RESULTS

Patient Characteristics and Drug Exposure

Between 11 December 2008 and 10 May 2009, eight patients were screened at four study sites. Six received L-BLP25 and were included in the safety population. Median (range) age was 63.5 (59–69) years and five were male. ECOG performance status was 0 in five patients and 1 in one patient. At first diagnosis, five had Stage IIIA disease and one had Stage IIIB disease. Four patients were diagnosed with adenocarcinoma and two with squamous cell carcinoma. The median (range) duration of NSCLC (from diagnosis) was 5.7 (4.4–9.4) months. Primary chemoradiotherapy was concomitant in four patients and sequential in two, and resulted in stable disease in one patient and objective responses (partial or complete) in five.

As of 12 June 2009, median (range) duration of treatment (L-BLP25 including cyclophosphamide) was 7.7 (4.4–13.6) weeks, with a median (range) of 8 (5–9) L-BLP25 vaccinations. The median (range) total dose of cyclophosphamide was 300.0 (299.4–300.0) mg/m^2.

Safety

Of the six patients, five (83.3%) reported at least one AE (Table 1), all of which were Grade 1. No serious AEs were observed. No AEs led to discontinuation. One patient discontinued because of disease progression.

AEs related to L-BLP25 treatment were myalgia and arthralgia in one patient, and nausea in another. AEs related to cyclophosphamide were dysgeusia in one patient, and anorexia and nausea in another.

No safety concerns were identified via serum cytokine monitoring (Fig. 1). Serum concentrations of IL-1β, sIL-2 Rα, IL-6, IL-8 and TNFα all fell within the normal range at baseline and during the study, except for two patients: one whose IL-6 levels normalized during treatment, from 12.8 (pre-treatment) to 11.3 pg/ml (normal range: 0.0–11.8 pg/ml), and another whose TNFα level increased from <2.2 (pre-treatment) to 44.49 pg/ml during treatment (normal range: 0.00–7.46 pg/ml). There were no clinically significant changes in other laboratory variables.

Table 1. Summary of adverse events (safety population)

(MedDRA preferred term)	Number of patients[a] ($n = 6$)	Related to cyclophosphamide	Related to L-BLP25
Anorexia	1	Yes	No
Arthralgia	1	No	Yes
Atrioventricular block	1	No	No
Back pain	1	No	No
Dysgeusia	1	Yes	No
Hyperuricemia	1	No	No
Injection site hematoma	1	No	No
Insomnia	1	No	No
Joint effusion	1	No	No
Myalgia	1	No	Yes
Nausea[b]	1	Yes	Yes
Radiation pneumonitis	2	No	No

L-BLP25, BLP25 liposome vaccine.
[a]Some patients experienced more than one adverse event.
[b]Experienced in the same patient on two separate occasions: the first event was considered to be related to cyclophosphamide and the second related to L-BLP25 treatment.

DISCUSSION

Preliminary safety data reported here, in six Japanese patients with unresectable Stage III NSCLC after primary chemoradiotherapy, suggest that L-BLP25 has an acceptable safety and tolerability profile in this patient population. These results were in accordance with previous findings in predominantly Caucasian populations (12,17). In a previous Phase IIb study of Caucasian patients with Stage IIIB or IV NSCLC, L-BLP25 was well tolerated with no unexpected safety issues. The most common side effects attributable to the vaccine were mild flu-like symptoms and mild injection site reactions (12). Follow-up of a subgroup of patients for ≥2 years showed that the good safety profile of L-BLP25 was maintained with prolonged treatment (14).

In March 2010 clinical trials of L-BLP25 were temporarily put on hold after a case of encephalitis occurred in a study of L-BLP25 for treatment of multiple myeloma. Subsequent work-up for the patient and overall safety analysis of L-BLP25 in NSCLC led to a lift of the clinical hold in June 2010. Trials of L-BLP25 in NSCLC restarted shortly afterwards. The data we present here were collected prior to, and so were not impacted by, the clinical hold.

Serum concentrations of pro-inflammatory cytokines were assessed in this study, in the expectation that according to its proposed mode of action, L-BLP25 induces an inflammatory and a T cell-driven immune response directed against the tumor. sIL-2 Rα is a cytokine receptor produced by activated T cells, while all other measured cytokines relate to inflammatory cells. All cytokines remained within the normal range for the majority of patients in this study, and these results did not indicate any safety concerns for L-BLP25.

Based on these Phase I findings, the Independent Safety Monitoring Board has recommended initiation of the Phase II Stage of this combined Phase I/II study without restrictions. In the Phase II component, 168 Japanese patients with unresectable Stage III NSCLC after primary

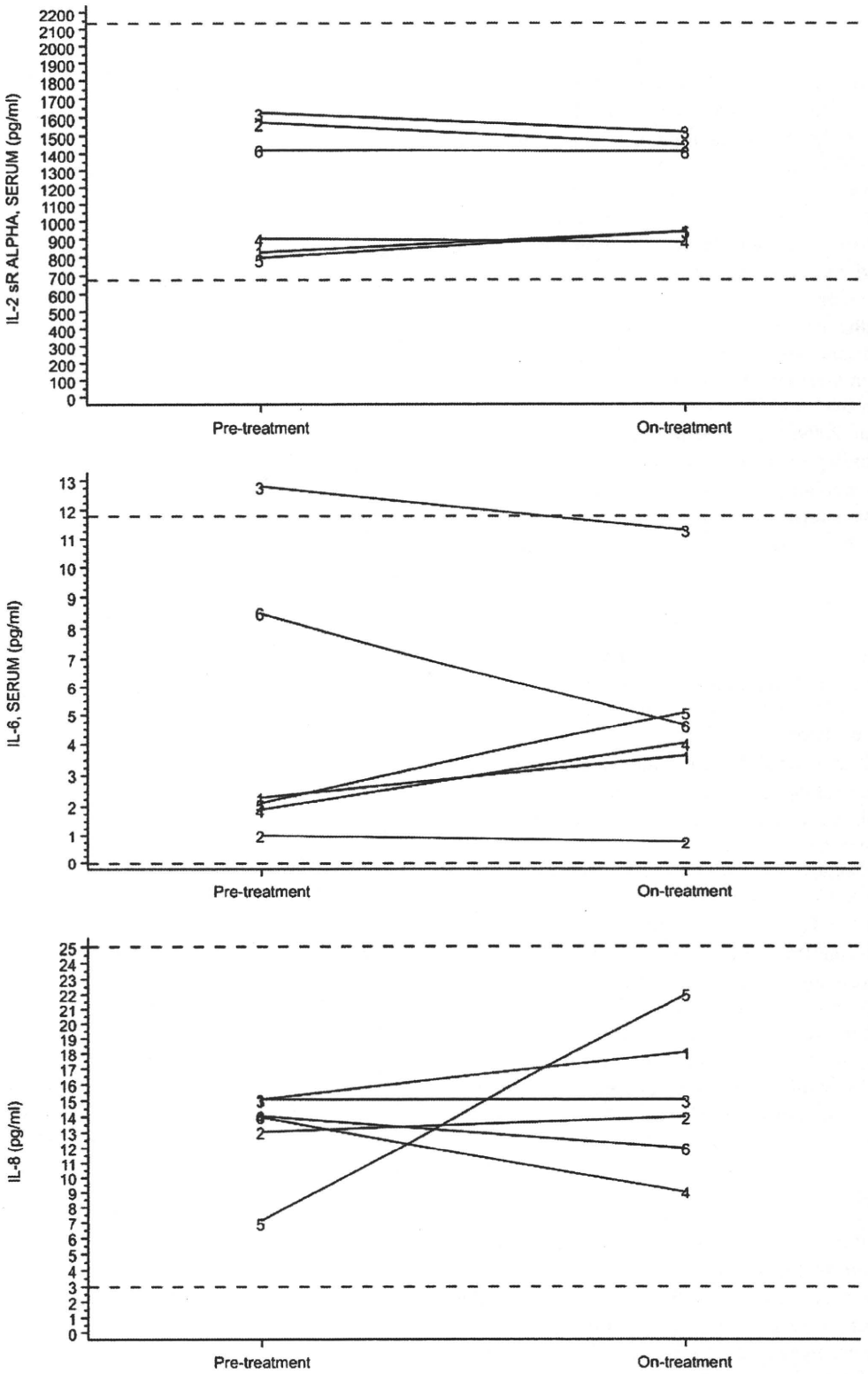

Figure 1. Serum concentrations of soluble interleukin (IL)-2 receptor alpha (sIL-2 Rα), IL-6 and IL-8 at the pretreatment evaluation visit and at week 5 of the open-label BLP25 liposome vaccine treatment period. Data not shown for serum concentrations of IL-1β (as all measurements were below the detection limit), or tumor necrosis factor alpha (as several measurements were below the detection limit). Dashed lines denote the corresponding normal ranges.

chemoradiotherapy will be randomized 2:1 to treatment with L-BLP25 plus BSC or placebo plus BSC, with once-weekly dosing for 8 weeks followed by maintenance doses every 6 weeks until disease progression or discontinuation (18). The primary objective of the Phase II stage is to compare overall survival time in the two treatment arms.

In conclusion, the first evaluation of L-BLP25 in Japanese patients with unresectable Stage III NSCLC after primary chemotherapy shows that it is well tolerated, and the safety profile is consistent with that seen in previous studies of Caucasian patients.

