factors. The resulting number of patients in each group was small.

Differences in the distribution of histological diagnoses between the two groups were found: the non-platinum group included a smaller number of patients with adenocarcinomas but a larger number of patients with large cell carcinomas than the platinum group. We performed subgroup analysis according to histological diagnosis to evaluate the effects of treatment. The overall response rate for patients with adenocarcinoma, squamous cell carcinoma, and large cell carcinoma were 67% (2/3), 29% (2/7) and 40% (2/5) respectively in the non-platinum group, and 50% (4/8), 34% (2/6) and 100% (1/1) respectively in the platinum group. There was no significant difference in overall response rate between the non-platinum and platinum groups when subgroups of patients with similar histological diagnoses were compared.

In previous studies regarding therapy for NSCLC, the response rate and MST were found to be 13% and 6 months respectively for treatment with DOC alone (Roszkowski *et al.* 2000), and 19% and 6.5 months respectively for treatment with VNR alone (The Elderly Lung Cancer Vinorelbine Italian Study Group 1999). As for combined regimens, the response rate and MST have been found to be 17% and 7.4 months (Schiller *et al.* 2002) and 37% and 11.7 months (Takeda *et al.* 2000) respectively for DOC + CDDP, and 30% and 9.3 months (Le Chevalier *et al.* 1994), and 26% and 8 months (Wozniak *et al.* 1998) respectively for VNR + CDDP. The response rate and MST of the platinum group in the present study (40%, 13.5 months respectively) were greater than those found in previous studies of combination regimens comprising CDDP and new anticancer drugs. In the present study, the MST of the platinum group was comparable to that of the non-platinum group. This might be partly due to additional treatments, such as radiotherapy and/or other chemotherapeutic agents, received by patients in the platinum group.

With respect to toxicity, some patients in the platinum group suffered from adverse reactions accompanied by symptoms such as leucopenia (one-third of patients) and nausea/vomiting (two-thirds or more). In addition, six patients who suffered a physical and/or mental burden from these toxicities refused further chemotherapy and withdrew from treatment early. It should be noted that

because the toxicity information in the platinum group was obtained from medical charts, a certain proportion of toxicity events may not have been reported, and thus the event rates may have been underestimated. In contrast, a thorough reporting system was used for patients in the non-platinum group because they were involved in a phase II trial. In the non-platinum group, one-fourth of patients had grade 3–4 neutropenia, but no patients presented with any severe non-haematological toxicity. In fact, the major reason for interruption of chemotherapy in the non-platinum group was progression of the primary cancer (8 cases). Furthermore, for five patients in the non-platinum group who began receiving chemotherapy in an ambulatory setting in the middle of the follow-up period, no severe adverse events were observed, and emergency hospital admission was not required.

In the subgroup of elderly patients (65 years or older), the overall response rate was higher than that in each group as a whole. No elderly patients in the non-platinum group suffered grade 3–4 non-haematological toxicity events, including nausea and vomiting or fatigue, whereas 55% and 17% of elderly patients in the platinum subgroup experienced these adverse reactions respectively.

As we have already seen, the incidence of toxic events in the non-platinum group was significantly lower than that in the platinum group, and in each group the incidence was similar in the subgroup of elderly patients and the group as a whole. We thus conclude that this new non-platinum regimen could be established as a standard treatment, especially for elderly patients or outpatients.

Because most participants in this study were inpatients, even in the non-platinum group, there was no difference in the cost of treatment between the two groups. The cost of hospitalization was also equal in each group. Because management of adverse events is required to a lesser extent for patients receiving non-platinum regimens, chemotherapy could be administered in an ambulatory setting rather than in an inpatient setting. If chemotherapy can be administered in an ambulatory setting, the medical cost would become substantially lower, much lower than that of CDDP-based chemotherapy, which usually requires hospitalization.