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Conflict of interest statement

S. Senger is employed by Merck KGaA and holds stock in Merck KGaA. N. Morsli is employed by Merck KGaA.

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CLINICAL INVESTIGATION

PHASE I STUDY OF CONCURRENT HIGH-DOSE THREE-DIMENSIONAL CONFORMAL RADIOTHERAPY WITH CHEMOTHERAPY USING CISPLATIN AND VINORELBINE FOR UNRESECTABLE STAGE III NON–SMALL-CELL LUNG CANCER

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Purpose: To determine the maximum tolerated dose in concurrent three-dimensional conformal radiotherapy (3D-CRT) with chemotherapy for unresectable Stage III non–small-cell lung cancer (NSCLC).

Patients and Methods: Eligible patients with unresectable Stage III NSCLC, age ≥20 years, performance status 0–1, percent of volume of normal lung receiving 20 GY or more (V_{20}) ≤30% received three to four cycles of cisplatin (80 mg/m^2 Day 1) and vinorelbine (20 mg/m^2 Days 1 and 8) repeated every 4 weeks. The doses of 3D-CRT were 66 Gy, 72 Gy, and 78 Gy at dose levels 1 to 3, respectively.

Results: Of the 17, 16, and 24 patients assessed for eligibility, 13 (76%), 12 (75%), and 6 (25%) were enrolled at dose levels 1 to 3, respectively. The main reasons for exclusion were V_{20} >30% (n = 10) and overdose to the esophagus (n = 8) and brachial plexus (n = 2). There were 26 men and 5 women, with a median age of 60 years (range, 41–75). The full planned dose of radiotherapy could be administered to all the patients. Grade 3–4 neutropenia and febrile neutropenia were noted in 24 (77%) and 5 (16%) of the 31 patients, respectively. Grade 4 infection, Grade 3 esophagitis, and Grade 3 pulmonary toxicity were noted in 1 patient, 2 patients, and 1 patient, respectively. The dose-limiting toxicity was noted in 17% of the patients at each dose level. The median survival and 3-year and 4-year survival rates were 41.9 months, 72.3%, and 49.2%, respectively.

Conclusions: 72 Gy was the maximum dose that could be achieved in most patients, given the predetermined normal tissue constraints. © 2011 Elsevier Inc.

Lung cancer, Chemotherapy, Radiotherapy, High dose, Conformal.

INTRODUCTION

Approximately one third of patients with non–small-cell lung cancer (NSCLC) present with locally advanced Stage III disease at the initial diagnosis (1). Of this category, Stage IIIA disease with bulky N2 and Stage IIIB disease without pleural effusion are characterized by a large primary lesion and/or involvement of the mediastinal or supraclavicular lymph nodes. In addition, the majority of these patients have occult systemic micrometastases. Concurrent thoracic radiotherapy and chemotherapy has been the standard care for these patients with unresectable disease (2, 3). A platinum doublet with a third-generation anticancer agent combined with thoracic radiotherapy was reported to yield a median overall survival time (OS) of more than 2 years and long-term survivors (4–6), but the effect of platinum-based chemotherapy has reached a plateau.

The failure pattern in patients with Stage III NSCLC treated by concurrent chemoradiotherapy was roughly local recurrence alone in one third of the patients, both local and distant recurrence in another third of patients, and distant metastasis without local failure in the remaining third of patients (2, 5).

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Thus, improvement of local control and suppression of distant metastasis are essential for prolongation of patient survival.

The conventional total dose of thoracic radiotherapy in patients with inoperable NSCLC has been 60 Gy administered in 30 fractions. This dose was established in 1987 by randomized Radiation Therapy Oncology Group trials that demonstrated better 3-year survival with a radiation dose of 60 Gy than with lower doses (7). In these trials, two-dimensional treatment planning was used, wherein the tumor volume was defined on kilovoltage radiographs (7). Thereafter, the standard initial target volume included the primary tumor, metastatic lymph nodes, and adjacent uninvolved ipsilateral hilar and mediastinal regions (elective nodal irradiation: ENI). Except for selected patients, excessive toxicity hampered an increase of the total dose to over 60 Gy in patients with locally advanced NSCLC.

It is, however, time now to reconsider the optimal dose of thoracic radiotherapy using new techniques in patients with locally advanced NSCLC, for the following reasons. First, positron emission tomography (PET) provides more accurate diagnosis of mediastinal lymph node metastases (8) and more accurate quantification of the tumor volumes, especially when atelectasis is present (9). Second, three-dimensional conformal radiation therapy (3D-CRT) enables radiation oncologists to delineate the tumor and adjacent normal tissue more sharply and to choose beam angles to maximize tumor coverage with minimum irradiation of normal tissues (10). Third, omission of the ENI resulted in improvement of radiation-associated toxicity without worsening the local control rate of the tumor (11, 12). Thus, by use of these new techniques, the optimal dose of thoracic radiation could exceed the conventional 60 Gy.

Two dose escalation studies in patients with locally advanced NSCLC showed that the total dose of thoracic radiotherapy could be increased up to 90 Gy in concurrent chemoradiotherapy using the 3D-CRT technique combined with weekly carboplatin and paclitaxel chemotherapy (13, 14). In these trials, chemoradiotherapy was administered after induction chemotherapy. However, it remained unclear whether these doses could be delivered safely to the majority of patients with locally advanced NSCLC, because it is not known how many patients were screened for the trials and how many of them were actually registered, and because some of the registered patients were excluded from the chemoradiotherapy phase after induction chemotherapy. The total number of patients evaluated in the two trials was also limited. Furthermore, chemotherapy other than weekly carboplatin and paclitaxel has not been evaluated in the setting of combined chemotherapy with high-dose thoracic radiotherapy, to our knowledge. The objectives of the current study were (1) to evaluate the toxicity of concurrent high-dose 3D-CRT without ENI with cisplatin and vinorelbine for unresectable Stage III NSCLC, (2) to determine the maximum tolerated dose (MTD) of thoracic radiotherapy, and (3) to observe the antitumor effects of this regimen.

PATIENTS AND METHODS

Study design

This study was designed as a Phase I study at the National Cancer Center Hospital. The protocol and consent form were approved by the Institutional Review Board of the National Cancer Center on July 28, 2005. We planned to treat 12 patients at a dose level and follow them up at least 6 months, and then escalate to the next level if 67% of the patients did not experience dose-limiting toxicity (DLT). We followed widely accepted normal tissue dose constraints. Patients with percent volume of the normal lung receiving 20 Gy or more (V_{20}) of greater than 30% were excluded and treated outside the study. Other dosimetric constraints were applied at the discretion of the treating radiation oncologist. Maximum doses exceeding 50 Gy to the spinal cord, 66 Gy to the esophagus, or 66 Gy to the brachial plexus were generally excluded.

Patient selection

Previously untreated patients with locally advanced NSCLC without effusion were screened for entry into this study. The eligibility criteria were (1) histologically or cytologically proven NSCLC, (2) unresectable Stage IIIA or IIIB disease confirmed by both computed tomography (CT) and PET, (3) no previous treatment, (4) measurable disease, (5) $V_{20} \leq 30\%$, (6) age ≥ 20 years, (7) Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, and (8) adequate bone marrow function (white blood cell [WBC] count $\geq 4.0 \times 10^9$/L, hemoglobin ≥ 9.5 g/dL, and platelet count $\geq 100 \times 10^9$/L), liver function (total bilirubin ≤ 1.5 mg/dL and transaminase ≤ 80 IU/L), renal function (serum creatinine ≤ 1.5 mg/dL), and pulmonary function ($PaO_2 \geq 70$ Torr under room air). Patients were excluded if (1) they had malignant pleural or pericardial effusion or (2) they had a concomitant serious illness such as uncontrolled angina pectoris, myocardial infarction in the previous 3 months, heart failure, uncontrolled diabetes mellitus, uncontrolled hypertension, interstitial pneumonitis or lung fibrosis identified by a chest x-ray, infection, or other diseases contraindicating chemotherapy or radiotherapy, or (3) they were pregnant or breast feeding. All patients gave their written informed consent.

Pretreatment evaluation

The pretreatment assessment included a complete blood cell count and differential count, routine chemistry determinations, creatinine clearance, blood gas analysis, electrocardiogram, lung function testing, chest x-rays, chest CT scan, brain CT scan or magnetic resonance imaging, abdominal CT, and PET.

Treatment schedule

Chemotherapy consisted of cisplatin 80 mg/m^2 on Day 1 and vinorelbine 20 mg/m^2 on Days 1 and 8, repeated every 4 weeks for three to four cycles. Cisplatin was administered by intravenous infusion for 60 minutes with 2,500 to 3,000 mL of intravenous fluid for hydration and prophylactic antiemetic therapy consisting of a 5-hydroxytriptamine-3 antagonist on Day 1 and a corticosteroid on Days 1 to 5. Vinorelbine, diluted in 50 mL of normal saline, was administered intravenously.

Radiation therapy started on Day 1 of the first cycle of chemotherapy and was delivered with megavoltage equipment (6–10 MV) once daily for 5 days a week. The total dose was 66 Gy in 33 fractions at level 1, 72 Gy in 36 fractions at level 2, and 78 Gy in 39 fractions at level 3. All patients underwent a 3D treatment planning CT 3 to 7 days before the start of the treatment, and the eligibility was finally confirmed based on evaluation using the

dose–volume histogram (DVH). The gross tumor volume (GTV) was defined as the primary tumor delineated on pulmonary windows of the chest CT or on the diagnostic PET scans. Atelectasis or secondary changes in the peripheral lung region of the primary tumor were not included. Metastatic lymph nodes defined as nodes of 1 cm or larger visualized on mediastinal windows of the CT images or PET-positive lymph nodes were also included in the GTV. The clinical target volume (CTV) was equivalent to the GTV. Uninvolved mediastinum or supraclavicular fossae were not included in the CTV. The planning target volume (PTV) was determined as the CTV plus 1.0 cm for the anterior, posterior, medial, and lateral margins and a 1.0 to 2.0 cm for the superior and inferior margins, taking account of setup variations and internal organ motion. The spinal cord dose was typically limited to 44 Gy, but a maximum of 50 Gy was allowed. The lung V_{20} was limited to 30% in all patients. The maximum dose to the brachial plexus and esophagus did not exceed 66 Gy. The 100% dose was prescribed to the reference point located in the central part of the PTV, and the entire PTV was covered with 95–107% of the prescribed dose principally, but variation of ±10% was allowed. Lung heterogeneity corrections using the equivalent path length algorithm were applied in all patients.

Toxicity assessment and treatment modification

Complete blood cell counts and differential counts, routine chemistry determinations, and a chest x-ray were performed once a week during the course of treatment. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE v3.0). The lung toxicity grade was defined as the highest grade among cough, dyspnea, obstruction/stenosis of airways, pneumonitis/pulmonary infiltrates, and pulmonary fibrosis in the pulmonary/upper respiratory section (15).

Vinorelbine administration on Day 8 was omitted if any of the following were noted: WBC count $<3.0 \times 10^9$/L, neutrophil count $<1.5 \times 10^9$/L, platelet count $<100 \times 10^9$/L, Grade 2–3 elevation of the serum hepatic transaminase level or total serum bilirubin levels, Grade 2–3 infection, Grade 2–3 pneumonitis, other ≥Grade 3 nonhematologic toxicity, body temperature ≥38°C, or PS of 2–3. Subsequent cycles of cisplatin and vinorelbine chemotherapy were delayed if any of the following toxicities were noted on Day 1: WBC count $<3.0 \times 10^9$/L, neutrophil count $<1.5 \times 10^9$/L, platelet count $<100 \times 10^9$/L, serum creatinine level ≥1.6 mg/dL, Grade 2–3 elevation of the serum hepatic transaminase level or total serum bilirubin levels, Grade 2–3 infection, Grade 2–3 pneumonitis, other ≥Grade 3 nonhematologic toxicity, body temperature ≥38°C, or PS of 2–3. If these toxicities did not recover within 6 weeks from Day 1 of the previous cycle of chemotherapy, subsequent cycles of chemotherapy were stopped. The dose of cisplatin was reduced by 25% in all subsequent cycles if the serum creatinine level rose to 2.0 mg/dL or higher. The dose of vinorelbine was reduced by 25% in all subsequent cycles if any of the following toxicities were noted: WBC count $<1.0 \times 10^9$/L, platelet count $<25 \times 10^9$/L, or Grade 3 infection or liver dysfunction. Thoracic radiotherapy was suspended if any of the following were noted: body temperature ≥38°C, Grade 3 esophagitis, PS of 3, or suspected radiation pneumonitis. Thoracic radiotherapy was terminated if any of the following were noted: Grade 4 esophagitis, Grade 3 or 4 pneumonitis, PS of 4, or duration of radiotherapy of over 62 days (level 1), 67 days (level 2), or 70 days (level 3). Any protocol-defined treatments were terminated if Grade 4 nonhematologic toxicities other than transient electrolyte disturbances or a PS of 4 was noted.

Dose-limiting toxicity and maximum tolerated dose

The DLT was defined as the following toxicities observed during a 6-month period from the start of treatment: (1) Grade 3 esophagitis, lung toxicity, myelitis, dermatitis associated with radiation, and cardiac toxicity associated with radiation, (2) Grade 4 nonhematologic toxicity, or (3) treatment termination due to prolonged toxicity. Twelve patients were enrolled at each dose level. All patients were followed up for at least 6 months to evaluate DLT. During the period, if none to 4 of the 12 patients experienced DLT, the next cohort of patients was treated at the next higher dose level. If 5 or more of the 12 patients experienced DLT, that level was considered to be the MTD. The recommended dose for Phase II trials was defined as the dose preceding the MTD.

Response evaluation

Objective tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.0 (16).

Follow-up

Patients who completed the protocol therapy were followed up to monitor toxicity, response, and recurrence. CT of the chest was performed every 2 to 4 months for 1 year, every 6 months for 2 years, and then yearly for 2 years. The relapse pattern was categorized into (1) local alone, including relapse from the primary site or the hilar, mediastinal, or supraclavicular lymph nodes, (2) distant metastasis alone, including pleural dissemination, pleural and pericardial effusions, and distant metastases, and (3) local and distant.

Statistical analyses

Progression-free survival time (PFS) and OS were estimated by the Kaplan-Meier method. The PFS was measured from the date of registration to the date of disease progression or death resulting from any cause or date of last follow-up. The OS was measured from the date of registration to the date of death resulting from any cause or date of last follow-up. Patients who were lost to follow-up without events were censored at the date of their last known follow-up. A confidence interval (CI) for the response rate was calculated by the method used for exact binomial CIs. The Dr. SPSS II 11.0 software package for Windows (SPSS Japan Inc., Tokyo, Japan) was used for the statistical analyses.

RESULTS

Registration and characteristics of the patients

From August 2005 to September 2008, 57 patients were deemed to initially be eligible. Of these, 3 patients were excluded because idiopathic interstitial pneumonitis ($n = 1$) and anemia ($n = 2$) developed. Explanation of the study using the consent form was given to 54 patients, and informed consent was obtained in 51 patients. The 51 patients underwent 3D treatment planning, and eligibility was finally confirmed in 31 patients. Those 31 were enrolled into this study. A total of 20 patients were excluded as a result of the DVH evaluation: because of V_{20} higher than 30% in 10 patients, overdose to the esophagus in 8 patients, and overdose to the brachial plexus in 2 patients. Eventually, of 17 patients assessed as to their eligibility for dose level 1, 16 patients for dose level 2, and 24 patients to dose level 3, 13 (76%), 12 (75%), and 6 (25%) patients were actually enrolled into levels 1 to 3, respectively (Fig. 1).

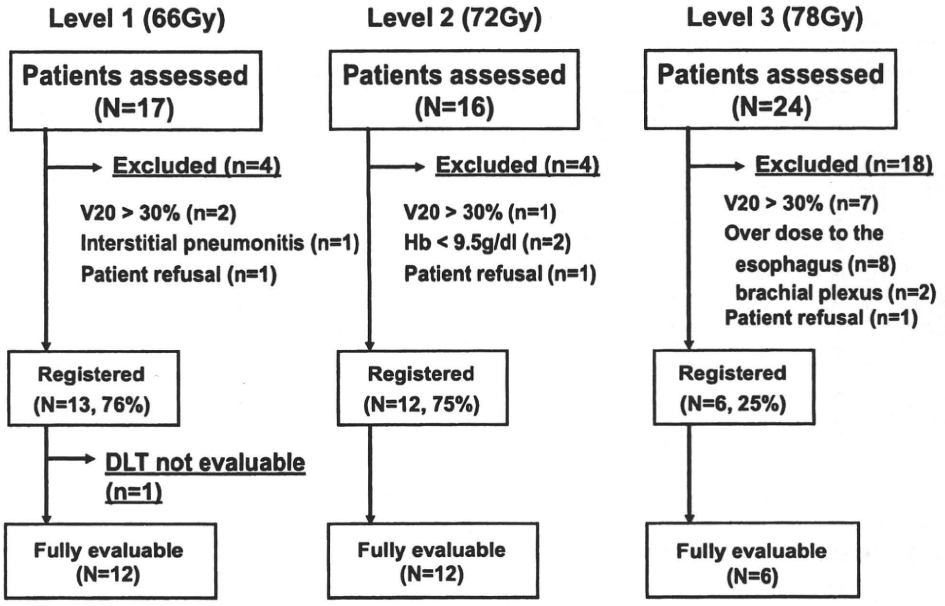

Fig. 1. Algorithm illustrating the flow of the patients. Of the 17, 16, and 24 patients assessed for eligibility, 13 (76%), 12 (75%), and 6 (25%) were actually enrolled at dose levels 1, 2, and 3, respectively.

The pretreatment characteristics of the patients enrolled in this trial are shown in Table 1. The majority of the patients were in good general condition, with a PS of 0 in 25 (81%) and no weight loss in 26 (84%) patients. Adenocarcinoma was the predominantly encountered histological characteristic, seen in 23 (74%) patients.

Treatment delivery

The treatment delivery to the patients was fairly good (Table 2). The planned dose of radiotherapy was administered to all patients of all the three dose levels. More than 80% of the patients received three to four cycles of chemotherapy without or with only one omission of vinorelbine on Day 8, regardless of the dose levels.