In an overall assessment of efficacy and toxicity, the number of responders who did not experience grade 3–4 toxic events, which represents one of the most positive outcomes for patients, was 3 (20%) in the non-platinum group and 0 (0%) in the platinum group. The number of non-responders who experienced grade 3–4 toxic events, which represents the worst outcome for patients, was 3 (20%) in the non-platinum group and 7 (47%) in the platinum group.

In conclusion, these results indicate that the chemotherapy regimen used for the non-platinum group was equally beneficial and less burdensome than those used for the platinum group. Although this study is retrospective and could be considered a preliminary study, given its limited small sample size, the results suggest that the new non-platinum sequential triplet combination could replace CDDP-based chemotherapy as first-line treatment for advanced NSCLC, and that this regimen would be particularly useful for elderly patients and outpatients.

REFERENCES

Beretta G.D., Michetti G., Belometti M.O., Gritti G., Quadri A., Poletti P. & Labianca R. (2000) Gemcitabine plus vinorelbine in elderly or unfit patients with non-small cell lung cancer. *British Journal of Cancer* **83**, 573–576.

Chen Y.M., Perng R.P., Yang K.Y., Liu T.W., Tsai C.M., Ming-Liu J. & Whang-Peng J. (2000) A multicenter phase II trial of vinorelbine plus gemcitabine in previously untreated inoperable (stage IIIB/IV) non-small cell lung cancer. *Chest* **117**, 1583–1589.

Feliu J., Lopez Gomez L., Madronal C., Espinosa E., Espinosa J., Giron C.G., Martinez B., Castro J., De la Gandara I. & Baron M.G. (1999) Gemcitabine plus vinorelbine in nonsmall cell lung carcinoma patients age 70 years or older or patients who cannot receive cisplatin. Oncopaz Cooperative Group. *Cancer* **86**, 1463–1469.

Frasci G., Lorusso V., Panza N., Comella P., Nicolella G., Bianco A., De Cataldis G., Iannelli A., Bilancia D., Belli M., Massidda B., Piantedosi F., Comella G. & De Lena M. (2000) Gemcitabine plus vinorelbine versus vinorelbine alone in elderly patients with advanced non-small-cell lung cancer. *Journal of Clinical Oncology* **18**, 2529–2536.

Gridelli C., Maione P., Castaldo V. & Rossi A. (2003) Gefitinib in elderly and unfit patients affected by advanced non-small-cell lung cancer. *British Journal of Cancer* **89**, 1827–1829.

Grilli R., Oxman A.D. & Julian J.A. (1993) Chemotherapy for advanced non-small-cell lung cancer: how much benefit is enough? *Journal of Clinical Oncology* **11**, 1866–1872.

Herbst R.S., Khuri F.R., Lu C., Liu D.D., Fossella F.V., Glisson B.S., Pisters K.M., Shin D.M., Papadimitrakopoulou V.A., Kurie J.M., Blumenschein G. Jr, Kies M.S., Zinner R., Jung M.S., Lu R., Lee J.J., Munden R.F., Hong W.K. & Lee J.S. (2002) The novel and effective nonplatinum, nontaxane combination of gemcitabine and vinorelbine in advanced nonsmall cell lung carcinoma: potential for decreased toxicity and combination with biological therapy. *Cancer* **95**, 340–353.

Hosoe S., Komuta K., Shibata K., Harada H., Iwamoto Y., Ohsaki Y., Morioka T., Origasa H., Fukushima M., Furuse K. & Kawahara M. (2003) Gemcitabine and vinorelbine followed by docetaxel in patients with advanced non-small-cell lung cancer: a multi-institutional phase II trial of nonplatinum sequential triplet combination chemotherapy (JMTO LC00-02). *British Journal of Cancer* **88**, 342–347.

Isokangas O.P., Knuuttila A., Halme M., Mantyla M., Lindstrom I., Nikkanen V., Viren M., Joensuu H. & Mattson K. (1999) Phase II study of vinorelbine and gemcitabine for inoperable stage IIIB-IV non-small-cell lung cancer. *Annals of Oncology* **10**, 1059–1063.