Toxicity and DLTs

The hematologic toxicity was comparable to that of other concurrent chemoradiotherapy (Table 3). Grade 4 septic shock was encountered during the fourth cycle of chemotherapy in 1 patient enrolled at dose level 1, but it was manageable by standard care with antibiotics. Other nonhematologic toxicities were mild and acceptable.

Table 1. Patient characteristics

Characteristic	*n*	(%)
Sex		
M	26	(84)
F	5	(16)
Age (y)		
Median (range)	60	(41–75)
Performance status		
0	25	(81)
1	6	(19)
Body weight loss (%)		
0	26	(84)
0.1–5.0	2	(6)
≤5.0	3	(10)
Histology		
Adenocarcinoma	23	(74)
Squamous cell carcinoma	4	(13)
NSCLC, not otherwise specified	4	(13)
Stage		
IIIA	20	(65)
IIIB	11	(35)

Abbreviation: NSCLC = non–small-cell lung cancer.

Table 2. Treatment delivery

	Level 1 (*n* = 13)	Level 2 (*n* = 12)	Level 3 (*n* = 6)
Radiotherapy			
Total dose (Gy)			
66	13 (100)	–	–
72	–	12 (100)	–
78	–	–	6 (100)
Delay (days)			
≤5	11 (85)	5 (42)	5 (83)
6–10	2 (15)	6 (50)	0
11–15	0	1 (8)	1 (17)
Chemotherapy			
No. of cycles			
4	6 (46)	6 (50)	4 (67)
3	6 (46)	4 (33)	2 (33)
2	0	1 (8)	0
1	1 (8)	1 (8)	0
No. of VNR omissions			
0	10 (77)	7 (58)	2 (33)
1	2 (15)	4 (33)	3 (50)
2	0	0	1 (17)
3	1 (8)	1 (8)	0

Abbreviation: VNR = vinorelbine administered on Day 8.

Table 3. Toxicity

	Grade										
	Level 1			(n = 13)	Level 2			(n = 12)	Level 3			(n = 6)
Toxicity	2	3	4	(3+4 %)	2	3	4	(3+4 %)	2	3	4	(3+4 %)
Leukopenia	4	6	2	(62)	1	3	8	(92)	1	3	2	(83)
Neutropenia	4	4	4	(62)	0	1	10	(92)	1	3	2	(83)
Anemia	8	2	2	(31)	7	3	1	(33)	2	2	0	(50)
Thrombocytopenia	0	0	0	(0)	1	1	0	(8)	0	0	0	(0)
Febrile neutropenia	–	1	0	(8)	–	3	0	(25)	–	1	0	(17)
Infection	0	0	1	(8)	0	1	0	(8)	2	0	0	(0)
Esophagitis	1	1	0	(8)	2	1	0	(8)	0	0	0	(0)
Lung toxicity	2	0	0	(0)	0	0	0	(0)	0	1	0	(17)
Anorexia	3	0	0	(0)	2	2	0	(17)	0	0	0	(0)
Nausea	3	0	0	(0)	3	0	0	(0)	0	0	0	(0)
ALT elevation	1	1	0	(8)	0	0	0	(0)	1	0	0	(0)
CRN elevation	7	0	0	(0)	4	0	0	(0)	0	0	0	(0)

Abbreviations: ALT = alanine aminotransferase; CRN = creatinine.

Of the 13 patients at dose level 1, one was excluded from the analysis of the DLT because he received only one cycle of chemotherapy as a result of the development of cisplatin-induced renal toxicity. Two (17%) of the remaining 12 patients at this dose level developed DLT: Grade 3 esophagitis in 1 patient and Grade 4 septic shock in the other. At dose level 2, two (17%) DLTs were noted: Grade 3 esophagitis in 1 patient and treatment delay by more than 15 days in the other. One (17%) of the 6 patients at dose level 3 developed Grade 3 bronchial stenosis without local recurrence of the disease. This was considered to be a Grade 3 lung toxicity and was counted as DLT. No other DLTs were noted. Thus, inasmuch as the incidence of DLT was below 33% at all dose levels, MTD was not reached.

Preliminary efficacy results

Objective responses and survival were evaluated in the 31 patients. Two patients showed complete responses and 27 showed partial responses, which represented a response rate (95% CI) of 94% (79–99). Disease progression was noted in 23 patients, and the median PFS was 11.6 months with a median duration of follow-up of 30.5 (range, 9.0–49.5) (Fig. 2). The first relapse sites aresummarized in Table 4. Brain metastasis alone as the first relapse site was noted in 7 (23%) patients. The median OS was 41.9 months, and the 2-, 3-, and 4-year survival rates (95% CI) were 83.6% (65.0–92.8), 72.3% (51.9–85.2), and 49.2% (26.2–68.7), respectively (Fig. 3).

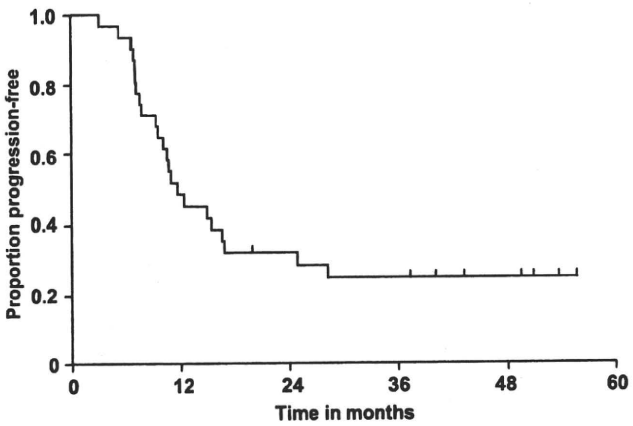

Fig. 2. Progression-free survival (n = 31). The median progression-free survival was 11.6 months, with a median duration of follow-up of 30.5 months (range, 9.0–49.5 months).

DISCUSSION

This study showed that concurrent 3D-CRT to the thorax with cisplatin plus vinorelbine chemotherapy was safe even up to 78 Gy in patients with unresectable Stage III NSCLC. This does not mean, however, that doses as high as 78 Gy can be given to all patients with this disease, because the safety in this study was shown only in highly selected patients by a PET/CT and DVH evaluation and by the standard staging procedure. Twenty-five of the 33 patients met the eligibility criteria for enrollment at dose levels 1 and 2, whereas only 6 of the 24 patients could be enrolled at dose level 3 in this study—that is, only one fourth of the patients could be treated with 78 Gy. Thus, this study showed that 72 Gy was the maximum dose that could be achieved in most patients given the predetermined normal tissue constraints, which forced three quarters of the enrolled patients at the 78-Gy level to not be eligible on the basis of those normal

Table 4. First relapse sites (n = 31)

Sites	n	(%)
Local recurrence alone	6	(19)
Local and distant metastasis	6	(19)
Distant metastasis alone	11	(35)
Brain alone	7	(23)
No relapse	8	(26)

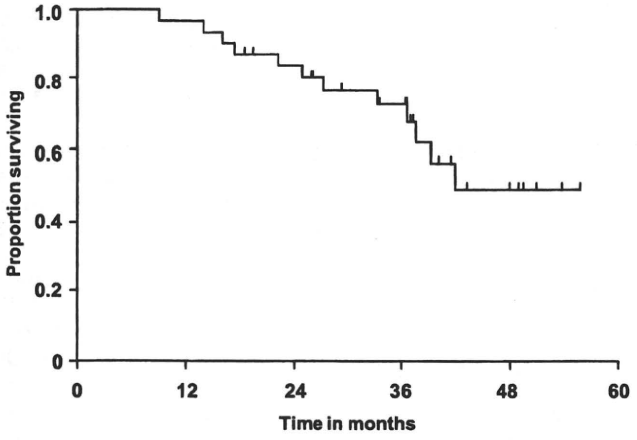

Fig. 3. The median overall survival was 41.9 months, and the 2-, 3-, and 4-year survival rates (95% CI) were 83.6% (65.0–92.8), 72.3% (51.9–85.2), and 49.2% (26.2–68.7), respectively.

tissue constraints, and that the maximum tolerated dose was not determined because of this issue.

One obstacle to enrolling patients at dose level 3 was that the lung V_{20} often exceeded 30% when the total dose was increased to 78 Gy. This lung V_{20} dose constraint might have been too strict. According to a recent review, it is prudent to limit V_{20} to ≤30–35% with conventional fractionation, but there is no sharp dose threshold below which there is no risk for severe radiation pneumonitis (17). This is partly because DVH-based parameters will change at specific phases of the respiratory cycle when CT images for DVH evaluation have been obtained, there is uncertainty regarding how much of the bronchus should be defined as lung, and the lung edges may vary with the CT window level setting. In addition, patient-associated factors such as age, smoking status, lung function, and preexisting lung damage may influence the incidence and severity of radiation pneumonitis (18). If the threshold of V_{20} were set at higher than 30% (*e.g.*, 35%), then more patients would meet the eligibility criteria, but safety might not be guaranteed. Given that the definite threshold cannot be determined, a strict constraint should be introduced. This study showed that the lung toxicity was acceptable when the V_{20} was kept within 30%; therefore, we decided to use this eligibility criterion for concurrent chemotherapy and high-dose radiotherapy for a subsequent Phase II study.

Another obstacle was overdose to the esophagus and brachial plexus, which were close to the subcarinal (No. 7) and supraclavicular lymph nodes, respectively, that were frequently involved in patients with advanced NSCLC; therefore, the volume of these serial organs were included, in part, in the PTV in many patients with Stage III disease. The radiation tolerance doses of these organs have been defined as no higher than 72 Gy when one third of the organs are included in the irradiation volume (19). However, few data are available on the radiation tolerance doses of normal organs in humans; therefore, whether or not radiation doses above 72 Gy may be tolerated is unknown, especially when only small percentages of the organs are actually included in the irradiation volume. Notwithstanding, we do not agree that the radiation dose can be increased close to the intolerable level, because serious radiation toxicity to these serial organs could be irreversible, frequently leaves severe sequelae, and is fatal in some cases.

The toxicity observed in this trial was comparable to that in our previous study of concurrent chemoradiotherapy with vinorelbine and cisplatin chemotherapy plus thoracic radiation at a total dose of 60 Gy administered in 30 fractions: Grade 3–4 neutropenia in 77% and 67% of patients, Grade 3–4 esophagitis in 6% and 12% of patients, and Grade 3–5 lung toxicity in 3% and 7% in the current and previous studies, respectively (5). This suggests that patient selection using PET/CT and DVH evaluation may be useful to keep the toxicity associated with high-dose thoracic radiation within the range of toxicity induced by conventional-dose thoracic radiation.

In this study, a remarkably high proportion (74%) of subjects had adenocarcinoma, which may provide an explanation for the high rate of subsequent brain metastases. Patient selection also affects the treatment efficacy considerably; therefore, it is difficult to compare it between the current and previous studies. However, the median PFS of 11.6 months and median OS of 41.9 months sound promising. We are conducting a Phase II study of concurrent 3D-CRT at a total dose of 72 Gy and chemotherapy with cisplatin and vinorelbine.

In conclusion, concurrent 3D-CRT with cisplatin and vinorelbine chemotherapy was feasible up to 72 Gy, in patients with unresectable Stage III NSCLC. At the level of 78 Gy, however, only 25% of the patients assessed for eligibility were found to be actually eligible. Thus, 72 Gy in 36 fractions was the maximum dose that could be achieved in most patients given the predetermined normal tissue constraints when administered concurrently with cisplatin and vinorelbine.

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ORIGINAL ARTICLE

Phase I and pharmacokinetic study of TSU-68, a novel multiple receptor tyrosine kinase inhibitor, by twice daily oral administration between meals in patients with advanced solid tumors

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Abstract

Purpose A single-agent dose-escalating phase I and pharmacokinetic study on TSU-68, a novel multiple receptor tyrosine kinase inhibitor, was performed to determine the safety profile, maximum-tolerated dose for Japanese patients with advanced solid tumors and to define the recommended dose of phase II studies.

Methods Study design was a dose escalation method on a three-patient cohort. TSU-68 was given orally twice daily (bid) between meals without interruption; the estimation of dose escalation was based on the toxicity within 4 week administration at each dose level.

Results Fifteen patients were enrolled into the study. Dose levels studied were 200, 400, 800, and 1,200 mg/m^2 bid. Grade 3 arrhythmia and anemia/thrombocytopenia were observed in 1 patient each at 800 mg/m^2 bid. Three patients discontinued continuous oral administration for 4 weeks at 400 and 800 mg/m^2 bid. At 1,200 mg/m^2 bid, 2 patients discontinued the treatment over 4 weeks for intolerable fatigue and abdominal pain, respectively. No serious drug-related toxicities have been observed. Grade 1–2 toxicity included urinary/feces discoloration, diarrhea, fatigue, anorexia, abdominal/chest pain, and edema. Tumor shrinkage was observed in 1 patient of NSCLC. In the pharmacokinetics, at any dose levels, C_{max} and AUC_{0-t} after repeated administration of TSU-68 on days 8 and 29 were ~2-fold lower that those after the first administration on day 1; these parameters are similar between days 8 and 28. In addition, no obvious dose-dependent increase in plasma exposure to TSU-68 repeatedly administered was observed over the four dose levels, including the higher dose levels.

Conclusions The tolerable dose in this administration schedule for continuing treatment is thought to be 800 mg/m^2 or less bid.

Keywords Receptor tyrosine kinase inhibitor · Solid tumors · Phase I · Pharmacokinetic

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Introduction

Angiogenesis, the growth of new blood vessels from existing host vasculature, plays a central role in a variety of physiologic and pathologic states. Several families of RTKs such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) receptors have been implicated in this process and

are involved in diverse developmental and oncogenic processes. VEGF has been shown to be the central positive regulator of tumor angiogenesis, since it is reported that the production of VEGF may be increased in many solid tumors and the ability of production may be correlated with the number of blood vessels and prognosis in breast caner, stomach cancer, colon cancer, lung cancer, and others [1–4]. VEGF signaling is an attractive therapeutic target, and the antibodies and inhibitors specifically directed against VEGF and/or its receptor VEGFR2 have been demonstrated to potently prevent vasculature and the growth of a large number of experimental tumor types [5, 6]. FGF and PDGF also play critical roles in angiogenesis, sometimes in concert with VEGF. The prototype FGF family member, FGF2, is a potent mitogen of different cell types including vascular endothelial cells, fibroblasts, and tumor cells, along with its receptors, in a variety of human tumor types [7, 8]. Additionally, FGF2 has been reported to be synergistic with VEGF and to induce the expression of VEGF [9]. FGF is one of the alternative angiogenesis factors during the resistance of VEGF-targeting therapy. PDGF and its receptors have been detected in diverse human cancers, and PDGFRs are expressed on tumor neovasculature and up-regulated during tumor progression [10, 11]. PDGF stimulates angiogenesis by up-regulating VEGF production and modulating the proliferation of pericytes and fibroblast-like cells surrounding the endothelium [12, 13]. Circulating PDGF has been associated with metastases and higher microvessel counts [14, 15].

The signaling cascades generated by these three ligands and their respective receptors are complex, directly and indirectly affecting tumor angiogenesis and tumor growth. In light of the important role of VEGF, FGF, and PDGF and their receptors in tumor angiogenesis and in survival of existing endothelial cells, it is reasonable to expect that simultaneously antagonizing the VEGF, FGF, and PDGF signaling pathways may be more effective than antagonizing one signal transduction alone. TSU-68 is a novel small molecule that competitively inhibits the tyrosine kinase of the receptors for VEGF, basic FGF, and PDGF. TSU-68 has significant antitumor activity against many types of tumor xenograft explants in athymic mice [16]. TSU-68 inhibits angiogenesis through several mechanisms, including the induction of apoptosis in vascular endothelial cells and tumor cells, resulting in perturbing existing tumor vascular function and also inhibiting newly synthesis angiogenesis for the tumor growth [17].

On the basis of these preclinical antiangiogenic and antitumor data, we planned a phase I study of TSU-68 in patients with advanced solid tumors using a continuous twice daily between meals oral administration regimen.

Patients and methods

Study population

Patients with a cytologically or histologically confirmed diagnosis of a solid tumor refractory to standard treatment or for whom no standard therapy was available were eligible for this study. Patients with symptomatic brain metastases were excluded. Further eligibility criteria included the following: age $\geq$20 years and $\leq$75 years; World Health Organization performance status of $\leq$2; life expectancy of $\geq$60 days; no anticancer therapy in the previous 4 weeks; no serious complication (ileus, myocardial infarction, lung fibrosis, and so on); no past history of thrombosis; adequate function of bone marrow (hemoglobin $\geq$8.0 mg/dl, absolute white blood cell count $\geq$4,000/mm^3 or neutrophil count $\geq$2,000/mm^3, and white blood cell count $\leq$12,000/mm^3); liver (bilirubin $\leq$1.5 mg/dl; AST and ALT $\leq$100 IU/ml); renal (creatinine clearance $\geq$50 ml/min); lung (PaO_2 $\geq$65 mmHg); and ability to take oral medication. Local ethics boards approved the protocol and informed-consent brochures in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. All patients gave written informed consent at study entry.

Study design

This study was an open, non-randomized, non-comparative, dose-escalation method on three-patient cohort to adult patients with advanced solid tumors. The trial was designed to comply with the ethical principals of Good Clinical Practice in accordance with the Declaration of Helsinki.

The estimation of dose escalation was based on the toxicity within 4 week administration at each dose level. If no grade 2 toxicity was observed at the previous dose level, a 100% dosage increment was allowed, and if no dose-limiting toxicity (DLT) was observed, a 40% dosage increment was allowed. However, if DLT was observed, a 33% dosage increment was allowed. At each dose level, the third patient was required to have 14 days of treatment before escalation was allowed. Once DLT was seen in one patient at a given dose level, an additional 3 patients had to be treated at that dose level before further dose escalation was allowed. DLT was defined as drug-related adverse events according to National Cancer Institute-Common Toxicity Criteria version 2.0 (NCI-CTC) [18] and comprised any grade 3/4 non-hematologic toxicity and grade 4 hematologic toxicity. The maximum-tolerated dose (MTD) was defined as the dose that induced DLTs in more than 33% of patients during 4 weeks. No intra-patient dose escalation was allowed.