Krajnik G., Mohn-Staudner A., Thaler J., Greil R., Schmeikal S., Marhold F., Deutsch J., Preiss P., Malayeri R., Schafer-Prokop

C., Wein W., Huber H. & Pirker R. (2000) Vinorelbine-gemcitabine in advanced non-small-cell lung cancer (NSCLC): an AASLC phase II trial. Austrian Association for the Study of Lung Cancer. *Annals of Oncology* **11**, 993–998.

Le Chevalier T., Brisgand D., Douillard J.Y., Pujol J.L., Alberola V., Monnier A., Riviere A., Lianes P., Chomy P. & Cigolari S. (1994) Randomized study of vinorelbine and cisplatin versus vindesine and cisplatin versus vinorelbine alone in advanced non-small-cell lung cancer: results of a European multicenter trial including 612 patients. *Journal of Clinical Oncology* **12**, 360–367.

Lorusso V., Mancarella S., Carpagnano F., Rienzo G.D., Cisternino L., Napoli G., Orlando S., Cinieri S., Brunetti C., Palazzo S., Catino A., Paradiso A., Panza N., Cataldis G.D., Frasci G. & Lena M.D. (1998) Gemcitabine plus vinorelbine in patients with stage IIIB–IV non small cell lung cancer (NSCLC). A phase II study. *Proceedings of the American Society for Clinical Oncology* **17**, 470a.

Lorusso V., Carpagnano F., Frasci G., Panza N., Di Rienzo G., Cisternino M.L., Napoli G., Orlando S., Cinieri S., Brunetti C., Palazzo S. & De Lena M. (2000) Phase I/II study of gemcitabine plus vinorelbine as first-line chemotherapy of non-small-cell lung cancer. *Journal of Clinical Oncology* **18**, 405–411.

Non-small Cell Lung Cancer Collaborative Group (1995) Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised trial. *British Medical Journal* **311**, 899–909.

Roszkowski K., Pluzanska A., Krzakowski M., Smith A.P., Saigi E., Aasebo U., Parisi A., Pham Tran N., Olivares R. & Berille J. (2000) A multicenter, randomized, phase III study of docetaxel plus best supportive care versus best supportive care in chemotherapy-naive patients with metastatic or non-resectable localized non-small cell lung cancer (NSCLC). *Lung Cancer* **27**, 145–157.

Schiller J.H., Harrington D., Belani C.P., Langer C., Sandler A., Krook J., Zhu J., Johnson D.H. & Eastern Cooperative Oncology Group (2002) Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *New England Journal of Medicine* **346**, 92–98.

Statistics and Information Department, Minister's Secretariat, Ministry of Health and Welfare (2000) Vital statistics of Japan 1998 (vol 3). Table 2. Death by causes (the condensed list of causes of death for Japan), sex and age. pp. 384–397. Tokyo.

Takeda K., Yamamoto N., Negoro S., Nakagawa K., Matsui K., Kawahara M., Takeda Y., Fukuoka M. & Aruyoshi Y. (2000) Randomized phase II study of docetaxel (DOC) plus cisplatin (CDDP) versus DOC plus irinotecan in advanced non-small-cell lung cancer (NSCLC); a West Japan Thoracic Oncology Group (WJTOG) Study. *Proceedings of the American Society for Clinical Oncology* **19**, 497a.

The Elderly Lung Cancer Vinorelbine Italian Study Group. (1999) Effects of vinorelbine on quality of life and survival of elderly patients with advanced non-small-cell lung cancer. *Journal of the National Cancer Institute* **91**, 66–72.

Therasse P., Arbuck S.G., Eisenhauer E.A., Wanders J., Kaplan R.S., Rubinstein L., Verweij J., Van Glabbeke M., van Oosterom A.T., Christian M.C. & Gwyther S.G. (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *Journal of the National Cancer Institute* **92**, 205–216.