Drug administration

TSU-68, (Z)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid was obtained from Taiho Pharmaceutical Co., Ltd. The starting dosing (200 mg/m^2 twice daily) of Japanese patients was calculated based on the safety results of 4 week toxicology studies in rats and referred to the precedence phase I study of Caucasian patients. At the first day of treatment, patients were given a single dose for pharmacokinetic purposes. The tablet was swallowed immediately more than 2 h after breakfast and supper. Patients were instructed to record their daily amount of tablets taken, the timing in relation to their meals. TSU-68 was taken for 28 consecutive days and was continued in case of stable disease or disease remission after this period for as long as no disease progression and/or no unacceptable drug-related toxicity were seen. Routine antiemetics were not prescribed. TSU-68 administration was immediately interrupted at the occurrence of DLT.

Patient assessment and follow-up treatment

Before therapy, a complete medical history was taken and a physical examination was performed. A complete blood count, including WBC differential, and serum chemistry, including sodium, potassium, chloride, calcium, urea, creatinine, protein, albumin, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, and lactate dehydrogenase, were performed, as were urine analysis, Electrocardiogram, and Chest X-ray. In addition, tumor markers related to the tumor type were measured. Weekly evaluations included history, physical examination, a toxicity assessment according to NCI-CTC, complete blood count, serum chemistries, and urine analysis. Tumor measurements were performed before treatment, at 4 weeks, and were evaluated according to the Japan Society for Cancer Therapy criteria [19], which was established based on World Health Organization (WHO) criteria. The criteria for the evaluation of antitumor effects were as follows: complete response (CR), eradication of all cancers and maintenance of the condition for 4 weeks or more; partial response (PR), 50% or more reduction in size of lesions and maintenance of the condition for 4 weeks or more; no change (NC), less than 50% reduction in size of lesions or enlargement of lesions within 25% and maintenance of the condition for 4 weeks or more; progressive disease (PD), 25% or more enlargement of lesions or appearance of new lesions. In case of progressive disease, patients were taken off the study.

Pharmacokinetic and biomarker sampling

For the pharmacokinetic evaluation, TSU-68 was administered once a day on days 1 and 29 and twice a daily during days 2–28. Blood samples were collected within about 30 min before dosing and at 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, and 12 h after dosing on days 1, 8 and 29. Regarding days 1 and 29, blood samples were also collected at 24 h after dosing. These samples were immediately centrifuged at 3,000 rpm for 10 min at 4°C, after which obtained plasma samples were frozen at −20°C until analysis. Urine samples were collected at the following intervals for measurement of TSU-68: before dosing on day 1, 0–12 and 12–24 h after dosing on days 1 and 29 and 0–12 h after the 1st dosing on day 8. In addition, angiogenesis-related factors such as plasma VEGF, E-selectin, tissue-type plasminogen activator (tPA), vascular cell adhesion molecule-1 (VCAM-1), plasminogen activator inhibitor-1 (PAI-1), and urine VEGF were measured on days 1, 8, and 29.

Pharmacokinetic and biomarker analyses

The pharmacokinetic samples were measured by MDS Pharma Services (Montreal, Canada), and biomarker samples were measured by SRL, Inc. (Tokyo, Japan). TSU-68 concentration was determined using a validated high-performance liquid chromatography method with UV detection, with a lower limit of quantification of 0.1 μg/mL. Non-compartmental pharmacokinetic parameters including area under the plasma concentration–time curves (AUC) from time 0 to the last measurable time (AUC_{0-t}), maximum concentration (C_{max}), time to maximum concentration (T_{max}), and elimination half-life ($T_{1/2}$) were calculated using PhAST (Ver.2.3, MDS Pharma Services, Montreal, Canada).

Statistical analysis

A regression approach was applied to evaluate dose proportionality in pharmacokinetic parameters. Variability in pharmacokinetics between administration days was evaluated by either the paired Student's t-test or an analysis of variance. Statistical significance was considered to be reached at $P < 0.05$. The analysis was performed using the SAS®.

Results

Patient characteristics

Fifteen patients were enrolled in this study between November 2000 and June 2001. All patients were Japanese. Details of dosage level were 3 patients at 200 mg/m^2 bid, 3 patients at 400 mg/m^2 bid, 6 patients at 800 mg/m^2 bid, and 3 patients at 1,200 mg/m^2 bid. Patient characteristics are listed in Table 1. There were 5 men and 10 women with a

Table 1 Patients characteristics

Characteristic		Patients number (%) (15 patients)
Gender		
Male	5	(33)
Female	10	(67)
Age (Years)		
Median	52.0	
Range	27–64	
Performance status		
0	1	(7)
1	14	(93)
2	0	(0)
Tumor type		
NSCLC	6	(40)
Colorectal	3	(20)
Thymoma	2	(13)
Others*	4	(27)

* Including cervical cancer, retroperitoneal cancer, intestinal cancer and sarcoma of uterus

median age of 52 years (range, 27–64 years). All patients were evaluated for safety and pharmacokinetic analyses. Three patients discontinued continuous oral administration for 4 weeks due to adverse events (one patient at 800 mg/m^2) and disease progress (two patients at 400 mg/m^2 and 800 mg/m^2). In addition, five patients continued the medication after 4 weeks, and these included two patients of dose reduction from 1,200 mg/m^2 to 800 mg/m^2.

Dose escalation and maximum-tolerated dose

The dosage was increased from 200 to 400, 800, and 1,200 mg/m^2 bid by cohort of three patients. At a dosage level of 1,200 mg/m^2 bid, two patients discontinued the treatment over 4 weeks for intolerable grade 2 fatigue or grade 2 abdominal pain, which were not critical in evaluating the dose escalation for 4 weeks. These two patients of grade 2 toxicities could continue drug administration for 4 weeks (one cycle); however, both patients had to reduce the dosage level to 800 mg/m^2 bid from the next cycle, and 1,200 mg/m^2 bid demonstrated unacceptable adverse events for an antitumor drug that is used by long-term consecutive oral administration. On the basis of the results of the pharmacokinetic data, increase in the dosage was terminated, and the three additional patients were administered at a lower dosage of 800 mg/m^2 bid. At a dosage level of 800 mg/m^2 bid, one patient was confirmed DLT into a total of six patients. In addition, two patients at 1,200 mg/m^2 could continue TSU-68 administration with a reduction to 800 mg/m^2 after 4 weeks. Thus, protocol-defined MTD was 1,200 mg/m^2 bid, and the acceptable dosage level was estimated 800 mg/m^2 bid in daily administration.

Dose-limiting toxicity and safety profile

There was one protocol-defined DLT as grade 3 arrhythmia at the dosage level of 800 mg/m^2 bid. The patient had a complication with supraventricular arrhythmia but had already been controlled by continuous administration of an antiarrhythmic agent. No other DLTs were recorded.

The major drug-related adverse events for 4 week administration with highest grade pre-event per patient are listed in Table 2. Main toxicities of subjective and objective symptom were diarrhea, fatigue, anorexia, nausea, and vomiting. At lower doses, these symptoms were usually mild and required no specific treatment. Six patients experienced grade 1/2 tumor pain in diverse pain events. Edema and/or pleural effusion progression were seen in 7 patients. In addition, other toxicities consisted of grade 1 urinary/feces discoloration based on drug color (such as saffron yellow).

Drug-related abnormal changes in laboratory values were observed in grade 3 anemia and thrombocytopenia from day 27 in one patient at 800 mg/m^2 bid. Other toxicities experienced in laboratory values were grade 1/2 hypoalbuminemia, grade 1 alkaline phosphatase elevation, and transient grade 1 transaminase elevation.

Four episodes of atrial rhythm abnormalities (atrial fibrillation in a patient and sinus tachycardia in 3 patients with previous cardiac history) were seen. Each patient at 200 mg/m^2 or 1,200 mg/m^2 experienced grade 2 supraventricular arrhythmia or grade 1 arrhythmia; these events were of a transient manner, and there was no relationship to TSU-68. One patient of grade 3 supraventricular arrhythmia at 800 mg/m^2 and one patient of grade 2 atrial fibrillation and grade 1 sinus tachycardia at 1,200 mg/m^2 were treated with medication, and there was a relationship to TSU-68. One patient of grade 3 supraventricular arrhythmia (DLT) with sinus tachycardia (heart rate over 160/min) occurred on day 6, although this patient had taken verapamil for a long time. TSU-68 treatment was discontinued, and he was treated with digoxin in addition to verapamil dose-up. Subsequently, heart rate was decreased to about 80/min.

Pharmacokinetics

Pharmacokinetic studies were performed in 15 patients at doses from 200 to 1,200 mg/m^2. The mean concentration–time profiles in each dosage are shown in Fig. 1. Pharmacokinetic results are presented in Table 3. The relationship between the dose and mean C_{max} or $AUC_{0\text{-}t}$ is plotted in Fig. 2. After the first dose, the plasma concentration of

Table 2 Number of patients with drug-related adverse events for 28-days administration (highest grads per event per patients)

Adverse events	200 mg/m^2 bid (N = 3)			400 mg/m^2 bid (N = 3)			800 mg/m^2 bid (N = 6)			1,200 mg/m^2 bid (N = 3)			Total* (N = 15)		Total** (N = 5)	
	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3	N	(%)	N	(%)
Urine discoloration	3	–	–	3	–	–	6	–	–	3	–	–	15	(100)	5	(100)
Diarrhea	3	–	–	1	–	–	2	2	–	1	1	–	10	(67)	3	(60)
Feces discolored	–	–	–	1	–	–	6	–	–	3	–	–	10	(67)	4	(80)
Fatigue	1	–	–	1	–	–	3	1	–	2	1	–	9	(60)	4	(80)
Anorexia	–	–	–	–	–	–	3	1	–	2	1	–	7	(47)	1	(20)
Abdominal pain	–	–	–	–	–	–	2	1	–	1	1	–	5	(33)	1	(20)
Face edema	1	–	–	1	–	–	1	–	–	2	–	–	5	(33)	3	(60)
Nausea	1	–	–	–	–	–	2	–	–	–	1	–	4	(27)	1	(20)
Upper abdominal pain	1	–	–	–	1	–	–	–	–	2	–	–	4	(27)	–	–
Chest pain	–	1	–	1	–	–	–	–	–	1	–	–	3	(20)	2	(40)
Vomiting	–	–	–	–	–	–	2	–	–	–	1	–	3	(20)	1	(20)
Alkaline phosphatase increased	1	–	–	1	–	–	3	–	–	1	–	–	6	(40)	2	(40)
Albumin decreased	–	–	–	–	1	–	4	–	–	–	1	–	6	(40)	–	–
Alanine aminotransferase increased	1	–	–	–	–	–	2	–	–	–	–	–	3	(20)	3	(60)
Total protein decreased	–	–	–	–	–	–	3	–	–	–	–	–	3	(20)	–	–
Arrhythmia	–	–	–	–	–	–	–	–	1	–	–	–	1	(7)	1	(20)
Anemia	–	–	–	–	–	–	–	–	1	–	–	–	1	(7)	–	–
Thrombocytopenia		–	–	–	–	–	–	–	1	–	–	–	1	(7)	–	–

G common toxicity criteria grade 1 is mild, a grade 2 is moderate, and grade 3 is severe

* The advanced events listed here were reported in >20% of patients and the grade 3 events of patient

** Number of patients with drug-related adverse events after 28 days (200 mg/m^2 bid; N = 1,400 mg/m^2 bid; N = 1,800 mg/m^2 bid; N = 3)

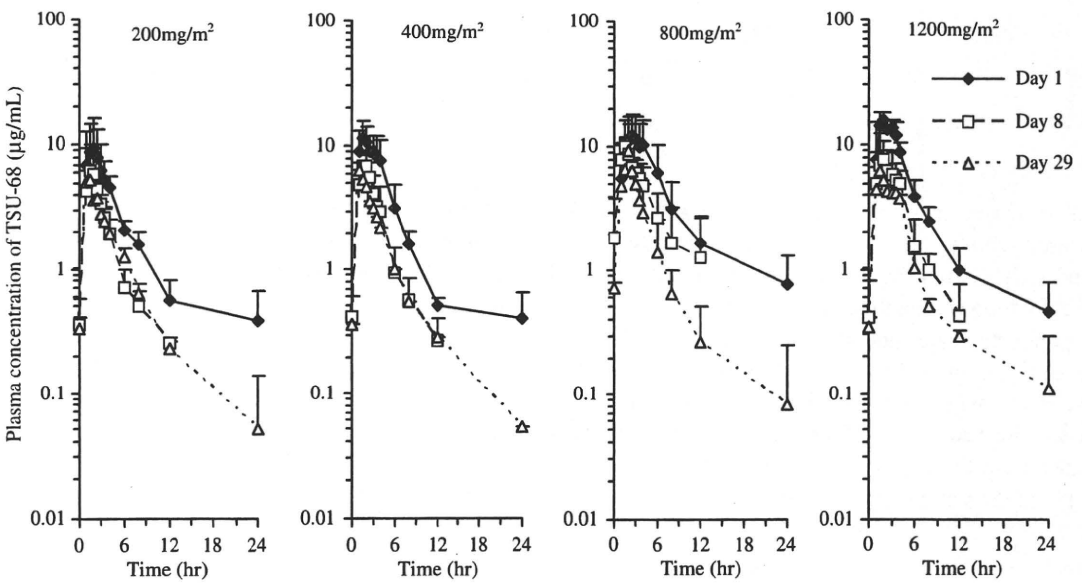

Fig. 1 Plasma concentration-versus-time profile of TSU-68

Table 3 Pharmacokinetic parameters of TSU-68 after day 1, day 8, or day 29 administration

PK Parameter	Dose (mg/m^2)	Pts. no.	Day 1	Day 8	Day 29
T_{max} (h)	200	3	2.3 ± 1.5	1.7 ± 0.3	1.7 ± 0.8
	400	3	2.5 ± 1.3	1.8 ± 0.3	1.0*
	800	6	2.8 ± 1.1	1.5 ± 0.4**	2.1 ± 0.3***
	1,200	3	2.2 ± 0.8	1.8 ± 0.3	1.7 ± 0.3
C_{max} (μg/mL)	200	3	10.552 ± 6.042	6.669 ± 3.698	6.354 ± 3.775
	400	3	11.667 ± 3.807	7.212 ± 1.060	6.351*
	800	6	14.620 ± 5.649	10.564 ± 1.831**	10.732 ± 4.690***
	1,200	3	16.004 ± 1.910	8.148 ± 4.219	6.706 ± 0.884
AUC_{0-t} (μg h/mL)	200	3	45.6 ± 21.0	20.6 ± 6.2	21.2 ± 9.5
	400	3	58.2 ± 16.8	25.0 ± 9.4	22.9*
	800	6	83.3 ± 32.7	44.6 ± 17.3**	30.0 ± 16.7***
	1,200	3	76.6 ± 1.0	32.9 ± 16.2	25.8 ± 2.4
$T_{1/2}$ (h)	200	3	8.03 ± 3.82	4.80 ± 2.14	3.59 ± 1.71
	400	3	7.89 ± 4.04	3.53 ± 0.43	5.36*
	800	6	9.93 ± 6.01	4.31 ± 2.72**	4.37 ± 4.05***
	1,200	3	8.33 ± 4.65	2.78 ± 0.90	4.84 ± 3.29

* 2 patients, ** 5 patients, *** 4 patients

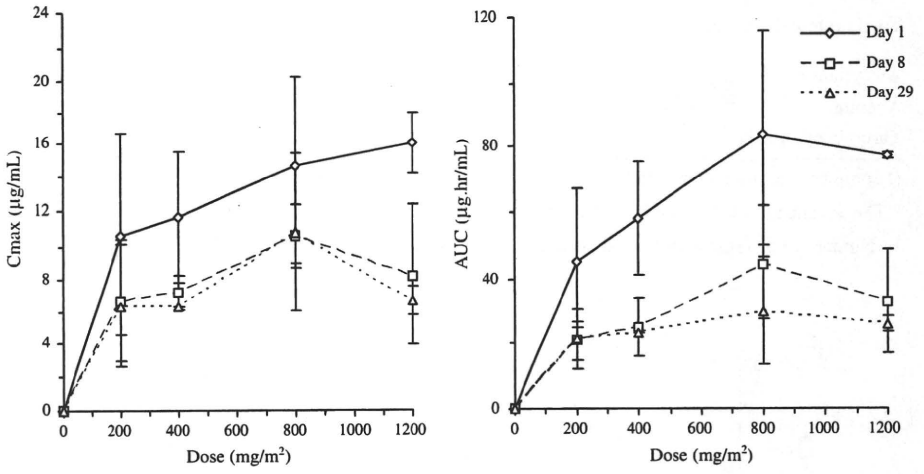

Fig. 2 Effect of dose on the C_{max} and AUC

TSU-68 increased to reach C_{max} at ~2.5 h and thereafter disappeared with $T_{1/2}$ in ~8–10 h. At the any dose levels, C_{max} and AUC_{0-t} after the repeated doses on days 8 and 29 were ~2-fold lower than those after the first doses on day 1. These parameters are not statistically different between days 8 and 29. In addition, no obvious dose-dependent increase in plasma exposure to TSU-68 repeatedly administered was observed over the four dose levels, most notably the higher dose levels. Urinary excretion of TSU-68 was below 1% of dose at the any dose levels.

Response

No partial or complete responses were seen. One patient with adenocarcinoma of the lung had a minor response by 4 week treatment (Fig. 3) and continued 3 months, but after that the treatment discontinued because of tumor progression and drug-related toxicity. The increased effusion was confirmed non-malignant, and this event was considered a drug-related toxicity. The size of the target lesion reduced from 5.5 cm × 5.0 cm of the base line to 4.5 cm × 4.3 cm of 4 week treatment (29.6% reduction). Reduction of tumor marker levels was seen in the same patient (CEA 22.6 → 12.6 ng/ml, SLX 74 → 58 U/ml). Four patients had stable disease (non-small cell lung cancer 2; thymoma 1; peritoneal sarcoma 1) and continued treatment over two cycles until disease progression (until 5 cycle for patients with peritoneal sarcoma and 2 cycles for the rest of the patients).