Williamson S.K., Crowley J.J., Lara P.N. Jr, McCoy J., Lau D.H., Tucker R.W., Mills G.M., Gandara D.R. & Southwest Oncology Group Trial S0003 (2005) Phase III trial of paclitaxel plus carboplatin with or without tirapazamine in advanced non-small-cell lung cancer: Southwest Oncology Group trial S0003. *Journal of Clinical Oncology* **23**, 9097–9104.

Wozniak A.J., Crowley J.J., Balcerzak S.P., Weiss G.R., Spiridonidis C.H., Baker L.H., Albain K.S., Kelly K., Taylor S.A., Gandara D.R. & Livingston R.B. (1998) Randomized trial comparing cisplatin with cisplatin plus vinorelbine in the treatment of advanced non-small-cell lung cancer: a Southwest Oncology Group study. *Journal of Clinical Oncology* **16**, 2459–2465.

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

Nobuyuki Yamamoto,[1,2,3] Masahiro Andoh,[1] Masaaki Kawahara,[1] Masahiro Fukuoka[1] and Hisanobu Niitani[1]

[1]TZT-1027 Cooperative Study Group; [2]Division of Thoracic Oncology, Shizuoka Cancer Center, Shimonagakubo 1007 Shunto-gun, Shizuoka 411-8777, Japan

(Received July 23, 2008/Revised September 19, 2008/Accepted October 6, 2008/Online publication December 8, 2008)

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

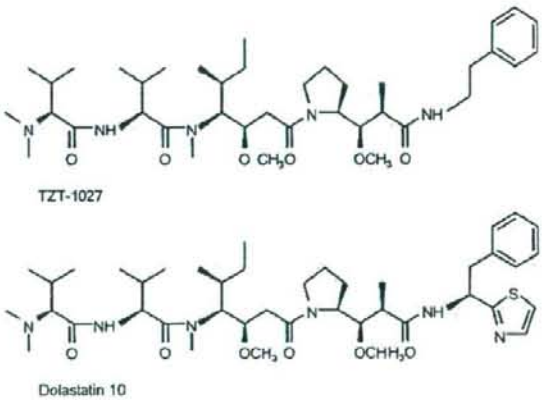

Fig. 1. Structural formulae of TZT-1027 and dolastatin 10.

[3]To whom correspondence should be addressed. E-mail: n.yamamoto@scchr.jp

doi: 10.1111/j.1349-7006.2008.01023.x

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

doi: 10.1111/j.1349-7006.2008.01023.x

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.

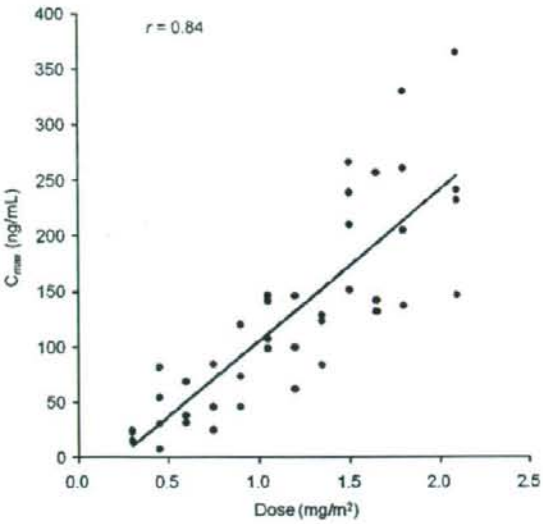

Fig. 2. Correlation between dose and maximum concentration (C_{max}) at the first administration.

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.