In the angiogenesis-related biomarker of the six tested factors with 14 patients, not including one patient of DLT,

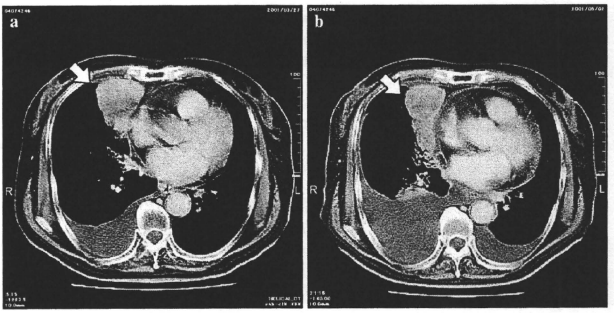

Fig. 3 Computed tomography scan of a tumor minor shrinkage in a patient with adenocarcinoma of the lung, and pleural effusion progression, treated with TSU-68. **a** Baseline; **b** week 4. *White arrow* primary tumor in the lung

an average of PAI-1 and urine VEGF saw an increase of more than 20% over the baseline (from 24.3 ± 6.7 ng/ml to 29.5 ± 11.3 ng/mL and from 96.6 ± 62.4 pg/ml to 132.1 ± 93.6 pg/mL, respectively), but plasma VEGF, E-selectin, tPA and VCAM-1 were not changed substantially.

Discussion

We performed a phase I and pharmacokinetic study to explore safety, tolerated dose, and pharmacokinetics of the oral multiple tyrosine kinase inhibitor TSU-68 in Japanese patients with advanced solid tumors. In this study using continuous oral bid administration between meals, side effects attributable to the study drug were subjective/objective symptom and abnormal changes in laboratory values, whereas DLT included arrhythmia.

The subjective and objective symptom toxicities of TSU-68 in our current study were predominantly gastrointestinal and consisted of dose-dependent, non-cumulative, and reversible diarrhea, fatigue, anorexia, and abdominal pain. At lower dose levels, these symptoms were mild and needed no additional treatment. At the highest dose level, 2 patients discontinued the treatment over 4 weeks for intolerable fatigue and abdominal pain, respectively (Table 2). Some patients with abdominal pain were given gastrointestinal endoscopy, but no remarkable finding was observed. It was considered a reasonable assessment to stop the dose escalation under grade 2 fatigue and grade 2 abdominal pain at a dosage level of 1,200 mg/m^2 bid, because of unacceptable adverse events for an antitumor drug that is used in long-term consecutive oral administration.

In 7 patients, edema and/or pleural effusion progression were seen as dose independent (for example: Fig. 3). Edema was remarkable on the face and eyelids. In addition, 11 patients experienced hypoalbuminemia dose dependently. Although this was seen from an early period, it improved immediately after therapy discontinuance. Although VEGF is also known as a vascular permeability factor [20, 21], the contribution of VEGF is unclear with no significant difference between patients with edema or effusion progression and patients without. VEGF level in pleural effusion was not elevated in some patients (data not shown). On the other hand, there is a possible contribution of PDGF in this phenomenon based on the report that edema was seen in about 40% patients including 7% grade 3/4 treated with Imatinib, which is an inhibitor of Bcr/Abl tyrosine kinase and PDGF/Kit tyrosine kinase and approved for chronic myeloid leukemia, Philadelphia chromosome positive acute lymphoblastic leukemia and gastrointestinal stromal tumor [22–24]. It was also reported that PDGF levels of pleural effusion with lung cancer were higher than in non-malignant pleural effusions [25].

Six patients experienced tumor pain progression such as chest pain from primary or metastatic lung cancer. This may be due to drug intake, because tumor pain was diminished after being taken off the drug. To clarify the mechanism of these symptoms, additional studies including basic research are needed.

The hematologic toxicity of TSU-68 in the current study was considered dose independent. Grade 3 anemia and thrombocytopenia were seen in the same patient at 800 mg/m^2 dose level. Thrombocytopenia was recovered to pretreatment level after treatment discontinuance, but anemia continued. This patient was given radiotherapy after 9 days due to bone metastasis. Therefore, anemia was not attributed to TSU-68 intake but tumor progression.

These toxicity findings, characterized as edema, were much different from the reports of recent approved and developing angiogenesis inhibitors showing bleeding, perforation, hypertension, hand-foot syndrome, and some leukocytopenia as the distinguishing toxicity profile [26–28].

In the pharmacokinetics, the C_{max} and AUC were similar between days 8 and 29, although decreased by half after the

repeated administration of TSU-68. This suggests that the decreased plasma exposure to TSU-68 rapidly reaches the steady state and is maintained over therapeutic cycles. This trend is consistent with a published clinical result showing that AUC of TSU-68 on day 56 was similar to that on day 28 [29]. In addition, at the higher dose levels, there was no dose-dependent increase in these parameters of TSU-68 repeatedly administered, which is probably due to a saturation of absorption. The trough plasma level in the steady-state exposure to 200 mg/m^2 TSU-68 is above the IC50 for VEGF and PDGF receptors cellular assay, and the exposure was also comparable with that showing efficacy in human cancer xenograft models [16]. The decrease in the exposure after repeated administration is probably due to autoinduction of TSU-68 metabolism. Since urinary excretion accounted for a very low percentage of the dose, predominant elimination of TSU-68 can be regarded as hepatic metabolism. Non-clinical studies [30, 31] suggest that TSU-68 causes induction of liver cytochrome P450, CYP1A1/2 involved in its own metabolism, leading to the decrease in the TSU-68 plasma concentrations.

A phase I study of the United States was reported before our Japanese study. Nineteen Caucasian patients were treated at doses ranging from 400–1,200 mg/m^2 fasted and 100–400 mg/m^2 fed by twice daily administration [32]. Dose-limiting toxicities including fatigue, pleuritic chest pain, shortness of breath, and pericardial effusions were seen in 2 patients. The AUC declined from 70 μg.h/ml on day 1 to 32 μg.h/ml on day 28. The results for TSU-68 in Japanese patients demonstrated a similar toxicity and pharmacokinetics profile to Caucasian patients.

Antitumor effect was observed in some patients in this clinical study. One patient with adenocarcinoma of the lung had a minor tumor shrinkage and reduction of tumor marker levels. In addition, four patients had no disease progression after one cycle treatment. Although angiogenesis inhibitors such as TSU-68 have been thought to have little or no tumor shrinkage, some drugs showed obvious tumor regression.

Although the primary objective of phase I clinical trial is not to see response but to determine recommended dose for phase II clinical trial, tumor response is thought to be an important factor. Therefore, further studies using this agent are expected. MTD was not reached in this study, because DLT was only seen in one patient at 800 mg/m^2. We suggest the highest dose level was not the tolerable dose, because two patients discontinued the treatment for drug-related toxicities in spite of their being low grade. In addition, pharmacokinetic data of this level showed no significant difference compared with other dose levels, and no dose-dependent increase on days 8 and 29.

In conclusion of this phase I and pharmacokinetic study with continuous oral bid between meals, TSU-68 has shown that a multiple receptor tyrosine kinase inhibitor can be safely administered. The recommended dose for further studies using this treatment schedule is less than 800 mg/m^2 bid.

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ORIGINAL ARTICLE

Phase I, pharmacokinetic, and biological studies of TSU-68, a novel multiple receptor tyrosine kinase inhibitor, administered after meals with solid tumors

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Abstract
Purpose TSU-68 is a low molecular weight inhibitor of the tyrosine kinases for vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor β, and fibroblast growth factors receptor 1. In this study, we assessed the recommended dose with TSU-68 administration of twice-daily (b.i.d.) or thrice-daily (t.i.d.) after meals for 4 weeks in Japanese patients with solid tumors based on the safety and tolerability and investigated the relationship between angiogenesis biomarker and clinical outcomes.
Methods The study design was a dose-escalation method with alternating enrollment of b.i.d. administration and t.i.d. administration after meal by traditional three-patient cohort.
Results We enrolled 24 patients at doses of 200, 400, and 500 mg/m^2 b.i.d. or 200 and 400 mg/m^2 t.i.d. No dose-limiting toxicity (DLT) occurred in the 200 mg/m^2 b.i.d. or t.i.d., and 3 patients experienced DLTs at 400 mg/m^2 b.i.d. or 400 mg/m^2 t.i.d. As main toxicity, blood albumin decreased, malaise, diarrhea, alkaline phosphatase increased, anorexia, abdominal pain, nausea, and vomiting were observed as almost all grade 1–2. There were no apparent differences in pharmacokinetic parameters between days 2 and 28 after the repeated b.i.d. and t.i.d. doses. Although tumor shrinkage was not observed, the disease control rate was 41.7%. As an angiogenesis-related factor of stratified analysis, plasma vascular endothelial growth factor and plasminogen activator inhibitor-1 were detected as a significant increase with progressive disease patients.
Conclusions A recommended dosage of TSU-68 for this administration schedules was estimated to be 400 mg/m^2 or less b.i.d.

Keywords Receptor tyrosine kinase inhibitor · Solid tumors · Phase I · Pharmacokinetic

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Introduction

Angiogenesis is essential for the proliferation of malignant tumors and development of its metastasis [1]. When a tumor grows to be 2–3 mm or more in the course of proliferation, it may produce angiogenesis-stimulating growth factors by acting on itself and its surrounding normal cells to supply oxygen and nutrition. Such growth factors may induce digestion, migration/proliferation, and formation of lumens of the basement membrane of endothelial cells, leading to formation of a new vascular nest. This may enlarge the lesion, resulting in infiltration