Acknowledgments

We thank the following investigators who, in addition to the authors, contributed patients to this study: Y. Nakai, S. Kudoh, T. Sasaki, N. Horikoshi, M. Kurihara, and M. Hoshiai, and M. Shibuya of the TZT-1027 Cooperative Study Group.

doi: 10.1111/j.1349-7006.2008.01023.x

References

1 Miyazaki K, Kobayashi M, Natsume T *et al.* Synthesis and antitumor activity of novel dolastatin 10 analogs. *Chem Pharm Bull* 1995; **43**: 1706–18.
2 Pettit GR, Kamano Y, Herald CL *et al.* The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10. *J Am Chem Soc* 1987; **109**: 6883–5.
3 Watanabe J, Natsume T, Fujio N, Miyasaka K, Kobayashi M. Induction of apoptosis in human cancer cells by TZT-1027, an antimicrotubule agent. *Apoptosis* 2000; **5**: 345–53.
4 Natsume T, Watanabe J, Tamaoki S, Fujio N, Miyasaka K, Kobayashi M. Characterization of the interaction of TZT-1027a potent antitumour agent, with tubulin. *Jpn J Cancer Res* 2000; **91**: 737–47.
5 Fujita F, Koike M, Fujita M, Sakamoto Y, Tsukagoshi S. Antitumor effects of TZT-1027a novel dolastatin 10 derivative, on human tumor xenografts in nude mice. *Jpn J Cancer Chemother* 2000; **27**: 451–8.
6 Hashiguchi N, Kubota T, Koh J *et al.* TZT-1027 elucidates antitumor activity through direct cytotoxicity and selective blockage of blood supply. *Anticancer Res* 2004; **24**: 2201–8.
7 Natsume T, Watanabe J, Koh Y *et al.* Antitumor activity of TZT-1027 (soblidotin) against endothelial growth factor-secreting human lung cancer in vivo. *Cancer Sci* 2003; **94**: 826–33.
8 Otani M, Natsume T, Watanabe JI *et al.* TZT-1027, an antimicrotubule agent, attacks tumor vasculature and induces tumor cell death. *Jpn J Cancer Res* 2000; **91**: 837–44.
9 Ogawa T, Mimura Y, Isowa K *et al.* An antimicrotubule agent, TZT-1027, does not induce neuropathologic alterations which are detected after administration of vincristine or paclitaxel in animal models. *Toxicol Lett* 2001; **121**: 97–106.
10 Niitani H, Hasegawa K, Furuse K, Fukuoka M, Horikoshi N, Kudoh S. Phase I studies of TZT-1027a novel inhibitor of tubulin polymerization (Abstract). *Ann Oncol* 1998; **9** (Suppl. 2): 360.
11 Shibuya M. Adverse drug reaction criteria of the Japan Society for Cancer Therapy. *Gan to Kagaku Ryoho* 1997; **24**: 2036–41.
12 Furue H, Hara Y, Imai Y *et al.* Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. *J Jpn Soc Cancer Ther* 1993; **28** (2): 101–30.
13 Mross K, Herbst K, Berdel WE *et al.* Phase I clinical and pharmacokinetic study of LU103793 (Cemadotin hydrochloride) as an intravenous bolus injection in patients with metastatic solid tumors. *Onkologie* 1996; **19**: 490–5.
14 Cunningham C, Appleman LJ, Kirvan-Visovatti M *et al.* Phase I and pharmacokinetic study of the dolastatin-15 analogue tasidotin (ILX651) administered intravenously on days 1, 3, and 5 every 3 weeks in patients with advanced solid tumors. *Clin Cancer Res* 2005; **11**: 7825–33.
15 Ebbinghaus S, Rubin E, Hersh E *et al.* A phase I study of the dolastatin-15 analogue tasidotin (ILX651) administered intravenously daily for 5 consecutive days every 3 weeks in patients with advanced solid tumors. *Clin Cancer Res* 2005; **11**: 7807–78.
16 Madden T, Tran HT, Beck D *et al.* Novel marine-derived anticancer agents: a phase I clinical, pharmacological, and pharmacodynamic study of dolastatin 10 (NSC 376128) in patients with advanced solid tumors. *Clin Cancer Res* 2000; **6**: 1293–301.
17 Pilot HC, McElroy EA, Reid JM *et al.* Phase I trial of dolastatin-10 (NSC 376128) in patients with advanced solid tumors. *Clin Cancer Res* 1999; **5**: 525–31.
18 de Jonge MJ, van der Gaast A, Planting AS *et al.* Phase I and pharmacokinetic study of the dolastatin 10 analogue TZT-1027, given on days 1 and 8 of a 3-week cycle in patients with advanced solid tumors. *Clin Cancer Res* 2005; **11**: 3806–13.
19 Kenji T, Kazuhiko N, Takayasu K *et al.* Phase I study of TZT-1027a novel synthetic dolastatin 10 derivative and inhibitor of tubulin polymerization, which was administered to patients with advanced solid tumors on days 1 and 8 in 3-week courses. *Cancer Chemother Pharmacol* 2007; **60**: 285–93.
20 Junichi W, Tsugitaka N, Motohiro K. Antivascular effects of TZT-1027 (Soblidotin) on murine Colon26 adenocarcinoma. *Cancer Sci* 2006; **97**: 1410–16.
21 Tsugitaka N, Junichi W, Kenji O, Kazuhiko Y, Motohiro K. Tumor-specific antivascular effect of TZT-1027 (Soblidotin) elucidated by magnetic resonance imaging and confocal laser scanning microscopy. *Cancer Sci* 2007; **98**: 598–604.
22 Akashi Y, Okamoto I, Suzuki M *et al.* The novel microtubule-interfering agent TZT-1027 enhances the anticancer effect of radiation in vitro and in vivo. *Br J Cancer* 2007; **96**: 1532–9.
23 Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981; **47**: 207–14.

Randomized phase II study of two different schedules of gemcitabine and oral TS-1 in chemo-naïve patients with advanced non-small cell lung cancer (NSCLC).

Sub-category: Metastatic Lung Cancer
Category: Lung Cancer--Metastatic Lung Cancer
Meeting: 2008 ASCO Annual Meeting

Abstract No: 8103
Citation: *J Clin Oncol* 26: 2008 (May 20 suppl; abstr 8103)
Author(s): M. Satouchi, Y. Kotani, N. Katakami, T. Shimada, Y. Urata, S. Yoshimura, Y. Funada, A. Hata, M. Ando, S. Negoro

Abstract: **Background:** TS-1, a novel oral fluorouracil derivative, has been shown to have anti-tumor activity with relatively mild adverse effects, and it is used in the treatment of NSCLC in Japan. The combination of gemcitabine (GEM) and 5-FU demonstrates a marked synergistic cytotoxic effect in a sequence-dependent manner in *in vitro* assay. This study was conducted in order to evaluate the efficacy and safety and to compare dosing schedules of gemcitabine combined with TS-1 in chemo-naïve NSCLC patients (pts). **Methods:** Pts with chemo-naive stage IIIB/IV NSCLC, an ECOG-PS of 0 or 1, and normal renal, liver, and bone marrow functions were randomized into 1 of 2 treatment arms. Oral TS-1 was administered daily from day 1 to 14, and GEM was given on days 1 and 8 (Arm A) or days 8 and 15 (Arm B). This cycle was repeated every 21 days. **Results:** A total of 80 pts were entered and 79 pts, treated in this trial. Randomization was well balanced across patient characteristics except for cell type (adenocarcinoma/squamous cell carcinoma = 37/4 (Arm A), 27/10 (Arm B)). Grade 3/4 hematological toxicities were neutropenia (54%), febrile neutropenia (9%), thrombocytopenia (11%) and anemia (4%). The hematological toxicity profiles did not differ very much between the two arms. Grade 3 pneumonitis was observed in 2 pts (3%). The response rate was 23.1% (95% confidence interval [CI]=11.1-39.3%) in Arm A and 30.6% (95% CI=16.3-46.1%), Arm B. Median time-to- progression (TTP) in Arm A was 4.1 months (95% CI=2.8-5.5) and Arm B, 5.4 months (95% CI=3.8-6.3) (p=0.75). Median survival time in Arm A was 15.7 months (95% CI=8.6-23.3) and Arm B, 22.4 months (95% CI=11.5-unknown) (p=0.32). **Conclusions:** The combination of GEM and TS-1 was determined to be feasible and effective for advanced NSCLC, and these results, particularly the favorable MST of Arm B, warrant further investigation of the Arm B dosing schedule for this combination for NSCLC.

Abstract Disclosures

Associated Presentation(s):

1. **Randomized phase II study of two different schedules of gemcitabine and oral TS-1 in chemo-naïve patients with advanced non-small cell lung cancer (NSCLC).**

   Meeting: 2008 ASCO Annual Meeting
   Presenter: Miyako Satouchi, MD
   Session: Lung Cancer — Metastatic (General Poster Session)

Other Abstracts in this Sub-Category

1. **FLEX: A randomized, multicenter, phase III study of cetuximab in combination with cisplatin/vinorelbine (CV) versus CV alone in the first-line treatment of patients with advanced non-small cell lung cancer (NSCLC).**

   Meeting: 2008 ASCO Annual Meeting Abstract No: 3 First Author: R. Pirker
   Category: Lung Cancer--Metastatic Lung Cancer - Metastatic Lung Cancer

2. **Characterization of NSCLC patients responding to anti-IGF-IR therapy.**

   Meeting: 2008 ASCO Annual Meeting Abstract No: 8000 First Author: A. Gualberto
   Category: Lung Cancer--Metastatic Lung Cancer - Metastatic Lung Cancer

3. **Molecular and clinical subgroup analyses from a phase III trial comparing gefitinib with docetaxel in previously treated non-small cell lung cancer (INTEREST).**

   Meeting: 2008 ASCO Annual Meeting Abstract No: 8001^ First Author: J. Douillard
   Category: Lung Cancer--Metastatic Lung Cancer - Metastatic Lung Cancer

More...

Abstracts by M. Satouchi

1. **Randomized phase II study of two different schedules of gemcitabine and oral TS-1 in chemo-naïve patients with advanced non-small cell lung cancer (NSCLC).**

   Meeting: 2008 ASCO Annual Meeting Abstract No: 8103 First Author: M. Satouchi
   Category: Lung Cancer--Metastatic Lung Cancer - Metastatic Lung Cancer

2. **Randomized phase III study of platinum-doublet chemotherapy followed by gefitinib versus continued platinum-doublet chemotherapy in patients (pts) with advanced non-small cell lung cancer (NSCLC): Results of West Japan Thoracic Oncology Group trial (WJTOG.**

Meeting: 2008 ASCO Annual Meeting Abstract No: LBA8012 First Author: T. Hida
Category: Lung Cancer--Metastatic Lung Cancer - Metastatic Lung Cancer

3. **Randomized, phase III study of mitomycin/vindesine/cisplatin (MVP) versus weekly irinotecan/carboplatin (IC) or weekly paclitaxel/carboplatin (PC) with concurrent thoracic radiotherapy (TRT) for unresectable stage III non-small cell lung cancer (NSCLC).**

Meeting: 2007 ASCO Annual Meeting Abstract No: 7530 First Author: T. Kimura
Category: Lung Cancer - Non-Small Cell Lung Cancer

More...

Presentations by M. Satouchi

1. **Randomized phase II study of two different schedules of gemcitabine and oral TS-1 in chemo-naïve patients with advanced non-small cell lung cancer (NSCLC).**

Meeting: 2008 ASCO Annual Meeting
Presenter: Miyako Satouchi, MD
Session: Lung Cancer — Metastatic (General Poster Session)

2. **Randomized Phase II Study Of Docetaxel (doc) Plus Cisplatin (cddp) Versus Doc Plus Irinotecan In Advanced Non-small Cell Lung Cancer (nsclc); A West Japan Thoracic Oncology Group (wjtog) Study**

Meeting: 2001 ASCO Annual Meeting
Presenter: Miyako Satouchi, MD, PhD
Session: Small Cell and Non-Small Cell Lung Cancer (General Poster)

More...
